Development and Evaluation of *Cassia fistula* Seed Gum as Drug Delivery Excipients

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PROFORMA – 1

"Statement of Originality"

I, **ABHIJIT CHANGDER** registered on 25.01.2017 do hereby declare that this thesis entitled **"Development and Evaluation of *Cassia fistula* Seed Gum as Drug Delivery Excipients."** contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iTenticate software is **9 %**.

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PROFORMA – 2

"Certificate from the Supervisors"

This is to certify that the thesis entitled **"Development and Evaluation of *Cassia fistula* Seed Gum as Drug Delivery Excipients."** submitted by **Mr. Abhijit Changder**, who got his name registered on 25.01.2017 for the award of **Ph. D. (Pharmacy)** degree of Jadavpur University is absolutely based upon his own work under the supervision of **Prof. (Dr.) L. K. Ghosh,** Professor and Former Head, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700032 and **Dr. Gouranga Nandi,** Assistant professor, Department of Pharmaceutical Technology, University of North Bengal, Raja Rammohunpur, Darjeeling, West Bengal, India, 734013 and that neither his thesis nor any part of the thesis has been submitted for any degree/ diploma or any other academic award anywhere before.

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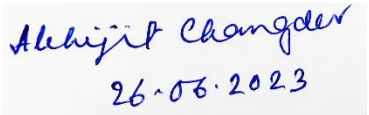


Preface

Nature has given us a vast range of resources that may either directly or indirectly be used to enhance and maintain the health of all living things. With the growing interest in using polymers and excipients of natural origin, gums and mucilage are often utilised natural materials for both traditional and new drug delivery methods; the pharmaceutical industry has agreed to employ the majority of them in their formulations. Due to their numerous pharmaceutical applications as diluents, binders, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspension, gelling agents in gels, and bases in suppositories, as well as their use in cosmetics, textiles, paints, and paper making, plant-derived polymers have attracted a lot of attention in recent years. The need for these compounds is always rising, and new sources are often being created. India has historically been a strong source for these items among the Asian nations due to its diverse geography and ecology. To keep up with the rising demand, huge amounts of these items are still being imported from Europe. These naturally available gums can be used without altered form and modified to obtain tailor-made materials for drug delivery systems, giving them an advantage over commercially available synthetic alternatives. Natural polysaccharides possess some advantages such as easy availability, low cost, physical and chemical compatibility with wide range of drugs, biocompatibility, and biodegradability. Being hydrophilic they also allow green manufacturing without use of harsh organic solvents. A significant number of natural polysaccharides such as gum acacia, sodium alginate, gelatin, guar gum, xanthan gum, starch, etc. have been widely used as excipients from very beginning.

Cassia fistula trees belonging to fabaceae family are native to Indian subcontinent and southeast countries. The endosperm of seeds of this plant is rich in galactomannan polysaccharide which is nonionic in nature and chemically composed of β-(1→4) linked poly-D-mannopyranose backbone

with a randomly distributed side chain of α-(1→6) linked D-galactopyranose unit. *Cassia fistula* seed galactomannan (CFSG) has been reported as film coating agent and its carboxymethylated form has been reported as disintegrating and controlled-release agent. Native form of CFSG has not been reported as tablet binding excipient to date and it is a promising galactomannan like tamarind seed gum and locust bean gum, which might be a potential drug delivery candidate after appropriate tailoring or derivatization. One of the aims of the present investigation was to explore the capability of native CFSG as tablet binder and another one was to chemically functionalize CFSG towards its application as gastro-retentive mucoadhesive sustained release polymer.

In this study one part evaluate the native CFSG as tablet binder polymeric excipient. Water-soluble diclofenac sodium was used as model drug. CFSG was extracted, purified and characterized with polysaccharide content determination, monosaccharide composition analysis, elemental analysis, FTIR, solid-state ^{13}C NMR, molecular weight, zeta potential, DSC, TGA-DTA, XRD, viscosity, pH and surface tension, rheology, SEM and acute oral toxicity study. Prior formulation, the drug-CFSG compatibility was checked by FTIR, DSC and XRD. Diclofenac sodium loaded granules were prepared by wet granulation method and evaluated for various granule properties such as granule-size, bulk density, true density, total porosity, angle of repose, Hausner ratio and Carr's compressibility index. Finally, granules were compressed into tablet and evaluated for apparent density, porosity, packing fraction, percent elastic recovery, weight and content uniformity, hardness, friability, disintegration time and drug dissolution. The binding capacity of CFSG was also compared with standard binder such as gum acacia and polyvinylpyrrolidone (PVP K-30).

Second part of this study, investigate the synthesis, characterizations and fabrication of CFSG-g-PSA into aceclofenac loaded gastric-mucosa-adhesive sustained-release tablet. The copolymer was synthesized by microwave-assisted free-radical initiation method using CAN as free-radical

initiator and then characterized by elemental analysis, FTIR, NMR, viscosity, DSC, TGA-DTA, PXRD, SEM and biodegradation study. Finally, aceclofenac sustained-release tablets were prepared with various batches of graft-copolymer by wet granulation method and evaluated for drug-release, mucoadhesion and other parameters. The release-kinetic and mucoadhesive strength were compared to marketed and established polymers such as HPMC and carbopol 974P.

In the chapter-I of this thesis, the concept of excipients and categorization of excipients by its route of administration, origin and functionality have been briefly discussed. In chapter-II discussed about Natural polysaccharide-based drug delivery excipients, in chapter-III, briefly discussed about chemically modified natural polysaccharide as drug delivery excipients. The chapter-IV, existing information about *Cassia fistula* seed gum along with its origin, phytochemical composition and chemical structure have been discussed. In Chapter V, different gastroretentive mucoadhesive dosage forms have been presented and the chapter VI covers the sustained release dosage forms. In chapter VII, includes a review of the previous works reported towards binding properties of natural gums and their application in sustained release drug delivery systems have been performed. The chapter-IX drugs' profiles and excipients profiles respectively. Experimental works and result discussion have been presented in chapter-X and chapter XI, including experimental design, preparation of gastro retentive-mucoadhesive tablets and their evaluations and optimization. Chapter-XII contains the summary and conclusion of the present study.

LIST OF FIGURES

LIST OF TABLES

CONTENTS	

CONTENTS	

CONTENTS	

CONTENTS	
CHAPTER X	
10. EXPERIMENTAL WORKS: MATERIALS & METHODS	**146**
10.1. Drug used	**146**
10.2. Chemicals used	**146**
10.3. Instrument handled	**147**
10.4. Evaluation of *Cassia fistula* seed gum (CFSG) as tablet binder	**149**
10.4.1. Determination of λ_{max} of Diclofenac sodium in phosphate buffer pH 7.5	**149**
10.4.2. Preparation of Standard calibration curve of Diclofenac sodium in phosphate buffer pH 7.5	**149**
10.4.3. Extraction and purification of CFSG	**152**
10.4.4. Characterization of CFSG	**152**
10.4.5. Preformulation studies	
10.4.6. Preparation and characterization of drug loaded granules	**158**
10.4.7. Compression and evaluation of diclofenac sodium loaded tablets	**159**
10.4.8. Statistical analysis	**162**
10.5. Chemical modifications of *Cassia fistula* seed gum through graft copolymerization with poly (sodium acrylate) and used as gastroretentive drug delivery excipients	**163**
10.5.1. Determination of λ_{max} of Aceclofenac in0.1 (N) Hydrochloric Acid with 0.2% sodium lauryl sulphate (SLS) Medium	**163**

CONTENTS	

LIST OF ABBREVIATIONS

F	Degree Fahrenheit
C	Degree Centigrade
&	And
<	Less than
>	Greater than
±	Plus or minus
θ	Theta
Σ	Summation
μ	Micro
β	Beta
Avg.	Average
e.g.	For example
i.e	That is
Fig.	Figure
mcg	Microgram
mg	Milligram
gm	Gram
ml	Milliliter
min	Minute
Hr.	Hour
no.	Number
rpm	Rotation per minute
USP	United States Pharmacopoeia
IP	Indian Pharmacopoeia

BP	British Pharmacopoeia
UV	Ultra violet
FTIR	Fourier Transform Infra-Red
PXRD	Powder X-ray diffraction
DSC	Differential scanning calorimetry
SEM	Scanning electron microscopy
HPLC	High performance liquid chromatography
FDA	Food and Drug Administration
ICH	International Commission for Harmonization
Amax	Maximum absorbance
HCI	Hydrochloric Acid
NaOH	Sodium Hydroxide

CHAPTER - I

INTRODUCTION

1. Introduction

Nature has given us a vast range of resources that may either directly or indirectly be used to enhance and maintain the health of all living things. With the growing interest in using polymers and excipients of natural origin, gums and mucilage are often utilised as natural materials for both traditional and new drug delivery methods; the pharmaceutical industry has agreed to employ the majority of them in their formulations. Due to their numerous pharmaceutical applications as diluents, binders, disintegrants in tablets, thickeners in oral liquids, protective colloids in suspension, gelling agents in gels, and bases in suppositories, as well as their use in cosmetics, textiles, paints, and paper making, plant-derived polymers have attracted a lot of attention in recent years. The need for these compounds is always rising, and new sources are often being created. India has historically been a strong source for these items among the Asian nations due to its diverse geography and ecology. To keep up with the rising demand, huge amounts of these items are still being imported from Europe. These naturally available gums can be used without altered form and modified to obtain tailor-made materials for drug delivery systems, giving them an advantage over commercially available synthetic alternatives. Natural polysaccharides possess some advantages such as easy availability, low cost, physical and chemical compatibility with a wide range of drugs, biocompatibility, and biodegradability. Being hydrophilic they also allow green manufacturing without use of harsh organic solvents. A significant number of natural polysaccharides such as gum acacia, sodium alginate, gelatin, guar gum, xanthan gum, starch, etc. have been widely used as excipients from very beginning.

Cassia fistula trees belonging to the Fabaceae family are native to the Indian subcontinent and southeast countries. The endosperm of seeds of this plant is rich in galactomannan polysaccharide which is non-ionic in nature and chemically composed of β-(1→4) linked poly-D-Mann pyranose backbone with a randomly distributed side chain of α-(1→6) linked D-galactopyranose unit. *Cassia fistula* seed galactomannan (CFSG) has been reported as film coating agent and its carboxymethylated form has been reported as disintegrating and controlled-release agent. Native form of CFSG has not been reported as tablet binding excipient to date and it is a promising galactomannan like tamarind seed gum and locust bean gum, which might be a potential drug delivery candidate after appropriate tailoring or derivatization.

Excipients are the ingredients which are applied with the active pharmaceutical ingredients to manufacture a perfect formulation. In maximum formulation these excipients are present in more amounts than the active pharmaceutical ingredient [1]. This word excipient it's actually a Latin word, which comes from this word excipere that means to receive, to gather, to take out. As time went on, we learned new explanations about the excipients, like in the year of 1957 the definition of excipient was; 'Excipients are the substance which is used as a medium for giving a medicament'. In the year of 1974 the term excipients are defined as 'any small or large amount of substance which is inert that is added to a prescription with suitable consistency or formulation of drug. According to the US' NF (United State of National Formulary) of 1994, the definition of the term excipient is any substance other than the API (active pharmaceutical ingredient) which is intentionally combined, or it is whole thing in the total formulation other than the API [2]. As it is previously said that excipients are used in the formulation in huge amounts than the APIs, so the selection of these excipients

must be done with care. We normally apply these excipients along with the active pharmaceutical ingredients for many reasons like;

- Excipients acts as a protecting agent, supporting agent or enhancing the stability of the formulation
- Excipients act as a bulking agent
- Sometimes excipients are used to increase the patient acceptability
- Excipients are used to improve the bioavailability of the active pharmaceutical ingredients
- Excipients are used to improve the safety and efficacy issue

1.1. Categorization of excipients:

Pharmaceutical excipients are categorized in different ways like:

1.1.1. Depending upon the route of administration

- Excipients for oral administration
- Excipients for topical use
- Excipients for parenteral preparation
- Other the above excipients

1.1.2. Depending upon the origin

- ***Inorganic chemicals***

  Calcium sulfate, calcium carbonate, halites, calcium phosphate metallic oxides

- ***Organic chemicals***
  - *Carbohydrate:* Sugar, sugar alcohol, unreal sweeteners
  - *Starch:* Modified starch as well as dried starch, starch which is converted

- *Cellulose:* Cellulose ethers as well as esters, carboxymethyl cellulose (CMC), croscarmellose sodium, MCC (microcrystalline cellulose)
- *Petrochemicals:* Poly ethylene glycols (PEG), propylene glycols (PG)
- *Povidones*
- *Mineral hydrocarbons:* Petrolatums, mineral waxes as well as oils
- *Acrylic polymers*
- Other petrochemical excipients
- *Oleochemicals:* Fatty alcohols, mineral stearates, glycerine, lipids
- *Proteins*

- ***Animal source:*** Lactose, Stearic acid, Beeswax, Musk, Lanolin, Honey
- ***Vegetable source:*** Starch, Acacia, Peppermint, Arginate, Turmeric, Guar gum

1.1.3. Depending upon the function:

- Fillers and diluents
- Binders or binding agents
- Suspending & emulsifying agents
- Viscosity enhancer
- Coating agents
- Disintegrating agents
- Lubricants & glidants
- Humectants
- Plasticizer
- Co-solvents
- Tonicity agents
- Chelating/sequestering agent
- Ointment/suppository base

- Preservatives
- Antioxidants
- Sweetening agent
- Flavouring agent
- Colouring agent
- Excipients for direct compression

1.2. Ideal characteristics of excipients:

Excipients which are used in pharmaceutical formulation must have some characteristics. Selection of these excipients are dependent on these characteristics.

- Excipients must be chemically stable
- They must be non-reactive with other material or ingredients used in the dosage form
- Excipients should not show any toxic activities
- Excipients must be acceptable regarding organoleptic characteristics
- They must be low in cost or in-expensive
- They must have efficacy regarding intended use
- They bust be inert
- They should not react with the drug component, the packaging material, the other excipients
- They must be easily available
- They must be stable and compatible [3].

1.2.1. Excipients used in solid dosage form and their purpose [4]:

Type of excipients	**Purpose of use**	**Working mechanism**	**Examples of excipients**
Diluent	Use to increase the volume of the formulation or acts as a filler	Where drug is insufficient to produce the bulk then diluent added to make up the volume	Lactose, dextrose, sorbitol, microcrystalline cellulose, direct compressible starches
Binder & adhesive	Used to form granule	Increase the cohesive force between the powder particles, improve the flow quality and form granule	Acacia, Starch paste, gelatin, PVP(polyvinyl pyrrolidone), glucose, CMC (carboxymethyl cellulose), povidone
Lubricant	Reduce the inter particle erosion between the, tablet and the die metal surface, induce the ejection of tablet from the die cavity	Makes a film around the tablet mass with reduce the stickiness between the tablet and the die cavity wall	Talc, stearic acid, magnesium stearate, polyethylene glycol, surfactants, vegetable oils

Glidant	Increase the flowability of the powder material in formulation	Decrease the friction between the powder particle	Colloidal silicon dioxide (carbosil), asbestos free starch, corn starch
Disintegrant	Breaking the dosage form into granules	They absorb water then swells up and finally bursts forming the granule	Starch, clay, cellulose, cross linked polymer, modified starch, cross carmalose, cross povidone
Super disintegrant	Increase the disintegrant efficacy		
Colouring agent	Improves the appearance and helps in identification		FD and C, D and C dyes and lakes
Flavouring agent	To mask the unpleasant taste		Spray dried and other flavours
Sweetening agent	Improve the sweet taste, musk the bitterness		Mannitol, saccharine, aspartame, sucrose
Sorbent	To make the formulation moisture proof	Taking up the liquids or gases by absorption or by adsorption	Silica gel, activated charcoal, clay
Coating material	To protect the tablet from deterioration,		Hydroxypropylmeth yl cellulose, shellac,

	makes the dosage form pH sensible, make the formulation-controlled release		synthetic polymers, gelatin, povidone, ethyl cellulose
Plasticizer	Used for formulation of soft gelatin capsule, gelatin-based suppositories	In case of tablet, it improves the elasticity and plasticity of the coating material, determine the hardness of the capsule, impart the softness of the suppositories	Castor oil, polyethylene glycol, propylene glycol

1.2.2. Excipients used in liquid dosage form and their purpose [5]:

Types of excipients	**Purpose of use**	**Working mechanism**	**Example**
Solvents	To solubilize all of the ingredients including active pharmaceutical materials	This agent breaks the bond and decrease the electric charge on ions and in this way the solvation process gets enhanced which is higher	Water, ethyl alcohol, anhydrous citric acid, syrups and acetone

		than the intermolecular attraction between solvent particles as well as solute particles	
Co-solvents	To enhance the solubilization capacity of the solutes	These agents actually reduce the interfacial tension between the hydrous solution and lipophilic solute ingredients	Ethyl alcohol, sorbitol solution(w/v), glycerine, PG
Buffers	For maintaining the whole formulation's pH to make the formulation stable	These agents bind with the H^+ ions in acids or take H^+ ions and gives H^+ ions to the base	Phosphate buffers, acetate buffers, citric acid
Antimicrobial & preservatives	To prevent the bacterial and microbial growth in the formulation in normal situation as well as in storage condition	These agents act by their bacteriostatic or bactericidal action	Sodium benzoate, benzyl alcohol, methyl paraben, propyl paraben, thiomersal
Antioxidants	To prevent the formulation from get oxidized	These agents block the oxidative chain reaction	Ascorbic acid, sodium bisulfide, thiourea, butyl

			hydroxyl toluene, tocopherols
Wetting agents	Used for wetting and dispersing the pharmaceutical ingredients which are hydrophobic	These agents decrease the interfacial tension between the solid and liquid in the suspension	Spans, tween 80, sodium lauryl sulphate, lecithin
Anti-foaming agents	To prevent foam formulation	These agents decrease the surface tension and cohesive bond of liquids	Simethicone, organic phosphates, alcohols, stearic acids, glycols, paraffin oils
Thickeners or viscosity modifying agents	To prevent the sedimentation or settling in case of suspensions and also maintain as well as modify the viscosity	This agent entraps the solid fragments	Hydroxyethyl as well as methyl cellulose, hydroxypropyl methyl cellulose, microcrystalline cellulose
Humectants	To prevent the dehydration of the hydrous vehicles from the formulation	As they are tending to absorb the moisture from the air, that's why these agents hold the water	Propylene glycol, poly ethyl glycol, glycerine

		molecule and decrease the evaporation of the solvent	
Chelating agents	To make complexes and prevent the drugs from catalysts	These agents form complexes with the metal ion which stops the catalytic activity in case of oxidation of active pharmaceutical ingredients	Disodium-EDTA, dihydroxy ethyl glycine, citric acid, tartaric acid
Emulsifier	To prevent agglomeration of the globules and make the emulsion stable	These agents reduce the interfacial tension between the oil and water by making the film around the globule which helps to disperse the globules in emulsion	Sodium lauryl sulphate, cetrimide, macrogol esters, sorbitol esters
Flocculating agents	To prevent the cake formulation and make the suspension stable	These agents are actually electrolytes which decrease the magnetic zeta-potential of dispersed solid particles	Starch, sodium alginate, Carbomer
Sweetening agents	To helps in taste of the formulation		Sucrose, saccharine, sorbitol, sucralose, sugar, aspartame

Colouring agents	To increasing the appearance and also identifying the formulation		Amaranth, erythrosine, eosin, tartrazine, sunset yellow, brilliant blue, caramel
Flavouring agents	Impart the flavour and increase patient acceptability		Aromatic waters, syrups, peppermint oil, cinnamon oil, honey, menthol
Excipients used in aerosol and propellant	Creating the pressure in the container which helps the product to expelling		Trichloromonofluoromethane, Dichlorodifluoromethane

1.2.3. Excipients used in liquid dosage form and their purpose [6]:

Types of excipients	**Purpose of use**	**Examples**
Structure forming excipient	To give the formulation a gel like thickened structure	Cetostearyl alcohol, sorbitol and some other surfactants which are water loving, fluid hydrocarbons like mineral oils
Preservative	To preserve the formulation normally as well as in storage condition	Benzyl alcohol, propylparaben, methyl

		paraben, chlorocresol, sodium benzoate
Antioxidant	To prevent the formulation from oxidation	Butyl hydroxy toluene (BHT), butyl hydroxy anisole (BHA), ascorbic acid
Solubilizer	To increase the solubility of active pharmaceutical ingredients in the formulation	Lanolin, cholesterol or cholesterol esters
Gelling agent	To forming the gel	Carbomer934, carboxymethyl cellulose (CMC), hydroxypropyl cellulose, xanthan gum
Emollients	To modify the characteristics involved in penetration through the skin of active pharmaceutical ingredients used in semisolid dosage form	Glycerin, mineral oil, petrolatum, isopropyl palmitate
Suppository base	To use as a base which helps to dissolve the active pharmaceutical ingredients	Cocoa butter, glycerine, coconut oil, gelatine, hydrogenated vegetable oil, polyethylene glycol

1.3. References:

[1] S.V. Sastry, J.R. Nyshadham, J.A. Fix, Recent technological advances in oral drug delivery–a review, pharmaceutical science & technology today 3(4) (2000) 138-145.

[2] G. Pifferi, P. Restani, The safety of pharmaceutical excipients, Il Farmaco 58(8) (2003) 541-550.

[3] S.P. Chaudhari, P.S. Patil, Pharmaceutical excipients: a review, Int J Adv Pharm Biol Chem 1(1) (2012) 21-34.

[4] P. Agrawala, Pharmaceutical dosage forms: Tablets. Volume 1. Edited by Herbert A. Lieberman, Leon Lachman, and Joseph B. Schwartz. Marcel Dekker: New York. 1989. 616 pages. 18× 26 cm. ISBN 0-8247-8044-2. $99.75, Wiley Online Library, 1990.

[5] L. Lachman, H.A. Lieberman, J.L. Kanig, The theory and practice of industrial pharmacy, Lea &Febiger Philadelphia1976.

[6] A. Katdare, M. Chaubal, Excipient development for pharmaceutical, biotechnology, and drug delivery systems, CRC Press2006.

CHAPTER - II

NATURAL POLYSACCHARIDE-BASED DRUG DELIVERY EXCIPIENTS

2. Natural polysaccharide-based drug delivery excipients

Nowadays polymers are widely used as excipients in pharmaceutical application. From recent research it's come to be known that most of the gums are generated from plant sources like plant parts like it can be internal part of plant tissue which can be disintegrated by some process that is known as gummosis. Through this process the plant tissue transforms into cavities which are further distilled and transformed into carbohydrates that are known to us as Gum. Gum can be also generated from the plant stem parts when there is any wound or damage like things on the plant stem, or any kind of attack on the plant part by bacteria, algae, or fungi [1]. These gums are actually made up from polymer. These gums or polymers are very much useful in solid, liquid and semisolid dosage form as well as a carrier in the novel drug delivery system (nanosomes, liposomes, niosomes, nanoparticles, nanospheres, microspheres, microparticle, microemulsion, nanosuspension, micelles), and apart from this these are also used in many kinds of formulation as a film coating agent, buccal films, and matrix-controlled system [2]. In recent days a huge number of polymers are being used because of their nature and important useful features like their economic property, availability, and non-toxic, capability of chemical modifications, potential biodegradability and also biocompatibility [3]. These gums or polymers are nothing but carbohydrates which consist of a long chain of monosaccharide, known to us as polysaccharide.

2.1. Isolation and Purification:

Polysaccharide or these types of gums are extracted from the parts of the plant body by applying temperature, solvent precipitation and microwave assisted extraction. From all of the previously mentioned methods, the simplest method is solvent precipitation. For this method first we have to choose the part of the plant which actually contains the

gum or polysaccharide, then we have to do the following steps like drying, grinding, sieving, stirring in distilled water, centrifugation, washing the residue and separation.

2.2. Characterization:

- Structural- gums are actually polysaccharides which actually contain the carbohydrate part sugar. Now different types of sugar is characterized by thin layer chromatography and high performance liquid chromatography, the structure of sugar part is confirmed by the spectroscopic method like Fourier-transformed infrared spectroscopy, mass spectroscopy, nuclear magnetic resonance spectroscopy
- Purity- purity of these polysaccharide we have to go for the test for alkaloids, glycosides, steroids, carbohydrate, flavonoids, terpanes, amino acids saponins, oils & fats, tannins and phenols
- Impurity- for a test of impurities we have to go for suitable analytical techniques.
- Physicochemical properties- for this we have to determine the colour, odour, shape, taste, texture, touch, solubility, swelling index, pH, LOD, hygroscopic nature, surface tension, rheological properties (because of their high viscous nature) and for bulk characteristics of powder we have to do the angle of repose, bulk and true densities, porosity. Determination of microbial load and presence of pathogens are also done.
- Toxicity- by the fixed dose method the acute toxicity is determined [4] [5] [6]

2.3. Classification of polysaccharide (gum and mucilage):

Most of the polysaccharide is available from the plant sources. Apart from the plant sources polysaccharides are also get from animal, seaweeds, fungi and other microbial sources. We can classify the polysaccharides depending upon various factors like presence of charge, depending on the origin like from where the polysaccharide can get available, depending upon their shape, their chemical structure.

2.3.1. Based on the charge- depending on the charge it can be anionic, cationic, non-ionic, amphoteric and hydrophobic.

- *Anionic polysaccharide:* Alginic acid, Xanthan gum, pectin, Gum Arabic, Gum Karaya, Gum Tragacanth these are the example of natural anionic polysaccharide and CMC (carboxymethyl cellulose), chitin gum these are the example of semisynthetic anionic polysaccharide.
- *Cationic polysaccharide:* chitosan is the example of natural cationic polysaccharide and guar gum is the example of semisynthetic cationic polysaccharide.
- *Non-ionic polysaccharide*: starch, dextrin these are the example of natural non-ionic polysaccharide and hydroxyethyl cellulose hydroxymethyl cellulose these are the example of semisynthetic non-ionic polysaccharide.
- *Amphoteric polysaccharide*: most of the amphoteric polysaccharides are semi synthetically like modified potato starch, carboxymethyl chitosan.
- *Hydrophobic polysaccharide*: most of hydrophobic polysaccharides are also semi synthetically like Cetylhydroxyethylcellulose, Polyquaternium.

2.3.2. Based on origin:

- Marine source: Agar, Carrageenans, Alginic acid, Laminarin
- Plant source: polysaccharides can get from different parts of plant like- Gum Arabica, Gum Karaya, Tragacanth these are found from shrubs/tree exudates; Guar Gum, Starch, Amylose, Cellulose these are found from seeds; Pectin, Larch gum these are like extracts; we can get the potato starch from root.
- Animal source: Chitin and chitosan, Hyaluronic acid these are the example of animal source.
- Microbial source: Xanthan gum, emulsan, scleroglucan Baker's yeast glycan, schizophyllan, lentinan and dextrin these are found from the bacteria and fungi

2.3.3. Semisynthetic:

- From starch: Heta-starch, Starch acetate, Starch phosphates these are synthesised from starch.
- From cellulose: Carboxymethyl cellulose (CMC), Hydroxyethyl cellulose, Hydroxypropyl methylcellulose (HPMC), methylcellulose (MC), Microcrystalline cellulose (MC) these are synthesised from cellulose.

2.3.4. Based on shape:

- Algins, Amylose, Cellulose, pectins these are linear in shape
- Xanthan, Xylan, Galactomannans (short); Amylopectin, Gum Arabic, Tragacanth (long) these are branched in shape

2.3.5. Based on chemical structure: presence of glycan structure these can be classified as

- Tri-heteroglycans- Arabinoxylans, Gellan, Xanthan
- Diheteroglycans- Algins, Galactomannans, Carragennans

- Homoglycans- Amylose, Cellulose, Arabinanas
- Tetra-heteroglycans- Gum of Psyllium seed
- Penta-heteroglycans- Gum ghutti, Gum Tragacanth [1]

2.4. Benefits: there are huge advantages of polysaccharide

2.4.1. Biodegradability: all the polysaccharides are generally generated from the living substances and for that they represent renewable sources and they have no harmful or bad effects on the human body.

2.4.2. Biocompatibility and non-toxic nature: if you notice carefully at the chemical structure of polysaccharides, they are nothing but the simple structure of carbohydrates. They are actually the repeating sugar unit or monosaccharide unit, that's why this compound easily gets biocompatible and they do not produce any toxic effect on human health.

2.4.3. Low cost effective: as all the polysaccharides are naturally available, these products are easily available from nature, for that reason they are very much cheap at cost. It is noticed that if comparison is done between other synthetic products and natural polysaccharide then natural polysaccharide is very much lower in cost.

2.4.4. Eco-friendly: this kind of polysaccharide can be collected in huge numbers from different regions from countries or areas in different seasons at any time for its simplicity of production process.

2.4.5. Easy availability: as the polysaccharides are used in huge amount in pharmaceutical industry that's why government promote the production of this kind of polysaccharide in the developing countries

2.4.6. Patient acceptance & compatibility: these polysaccharides are naturally found from plant source (mostly), animal source that's why they are very much compatible with patient health and patient acceptability is also high compared to other products.

2.4.7 Edible sources: maximum amount of polysaccharides are collected from the edible sources.

2.5. Disadvantages: besides the advantages there are some kind of disadvantages is present in case of polysaccharide

2.5.1. Microbial contamination: previously it is already known to us that these polysaccharides are simple structure of carbohydrate and equilibrium moisture content present these polysaccharides is 10% approximately, beside that during manufacturing process these polysaccharides are expose to the external environment where moisture is present, that's why there is a chance of microbial attack or contamination on these polysaccharides. But this kind of contamination or impurities can be avoided by using the proper amount of preservatives and proper corrective handling processes.

2.5.2. Alteration between batches: manufacture of material which is synthetic is a maintained procedure where a predetermined amount of ingredient is used while the manufacture of polysaccharide is depend upon the seasonal as well as environmental elements.

2.5.3. Overhydration: collection of natural polysaccharides is done at various time periods and from various regions also. That's why the climatic zones are different. For that reason, the chemical constituents present in polysaccharides are different, that's why the rate of hydration is uncontrolled. But this can be avoided by developing a suitable monograph of different types of polysaccharides.

2.5.4. Reduction of viscosity in storage condition: it has shown that when the polysaccharides are got contact with water, they represent higher viscosity. But as the structure of polysaccharides are very much complex like branched monosaccharides of carbohydrate that's why on storage conditions their viscosity decreases. But this condition can be reduced by proper storage conditions [7] [8] [9].

2.6. Application of polysaccharide:

2.6.1. Application of polysaccharide in tablet formulation: polysaccharides are adhesive in nature that's why they are used in tablet formulation as a binder. They increase the cohesiveness of powder particles and help to form granules. Polysaccharides also absorb water which helps the tablet to get swell, and for this swelling the tablet bursts and converts into smaller particles for this nature of polysaccharide it's also used as a disintegrant in tablet formulation which further helps to increase the rate of dissolution.

2.6.2. Application of polysaccharide as emulsifying and suspending agent: polysaccharides are actually acts as a protective colloid, in case of emulsion it makes a film around the droplets with interfacial absorption which prevents the coagulation of the globules, this makes dispersion of globules easier. This is how the polysaccharides build a strong multi molecular film around each globule. This is how the polysaccharides stabilize the oil in water type emulsion by making the hydrophilic barrier around the globule. For this character polysaccharide is used as an emulsifying as well as suspending agent.

2.6.3. Application of polysaccharide as a sustaining material in dosage form: polysaccharides are also used as a sustaining material because of their nature, like-when polysaccharide comes to contacts with water it forming a material like gel,

so when we use it in the formulation like tablet or suspension and it comes contact with water and hydrated, then it forms a gel and the release of active pharmaceutical ingredients or the drug materials somehow extended or delayed or controlled.

2.6.4. Application of polysaccharide as a coating agent: sometimes the polysaccharides are also used as a coating agent. If the formulation is coated with the polysaccharide, it prevents the degradation of drug in the stomach thus decreasing the release of drug is noticed.

2.6.5. Application of gums in microencapsulation: polysaccharides have the ability of coating and also the sustaining property that's why it is also used in microencapsulation.

2.6.6. Application of polysaccharide as a gelling agent: polymers are highly cross-linked material in which some of the atom valencies are satisfied by the bond that results intra and intra molecular association and form three-dimensional structure. Because of this nature polysaccharides when comes to contact with water it forms a gel and this may happen by the single polysaccharide or with the combination of two or more polysaccharide, this changes the physical characteristics like change in pH, change in temperature, change in chemical characteristics. For this reason, the polysaccharides are also used as a gelling agent [1].

2.6.7. List of some polysaccharides and their application:

Name of the polysaccharide	**Application**
Agar	Emulsifier & Suspending agent, gelling agent in suppositories, lubricating agent for surgical use, disintegrating agent in tablet, medium for bacterial culture, laxative
Albizia gum	Binding agent in tablets
Carrageenan	Gelling agent, stabilising agent in suspension as well as emulsions, in paste, laxative etc.
Cashew gum	Dispersing agent
Cassia tora	Binding agent
Guar gum	Binding agent, disintegrating agent, thickening agent, emulsifying agent, laxative, sustaining agent
Gum acacia	Dispersing agent, emulgent, binder in solid oral formulation, emollient as well as demulcent in cosmetic preparation
Gum ghatti	Binder, emulgent, dispersing agent
Gum Tragacanth	Emulsifier, suspending agent, emollient in cosmetics and sustainer
Karaya gum	Emulsifier and suspending agent, adhesive, sustain release agent in tablets

Khaya gum	Binder
Luecaena seed gum	Emulsifying agent, suspending agent, binding agents in tablets, disintegrant in tablets
Sodium alginate	Suspending agent, stabilizing agent, coating agent for tablet and gelation for dental film
Tamarind seed polysaccharide	Sustain release agent, emulsifying and suspending agent, and binding agent.
Xanthan gum	Suspending and emulsifying agent, stabilising agent in semisolid dosage form and sustaining agent
Gellan gum	Disintegrant

2.7 References:

[1] S. Goswami, S. Naik, Natural gums and its pharmaceutical application, Journal of Scientific and Innovative Research 3(1) (2014) 112-121.

[2] A.M. Avachat, R.R. Dash, S.N. Shrotriya, Recent investigations of plant based natural gums, mucilages and resins in novel drug delivery systems, Ind J Pharm Edu Res 45(1) (2011) 86-99.

[3] C.E. Beneke, A.M. Viljoen, J.H. Hamman, Polymeric plant-derived excipients in drug delivery, Molecules 14(7) (2009) 2602-2620.

[4] P.D. Choudhary, H.A. Pawar, Recently investigated natural gums and mucilages as pharmaceutical excipients: an overview, Journal of pharmaceutics 2014 (2014).

[5] S. Baveja, Examination of natural gums and mucilages as sustaining materials in tablets dosage forms, Ind. J. Pharma. Sci. 50 (1988) 89-92.

[6] S. Wahi, V. Sharma, V. Jain, P. Sinha, Studies on suspending property of mucilages of Hygrophila spinosa T anders and Hibiscus esculentus Linn, Indian Drug 22(9) (1985) 500-502.

[7] B.W. Tekade, Y.A. Chaudhari, Gums and mucilages: Excipients for modified drug delivery system, J. Adv. Pharm. Edu. & Res 3(4) (2013).

[8] G.K. Jani, D.P. Shah, V.D. Prajapati, V.C. Jain, Gums and mucilages: versatile excipients for pharmaceutical formulations, Asian J Pharm Sci 4(5) (2009) 309-323.

[9] K. Hizawa, H. Inaba, S. Nakanishi, H. Otsuka, K. Izumi, Subcutaneous pseudosarcomatous polyvinylpyrrolidone granuloma, The American journal of surgical pathology 8(5) (1984) 393-398.

CHAPTER - III

CHEMICALLY MODIFIED NATURAL POLYSACCHARIDE AS DRUG DELIVERY EXCIPIENTS

3. Chemically modified natural polysaccharide as drug delivery excipients

3.1. Introduction:

For the vast application of natural polysaccharide, they are combined with new chemical ingredients to modify their characteristics and bring themselves to new features. In case, it has been noticed that there is a difference between the modified version of natural polysaccharide and the generatrix natural polysaccharide and the modification can be done in various steps which produce the new material in a particular range. The new materials with new structure have different specific features because of their extra special properties. By this modification we can replace one polysaccharide's nature into another polysaccharide. Normally polysaccharides are highly hydroxylated in nature, each chain contains three hydroxyl groups which further conjugate with the ring and glycosidic O_2 atoms, this conjugation produces in each chain five each site for hydrogen bonding. These polysaccharides are very much water loving in nature, in general case in immanent condition (Temperature: 25^0C and Relative humidity: 60-80%) dry polysaccharide holds 8-12% of water because of their hydrophilicity. Polysaccharide causes monodisperse solution by fully dissolving in a water dispersion. They are completely dissolved and solvated because they enclose themselves with water molecules. Sometimes for reciprocal intermolecular bonding polysaccharides are not easily dissolved. This intermolecular coalition grows via hydrogen bonding which further turns into a crystalline field. Crystallinity is the innate nature of the polysaccharide which is produced by bigger

structure and this delivers the chains to befit into H-bond. For its complex structure these polysaccharides are insoluble in general condition [1].

3.2. Carboxymethylation

This process is the most currently used chemical modification method by which the solubility of polysaccharide and their biological activities can be increased.

There are some examples of natural polymers which have already been carboxymethylated. They are: inulin [2], cashew gum [3], xyloglucan gum [4] locust bean gum [5], starch [6], hemicelluloses [7], konjac glucomannan [8], xylan [9], guar gum and tara gum [10].

This process of carboxymethylation can be commenced by both procedures like aqueous and non-aqueous [4-10].

In this case, applying aqueous method in carboxymethylation the process can be affected by the composition of solvent, the solvent system, NAOH concentration, monochloroacetic acid concentration, time of the reaction, temperature of the reaction [4]. Carboxymethylation of the polysaccharides like mucilage and gums is commenced with acrylamide with NaOH and in this method this process can be affected by the ratio of gum and liquor, acrylamide and NaOH concentration time period of the reaction, temperature of the reaction [11]. This process increases the solubility in water and as well as the stability & clarity. There is some mucilage and gum which have been carboxymethylated encircle guar gum [11] and *Cassia Tora* gum [12].

A schematic diagram of carboxymethylation is shown below in figure [13].

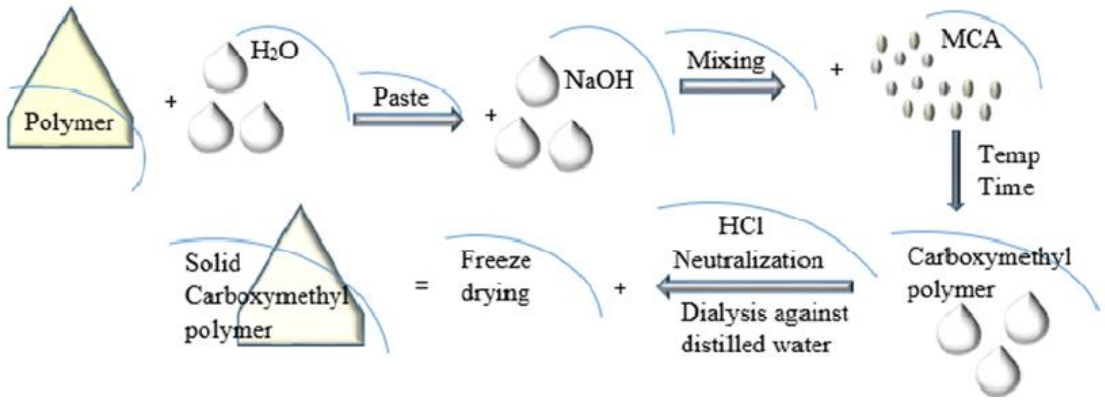


Figure 3.1. A schematic diagram of carboxymethylation

3.2.1. Example

According to P. Goyal et al [4], tamarind kernel powder has a high concentration of xyloglucan gum. Because of its diverse uses, this gum is used in a variety of sectors. Tamarind kernel powder is used for carboxymethylation. This reaction is carried out at its maximal level with the concentration of NaOH, monochloroacetic acid, solvent ratio, reaction duration, and reaction temperature. This chemical change improves the solubility and stability in cold water. Carboxymethylation of tamarind kernel powder was carried out with monochloroacetic acid as a catalyst and the reaction state was heterogeneous. The best degree of substitution obtained is 0.649 by using 0.050 M tamarind kernel powder, 0.158 M NaOH, and 0.090 M monochloroacetic acid in a 4:1 methanol/water ratio at 70 ^{0}C for 60 minutes. The carboxymethylated tamarind kernel powder has considerably superior paste in quality and microbiological resistance than the parent tamarind kernel powder after chemical modification. The modified tamarind kernel powder has a greater viscosity in 2% solution than the original gum. The rheological properties of modified tamarind kernel powder were discovered to be non-Newtonian pseudoplastic in nature.

D.A. Silva et al [3] developed cashew tree gum modified by carboxymethylation in an aqueous alkaline media using monochloroacetic acid as an etherifying agent. The quantity of alkali, the ratio of monochloroacetic acid and cashew gum, the temperature of the reaction, the yield, and the degree of substitution were all measured. The substance is characterized using 13C NMR, FTIR, gel permeation chromatography, and flow capillary viscosity. Chemically modified cashew tree gum with substitution levels ranging from 0.10 to 2.21 was created. The gum yield ranges from 31 to 75%. At the peak of the reaction, the NaOH concentration was 5.5M, the reaction temperature was 55^{o} C, the reaction time was 3 hours, and the ratio of monochloroacetic acid to cashew gum was 1:1. The deterioration of chain length in structure was seen during the carboxymethylation process at the beginning site, which relied on the reaction condition. To make carboxymethylated cashew gum in the acidic form, cashew gum was carboxymethylated in the presence of monochloroacetic acid in alkaline solution. The degree of substitution ranged from 0.10 to 2.21, depending on the quantity of NaOH, the ratio of monochloroacetic acid in the gum, and the temperature of the reaction.
This degree of substitution increases with alkali concentration up to 6.2N and then decrea ses automatically. The degree of substitution has an inverse relationship with the yield.

3.3. Grafting:

3.3.1. Brief introduction of Grafting:

- ✓ **Monomer:**

  These are the tiny molecules which perhaps bind with each other in a simultaneous fashion to compose more complex molecular structure known as polymers

- ✓ **Polymer:**

  Polymers, possibly a natural or synthetic macromolecule, consist of simultaneous units of a tiny molecule known as monomers. One concept is that 'polymer' and ‘plastic’ these two terms might be exchangeable but polymers are a very huge class of molecules which consists of plastics and many other materials like cellulose, amber, and natural rubber.

- ✓ **Grafted copolymer:**

A grafted copolymer is macromolecular chains consisting of various species of block attached to the main body or chain as partial chain(s). Thus, grafted copolymers can be narrated as having the common structure, where the prime polymer backbone, generally referred to as the torso polymer, has branches of different polymeric chains arising from various points along its range [14]. There is a very suitable way we can utilize the polysaccharide’s benefits for controlled or modified release drug delivery by grafting or graft copolymerization of synthetic polymers onto polysaccharide spine. Graft copolymerization which is very much simple method for modification of the features of the natural polymer and by this process these are easily converted into fascinating bio materials which is more applicable for controlled released dosage form compare to parent

polysaccharide because of their tangible swelling and fast enzymatic downfall in the biological fluid [15].

3.3.2. Types of grafting:

Grafting can be classified by-

- ✓ Grafting with a single monomer
- ✓ Grafting with a mixture of two (or more) monomers

First one commonly appeared by one step, but another one may arise with either the repetition or pursuant use of the two monomers. Mosaic grafting has devoted much remark for binary monomer grafting. Two other monomers are grafted edgewise to attain the requisite property. This is the genesis of bipolar membranes. The first part of the review discusses individual procedures of grafting, and the primary elements, which maintain the process of grafting. Subsequently, two applications are talked about, viz. membrane separation science and conducting polymers. Various grafting procedures are there like chemical, radiation, photochemical, plasma induced techniques and enzymatic grafting.

3.3.3. Techniques of grafting:

There are various procedures by which we can graft the polysaccharide. Those techniques are chemical grafting, radiation grafting, photochemical grafting, plasma induced grafting and enzymatic grafting.

3.3.3.1. Chemical grafting or Grafting initiated by chemical means:

Free radical and ionic are the main two tracks by which chemical grafting can be done. In case of chemical grafting, the main character is the initiator which is very much important to determine the grafting procedure. Except for the common free radical process or

technique melt and atom transfer radical polymerization (ATRP) these are also very much fascinating for grafting procedures.

3.3.3.2. Radiation grafting or Free-radical grafting:

Free radicals are produced from the instigator and then pass on to the substrate to respond with the monomer to convert into newly grafted copolymer in case of chemical grafting process. In the chemical grafting technique free radicals are generated by direct or indirect methods. For example, free radicals generated by an indirect process is the production via redox reaction, viz. Mn^{n+}/H_2O_2, per-sulphates [15-18].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

$$Fe^{2+} + {}^-O_3S\text{-}OO\text{-}SO_3^- \rightarrow Fe^{3+}SO_4^{2-} + SO_4^{-\cdot}$$

3.3.3.3. Grafting through living polymerization:

In the last few years supplies of a prospective for grafting reaction ‘Living Polymerization’ have developed. In the view of Szwarc*et al*. [19], the most feasible definition of a ‘living polymer’ is ‘that hold on to their ability to breed for a huge time and spread to a preferred topmost size while their degree of ending or chain transfer is still negotiable’. There are two types of technique like conventional free radical and ionic polymerization. By combining these two techniques controlled free-radical polymerization is done. Activation of unreactive polymers or dead polymers, essentially time constant degree of polymerization and broad molecular weight distribution these all happen by coupled reaction of spreading chain with its ending and this conventional free radical

polymerization needs repeating initiation. For living polymerization, it gives living polymers with regulated molecular weights and low poly-dispersities [20-22].

3.3.3.4. Photochemical grafting:

In case of photochemical grafting, free radicals are generated by absorbing the light in the chromophore present on a macromolecule, for absorbing the light it turns active from ground state to excited state. If forming of free radical with bond rupture by absorbing the light on the macromolecule's chromophore is not happened then the technique can be encouraged by adding of some photo sensitizer like benzoin ethyl ether, dyes, such as Na-2,7 anthraquinone sulfonate or acrylate azo dye, aromatic ketones (such as benzophenone, xanthone) or metal ions UO_2^{2+}. From the above discussion we can conclude that this photochemical grafting can be done by two techniques: with sensitizer and without sensitizer. The technique without sensitizer generates free radicals in the spine of the molecule and these free radical further transform into grafted copolymers by the reaction with the monomer. The technique with sensitizer generates free radicals, abstruse H+ atoms from the main polymer and produce free radical sites which is necessary for the grafting by diffusion process [23-25].

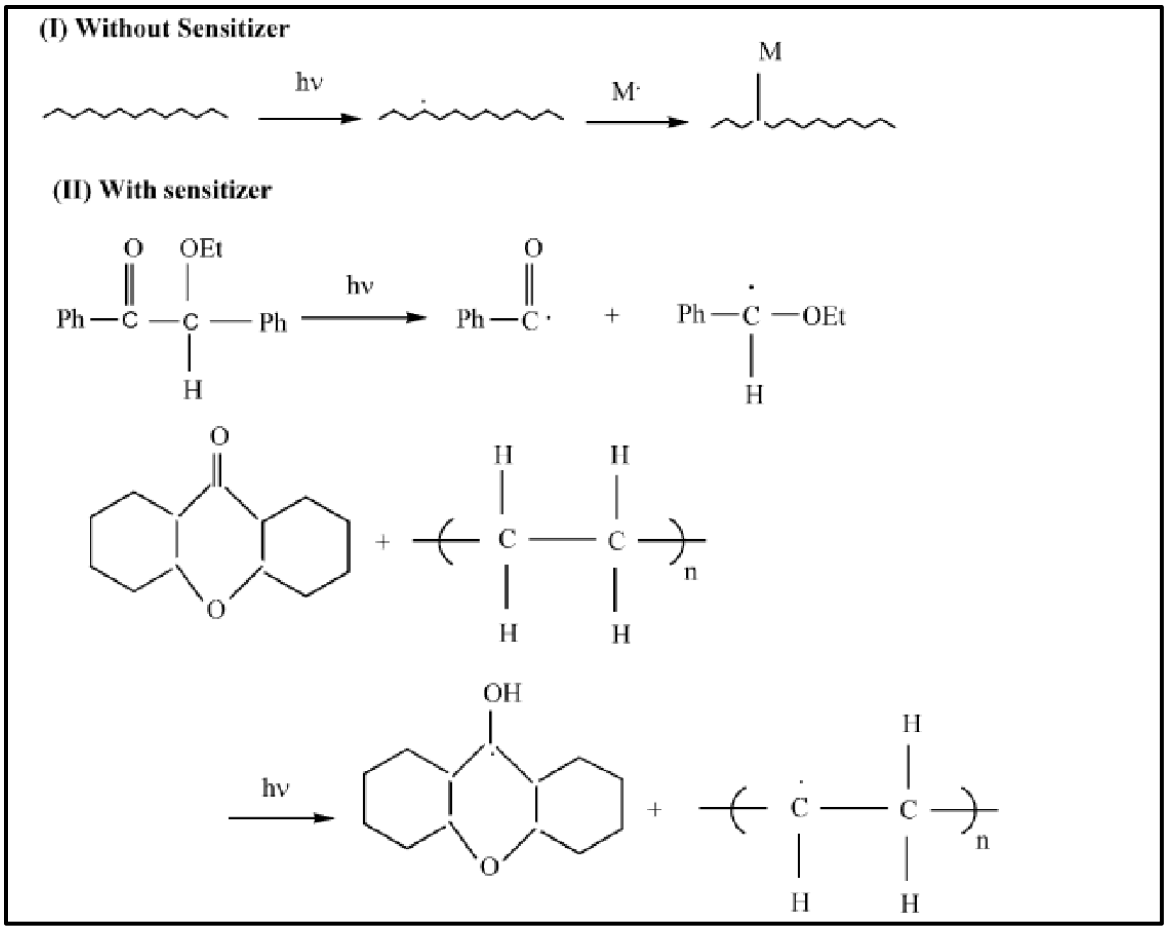


Figure 3.2. Process involved in photochemical grafting

3.3.3.5. Example

According to D.A. da Silva et al. 's theory [26], the radical polymerization method was used to create cashew gum-g-polyacrylamide at 60 °C while employing potassium persulfate as the redox initiator in a N_2 environment. A sequence of graft co-polymers with variable acrylamide concentrations while maintaining constant initiator and polysaccharide concentrations. Elemental analyses, infrared and 13C NMR spectroscopy, rheological tests, differential scanning calorimetry, and thermogravimetric analysis were used to characterize these graft copolymers. There were comparisons made between the grafting parameters of the reaction of several natural polysaccharides with polyacrylamide (PAM).

Even with a low acrylamide/gum ratio, high acrylamide conversion (%C) and grafting efficiency (%E) percentages were still attained for cashew gum (CG). Due to the inherent viscosity of all copolymers, their hydrodynamic volumes were substantially larger than the CG value and much closer to the PAM. When compared to the gum, the viscosity of CG-g- PAM increased by up to 27.3 times. Even with a smaller quantity of acrylamide (56%) grafted onto cashew gum, this enhancement factor is larger than that reported for several polysaccharides. The thermal stability of the polysaccharide is improved through PAM chain grafting.

3.4. Cross-linking of gums

By constructing tridimensional networks, crosslinking involves joining polymer chains together with covalent or noncovalent linkages. Physical crosslinking utilizes non covalent connections, such as ionic contacts, hydrogen bonds, or hydrophobic interactions, whereas chemical crosslinking arises from covalent bonding between polymer chains achieved by irradiation, sulfur vulcanization, or chemical processes. One of the most important methods for modifying polymers is crosslinking, which can take either intra- or intermolecular. Proteins and polysaccharides have been crosslinked by a variety of substances with varying processes, properties, and outcomes. The most popular crosslinkers for protein films are probably aldehydes. Aldehyde residues still present in the films have raised questions about their toxicity, which might be an issue if they migrate into food items. Thus, it has been investigated if there are any alternate harmless or less toxic crosslinking agents that might be preferable for applications involving food [28].

3.4.1. Example:

Z. Petronijevic et al [29] described how to use a straightforward technique to crosslink dextran, starch, and various other polysaccharides. Dimethyl sulfoxide (DMSO) activated with organic or inorganic acid halogenides or phosphorus pentoxide is used to crosslink polysaccharides. With an increase in acid chloride concentration, temperature, and reaction time, the crosslinking level rises. The resulting compounds swell in water yet are insoluble in water, DMSO, and other solvents. Additionally, the findings of periodate oxidation tests and the products' resistance to hydrolytic enzyme activity provided further evidence of crosslinking. Other non-ionic polysaccharides and other compounds having hydroxyl groups may be crosslinked using this technique. The creation of carriers for chromatography as well as the immobilization of enzymes and cells are also possible uses for this cross-linking technique.

C. Sandolo et al [30] evaluate, At various temperatures (7, 15, 25 and 37 oC), the viscoelasticity inside a solution of guar gum (GG) in the presence of an excess of cross-linker (glutaraldehyde, Ga) has been measured, assessing the sol-gel transition by dynamic mechanical studies. The samples were also characterized at 25 oC in relation to the various cross-linker concentrations. With rising temperature and, at a given temperature (25^{o}C), with increasing cross-linker concentration, the systems' gelation times, as measured by the loss tangent, reduced. At the gel point a power-law frequency dependence of the dynamic storage modulus ($G' \approx \omega^{n}$) and loss modulus ($G'' \approx \omega^{n}$) was observed. The value of n, evaluated according to three different methods (n_{slope}, considering the parallelism of the two moduli, n_{tan}, from the frequency-independence of tan δ, and from the crossing point between the apparent exponents of G′ and G″, n_{app}), decreases with increasing cross-linking

densities and with increasing temperature. The fractal dimension (df) of the developing GG/Ga hydrogels was identified. When the cross-linker concentration is increased, the value of df goes up; when the temperature is raised, it goes down. This df trend indicates that the network has a tighter structure at lower temperatures and higher cross-linking density. The activation energy (Ea) of the chemical reaction involving GG and Ga was calculated; the experimental value was close to that found in the case of chemical reactions involving polymers and various cross-linkers that included comparable polymers and cross-linkers.

3.4. References:

[1] J.N. BeMiller, R.L. Whistler, Industrial gums: polysaccharides and their derivatives, Academic Press2012.

[2] D.L. Verraest, J.A. Peters, J.G. Batelaan, H. van Bekkum, Carboxymethylation of inulin, Carbohydrate Research 271(1) (1995) 101-112.

[3] D.A. Silva, R.C. De Paula, J.P. Feitosa, A.C. De Brito, J.S. Maciel, H.C. Paula, Carboxymethylation of cashew tree exudate polysaccharide, Carbohydrate Polymers 58(2) (2004) 163-171.

[4] P. Goyal, V. Kumar, P. Sharma, Carboxymethylation of tamarind kernel powder, Carbohydrate Polymers 69(2) (2007) 251-255.

[5] P. Dey, B. Sa, S. Maiti, Carboxymethyl ethers of locust bean gum-a review, Int J Pharm Pharm Sci 3(2) (2011) 4-7.

[6] G. Sen, S. Pal, A novel polymeric biomaterial based on carboxymethylstarch and its application in controlled drug release, Journal of applied polymer science 114(5) (2009) 2798-2805.

[7] J.-L. Ren, R.-C. Sun, F. Peng, Carboxymethylation of hemicelluloses isolated from sugarcane bagasse, Polymer Degradation and Stability 93(4) (2008) 786-793.

[8] S. Kobayashi, S. Tsujihata, N. Hibi, Y. Tsukamoto, Preparation and rheological characterization of carboxymethyl konjac glucomannan, Food Hydrocolloids 16(4) (2002) 289-294.

[9] K. Petzold, K. Schwikal, W. Günther, T. Heinze, Carboxymethyl Xylan-Control of Properties by Synthesis, Macromolecular symposia, Wiley Online Library, 2005, pp. 27-36.

[10] K. Parvathy, N. Susheelamma, R. Tharanathan, A.K. Gaonkar, A simple non-aqueous method for carboxymethylation of galactomannans, Carbohydrate Polymers 62(2) (2005) 137-141.

[11] S. Gupta, P. Sharma, P. Soni, Chemical modification of Cassia occidentalis seed gum: carbamoylethylation, Carbohydrate polymers 59(4) (2005) 501-506.

[12] B.R. Sharma, V. Kumar, P. Soni, Carbamoylethylation of Cassia tora gum, Carbohydrate polymers 54(2) (2003) 143-147.

[13] N.C. Ngwuluka, N.A. Ochekpe, O.I. Aruoma, Naturapolyceutics: the science of utilizing natural polymers for drug delivery, polymers 6(5) (2014) 1312-1332.

[14] M.J. Zohuriaan-Mehr, Advances in chitin and chitosan modification through graft copolymerization: a comprehensive review, Iran Polym J 14(3) (2005) 235-265.

[15] M. Pepenzhik, A. Virnik, Z. Rogovin, Synthesis of graft cellulose copolymers and calcium salt of poly (acrylic acid), VysokomolSoedin Ser B 11 (1969) 245-50.

[16] B. Misra, R. Dogra, I. Mehta, Grafting onto cellulose. V. Effect of complexing agents on Fenton's reagent (fe2+− H2O2)-initiated grafting of poly (ethyl acrylate), Journal of Polymer Science: Polymer Chemistry Edition 18(2) (1980) 749-752.

[17] B. Misra, I. Mehta, R. Khetarpal, Grafting onto cellulose. VIII. Graft copolymerization of poly (ethylacrylate) onto cellulose by use of redox initiators. Comparison of initiator reactivities, Journal of Polymer Science: Polymer Chemistry Edition 22(11) (1984) 2767-2775.

[18] K.H. Prashanth, R. Tharanathan, Studies on graft copolymerization of chitosan with synthetic monomers, Carbohydrate Polymers 54(3) (2003) 343-351.

[19] M. Szwarc, Living polymers. Their discovery, characterization, and properties, Journal of Polymer Science Part A: Polymer Chemistry 36(1) (1998) IX-XV.

[20] K. Russell, Free radical graft polymerization and copolymerization at higher temperatures, Progress in Polymer Science 27(6) (2002) 1007-1038.

[21] U.M. Stehling, E.E. Malmström, R.M. Waymouth, C.J. Hawker, Synthesis of poly (olefin) graft copolymers by a combination of metallocene and “living” free radical polymerization techniques, Macromolecules 31(13) (1998) 4396-4398.

[22] V. Percec, B. Barboiu, " Living" radical polymerization of styrene initiated by arenesulfonyl chlorides and CuI (bpy) nCl, Macromolecules 28(23) (1995) 7970-7972.

[23] D. Hada, K. Rathore, T. Barupal, N.S. Chundawat, K. Sharma, N.P.S. Chauhan, Grafted biopolymers I: methodology and factors affecting grafting, Advanced functional polymers for biomedical applications, Elsevier2019, pp. 21-42.

[24] H. Kubota, I.G. Suka, S.-i. Kuroda, T. Kondo, Introduction of stimuli-responsive polymers into regenerated cellulose film by means of photografting, European polymer journal 37(7) (2001) 1367-1372.

[25] T. Peng, Y.-L. Cheng, PNIPAAm and PMAA co-grafted porous PE membranes: living radical co-grafting mechanism and multi-stimuli responsive permeability, Polymer 42(5) (2001) 2091-2100.

[26] D.A. da Silva, R.C. de Paula, J.P. Feitosa, Graft copolymerisation of acrylamide onto cashew gum, European Polymer Journal 43(6) (2007) 2620-2629.

[27] M. Shahid, S.A. Bukhari, Y. Gul, H. Munir, F. Anjum, M. Zuber, T. Jamil, K.M. Zia, Graft polymerization of guar gum with acryl amide irradiated by microwaves for colonic drug delivery, International journal of biological macromolecules 62 (2013) 172-179.

[28] H.M. Azeredo, K.W. Waldron, Crosslinking in polysaccharide and protein films and coatings for food contact–A review, Trends in Food Science & Technology 52 (2016) 109-122.

[29] Z. Petronijevic, B. Maluckov, A. Smelcerovic, Crosslinking of polysaccharides with activated dimethylsulfoxide, Tetrahedron Letters 54(24) (2013) 3210-3214.

[30] C. Sandolo, P. Matricardi, F. Alhaique, T. Coviello, Effect of temperature and cross-linking density on rheology of chemical cross-linked guar gum at the gel point, Food hydrocolloids 23(1) (2009) 210-220.

CHAPTER - IV

CASSIA FISTULA SEED GUM

4. Cassia fistula seed gum

4.1. Origin: Native to Asia, the *Cassia fistula* (CF) plant may be found in a number of nations, including Mexico, South Africa, India, and Brazil [1]. It is a beautiful shrub that proliferates erratically in mixed monsoon forests over most of India and climbs to a height of 1300 m in the outer Himalaya. It grows as a sporadic tree in Maharashtra's Deccan and Konkan regions. It is 6–9 m tall with a straight trunk, smooth, pale grey bark when young, and rough, dark brown bark when it is older [2].

4.2. Cassia fistula seed gum: Mucoadhesion has received a lot of attention over the past 20 years for a variety of reasons, including its potential to improve localised drug delivery by keeping a dosage form at the site of action (such as the gastrointestinal tract) and, in the case of systemic delivery, by keeping the formulation in close contact with the absorption site (such as the stomach or buccal cavity). Several research on the buccal administration of medications employing muco-adhesive polymers, mostly polysaccharides, have been undertaken [3]. Carbohydrates that are relatively complicated include polysaccharides. For use as fibres, films, adhesives, rheology modifiers, hydrogels, emulsifiers, and drug delivery agents, they offer good mechanical qualities. For instance, certain polysaccharides have demonstrated that their high mucoadhesive qualities improve the interface between drugs and human mucosa [4]. Polysaccharides, such as cellulose ethers [5]. xanthan gum [6], scleroglucan [7], locust bean gum [8] and gaur gum [9] have been evaluated as hydrophilic matrices for delivering the drug. Although the use of cassia fistula seed gum in medicinal compositions has not yet received a thorough evaluation. A galactoxyloglucan called CFSG was isolated from the cassia fistula seed kernel.

4.3. Phytochemical composition and structure of *cassia fistula* seed gum:

Extensive research work has been performed on primary and secondary metabolites of *C. fistula* contains a combination of glycosides, flavonoids, proanthocyanidins, polyphenols essential oils and terpenoids. Seeds mainly contain eight compounds, (Table 1) namely 5-(2-hydroxyphenoxymethyl) furfural **(1)**, (2′S) -7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl) chromone **(2)**, benzyl-2-hydroxy-3,6-dimethoxybenzoate**(3)**,benzyl 2â-O-D-gluco-pyranosyl-3,6-dimethoxybenzoate **(4)**, 5-hydroxymethylfurfural **(5)**, (2′S) -7-hydroxy-2-(2′-hydroxypropyl) -5-methylchromone **(6)**, chrysophanol **(7)** and chrysophanein **(8)[10]**. Seeds were also reported to have bioactive flavones glycoside 5,3', 4'-tri-hydroxy-6-methoxy-7-O-α-L-rhamnopyranosyl-(1-2) -O-β-D-galactopyranoside **(9)**, 5,7,3', 4'-tetrahydroxy-6-methoxyflavone **(10)[11]**.

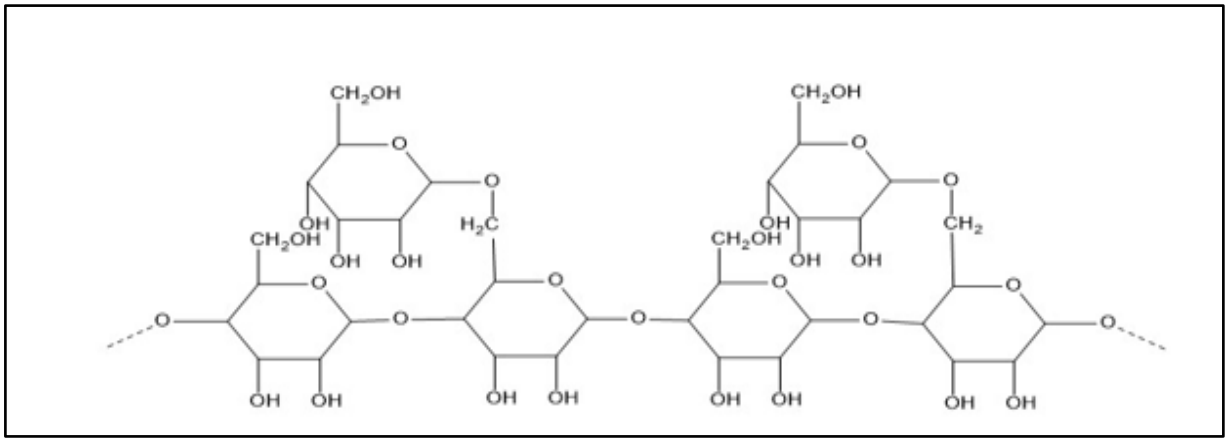


Figure 4.1. Chemical structure of cassia fistula seed gum

4.4. Properties of Cassia fistula gum: [12]

- ✓ D – mannopyranose, D-galactopyranose
- ✓ This Powder is odor free and is off white in color
- ✓ **Moisture Content:** 08.87±0.21%
- ✓ **Crude Fibre:** 01.5 to 02 %

- ✓ **Protein:** 1.03 %
- ✓ **Fat:** 10.04%
- ✓ **Polysaccharide:** 88.92%
- ✓ **Viscosity:** 2400 CPS in 6%(w/v) solution
- ✓ **Color:** off white
- ✓ **pH of 6% slurry:** 6.0 to 7.0
- ✓ **Sieve Value:** 100 mesh 100% w/w passing
- ✓ **Sieve Value:** 200 mesh 99% w/w passing
- ✓ **Ash content:** 0.10±0.00%

4.5. Extraction of polymer:

4.5.1. From pulp

Chloroform water IP solution was used for the extraction. For the leaching out of carbohydrate polymers from pulp, pulp was put in chloroform water IP and constantly stirred for 24 hours in an orbital shaker at 37°C and 60 rpm. Polysaccharides were precipitated after the extract was filtered using Whatman filter paper and several litres of acetone. For additional research, these precipitated polymers were dried at 50°C and stored in desiccators [13].

4.5.2. From seeds

1. To remove the oil, dried powdered C. fistula seeds were first extracted with n-hexane. Additionally, this de-oiled powder was agitated continuously for 12 hours at 40 degrees Celsius and 70 revolutions per minute in an orbital shaker in order to test for the leaching of proteins from the seed powder. The dark sodium chloride extract was extracted after being filtered with Whatman filter paper. This extract, which was a separated crude protein that was further refined by dialysis, was boiled until no white

precipitate formed at the bottom of the solution. We have utilized immediately de-oiled seed powder to prevent having to remove oil each time.

2. For the extraction of the gum, 20 g of the seeds from the cassia fistula were employed. The powder was dissolved in 220 ml of ice-cold, distilled water to create the powder slurry. 900 ml of boiling distilled water was then added to the slurry, which was then cooked for 20 minutes over a water bath with constant stirring. To aid in the sedimentation of protein and fibres, the boiling was halted after the allotted amount of time and the system was left unattended overnight. After 20 minutes at 5000 rpm of centrifugation, the clear supernatant was collected. By adding twice as much ethanol to the supernatant while stirring, the polysaccharides were precipitated. Following ethanol, diethyl ether, and petroleum ether washes in order, the precipitate was dried at 45 to 50 °C. The finished product was desiccated until use after being filtered via sieve number 100 [12].

4.6. References:

[1] L.M. da Silva, L.F.S. Araújo, R.C. Alvez, L. Ono, D.A.T. Sá, P.L. da Cunha, R.C.M. de Paula, J.S. Maciel, Promising alternative gum: Extraction, characterization, and oxidation of the galactomannan of Cassia fistula, International Journal of Biological Macromolecules 165 (2020) 436-444.

[2] M. Srivastava, V. Kapoor, Seed galactomannans: an overview, Chemistry & Biodiversity 2(3) (2005) 295-317.

[3] F. Bottari, G. Di Colo, E. Nannipieri, M. Saettone, M. Serafini, Evaluation of a dynamic permeation technique for studying drug–macromolecule interactions, Journal of pharmaceutical sciences 64(6) (1975) 946-949.

[4] G. Uccello-Barretta, S. Nazzi, F. Balzano, G. Di Colo, Y. Zambito, C. Zaino, M. Sansò, E. Salvadori, M. Benvenuti, Enhanced affinity of ketotifen toward tamarind seed polysaccharide in comparison with hydroxyethylcellulose and hyaluronic acid: a nuclear magnetic resonance investigation, Bioorganic & medicinal chemistry 16(15) (2008) 7371-7376.

[5] J.L. Ford, M.H. Rubinstein, F. McCaul, J.E. Hogan, P.J. Edgar, Importance of drug type, tablet shape and added diluents on drug release kinetics from hydroxypropylmethylcellulose matrix tablets, International journal of pharmaceutics 40(3) (1987) 223-234.

[6] M.M. Talukdar, J. Plaizier-Vercammen, Evaluation of xanthan cum as a hydrophilic matrix for controlled-release dosage form preparations, Drug development and industrial pharmacy 19(9) (1993) 1037-1046.

[7] S. Rizk, C. Duru, D. Gaudy, M. Jacob, P. Colombo, G. Massimo, Natural polymer hydrophilic matrix: influencing drug release factors, Drug development and industrial pharmacy 20(16) (1994) 2563-2574.

[8] J. Sujja-Areevath, D. Munday, P. Cox, K. Khan, Release characteristics of diclofenac sodium from encapsulated natural gum mini-matrix formulations, International Journal of Pharmaceutics 139(1-2) (1996) 53-62.

[9] P. Khullar, R.K. Khar, S.P. Agarwal, Evaluation of guar gum in the preparation of sustained-release matrix tablets, Drug development and industrial pharmacy 24(11) (1998) 1095-1099.

[10] Y.-H. Kuo, P.-H. Lee, Y.-S. Wein, Four New Compounds from the Seeds of Cassia f istula, Journal of Natural Products 65(8) (2002) 1165-1167.

[11] R. Yadava, V. Verma, A new biologically active flavone glycoside from the seeds of Cassia fistula (Linn.), Journal of Asian natural products research 5(1) (2003) 57-61.

[12] K. Huanbutta, W. Sittikijyothin, Use of seed gums from Tamarindus indica and Cassia fistula as controlled-release agents, Asian journal of pharmaceutical sciences 13(5) (2018) 398-408.

[13] S.G. Killedar, A.B. Nale, S.J. Nadaf, A.A. Pawar, U.S. Tamboli, Isolation, characterization, and evaluation of Cassia fistula Linn. seed and pulp polymer for pharmaceutical application, International Journal of Pharmaceutical Investigation 4(4) (2014).

CHAPTER - V

GASTRO RETENTIVE MUCOADHESIVE DOSAGE FORM

5. Gastro retentive mucoadhesive dosage form

5.1. Introduction:

This gastro retentive drug delivery system this approach first started after discovering the *Helicobacter pylori* by Warren and Marshall. *Helicobacter pylori* causes gastric ulcer which is treated by anti- *Helicobacter pylori* drugs. These drugs are given by the formulation which acts locally over a prolonged period of time to eradicate the bacteria [1]. After some time period this technique helps to develop rate control oral dosage form which increases the bioavailability with predictable and reproducible plasma drug concentration vs. time profile, this technique also increases the other pharmacokinetic parameters from the delivery system [2]. The bioavailability of the dosage form depends on the profile or the characteristics of the drug molecule, the physiological environment of the gastrointestinal tract or absorption site, residential time of the delivery device at the absorption site. So, if we can produce a proper environment mentioned above for the entire period of the drug release therefore a uniform drug release from a particular drug can be obtained. But in that case many difficulties may arise because of the highly variable nature of gastric emptying, and also there are different pH regions present in the whole gastrointestinal system. But these types of problems can be avoided by submitting the drug delivery device in the stomach for the whole time period of drug release, and for this design and development some important factors should be appraised like the physiological condition of the stomach.

5.2. Physiological aspects:

The factors which actually help to determine the rate and extent of drug absorption are the innate property of the drug molecule and the target environment of the drug delivery. There are some factors which are actually play an important role in delivery and drug absorption like pH of the GIT, enzymes present in GIT, nature and amount of secretion, residence time, effective

absorbing surface area etc. many types of cells are present in stomach secrets approximately 2-3 litres of gastric juice per day. As an example, hydrochloric acid is secreted by the parietal cell, mucus secreted by goblet cells, pepsinogen is secreted by the chief cell. Stomach stirs the chime and mix it thoroughly [3].

5.2.1. Gastric pH:

pH of the GIT is not constant for all the time. It is fluctuated by different factors like diet, disease, presence of gases, fatty acids, and other many fermented products [4]. Gastric pH exhibits inter subject and intra subject variation which influence the activity of orally administered drugs. Gastrointestinal pH can be measured by the non-invasive procedure called radiotelemetry. It has been reported that in the fasting condition of a healthy human subject the average pH is 1.1±0.15 [5-7], in the fed state of male human subject the pH is 3.6±0.4 and within 2 to 4 hours this pH comes back. In the fed state the pH of the female subject is a little bit lower than male subject. Gastric pH may be affected by age, pathological condition and drugs.

Age: gastric acid secretion can be affected by the age like it has been observed that 20% of aged people shows either less amount of gastric acid secretion which is called (hypochlorhydria) or no gastric acid secretion (achlorhydria) which results in pH over 5.0.

Pathological condition: pathological condition or disease can affect the gastric acid secretion like pernicious anaemia, AIDS this condition lowers the gastric acid secretion apparently increases the pH.

Presence of drug: H_2 receptor antagonist, proton pump inhibitor these drugs decrease the gastric acid secretion.

Acidic chyme peristalsis from the stomach can be neutralized by the bicarbonate secretion by the pancreas and duodenal mucosa, this results the mean pH of healthy subject 5.8±0.3. pH of

the fasted state of small intestine is 6.0±0.14, passing from jejunum, mid intestine and ileum pH rises about 6.6 – 7.5. For an intragastric drug delivery system the selection of drug molecules, excipients, drug carrier(s) all depend on the pH of the GIT.

5.2.2. Gastrointestinal motility and transit time [3]:

Gastrointestinal motility, secretion, and transit time all depend on the state of the stomach, whether the stomach is in fed state or empty or fasted state. In case of fasted state saliva mucus, cellular debris like things are seen in stomach association with migrating myoelectric complex (MMC) which are kind of contractile events. In case of a fed state this contractility is changed. This MMC controls or regulates gastrointestinal motility. This MMC is associated with alternating cycles of activity and quiescence. Evidently in MMC are four successive phases of activity

Phase I: It is an inactive period which lasts for 30 to 60 minutes without contractions.

Phase II: It consists of spasmodic contractions which gradually increase in intensity as the phase proceeds, and it stays about 20 to 40 minutes. In this phase gastric discharge of fluid and very small particles begins later.

Phase III: This phase is very short in which distal and proximal gastric contractions take place (4–5 contractions per minute) which lasts for 10 to 20 minutes; this is also known as ‘‘house-keeper wave’’ sweep gastric contents down the small intestine.

Phase IV: The contractions subside between the latter portion of phase III and the quiescence of phase I during this brief transitional phase, which lasts for 0 to 5 minutes. A simplified schematic representation of the motility pattern, frequency of contraction forces during each phase.

Figure 5.1.

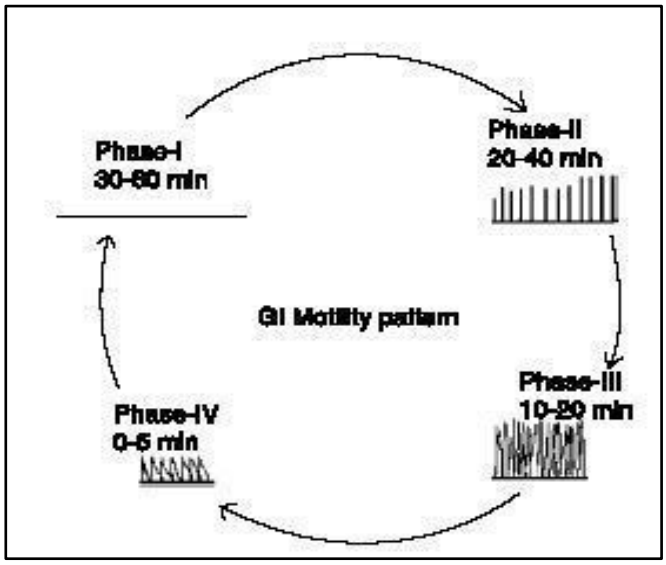


Figure 5.1.: Schematic representation of interdigestive motility pattern

5.2.3. Factors affecting gastric retention [2, 8]:

Gastric emptying and gastric retention time of an oral dosage form can be affected by various factors which are discuss as follows

1. *Density:* for producing perfect gastric retention floating system's density should be less than 1.0 g/ml and high-density system's density should be near about 2.5g/ml.
2. *Size and shape:* In case of size gastric retention time of unit dosage form having approximately 7.5mm diameter is more than unit dosage form having 9.9mm diameter. In case of shape the devices with tetrahedron and ring shape have superior gastric retention time than the device having other shapes.
3. *Single unit dosage form or multiple unit dosage form:* from the comparison of single unit dosage form with the multiple unit dosage form, multiple unit dosage form have higher safety and efficacy profile and much more expected floating profile.
4. *Fed and fasted state:* in case of fasting state gastric retention time is short and in case of fed state gastric retention time is comparatively longer because of delayed MMC.

5. *Nature of the food taken:* in fed state gastric retention time can be changed (increased) by taking the food, like taking indigestible polymer of fatty acid salts resulting reduced gastric emptying rate.
6. *Caloric content of food material:* gastric retention time is got higher with taking high caloric food
7. *Time interval of taking food:* the greater gastric retention time observed over 6.5h when consecutive meals are taken compared with a single meal because of larger time interval of MMC
8. *Gender/sex:* gastric retention time is low for male.
9. *Age:* people with more age have greater gastric retention time.
10. *Posture:* gastric retention time varies with changing the posture.
11. *Association or presence of drug material:* gastric retention time is increased with presence of drugs like anticholinergic, opiates which reduce the peristalsis & gastric retention time decreases with presence of cisapride, metoclopramide.
12. *Biological factor:* gastric retention time can be affected by some diseases like Crohn's disease diabetes.

5.2.4. List of devices for gastric retention:

A. High density systems: these devices utilize weight as a retention mechanism. Because of the density then gastric juice goes down to the bottom of the stomach.by based on the high-density mechanism.
B. Floating system: this device floats on the top of the stomach for its lower density than the gastric juice which increases the rate and extent of the absorption of the drug because of its prolonged gastric residence time. There are various types of floating devices are present like,
    1. Hydrodynamically balanced system- HBS^{TM}

2. Gas generating system
3. Raft forming systems
4. Low density core systems

C. Unfoldable, Extendible and Expandable systems

D. Superporous hydrogels

E. Mucoadhesive systems

F. Magnetic system

5.3. Mucoadhesive system:

An attraction between the pharmaceutical dosage form or formulation and the mucosal membrane is known as mucoadhesion. The system by which drug can be deliver to the body with the help of the mucoadhesion is called mucoadhesive system which can be given in various s region in the body like ocular, buccal, nasal, gingival, oral or gastrointestinal, vaginal rectal etc.

5.3.1. Advantages of mucoadhesive drug delivery systems:

i. Prolong residence time of the drug in gastrointestinal tract
ii. Targeting and localization of the dosage form at a specific site
iii. High drug flux at the absorbing site
iv. This mucoadhesive drug delivery system serves both the purposes of sustain release and presence of dosage form at the site of absorption
v. Excellent accessibility
vi. Painless administration
vii. Low enzymatic activity and avoid first pass metabolism

For mucoadhesion, bio adhesion takes place where a synthetic or biological product or material is attached to a biological tissue which is actually a mucous membrane for a long period of time.

5.3.2. Stages of mucoadhesion:

In case of mucoadhesion there are three steps:

i. Contact stage- in this stage a strong interaction between bio adhesive material and the biological membrane
ii. Penetration- interpenetration between the polymer chains of bio adhesive material and the mucosal membrane
iii. Consolidation stage- formation bond between the chains like mucin and polymer. And this bond can be ionic bonds, covalent bonds, hydrogen bonds, van-der waal's bonds, hydrophobic bonds etc.

5.3.3. Types of mucoadhesion:

In biological system there are four types of mucoadhesion is present

i. Between normal cell and another cell
ii. Between normal cell and foreign substance
iii. Between normal cell and pathological cell
iv. Between adhesive and biological substance

5.3.4. Factors affecting on mucoadhesion:[9]

i. Molecular weight of the polymer
ii. Mucin turnover rate
iii. pH at polymer-substrate interaction
iv. concentration of the polymer used in mucoadhesion

v. flexibility of the polymer chain

vi. swelling factor of the polymer

vii. stereochemistry of the polymer

5.3.5. Mucoadhesive Polymer

These mucoadhesive polymers can be hydrophilic as well as hydrophobic which have swelling properties and forming networks by cross-linking agents. This polymer can reflect optimal polarity which confirms that they can produce adequate wetting by the mucus and optimal fluidity which allows the mutual absorption to occur the diffusion of the polymer and the mucus.

5.3.6. Classification of mucoadhesive polymer [9, 10]

Based on rheological property-

i. *Hydrophilic polymer:* Consist carboxylic group and shows great mucoadhesive property. As an example- PVP (Poly vinyl pyrrolidone), MC (Methyl cellulose), NaCMC or SCMC (Sodium carboxymethyl cellulose), HPC (Hydroxy methyl cellulose)

ii. *Hydrogels:* When contact with water these types of polymers get swelled and attached to the mucus membrane. Depending upon the charge present they can be classified into three groups.

   a. Anionic – Carbopol, polyacrylates

   b. Cationic- Chitosan

   c. Non-ionic- Eudragit analogues

Based on the sources-

i. *Natural polymer:* Tragacanth, pectin, gelatine etc

ii. *Synthetic polymer:* Cellulose derivatives, Carbopol etc

5.3.7. Ideal characteristics:[11]

1. Molecular weight: The polymer must consist of a high molecular weight up to 10000 or more than that. This high molecular weight increases the adhesiveness or binding between the mucus and the polymer
2. Long chain polymer: the length of the polymer should be sufficient which can induce the interpenetration.
3. Viscosity: The viscosity must be sufficient to produce the comfort adhesion
4. Degree of crosslinking: This crosslinking promotes mobility and causes the resistance to the dissolution.
5. Spatial confirmation
6. Flexibility of the polymer chain
7. Concentration of the polymer: This is an important characteristic because the concentration should be optimum to produce adequate adhesiveness and this concentration is dependent on the types of dosage form. In case of solid dosage form greater the concentration greater the adhesion. But in case of semisolid dosage form after a certain concentration the adhesion may decrease.
8. Charge and degree of ionization: This can change the adhesiveness. (anion> cation> non-ionic)
9. Optimum hydration: hydration should be sufficient because excess hydration causes reduction in adhesion
10. Optimum pH: In low pH adhesion is optimum but higher pH produce rod like structure which promote the interdiffusion and interpenetration
11. High applied strength and initial contact time
12. It should not have any toxic effect

13. Polymers must be economic
14. They must be biodegradable
15. They must be biocompatible

5.4. References:

[1] P. Bardonnet, V. Faivre, W. Pugh, J. Piffaretti, F. Falson, Gastroretentive dosage forms: Overview and special case of Helicobacter pylori, Journal of controlled release 111(1-2) (2006) 1-18.

[2] B.N. Singh, K.H. Kim, Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention, Journal of Controlled release 63(3) (2000) 235-259.

[3] R. Talukder, R. Fassihi, Gastroretentive delivery systems: A mini review, Drug development and industrial pharmacy 30(10) (2004) 1019-1028.

[4] A. Rubinstein, Microbially controlled drug delivery to the colon, Biopharmaceutics & drug disposition 11(6) (1990) 465-475.

[5] J.B. Dressman, R.R. Berardi, L.C. Dermentzoglou, T.L. Russell, S.P. Schmaltz, J.L. Barnett, K.M. Jarvenpaa, Upper gastrointestinal (GI) pH in young, healthy men and women, Pharmaceutical research 7(7) (1990) 756-761.

[6] T.L. Russell, R.R. Berardi, J.L. Barnett, L.C. Dermentzoglou, K.M. Jarvenpaa, S.P. Schmaltz, J.B. Dressman, Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women, Pharmaceutical research 10(2) (1993) 187-196.

[7] C.Y. Lui, G.L. Amidon, R.R. Berardi, D. Fleisher, C. Youngberg, J.B. Dressman, Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans, Journal of pharmaceutical sciences 75(3) (1986) 271-274.

[8] S. Arora, J. Ali, A. Ahuja, R.K. Khar, S. Baboota, Floating drug delivery systems: a review, AapsPharmSciTech 6(3) (2005) E372-E390.

[9] P. Rathee, M. Jain, A. Garg, A. Nanda, A. Hooda, Gastrointestinal mucoadhesive drug delivery system. A review, J Pharm Res 4(5) (2011) 1488-1453.

[10] D. Accili, G. Menghi, G. Bona Cucina, P. Di Martino, G.F. Palmieri, Mucoadhesion dependence of pharmaceutical polymers on mucosa characteristics, European journal of pharmaceutical sciences 22(4) (2004) 225-234.

[11] F. Madsen, K. Eberth, J.D. Smart, A rheological assessment of the nature of interactions between mucoadhesive polymers and a homogenised mucus gel, Biomaterials 19(11-12) (1998) 1083-1092.

CHAPTER - VI

SUSTAINED RELEASE DRUG DELIVERY SYSTEM

6. Sustained release drug delivery system

6.1. Introduction:

A control release formulation that maintains a constant level of blood circulation is required because the early discharge and recurrent dosing of drugs in a conventional drug delivery system put any medication at danger of dosage variations. Sustains released with the induction of elongated release of matrix tablets has proved to be an efficient tool of the control release of drugs without involvement in the procedures of complex production.A sustained release approach allows for the longer-lasting accumulation of therapeutically active concentration in the systemic circulation, which improves patient compliance. There are several sustained release oral dose forms, such as that matrix with a plasma membrane-controlled system and the solubility or insolubility of polymers or waxes in water, have recently focused on the consciousness of sustained release systems for the drugs, which are poorly soluble in water. Nevertheless, it makes an idea about the problems/disadvantages shown by the traditional drug delivery system and the sustain release helps to perform the following goals:

i) Invariant release of the drugs by increasing the time-period.

ii) Reducing the dosing frequency of any drug.

iii) Reduce the range of blood levels.

In many cases, common methods are more preferable for administering the drug, but some the drugs are not stable and toxic by frequent dosing. This type of drugs has less therapeutic effects and also faces some solubility niceties. In this type of cases, we can use a sustained release drug delivery system, which controls the concentration of drug in plasma and the therapeutic index [1-4].

This early release of drug dosage forms has some limitations:

1) Sustained release pharmaceutical administration prevents early conclusion of therapy.

2) Compared to normal dosage forms, controlled release tablets are more expensive per unit dose.

3) It cannot derive ionic drugs, and cannot develop drugs of large molecular size [5].

6.2. Advantages of Controlled Release Drug Delivery System [4, 6, 7]

6.2.1. Clinical merits

1. Reduced the systemic and local adverse effects:
    - Decreased the stomach as well as gastrointestinal irritation.
2. Better uses of drug:
    - Decrease the amount of drug used in systemic circulation.
3. Increased the effectiveness of drugs, which are used in treatment:
    - Optimized therapy.
    - Reducing the activity of drugs for long time use.
4. Increased the consent of the patient:
    - Reduced the frequent dose of drugs.

6.2.2. Commercial merits

1. Improved the possibilities of description of innovative or technological direction.
2. Increased the half-life of products.
3. Differentiate between two products.
4. Extension of market and patents.

6.3. Disadvantages of Controlled Release Drug Delivery System [4, 6-8].

1. Drugs with short half-life, metabolism rapidly and it has to be given repeatedly, so there are chances of missing any dose of drug.
2. Sustained release drug administration prevents early discontinuation of therapy.

3. Decreased systemic availability in comparison to immediate release conventional dosage forms.
4. Not all drugs are suitable candidates for formulation as prolonged action medication.

6.4. Drug properties of drugs affecting the dosage form [9, 10].

The design of a controlled release system depends on a number of drug-related variables, including the drug's method of administration, kind of dose, target disease, timing of therapy, and other qualities. The following are the key characteristics of medications that have the greatest impact on their effects.

6.4.1. Physicochemical properties:

1] Aqueous solubility and pka: The drug must first dissolve in the watery environment around the administration site before partitioning into the absorbing membrane.

2] Partition coefficient: Drugs with higher partition coefficients—those that are more lipid or water soluble—will exhibit either slower or faster tissue flux. For a sustained release medication delivery system, neither of the circumstances are desirable.

3] Stability of the drug: Drugs that are unstable in the intestinal environment may be difficult to turn into a sustained release drug delivery system since the majority of oral controlled release systems are designed to relieve their goals of excessive length of gastrointestinal tract.

4] Size of the dose: It may be necessary to use a controlled release dosage form to prevent the fast administration of medications with an elimination half-life of less than 2 hours.

5] Molecular size and diffusivity: Drugs in sustained release systems must also diffuse with a matrix or membrane that controls the rate of diffusion in addition to diffusing with various biological membrane types. The capacity of a material or energy to disperse or to let anything to pass through diffusion is measured by its diffusivity.

6.4.2. Biological properties:

1] Absorption: Before reaching the systemic circulation, medicines must first be dissolved in fluid. When deciding whether to formulate a medicine into a controlled release system, the pace, extent, and consistency of drug absorption are crucial considerations.

2] Distribution: The total drug elimination kinetics may be significantly influenced by the medication's distribution throughout tissues. One needs knowledge of drug disposition in order to build controlled release products.

3] Metabolism: In order to create a sustained release mechanism for the medications, metabolism—the process by which a drug is changed into another chemical form—must be taken into account. As a result, the route of administration of a medicine may be impacted by its metabolic activity.

4] Elimination or Biological half-life: The biological half-life of a substance is used to gauge its rate of elimination. Drugs with a shorter half-life need to be dosed more often, hence it would be advantageous to create SDRS. Drugs having longer half-lives will exhibit the opposite of this.

5] Safety considerations and Side effects: A specific medicine's negative effects can be reduced by managing its plasma levels and gradually using less of the drug overall throughout treatment. The therapeutic index (TI) is the most often used indicator of a drug's safety margin.

This is defined as,

$$TI = TD50/ED50$$

Where,

TD50 is median toxic dose

ED50 is median effective dose

In general, the larger the value of Therapeutic Index means the drug is safer.

6] Protein binding: Drug compound formation with proteins is referred to as protein drug binding. Particularly responsible for such a connection are the proteins.

7] Disease state: Disease is the abnormal state of the body; it may affect the physiological immunology & movement of the body, and also can lead to death. As the function of the body is altered, it can also affect the physic-chemical properties of the drugs, Pharmacokinetics & Pharmacodynamics of the drugs.

As an example, for rheumatoid arthritis, aspirin, which is in, form of sustained release, delivers the expected blood levels, especially through the whole night resulting in morning firmness.

6.5. Oral sustained release drug delivery design

The oral route of drug administration is commonly accepted because it is the most facile dosage form, design and caring patient. For formulation of sustained release dosage form we should always remember the several parameters like different pH in gastrointestinal tract, peristaltic movement, enzyme present in gastrointestinal tract and their effects on the dosage form and as well as the drug present in the dosage form. There are many sustain release dosage forms which chase the several steps like diffusion, dissolution or diffusion and dissolution both which produce gradual release of the drug at a rate which is already predetermined. Speculatively, the release of sustain release dosage form follows zero order which carry on the time of plasma-drug level as same as in IV infusion

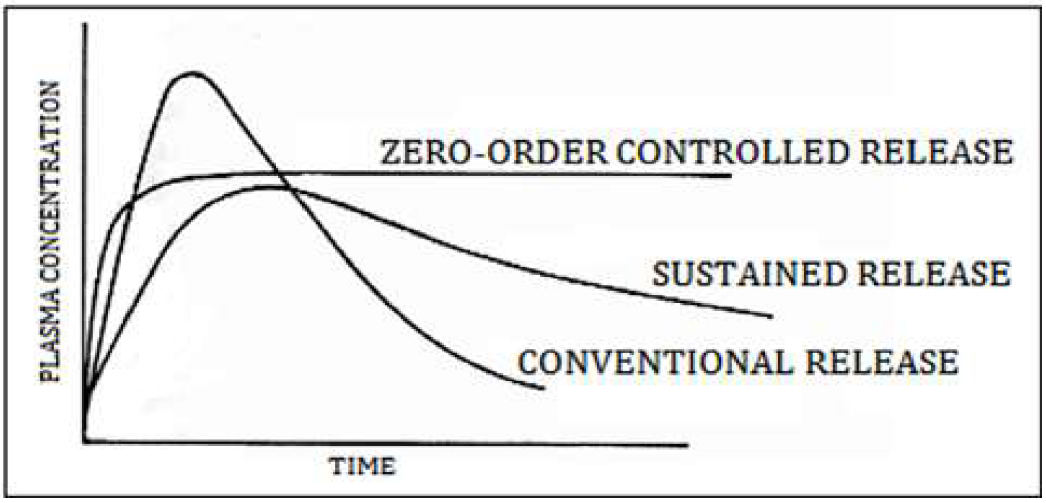


Figure 6.1. Plasma drug concentration profile for conventional release, a sustained release and zero order controlled release formulation

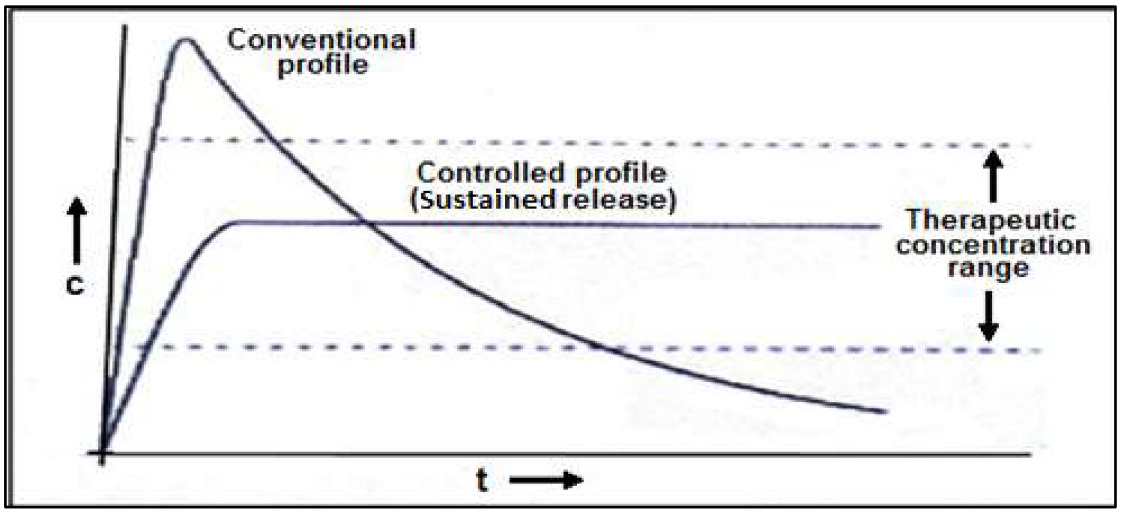


Figure 6.2. Comparison of conventional and controlled release profiles

6.6. Approaches to sustain release drug delivery system [4, 11, 12]

6.6.1. Dissolution controlled release systems:

keep the release The idea applied in this system is oral products using rate-limiting processes called dissolving. The system's categorization:

A. Membrane dissolution-controlled release system: Membrane dissolution systems are sometimes referred to as monolithic systems because the pharmaceuticals contained in the membrane are completely dissolved in the medium that regulates the release

of the medications. For membrane dissolution-controlled release devices, drug releases typically follow first order kinetics.

B. System of controlled drug release based on reservoir dissolution: In reservoir-based systems, the drug reservoir is encased in the polymer coatings. The rate-regulating porous polymeric membrane releases the medication.

6.6.2. Diffusion controlled release systems:

Pharmaceutical compounds diffuse across a polymeric membrane. In order to create them, drug particles are either dispersed in a polymeric membrane or encapsulated in a polymeric membrane. They demonstrate how drug molecules travel from an area of greater concentration to one of lower concentration in the diffusion mechanism. According to Fick's law,

$$J = -D \frac{dc}{dx}$$

Where,

J = flux of the drug across a membrane in the direction of reducing concentration,

D = Diffusion coefficient of the drug

dc /dx = Change in the concentration of the drug in the membrane

Whereas when drug is present in a water insoluble membrane, it must diffuse through the membrane. The drug release rate dm/dt is given by,

$$\frac{dm}{dt} = \frac{ADK\Delta C}{L}$$

Where,

A = Area.

K = Partition coefficient of drug between the membrane and drug core.

L = Diffusion path length which is known as coating thickness

ΔC = Difference in concentration across the membrane.

6.6.3. Dissolution and diffusion-controlled release systems:

The medicine is surrounded by membranes in the body that are only partly soluble in water. The primary characteristic of this system is the presence of a membrane that is only partly soluble around the drug core.

6.6.4. Ion exchange resin- drug complexes:

The approach uses chug release properties that depend on the resin-containing drug's ionic environment and should be less successful in environments with high enzyme concentration and low pH levels. By employing one of the microencapsulation methods to coat the drug-resin combination, the release rate may be regulated.

6.6.5. pH dependent formulation:

Some drugs are absorbed in the gastrointestinal tract, when we change the pH of gastrointestinal tract, then the formulation of dosage forms is prepared using an adequate amount of buffering agents like carbonates and bicarbonates etc. The buffering agents alter the proper constant pH as GI fluid passes over the membrane, which results in a consistent rate of medication release.

6.6.6. Osmotic pressure-controlled systems:

The osmotic is the pressure driving force that creates consistent medication release in the kind of drug delivery system. To regulate the flow of GI tract fluid to penetrate through the permeable membrane. This type of system requires osmotic pressure to be effective and is independent of its environments.

6.7. References:

[1] L. Deb, recent trends in scope and opportunities of control release oral drug delivery systems.

[2] K. Vamsy, K. Srinath, P. Chowdary, P. Planisamy, G. VIjayasanker, Formulation development and evaluation of Divalproex sodium extended release tablet, International Journal of Research pharmaceutical and Biomedical Science 2 (2011) 809-832.

[3] H. Patel, D.R. Panchal, U. Patel, T. Brahmbhatt, M. Suthar, Matrix type drug delivery system: A review, J Pharm Sci Biosci Res 1(3) (2011) 143-51.

[4] B. Ankit, R. Rathore, Y. Tanwar, S. Gupta, G. Bhaduka, Oral sustained release dosage form: an opportunity to prolong the release of drug, Res. Pharm. Bio. Sci 8 (2013) 7-14.

[5] A. Anal, D. Bhowmik, H. Gopinath, B. Kumar, S. Duraivel, K. Kumar, Overview on controlled release dosage forms, Int J Pharma Sci 3 (2013) 258-269.

[6] J.H. Perrin, Sustained and controlled release drug delivery systems, vol. 6. Edited by JOSEPH R. ROBINSON. Dekker, 270 Madison Ave., New York, NY 10016. 1978. 773 pp. 15× 23 cm. Price $59.75, Wiley Online Library, 1980.

[7] N. Bhalla, A. Deep, M. Goswami, An overview on various approaches to the oral controlled drug delivery system via gastroretentive drug delivery system, International research journal of pharmacy 3(4) (2012).

[8] H. Bechgaard, G.H. Nielsen, Controlled-release multiple-units and single-unit doses a literature review, Drug Development and Industrial Pharmacy 4(1) (1978) 53-67.

[9] H.C. Ansel, N.G. Popovich, L.V. Allen, Pharmaceutical dosage forms and drug delivery systems, Lippincott Williams & Wilkins1995.

[10] B. Narwate, Sustained release dosage form: a concise review, International journal of pharmaceutics and drug analysis (2017) 153-160.

[11] D. Bhatt, Studies on enteric coated sustained timed-release tablets of metronidazole, Journal of Chemical and Pharmaceutical Research 2(2) (2010) 226-232.

[12] T. Parashar, S.V. Soniya, G. Singh, S. Tyagi, C. Patel, A. Gupta, Novel oral sustained release technology: a concise review, Int J Res Dev Pharm Life Sci 2(2) (2013) 262-269.

CHAPTER - VII

REVIEW OF LITERATURE

7. Review of Literature

Sarojini et al. [1] undertook the current study, to learn whether almond gum has the ability to function as a binder and release inhibitor in tablet formulations. There hasn't been any noteworthy work documented using it as a tablet binder. It was investigated how almond gum and PVP K-30 affected the release of diclofenac sodium. No chemical interaction was found in the medication, gum, or combo according to FT-IR spectroscopic measurements. Seven formulations using Microcrystalline cellulose as diluents, diclofenac sodium as the model drug, and solutions of 2%, 4%, 6%, 8%, and 10% w/v of almond gum and 2%, 4% w/v of PVP K-30 were made using the wet granulation process. This research was done to determine the differences between synthetic and natural gums and to determine whether natural gums could take the place of synthetic gum. Studies on granules and tablets' physical and technical properties, such as flow rate, Carr's index, Hausner's ratio, angle of repose, friability, and disintegration time, were conducted and found to be satisfactory. When compared to the 2% and 4% concentrations of synthetic gum, the drug release was greater with almond gum. It was discovered that the release exponent values were less than 0.5. This suggests that non-fickian diffusion is the releasing mechanism. Tablets with a binder content of 2% w/v produced the best results. The creation of uncoated tablet dosage forms was shown to benefit from the usage of almond gum. This research can be expanded further to look at cutting-edge sustained release formulations.

Rahim et al. [2] analyse the gum from Prunus domestica's binding capacity in tablet formulations. Wet granulation was utilised to create six batches of tablets (F- 1B to F-6B), with Avicel pH 101 serving as the diluent, sodium diclofenac serving as the model drug and 10, 15, and 20 mg of Prunus domestica gum serving as the binder. PVP K30 served as the standard binder. As

a lubricant, magnesium stearate was utilised. The bulk density, tapped density, Carr index, Hausner's ratio, angle of repose, and physical characteristics of the compressed tablets, such as hardness, friability, thickness, and disintegration time, were assessed and found to be suitable in terms of granule flow properties. The formulation comprising plant gum is compatible with the medication and other excipients used in tablet formulation, according to the results of the FTIR spectroscopic investigation. As a result, the plant gum may be used as a binder in tablet formulations. According to the dissolving profile, tablet formulations using Prunus domestica gum 15 mg/200 mg of the total weight of the tablet as a binder performed better than those using PVP K30.

Tavakoli et al. [3] The purpose of this study was to assess the performance of a novel tabletting binder made from Hibiscus esculentus (okra gum). Okra gum was extracted from the fruit's pods by macerating it in distilled water, filtering the resulting viscous solution, and then precipitating the gum extract with acetone. Two models, comprising a placebo formulation (lactose) and a medication formulation (acetaminophen, ibuprofen, and/or calcium acetate), were examined to assess the efficacy of the binder. Okra gum was used to make granules in various concentrations (0.5 -- 6%w/w) and was tableted in a Kilian single punch press. As the reference binders, cornflour (12.5% w/w) and P.V.P (22% w/w) were used. Disintegration time and dissolving rate, among other physical characteristics of the granules and tablets, were investigated. Both the tablet's (hardness, friability, and disintegration time) and placebo granulate's (bulk and tapped density, granule strength, and flowability) physical characteristics were generally favourable. Additionally, the Ibuprofen and Calcium acetate tablets with Okra gum demonstrated adequate hardness, a desired disintegration time, and minimal friability. For pills of

acetaminophen, ibuprofen, and calcium acetate, the percentage of medication released after 45 minutes was 15%, 44%, and 96%, respectively. Some pill formulations with good hardness and friability are made with okra gum. However, this binder slows down the pace at which some hardly soluble medicines dissolve, making it a potential contender for formulations with prolonged release.

Singh AK et al [4] Mangifera indica (mango) gum was tested as a tablet binder using paracetamol as a model medication. Natural gums are inexpensive, widely accessible, and effective as a tablet binder. To the best of our knowledge, no major research on mango gum as a tablet binder has been published. Mangifera indica gum was used as a tablet binder during the wet granulation process to create paracetamol tablets. The produced tablet's physicochemical properties were assessed. It's friability ranges from 1.12 to 0.26%, and their duration to dissolve from 3 to 8 minutes. At a comparable concentration (5% w/w), the Mangifera indica gum's binding effectiveness was compared to that of the industry-standard binder gum acacia. Mangifera indica gum is used to make tablets that range in hardness from 6.3 to 6.8 kg/cm2, which is equivalent to gum acacia, the industry standard binder (4.8 kg/cm2). MIG may work effectively as a binding agent in the creation of tablet dosage forms, in conclusion.

Natural gums work well as tablet binders because they are affordable and accessible. Within this inquiry, Mistry AK et al [5] produced a formulation of ofloxacin tablets utilising xanthan gum, hibiscus esculentus, and three natural binders: acacia arabica. These six batches of ofloxacin tablets were created utilising a wet granulation technique and a variety of natural binders in varying kinds and concentrations. The tablets' hardness, friability, and weight fluctuation were examined, and an in vitro release experiment was carried out in a phosphate buffer with a pH of

6.8. The produced pills' diverse release kinetics and similarity factors (f2) were also assessed. The natural binders in the tablets gave them enough hardness, a suitable disintegration period, and little friability. They were shown to have a higher percentage of medication release than the commercial formulation, which showed more than 85% drug release in just 45 minutes. The in vitro release data fit the zero-order distribution well, and the values of the release exponent 'n' ranged from 0.303 to 0.514. In comparison to the commercially available tablets, the best batch had a high similarity factor f2 of 64.50. The outcomes showed that, in comparison to the other binders used in the formulation of the Ofloxacin tablet, the gum Acacia arabica performed just as well as gelatin.

Prajapati et al [6] reported formulation and evaluation of matrix tablets of aceclofenac prepared by using different proportions of xanthan and karaya natural gum. And measurement of physical parameter like hardness, weight variation, friability, swelling index, and drug content, in vitro drug release in phosphate buffer at pH 7.4 for 24 hours. Diffusion of drug molecules into aqueous medium controlled by gelatinous swollen masses of aceclofenac. Because of burst effect and fast release, combination formulation of karaya and xanthan shows better controlled release than individual FX and FK formulation and FXK shows synergistic interaction. FTIR study clearly revealed there was no chemical interaction between drug and polymer. This sustained release matrix tablet followed zero order, first order, Higuchi and korsmeyer peppas equation.

Metal ion induced alginate – locust bean gum interpenetrating microsphere for sustained oral delivery of aceclofenac, an have been reported by Jana et al [7]. Stability and rapid release of drug at higher pH value was the main drawback of alginate microsphere. For this reason, ionotropic gelation techniques were applied for prolonged release of aceclofenac. Alginate and locust bean gum were used for prepared interpenetrating microspheres with entrapment efficiency 59 – 93% and calcium ion used as an inducing agent. The interaction of drug and polymer was confirmed by

FTIR study. The optimized formulation followed the korsmeyer-peppas model with an anomalous (non-Fickian) diffusion mechanism. And diffusion of drugs at pH 6.8 over a period of 8 hours. Also, the carrageenan induced rat paw model showed anti-inflammatory activity.

Prajapati et al [8] reported that Ionotropic-gelation technique with 3^2 factorial designs applied for development aceclofenac containing Locust bean gum (LBG)-alginate mucoadhesive macromolecules and optimized the drug entrapment efficiency(%DEE), % of mucoadhesion at 8 hr(M8) and % in vitro drug release at 10 hr was 84.95±0.02 to 95.33±1.56. The percentage yield, average size and DEE of macromolecules were found within the range of 93.19 to 96.65%, 1.328±0.11 to 1.428±0.13µm and 56.37 to 68.54% respectively. The data obtained from the study was fitted first order pattern with super-ll transport mechanism and the optimized formulation was characterized by FTIR, SEM and DSC. pH of the medium affects the swelling and mucoadhesive of the formulation.

Ghosh et al [9] reported the various viscosity of HPMC in two different ratios, hydrophobic polymer ethyl cellulose and guar gum were used to prepare matrix tablets of aceclofenac. The in vitro drug release (at pH 7.5) of wet granulated matrix tablet formulation (F-7) exhibited controlled release pattern (Higuchi kinetic) and compared with marketed sustained release formulation (Aeroff-SR). Stability study data at 40ºC with 75% RH for 6 months provided no changes of physical appearance, drug content or dissolution pattern. S0, it was observed the aceclofenac containing HPMC matrix tablet exhibit oral controlled release.

Formulation and evaluation of xanthan gum based aceclofenac tablet for colon targeted drug delivery reported by ramasamy et al [10]. Aceclofenac containing colon targeted tablets was prepared using xanthan gum as a carrier and Eudragit multilayer coating agent used to resist the drug release in stomach and small intestine. The prepared tablet was tested for physicochemical properties, drug content uniformity, in vitro drug release pattern. The interaction of drug and gum mixture was confirmed by FTIR and DSC study. Release of Eudragit coated aceclofenac tablet in simulated colonic fluid was shown due to the degradation of the xanthan gum membrane by bacterial enzymes.

Kaur et al [11] reported the solvent emulsification method applied to prepared aceclofenac containing poly (lactic-co-glycolic acid) (PLGA) (75:25) and polycaprolactone (PCL) biodegradable controlled release microsphere for parenteral administration. Polymer drug ratio (1:1,2:1,3:1) affects the entrapment efficiency and drug loading of PLGA and PCL microspheres. The drug entrapment efficiency, average size of PLGA and PCL microsphere were found within the range of 90±0.72% to 91.06±4.01% with PLGA and 83.01±2.13 to 90.40±2.11% with PCL, and the average size was 11.75µm, 3.81µm respectively. SEM study clearly revealed the good spherical structure of microsphere and drug polymer interaction confirmed by FTIR and DSC study. The drug release from the PLGA microsphere followed the Higuchi model and PCL microsphere followed the korsmeyer-peppas pattern. The in vivo result showed excellent anti-inflammatory activity in carrageenan induced rat after parenteral administration over a prolonged period(48hr). Stability study data at 2-8ºc for 30, 60 and days provided no change of entrapment efficiency, residual drug content and polymer drug compatibility.

The aim of the study was to prepare and evaluate once daily sustained release tablets of aceclofenac by using polyethylene oxide (PEOs) of different molecular weight as matrix polymers

as reported by Gupta et al [12]. Different proportions of PEO in direct compression tablets affect the aceclofenac release profile. 28% PEO (80%PEO WSR303 and 20% of PEO WSR NboK) containing direct compressed matrix tablets showed a similar release profile as estimated by similarity factor (f2), to a marketed product Hifenac SR. Optimized formulation and marketed formulation (Hifenac SR) exhibited the same pharmacokinetics parameters such as Tmax and Cmax when bioavailability study was done in human volunteers.

Deshmukh et al [13] reported the aceclofenac containing polymeric microsphere was prepared by using ethylcellulose and eudragit RS100 two biodegradable polymer by utilizing the single (oil in water,o/w) solvent evaporation method. A plackett Burman design (PBD) was employed by using the design-expert $^{(R)}$ software (8.0.2.1) to enhance the encapsulation efficiency. Evaluation of all batches of the microsphere for their size, morphology, encapsulation efficiency and drug release. FTIR and XRD study done for analysis of the drug polymer interaction and SEM study done for imagining the particle. Graphical and mathematical analysis of Plackett-Burman model optimize the formulation which prepared by eudragit RS100 and polyvinyl alcohol (PVA) had a significant negative effect on encapsulation efficiency. From this study identified the major significant factor that influences the encapsulation efficiency of the microsphere. So, the optimized formulation of eudragitRS100 and ethyl cellulose microsphere showed high encapsulation efficiency (70.15% to 83.82%). SEM study clearly revealed the oval and smooth surface of the microsphere. FTIR study showed no drug polymer interaction and equal dispersion of drug in polymer is confirmed by XRPD study. Optimized formulation prepared by ethyl cellulose and eudragitRS100 polymer shows 12-hour drug release. So, by implementing the experimental design technique to increase the encapsulation efficiency of aceclofenac containing the polymeric microsphere by optimizing the formulation variable.

Ganesh et al [14] investigated to increase the bioavailability of aceclofenac, chitosan co-crystal of aceclofenac was prepared by solvent exchange method and its entrapment into alginate matrix a supersaturated drug delivery system (SDDS). Particle size of co-crystal was determined by laser-light scattering methodology using Malvern particle size analyzer. Aceclofenac co-crystal, SDDS and pure drug characterized by FTIR, SEM, XRD and DSC studies. FTIR and DSC study shows there was no drug polymer interaction and SEM study clearly revealed the fluffy, porous, and rough surface of aceclofenac co-crystal and SDDS particle. Optimized formulation of aceclofenac co-crystal shows high solubility of drug in co crystal form and its entrapment calcium alginate beads shows continuous and sustained drug release by super saturated drug diffusion. Pharmacokinetics parameter of aceclofenac co-crystal and SDDS were 2.06±0.42µg/ml, 1hr, 159.72±10.84 and 2.01µg/ml, 1hr, 352.76±12.91 respectively of Cmax, Tmax and relative bioavailability. Anti-inflammatory activity was determined by carrageenan induced rat paw edema model. Respectfully, all of the aforementioned findings supported the notion that SDDS describe may be a useful method for the oral sustained release of aceclofenac and perhaps for a number of other poorly soluble medications.

Jana elal[15] reported through maleic anhydride induced esterification, aceclofenac containing microspheres were prepared by using sodium alginate and gellan gum polymer. Optimum formulation (F-10) shows highest entrapment efficiency and percentage of yield were 98.46±0.10% and 91.66±2.18% respectively, which contain 1%(w/v) sodium alginate, gellan gum and aceclofenac and 3% maleic anhydride and 1%(v/v) concentration sulphuric acid. Particle size of optimized formulation was found to be 270-490µm and spherical, rough surface of the microsphere was revealed by SEM analysis. No significant shifting of peak observed by FTIR

study. In-vitro drug release study performed using USP-II rotating basket type dissolution indicating prolonged sustained release of aceclofenac over 6 hr and followed the korsmeyer-pappas model (R^2= 0.9571-0.9952). New Zealand white rabbit (reg no- CPCSEA/06/a/955) used to perform in-vivo studies which showed that drug sustained in plasma around 7hr. Formulation shows good anti-inflammatory activity done by using carrageenan induced rat paw model after oral administration of 10mg/kg dose of aceclofenac.

Oil entrapped sterculia gum-alginate buoyant system of aceclofenac: development and in-vitro evaluation have been reported by Guru et al [16]. Three level factorial design (3^2) was employed to develop and optimize aceclofenac containing liquid paraffin-entrapped sterculia gum alginate beads prepared by using ionotropic emulsion-gelation technique. Polymer drug ratio affects the entrapment efficiency and cumulative release after 7hr. The drug entrapment efficiency of these formulations was found 63.28±0.55 to 90.92±2.34% with average bead size 1.32±0.04 to 1.72±0.12mm. The microsphere was characterized by FTIR, DSC, P-XRD and SEM analysis. The cumulative drug release of optimized formulation(F-0) in gastric fluid (P^H 1.2) was found 41.65±3.97% after 7hr and well floated over 8hr with 5.20min log time. The drug release of the formulation followed karsmeyer-pappas (R^2-0.9866 – 0.9995) model with anomalous (Non-Fickian) diffusion mechanism

Deshmukh et al [17] reported the aceclofenac microsphere was prepared by using surface methodology and by applying a single emulsion (o/w) solvent evaporation method. Amount of different variables like Eudragit RS-100 and polyvinyl alcohol having the significant effect on

entrapment efficiency and in vitro drug release of the polymorphic microsphere. A plackett-Burman (PBD) screening design was employed by using design expert(R) software to increase the entrapment efficiency of the microsphere and optimum entrapment efficiency was predict by utilizing the linear mathematical model equation. Resultant formulation was characterized by FTIR, SEM and PXRD analysis and evaluation of all batches for their size, entrapment efficiency and in vitro drug release. FTIR study shows there was no drug polymer interaction and drug dispersion within the microsphere shown by DSC and PXRD analysis. SEM study clearly revealed the spherical and smooth surface of the microsphere. Optimized formulation shows high entrapment efficiency (84.87±0.005) with suitable drug release over a period of 12 hr and shows small error value (1.39). The results show that these microspheres might be a potential drug delivery technology with improved entrapment effectiveness and prolonged drug release. These prolonged actions provide the greatest healing impact while having the fewest gastrointestinal side effects. In vitro release kinetics followed korsmeyer-peppas equation and drug release by simple diffusion-controlled mechanism with good linearity (R^2 0.9906-0.9949)

Ravella et al [18] reported the preparation of sustained release pellets of aceclofenac using pelletization technique. Coating of the drug layered pellets with ethyl cellulose N50 and hydroxypropyl methylcellulose E5 ensured the sustained release of this preparation. It was investigated how varied weight ratios of the film-forming agent HPMC and the rate-retarding polymer ethyl cellulose affected the kinetics of drug release. Statistically significant differences were found among the drug release profile from different formulations. The dissolution profile which was carried out in pH 6.8 phosphate buffer using USP type I apparatus and Aceclofenac pellets appeared to have promise for long-lasting drug administration, according to in vitro release kinetics. The micromeritic properties evaluated showed that the optimized formulation have

passable flow properties, Drug release from pellets appeared to follow first order kinetics, according to a kinetic investigation, and pharmacological analyses suggested that it had a sizable analgesic effect. and FTIR study clearly revealed there was no chemical interaction between the drug and the polymer.

Aceclofenac prodrugs based on hydroxyethylcellulose were created, characterised, and evaluated for their bioavailability and immunomodulatory activity by abbas et al[19]. Investigated the synthesis of aceclofenac (ACL) macromolecular prodrugs which were found organo-soluble on to hydrophilic polysaccharide hydroxyethylcellulose (HEC).The esterified prodrugs emerged as 200–550 nm diameter nanoparticles and exhibited self-assembly behaviour at the DMSO/water solvent interface.Spectrophotometric study FTIR showed the structural characterization and ester signal of HEC-ACL conjugates at 1744 cm−1 which indicate the success of esterification reaction between polymer and ACL.Interleukin 6 (IL-6) levels were significantly reduced (43%), indicating the HEC-ACL conjugates' potential for immunomodulation. The edema that resulted from the infusion of carrageenan was 81% inhibited by the conjugates. In cell viability experiments, HEC-aceclofenac conjugates seemed to be biocompatible. According to bioavailability tests, the conjugate's ACL has a considerably improved bioavailability compared to unmodified ACL (3.93 h), with improved pharmacokinetic properties, as evidenced by its plasma half-life of 7.90 h, tmax of 4.0 h, and peak plasma concentration (Cmax) of 8.27 μgmL−1.

Interpenetrating polymeric network (IPN) microparticles with glutaraldehyde cross-linked chitosan and tamarind seed polysaccharide (TSP) for extended aceclofenac release have been prepared, characterised, and evaluated by jana et al [20]. The average particle diameters of these

microparticles varied from 490.55 23.24 to 621.60 53.57 µm, and the drug entrapment effectiveness ranged from 85.84 1.75 to 91.97 1.30%. The formulation F-5, which was made with 400 mg TSP, 200 mg chitosan, 2 ml glutaraldehyde, and 400 mg aceclofenac, had the highest drug entrapment efficiency. Increased drug entrapment was seen with increasing glutaraldehyde concentrations, which may be related to the compound's strong degree of cross-linking. These chitosan-TSP IPN micro particles were characterized by FTIR which revealed the absence of any chemical interaction of aceclofenac with IPN polymeric structure, The presence of aceclofenac in the IPN polymeric matrix as a separate entity and in a stable state was confirmed by DSC, indicating that there was no physical or chemical interaction between the aceclofenac and the IPN polymeric matrix. Additionally, SEM analyses of the aceclofenac-loaded chitosan-TSP micro particles revealed rough surfaces with wrinkles, which may be caused by partly collapsing the polymeric gel network during drying. These chitosan-TSP IPN microparticles were loaded with aceclofenac, and the in vitro drug release from them demonstrated sustained aceclofenac release over an 8-hour period and followed the Korsmeyer-Peppas model (R2 = 0.9809-0.9828) with an anomalous (non-Fickian) diffusion drug release mechanism. The newly discovered aceclofenac-loaded IPN microparticles showed persistent anti-inflammatory effect in vivo experiments in carrageenan-induced rats for a lengthy period after oral administration.

Deshmukh et al [21] investigated that Aceclofenac containing polymeric (Ethyl Cellulose:Eudragit) based microspheres were prepared by o/w single emulsion solvent evaporation method. The IV decimal design was employed using the design Expert^{R}software(version 8.0.7.1 stat-easy Inc, Minneapolis MN) to evaluate the formulation variables (amt of PVA,amt of polymer with respect to drug) and process variables(speed of stirr) to optimize the formulation. The

resulting formulations were evaluated for their size, Entrapment efficiency and in vitro drug release. FTIR of optimized formulation shows there was no incompatibility with drug and polymer and SEM study shows discrete, spherical porous and with uniform surface microsphere. Drug dispersion within polymers was revealed by XRD study. Release kinetics data fitted with higuchi model(R^2 =0.9692) and korsmeyer – peppas equation(R^2 = 0.9218 – 0.9674). Entrapment efficiency of the optimized formulation depends on the amount of PVA and stirrer speed. Entrapment efficiency ranges from 60.86±0.76% to 84.07±0.88 with decrease of PVA and increase the stirrer speed. Invitro study shows that optimized formulation of combined polymer exhibits 12 hour sustained drug release at pH- 6.8. The results show that these microspheres might be a potential drug delivery technology to extend drug activity and enhance encapsulation effectiveness, hence achieving the best healing benefit with the least amount of GIT impact.

7.1. References:

[1] S. Sarojini, D.S. Kunam, R. Manavalan, B. Jayanthi, Effect of natural almond gum as a binder in the formulation of diclofenac sodium tablets, International Journal of Pharmaceutical Sciences and Research (IJPSR) 1(3) (2010) 55-60.

[2] H. Rahim, M.A. Khan, A. Badshah, K.A. Chishti, S. Khan, M. Junaid, Evaluation of Prunus domestica gum as a novel tablet binder, Brazilian Journal of Pharmaceutical Sciences 50 (2014) 195-202.

[3] N. Tavakoli, N.G. Dehkordi, R. Teimouri, H. Hamishehkar, Characterization and evaluation of okra gum as a tablet binder, Jundishapur Journal of Natural Pharmaceutical Products 3(1) (2008) 33-38.

[4] A.K. Singh, V.K. Shingala, R.P. Selvam, T. Sivakumar, Evaluation of Mangifera indica gum as tablet binder, International Journal of PharmTech Research 2(3) (2010) 2098-2100.

[5] A.K. Mistry, C.D. Nagda, D.C. Nagda, B.C. Dixit, R.B. Dixit, Formulation and in vitro evaluation of ofloxacin tablets using natural gums as binders, Scientia pharmaceutica 82(2) (2014) 441-448.

[6] S. Kumar, A.K. Singh, S. Prajapati, V.K. Singh, Formulation and evaluation of once daily sustained release matrix tablets of aceclofenac using natural gums, Journal of Drug Delivery and Therapeutics 2(1) (2012).

[7] S. Jana, A. Gandhi, S. Sheet, K.K. Sen, Metal ion-induced alginate–locust bean gum IPN microspheres for sustained oral delivery of aceclofenac, International journal of biological macromolecules 72 (2015) 47-53.

[8] V.D. Prajapati, G.K. Jani, N.G. Moradiya, N.P. Randeria, P.M. Maheriya, B.J. Nagar, Locust bean gum in the development of sustained release mucoadhesive macromolecules of aceclofenac, Carbohydrate polymers 113 (2014) 138-148.

[9] S. Ghosh, B. Barik, Preparation and evaluation of aceclofenac sustained release formulation and comparison of formulated and marketed product, International Journal of Medicine and Medical Sciences 1(9) (2009) 375-382.

[10] T. Ramasamy, U.D.S. Kandhasami, H. Ruttala, S. Shanmugam, Formulation and evaluation of xanthan gum based aceclofenac tablets for colon targeted drug delivery, Brazilian Journal of Pharmaceutical Sciences 47(2) (2011) 299-311.

[11] M. Kaur, S. Sharma, V. Sinha, Polymer based microspheres of aceclofenac as sustained release parenterals for prolonged anti-inflammatory effect, Materials Science and Engineering: C 72 (2017) 492-500.

[12] C.R. Gupta, G.K. Kishore, J.V. Ratna, Development and evaluation of aceclofenac matrix tablets using polyethylene oxides as sustained release polymers, Journal of pharmacy research 6(2) (2013) 249-254.

[13] R.K. Deshmukh, J.B. Naik, Aceclofenac microspheres: Quality by design approach, Materials Science and Engineering: C 36 (2014) 320-328.

[14] M. Ganesh, U.J. Jeon, U. Ubaidulla, P. Hemalatha, A. Saravanakumar, M.M. Peng, H.T. Jang, Chitosan cocrystals embedded alginate beads for enhancing the solubility and bioavailability of aceclofenac, International journal of biological macromolecules 74 (2015) 310-317.

[15] S. Jana, A. Das, A.K. Nayak, K.K. Sen, S.K. Basu, Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: In vitro and in vivo assessment, International journal of biological macromolecules 57 (2013) 129-137.

[16] P.R. Guru, A.K. Nayak, R.K. Sahu, Oil-entrapped sterculia gum–alginate buoyant systems of aceclofenac: development and in vitro evaluation, Colloids and Surfaces B: Biointerfaces 104 (2013) 268-275.

[17] R.K. Deshmukh, J.B. Naik, Optimization of sustained release aceclofenac microspheres using response surface methodology, Materials Science and Engineering: C 48 (2015) 197-204.

[18] V.N. Ravella, R.R. Nadendla, N.C. Kesari, Design and evaluation of sustained release pellets of aceclofenac, Journal of pharmacy research 6(5) (2013) 525-531.

[19] K. Abbas, M.A. Hussain, S.N.A. Bukhari, M. Amin, M. Zaman, Fabrication, characterization, appraisal of bioavailability and immunomodulatory potential of aceclofenac prodrugs based on hydroxyethylcellulose, Journal of Drug Delivery Science and Technology 52 (2019) 856-862.

[20] S. Jana, A. Saha, A.K. Nayak, K.K. Sen, S.K. Basu, Aceclofenac-loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microparticles, Colloids and Surfaces B: Biointerfaces 105 (2013) 303-309.

[21] R.K. Deshmukh, J.B. Naik, The impact of preparation parameters on sustained release aceclofenac microspheres: A design of experiments, Advanced Powder Technology 26(1) (2015) 244-252.

CHAPTER - VIII

OBJECTIVE OF RESEARCH WORK

8. Objective of research work

The polysaccharides obtained from natural sources have been gaining popularity in fabrication of various pharmaceutical dosage forms such as immediate-release and controlled-release peroral tablets, beads, microspheres, microcapsules, nanoparticles, mucoadhesive gels, transdermal patches, etc. A wide range of natural polysaccharides has been substantially reported as potential drug-carriers, which includes chitosan, alginates, pectin, gellan, gum acacia, gum tragacanth, xanthan gum etc. Physiological compatibility, low cost, biodegradability, easy availability, relatively higher green fabricability and environment friendliness make these natural polysaccharides preferable over synthetic polymers as vehicles for drug delivery and other biomedical applications such as wound healing, tissue engineering, etc.

The main objective of the present investigation was to evaluate Cassia fistula seed gum (CFSG) as tablet binder and chemically modify CFSG through graft-copolymerization towards its application as excipient in fabrication of mucoadhesive-cum-gastroretentive sustained-release drug delivery system.

In the first part, for the purpose of evaluation of *Cassia fistula* seed gum (CFSG) as tablet binder, the following experiments and investigations were planned to perform:

- Extraction and purification of CFSG
- Characterization of CFSG
  - Phytochemical screening
  - Polysaccharide content
  - Monosaccharide composition analysis by HPLC
  - Elemental analysis
  - FTIR and solid state ^{13}C NMR study

- ➢ Molecular weight, zeta potential, viscosity, pH and surface tension
- ➢ Rheology study
- ➢ DSC, TGA and DTA study
- ➢ Powder X-ray Diffraction (PXRD) study
- ➢ Scanning Electron Microscopy
- ➢ Acute oral toxicity and histological study

- ❖ Preformulation studies
- ❖ Preparation and characterization of drug loaded granules
- ❖ Preparation and evaluation of granules of CFSG with diclofenac sodium.
- ❖ Compression and evaluation of diclofenac sodium loaded tablets
- ❖ Hydration kinetic study
- ❖ Accelerated stability study

After extraction of the native CFSG gum by the scientific extraction procedure and step by step evaluation of the gum by the tests mentioned above, suitableness of the native CFSG gum as binder will be established.

In the second part of this investigation, native CFSG was grafted with poly (sodium acrylate) to synthesize a graft-copolymer having mucoadhesive and sustained-release capability. Microwave-assisted free-radical initiated graft-copolymerization was employed for the synthesis. The following studies were performed in order to characterize the resultant graft-copolymer and evaluate its potential as mucoadhesive sustained-release polymer:

- ❖ Synthesis of *Cassia fistula* seed gum-g-poly (sodium acrylate) (CFSG-g-PSA)
- ❖ Characterizations of CFSG-g-PSA
  - ➢ *Elemental analysis*
  - ➢ *FTIR*

- ➢ *^{13}C Solid state NMR spectroscopy*
- ➢ *Determination of molar mass and zeta potential*
- ➢ *Thermal study (DSC, TGA and DTA)*
- ➢ *Powder XRD*
- ➢ *SEM study*
- ➢ *Viscosity measurement*
- ➢ *Acute oral toxicity study*
- ➢ *Biodegradation study*

❖ **Preparation of gastroretentive mucoadhesive tablets of aceclofenac with CFSG-g-PSA**

❖ ***Evaluations of tablets***

- ➢ Evaluation of tablet properties
- ➢ *Ex-vivo mucoadhesion study*
- ➢ *In-vitro drug-release study*
- ➢ *Kinetic modelling of drug release profile*
- ➢ *Accelerated stability study*

Chemically modified native CFSG gum will be characterized by the above-mentioned tests and results of the studies of prepared gastroretentive mucoadhesive tablets of aceclofenac with CFSG-g-PSA will be scientifically analysed and evaluated to establish the suitableness of modified CFSG as excipient in fabrication of mucoadhesive-cum-gastroretentive sustained-release drug delivery system.

CHAPTER - IX

DRUGS AND EXCIPIENTS PROFILE

9. Drugs and Excipients profile

9.1. Profile of the Drugs

The current study's objectives were to assess *Cassia fistula* seed gum (CFSG) as a tablet binder and chemically alter CFSG using graft-copolymerization in preparation for its use in the creation of a sustained-release mucoadhesive-cum-gastroretentive drug delivery system. The tablet must first be prepared for assessment as a tablet binder as well as sustained-release mucoadhesive-cum-gastroretentive drug delivery system, and aceclofenac and diclofenac sodium are utilized as model medications for tablet production. Below is a monograph for diclofenac sodium and aceclofenac.

9.1.1. Monograph of diclofenac sodium. [1-3]

- **Synonyms: Voltaren, Voltarol, Voldal, Orthophen**
- **Chemical name:** Sodium 2[(2, 6-dichlorophenyl)-amino] phenyl acetic acid monosodium salt.
- **Structure of Diclofenac sodium**

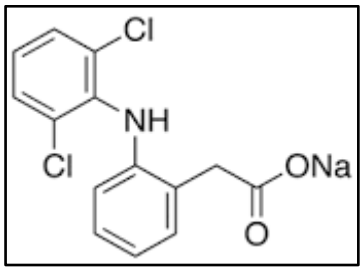


Figure 9.1. Structure of Diclofenac sodium

- **Molecular weight:** 318.13.
- **Category:** Synthetic nonsteroidal anti-inflammatory and analgesic compound
- **Dose:** 25 to 75 milligrams given orally or through intramuscular injection.

- **Appearance:** Powder that is crystalline, somewhat hygroscopic, and odorless.
- **Solubility:** The equilibrium solubility at Room temperature in different solvent are shown below.

Solvent	Solubility(mg/ml)
Deionized Water (pH 5.2)	>9
Methanol	>24
Acetone	6
Acetonitrile	<1
Cyclohexane	<1
pH 1.1 (HCl)	<1
pH 7.2 (phosphate buffer)	6

- **Dissociation constant:** pKa value in water is 4.
- **Partition coefficient:** In n-octane/aqueous buffer pH is 13.4
- **Storage:** Store in well-closed, light-resistant containers.
- **Standards:**

  Not less than 98.5 percent nor more than 101.0 percent of diclofenac sodium is present of C_{14} H_{10} N NaO_2, computed using the dried material as a reference.

- **Identification:**

  A: The infrared absorption spectrum agrees with either the diclofenac sodium reference spectrum or the spectrum produced by diclofenac sodium RS.

B: Nitric acid is added to 1 ml of a 0.4% w/v solution in methanol, and a dark red color result.

C: A 1% w/v solution gives the reaction of sodium salts.

D: Melts with decomposition at around 280°.

- **Clarity and Color of solution**: In comparison to the reference solution, the 5% w/v solution in methanol is clearer and brightly colored.
- **Light absorption**: Absorbance at 440 nm of a 5% w/v solution in methanol, not to exceed 0.050.
- **Related substances**: Use silica gel GF254 as the coating material and a mixture of 100 volumes of toluene, 10 volumes of hexane, and 10 volumes of anhydrous formic acid as the mobile phase while performing the thin-layer chromatography procedure. Apply 2 µl of each of the solutions in methanol individually to the plate. A 5.0% w/v solution of the material under investigation is Solution (1). Diclofenac sodium RS is present in solutions (2) and (3) in amounts of 5.0% w/v and 0.010% w/v, respectively. A 0.010% w/v solution of 1-(2, 6-dichlorophenyl)-2-indolinone RS in water is solution (4) . The plate should be removed, dried in warm air for ten minutes, and then examined under ultraviolet light (254 nm) after having been exposed to the light for around twenty minutes. The spot in the chromatogram produced by solution (1) that corresponds to 1-(2, 6-dichlorophenyl)-2-indolinone is not more intense than the spot in the chromatogram produced by solution (3).
- **Heavy metals:** Not more than 20 ppm, as calculated on 1g using Method B and 2.0 ml of lead standard solution (10 ppm Pb).
- **Loss on drying:** Not more than 0.5%, as measured on 1 g by drying for 3 hours at 105 degrees.

- **Assay**: To do Method A for non-aqueous titration, properly weigh 0.2 g, dissolve in 50 ml of anhydrous glacial acetic acid, and potentiometrically determine the end-point. Complete a blank calculation, then rectify any errors. The chemical formula for C14H10Cl2NNaO2 is 0.03181 g per ml of 0.1 M perchloric acid.

- **Mechanism of Action:**

  A non-steroidal anti-inflammatory medication (NSAID) is diclofenac. Diclofenac has demonstrated anti-inflammatory, analgesic, and antipyretic efficacy in pharmacologic tests. As with other NSAIDs, the exact mechanism of action is unknown; however, the anti-inflammatory effect may be influenced by its capacity to block prostaglandin formation. Fever, discomfort, and inflammation are all significantly influenced by prostaglandins. At dosages comparable to those found in people, diclofenac sodium does not inhibit proteoglycan production in cartilage when used in vitro.

- **Pharmacokinetics:**

  - **Absorption:**

  After passing through the stomach, the gastro-resistant pills' diclofenac is entirely absorbed. The tablet's gastro-resistant coating may cause its quick absorption to have a delayed start. After ingesting one 50 mg tablet, mean peak plasma concentrations of 1.5 micrograms/mL (5 micromol/L) are typically reached in 2 hours. The magnitude of the dosage has a linear relationship with the amount absorbed. When taken with or after food, a tablet passes through the stomach more slowly than when taken before, but the amount of diclofenac absorbed is the same. The area under the concentration curve (AUC) after oral or rectal administration is roughly half that after an identical parenteral

dosage because approximately half of diclofenac is metabolized during its first transit through the liver ("first pass" effect).

Repeated dosing has no effect on pharmacokinetic behavior. As long as the appropriate dose intervals are followed, no buildup happens. Children given equivalent dosages (mg/kg body weight) of medication achieve plasma concentrations that are comparable to those acquired by adults.

- **Distribution:**

The majority of diclofenac (99.7%) is linked to albumin (99.4%) in serum proteins. 0.12-0.17 L/kg is the predicted apparent volume of distribution per kilogram. After reaching peak plasma levels, diclofenac reaches the synovial fluid, where maximum concentrations are detected 2 to 4 hours later. Three to six hours is the apparent half-life for removal from synovial fluid. Concentrations of the active ingredient are greater in the synovial fluid than in the plasma two hours after achieving peak plasma levels, and they continue to be higher for up to 12 hours.

- **Biotransformation:**

The biotransformation of diclofenac primarily involves single and multiple hydroxylation and methoxylation, which produces a number of phenolic metabolites, the majority of which are converted to glucuronide conjugates (3'-hydroxy-, 4'-hydroxy-, 5'-hydroxy-, 4', 5'-dihydroxy-, and 3'-hydroxy-4'-methoxy-diclofenac). Compared to diclofenac, two of these phenolic metabolites are physiologically active to a substantially lesser level.

- **Elimination:**

Diclofenac has a mean systemic clearance rate of 263 56 mL/min (mean standard deviation). In plasma, the terminal half-life lasts one to two hours. The plasma half-lives of four of the metabolites, including the two active ones, range from one to three hours. One metabolite, 3'-hydroxy-4'-methoxy-diclofenac, has a plasma half-life that is significantly longer. This metabolite is essentially inactive, though. The intact molecule's glucuronide conjugate and metabolites, the majority of which are also transformed to glucuronide conjugates, account for around 60% of the injected dosage that is eliminated in the urine. Less than 1% of the chemical is excreted unaltered. The remaining dosage is removed by bile in the feces as metabolites.

- **Indication:**
  - Rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and spondylarthritis, painful syndromes of the vertebral column, and non-articular rheumatism are among the inflammatory and degenerative types of rheumatism that it is used to treat-
  - acute gout episodes.
  - painful post-surgical and post-traumatic edema and inflammation.
  - primary dysmenorrhea.

- **Contraindications:**
  - Hypersensitivity to the active ingredient or any of the excipients is known.
  - active bleeding or perforation from an intestinal or stomach ulcer.
  - last stage of pregnancy.
  - severe renal, cardiac, or hepatic failure.

- ❖ Diclofenac sodium is contraindicated in individuals whose bouts of asthma, urticaria, or acute rhinitis are exacerbated by acetylsalicylic acid or other NSAIDs, just like other non-steroidal anti-inflammatory medicines (NSAIDs).

- **Interactions with Other Medicaments:**

The interactions that have been reported with enteric-coated diclofenac sodium tablets and/or other diclofenac-containing medications are listed below.

- ❖ **Lithium:** Lithium plasma concentrations may increase if diclofenac is being taken at the same time. It is advised to monitor the serum lithium level.
- ❖ **Digoxin:** Digoxin plasma concentrations may be increased by simultaneous use of diclofenac. It is advised to monitor the serum digoxin level.
- ❖ **Diuretics and antihypertensive agents:** Like other NSAIDs, diclofenac may have a reduced antihypertensive impact when used concurrently with diuretics or other antihypertensive medications (such as beta-blockers and ACE inhibitors). As a result, the combination should be provided carefully, and patients' blood pressure should be routinely checked, especially in the elderly. Patients should drink enough water, and monitoring of renal function should be taken into mind when starting concurrent medication and on a regular basis thereafter, especially for diuretics and ACE inhibitors due to the increased risk of nephrotoxicity. Increased blood potassium levels may result from concurrent therapy with potassium-sparing medications;thus, these levels should be regularly checked.
- ❖ **Other NSAIDs and corticosteroids:** Taking diclofenac together with other systemic NSAIDs or corticosteroids simultaneously may make gastrointestinal side effects more common.

- **Anticoagulants and antiplatelet agents:** Due to the potential for increased bleeding risk from concurrent administration, caution is advised. Despite sporadic reports of an increased risk of hemorrhage in individuals receiving diclofenac and anticoagulants concurrently, clinical research does not seem to show that diclofenac alters the action of anticoagulants. Therefore, it is advised that these patients be closely watched.
- **Selective serotonin reuptake inhibitors (SSRIs):** The risk of gastrointestinal bleeding may be increased when SSRIs and systemic NSAIDs, such as diclofenac, are used together.
- **Antidiabetics:** Diclofenac can be used together with oral antidiabetic medications without affecting their therapeutic impact, according to clinical research. Nevertheless, there have been a few isolated cases of hypoglycemia and hyperglycemic reactions that called for adjusting the dosage of the antidiabetic medications while using diclofenac. Because of this, monitoring blood glucose levels while receiving concurrent medication is advised as a precaution.
- **Methotrexate:** When NSAIDs, such as diclofenac, are taken less than 24 hours before or after methotrexate treatment, caution is advised since methotrexate blood concentrations may increase and its toxicity may be enhanced.
- **Ciclosporin:** Due to its impact on renal prostaglandins, diclofenac, like other NSAIDs, may enhance the nephrotoxicity of cyclosporine. As a result, it ought to be administered at lower levels than would be provided to individuals not taking ciclosporin.
- **Quinolone antibacterials:** There have been a few sporadic reports of convulsions, which may have been caused by taking quinolones and NSAIDs at the same time.
- **Potent CYP2C9 inhibitors:** When co-prescribing diclofenac with strong CYP2C9 inhibitors (such sulfinpyrazone and voriconazole), caution is advised since this might

lead to an increase in peak plasma concentrations and exposure to diclofenac because the metabolism of diclofenac is inhibited.

- **Phenytoin:** Due to an anticipated increase in exposure to phenytoin while taking phenytoin with diclofenac, monitoring of phenytoin plasma concentrations is advised.

- **Statement on usage during pregnancy and lactation:**

- **Pregnancy:** Diclofenac dosage during pregnancy has not been investigated. Therefore, unless the possible benefit to the mother surpasses the danger to the fetus, diclofenac sodium should not be administered during the first two trimesters of pregnancy. Diclofenac usage is not advised during the third trimester of pregnancy, since it increases the risk of uterine inertia and/or premature ductus arteriosus closure. Animal studies have not revealed any adverse effects on fecundity, parturition, pregnancy, embryonal/fetal development, or postnatal growth.
- **Lactation:** Diclofenac enters into breast milk in trace levels, much like other NSAIDs do. Therefore, in order to prevent negative effects on the baby, diclofenac sodium shouldn't be given when breastfeeding.
- **Fertility:** Diclofenac sodium usage may reduce female fertility, like with other NSAIDs, hence it is not advised for women who are trying to get pregnant. Withholding diclofenac sodium should be explored in women who are having trouble becoming pregnant or who are being investigated for infertility.

- **Adverse Effects / Undesirable Effects:**

❖ **Dermatological disorders:**

Common: Rash.

Rare: Urticaria.

Very rare: Bullous eruptions, eczema, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis (Lyell's syndrome), exfoliative dermatitis, hair loss, photosensitivity response, purpura, allergic purpura, and pruritus are some of the skin conditions that can cause these symptoms.

❖ **Blood and lymphatic system disorders:**

Very rare: leukopenia, aplastic and hemolytic anaemias, thrombocytopenia, and agranulocytosis.

❖ **Immune system disorders:**

Rare: hypersensitivity, anaphylactic responses, and anaphylactoid shock and hypotension.

Very rare: Face oedema due to angioneurotic oedema.

❖ **Psychiatric disorders:**

Very rare: sleeplessness, nightmares, irritability, disorientation, sadness, and psychotic disorder.

❖ **Nervous system disorders:**

Common: Headache, dizziness.

Rare: Somnolence.

Very rare: Memory loss, convulsion, anxiety, tremor, meningitis, taste changes, and cerebrovascular accidents are some of the symptoms.

- **Eye disorders:**

  Very rare: diplopia, hazy vision, and visual disturbance.

- **Ear and labyrinth disorders:**

  Very rare: Tinnitus and hearing loss.

- **Cardiac disorders:**

  Very rare: heart failure, chest discomfort, myocardial infarction, palpitations, etc.

- **Vascular disorders:**

  Very rare: Vasculitis with hypertension.

- **Respiratory, thoracic and mediastinal disorders:**

  Rare: Asthma (including dyspnoea).

  Very rare: Pneumonitis.

- **Gastrointestinal disorders:**

  Common: Anorexia, dyspepsia, stomach discomfort, vomiting, diarrhea, and nausea.

  Rare: Gastritis, gastrointestinal ulcers (with or without bleeding or perforation), haematemesis, hemorrhagic diarrhea, and melaena.

Very rare: Constipation, stomatitis, glossitis, esophageal dysfunction, intestinal strictures that resemble a diaphragm, pancreatitis, and colitis (including hemorrhagic colitis and an aggravation of ulcerative colitis or Crohn's disease).

❖ **Hepatobiliary disorders:**

Common: Transaminases increased.

Rare: Hepatitis, jaundice, liver disorder.

Very rare: Hepatic failure, hepatic necrosis, and fulminant hepatitis.

❖ **Renal and urinary disorders:**

Rare: Oedema

Very rare: Haematuria, proteinuria, nephrotic syndrome, interstitial nephritis, and renal papillary necrosis are symptoms of acute renal failure. Conditions at the administration site and general problems

.

- **Overdose and treatment:**

❖ **Symptoms:**

An overdose of diclofenac does not often present with a particular clinical picture. Vomiting, gastrointestinal hemorrhage, diarrhea, lightheadedness, tinnitus, and convulsions are only a few of the signs and symptoms of an overdose. Acute renal failure and liver damage are potential side effects of severe poisoning.

❖ **Therapeutic measures:**

The mainstays of the management of acute poisoning from NSAIDs, particularly diclofenac, are supportive care and symptomatic therapy. For problems including hypotension, renal failure, seizures, gastrointestinal dysfunction, and respiratory depression, supportive measures and symptomatic therapy should be administered. Due to the strong protein binding and extensive metabolism, special techniques like forced diuresis, dialysis, or hemoperfusion are probably ineffective in getting rid of NSAIDs, including diclofenac. After ingesting a potentially hazardous overdose, activated charcoal may be explored, as well as stomach decontamination (such as vomiting or gastric lavage) after ingesting a possibly fatal overdose.

9.1.2. Monograph of Aceclofenac. [4-8]

- **Chemical Structure**

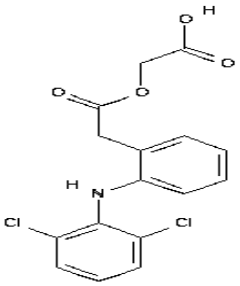


Figure 9.2. Structure of Aceclofenac

- **Chemical formula:** $C_{16}H_{13}Cl_2NO_4$
- **Molecular weight:** 354.2
- **CAS Number:** 89796-99-6
- **Therapeutic Category:** Nonsteroidal anti-inflammatory
- **BCS Classification:** BCS CLASS II

- ***Half-life:*** The mean plasma elimination half-life is approximately 4 hours.
- **Appearance:** a crystalline powder that is white or almost white.
- **Solubility:** Freely soluble in acetone, practically insoluble in water, and soluble in alcohol
- **Partition coefficient:**
- **Melting Point:** 149-150°C
- **Dissociation constant:** 3.37 in acidic medium, 1.38 in alkaline medium
- ***Preparation:*** In 300 ml of N, N- dimethylformamide, which had been heated to 50°C, 50 g of sodium 2- [(2, 6- dichlorophenyl) amino] phenylacetate was dissolved. 44.22 g of benzyl bromoacetate was then added. In these circumstances, stirring went on for 9 hours. The reaction was finished, the solvent was withdrawn under reduced pressure, 400 ml of ether was added to precipitate the sodium salt, the mixture was filtered, and the ether phase was twice washed with 100 ml of hexane. The end result was crystallized from hexane/ether, followed by acetone/chloroform (1:9), yielding 44.1 g (61%) of benzyl 2- [(2, 6- dichlorophenyl) amino] phenylmethyl acetate in the form of white crystals with a melting point of 67-69° C. 1500 ml of ethyl acetate was used to dissolve 45.28 g of benzyl 2- [(2, 6-dichlorophenyl) amino] phenylmethyl acetate. The resultant mixture was then combined with 7 g of Pd/C and hydrogenated at atmospheric pressure for 14 hours. Filtration, concentration, and crystallization were used to extract 23.51 g (65%) of 2- [92, 6-dichlorophenyl) amino] phenyl acetoxyacetic acid from the solution.
- ***Description:*** Aceclofenac is an oral non-steroidal anti-inflammatory medication (NSAID) used to treat osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. It has notable anti-inflammatory and analgesic characteristics. In double-blind trials, it is said to have a stronger anti-inflammatory impact than traditional NSAIDs, or at least

effects that are equivalent to them. The cyclo-oxygenase enzyme (COX), which is necessary for the production of prostaglandins, an inflammatory mediator that contributes to pain, swelling, inflammation, and fever, is strongly inhibited by aceclofenac. Aceclofenac's limited water solubility places it in BCS class II. It has strong permeability to enter synovial joints, where the loss of articular cartilage in the region produces joint discomfort, soreness, stiffness, crepitus, and local inflammation in individuals with osteoarthritis and associated disorders. Other painful disorders including dental and gynecological problems are said to respond well to aceclofenac treatment. Aceclofenac was created in 1991 as a chemically modified analogue of the widely used NSAID diclofenac in an effort to increase the drug's gastrointestinal tolerance.

- ***Pharmacodynamics:*** Aceclofenac is an NSAID that blocks both COX enzyme isoforms, a crucial enzyme in the inflammatory cascade. While COX-2 is an inducible enzyme engaged in the synthesis of inflammatory mediators in response to inflammatory stimuli, COX-1 enzyme is a constitutive enzyme involved in prostacyclin production and protective activities of the gastric mucosa. When compared to other NSAIDs, aceclofenac has a higher selectivity towards COX-2 (IC50 of 0.77 M) than COX-1 (IC50 of >100 M). Its major metabolite, 4-hydroxy aceclofenac, similarly only slightly inhibits COX-2 with an IC50 value of 36 M. Although prostaglandin synthesis suppression is assumed to be the primary mechanism of action of aceclofenac, the drug also suppresses the production of inflammatory cytokines, interleukins (IL-1β, IL-6), and tumor necrosis factors (TNF). Additionally, aceclofenac is said to have an impact on neutrophil cell adhesion molecules. Aceclofenac also affects glycosaminoglycan production and exerts chondroprotective effects.

- ***Mechanism of Action:*** Aceclofenac reduces the generation of a number of inflammatory mediators, including prostaglandin E2, IL-1β, and TNF from the arachidonic acid route, by inhibiting COX-2. The conversion of aceclofenac to diclofenac is assumed to be the mechanism through which IL-6 is inhibited. Reactive oxygen species are produced less when the inflammatory cytokines are suppressed. Human articular chondrocytes have demonstrated to produce less nitrous oxide when treated with aceclofenac. Aceclofenac also inhibits neutrophil adherence to endothelium by lowering the production of L-selectin, a lymphocyte-expressed cell adhesion molecule. It is hypothesized that aceclofenac, through inhibiting IL-1 production and activity, can increase the synthesis of glycosaminoglycan in human osteoarthritis cartilage. The chondroprotective effects are produced by 4-hydroxy aceclofenac, which blocks chondrocyte proteoglycan release and inhibits IL-1-mediated promatrix metalloproteinase-1 and metalloproteinase-3 synthesis.
- ***Pharmacokinetics:*** Peak plasma concentrations of aceclofenac are attained 1 to 3 hours after an oral administration because of the drug's good absorption from the digestive system. More than 99% of aceclofenac is bound to plasma proteins. The half-life of plasma elimination is around 4 hours. A dosage is mostly eliminated in the urine as hydroxy metabolites, around two thirds of it.
- ***Uses and Administration:*** Aceclofenac is a non-steroidal anti-inflammatory medicine (NSAID) similar to diclofenac. It is a phenylacetic acid derivative. It is utilised in the treatment of rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis.
- ***Adverse effects:*** Gastrointestinal issues (dyspepsia, stomach discomfort, and nausea), rash, urticaria, signs of enuresis, headache, dizziness, and sleepiness are a few examples of typical side effects.

The most frequent side effects of NSAIDs are often digestive issues including dyspepsia, stomach discomfort, and nausea. These are typically moderate and treatable, but in some patients, peptic ulceration and serious gastrointestinal bleeding may happen. The prevailing consensus is that the gastrointestinal side effects of NSAIDs are significantly influenced by the inhibition of cyclo-oxygenase 1 (COX-1); the specific inhibition of COX-2 increases gastrointestinal tolerance.

Headache, vertigo, dizziness, anxiousness, tinnitus, depression, sleepiness, and sleeplessness are CNS-related side effects. Occasionally, hypersensitivity responses might lead to fever, angioedema, bronchospasm, and rashes. Rarely occurring conditions like aseptic meningitis and hepatotoxicity may potentially be hypersensitivity responses.

- ***Interactions:*** NSAID interactions include raising plasma concentrations of lithium, methotrexate, and cardiac glycosides as well as improving the effects of oral anticoagulants (particularly those caused by azapropazone and phenylbutazone). If administered together with ACE inhibitors, ciclosporin, tacrolimus, or diuretics, the risk of nephrotoxicity may rise. An increased risk of hyperkalemia with ACE inhibitors and potassium-sparing diuretics may potentially have an impact on renal function. Some antihypertensives, such as ACE inhibitors, beta blockers, and diuretics, may have decreased antihypertensive effects. Quinolone interactions might result in convulsions. Phenytoin and sulfonylurea anti-diabetics may have an enhanced impact when used with NSAIDs.

- **Drug Interactions**

Drug	**Interaction**
Acyclovir	Combining acyclovir with aceclofenac has the potential to enhance the risk or severity of nephrotoxicity.
Acetaminophen	When acetaminophen and aceclofenac are used together, the chance or intensity of side effects may rise.
Acetazolamide	Aceclofenac's excretion rate may be accelerated by acetazolamide, which might lower the drug's blood level and perhaps reduce its effectiveness.
Acetylsalicylic acid	The risk or severity of adverse effects can be increased when Acetylsalicylic acid is combined with aceclofenac.
Allopurinol	Aceclofenac may slow down Allopurinol's elimination, which might raise the serum level.
Amiloride	When aceclofenac and amiloride are used together, the likelihood or severity of renal failure, hyperkalemia, and hypertension can all be raised.
Amphotericin B	Combining Amphotericin B with aceclofenac can enhance the risk or severity of nephrotoxicity.
Amlodipine	Amlodipine's antihypertensive effects may be lessened by aceclofenac.
Atropine	Combining atropine and aceclofenac may enhance the risk or severity of hypertension.
Beclomethasone dipropionate	When Beclomethasone dipropionate and Aceclofenac are used together, there is a chance that the risk or intensity of gastrointestinal discomfort will rise.
Warfarin	When Warfarin and aceclofenac are taken together, there is a potential increase in the risk or severity of bleeding and haemorrhage.

Streptokinase	Aceclofenac with Streptokinase may enhance the likelihood or severity of bleeding and haemorrhage.
Spironolactone	When Spironolactone and aceclofenac are combined, there is a potential for increased risk or severity of renal failure, hyperkalemia, and hypertension.
Succinylcholine	Combining succinylcholine with aceclofenac can enhance the risk or severity of hyperkalemia.
Ramipril	When aceclofenac and Ramipril are taken together, there is a potential for an increase in the risk or severity of renal failure, hyperkalemia, and hypertension.
Prednisolone	When Prednisolone and Aceclofenac are used together, there is a chance that the risk or intensity of gastrointestinal discomfort will rise.
Ofloxacin	Aceclofenac may increase the neuroexcitatory activities of ofloxacin.

Precautions: All NSAIDs should not be used in individuals with current peptic ulcers, and non-selective NSAIDs should only be used sparingly in patients with a history of such conditions. NSAIDs can be taken before, with, or after food or milk to lessen the possibility of adverse gastrointestinal effects. Omeprazole or misoprostol, histamine H2-antagonists, may be used for a related purpose in high-risk patients taking non-selective NSAIDs.

9.2. Profile of the excipients

Apart from APIs, various excipients have also been utilized for the preparation of tablet. Detailed information about those excipients have been summarized below.

9.2.1. Polyvinylpyrrolidone (PVP K-30) [9]

- **Empirical formula**
- **$(C_6H_9NO)_n$ (Where n = 22- 27000)**
- **Molecular weight 2500-3000000**

  According to the USP 25, Povidone is a synthetic polymer mostly made up of linear 1-vinyl-2-pyrrolidinone groups, the degree at which these groups are polymerized determining the polymers' molecular weights. Its K value, which measures its relative viscosity in aqueous solutions to that of water, defines it.

- **Functional category**

  Disintegrate dissolution aid, suspending agent and tablet binder.

- **Application in Pharmaceutical Formulation or Technology**

  Despite being utilized in many different pharmacological formulations, it is most frequently employed in solid dosage forms. Povidone solutions are employed in the wet granulation process of tableting as binders. Additionally, it is added to dry powder mixes and in-situ granulation by mixing it with water, alcohol, or hydroalcoholic solutions. Povidone is utilized as a solubilizer in oral and parenteral formulations because it has been demonstrated to improve the dissolving of medications from solid dosage forms that are poorly soluble. As coating agents, povidone solutions can also be utilized.

In a number of topical and oral suspensions and solutions, it is also utilized as a suspending, stabilizing, or viscosity-increasing agent. When used with povidone, a variety of active medications that aren't very soluble may become more soluble.

- **Description**

It appears as a fine, Odourless or almost Odourless, hygroscopic powder that ranges in hue from white to creamy-white. It is produced by spray drying and comes in the form of spheres. In the form of plates, povidone K-90 and higher K-value povidone are produced by drum drying.

- **Properties**

  - **Moisture content**

Not to go beyond 15% w/w. It absorbs a substantial quantity of moisture at low relative humidity levels due to its high hygroscopicity.

  - **Solubility**

Practically insoluble in ether, hydrocarbons, and mineral oil; freely soluble in acids, chloroforms, ethanol, ketones, methanol, and water. In water, the concentration of a solution is solely constrained by the resultant solution's viscosity, which is determined by the K value.

  - **Incompatibilities**

It may be dissolved in a variety of inorganic salts, synthetic or natural resins, and other compounds. In a solution, it forms molecular adducts with tannin, phenobarbital, sodium salicylate, and salicylic acid. The creation of complexes with povidone may negatively impact the efficacy of various preservatives, such as thimerosal.

❖ **Safety**

When taken orally, povidone may be regarded as essentially harmless since it is not absorbed from the gastrointestinal system or mucous membrane. It is commonly used as excipients, especially in oral tablets and solutions. Povidone also doesn't irritate the skin and doesn't create sensitization.

The WHO has established a temporary tolerable daily dose for povidone of up to 25 mg/kg body weight.

9.2.2. Talcum [9]

- **Empirical formula**

  A pure, hydrated magnesium silicate with a formula that resembles $Mg_6(Si_2O_5)_4(OH)_4$ is talc. Iron and aluminium silicate may be present in trace levels.

- **Functional category**

  Alkylating agent, glider, diluent for tablets and capsules, and lubricant for tablets and capsules.

- **Application in Pharmaceutical Formulation or Technology**

  Talc is widely used as a dissolution retardant in the development of controlled release products.

  Talc is utilised as a dusting powder in topical medicines; however, it shouldn't be used to dust surgical gloves. Since talc is a natural product, it frequently contains bacteria and needs to be sterilised before being used as a dusting powder.

Talc is also utilised in culinary and cosmetic items, mostly for its lubricating characteristics, and to clarify liquids.

- **Uses of talc**

Use	**Concentration (%)**
Dusting powder	90.0-99.0
Glidant and tablet lubricant	1.0-10.0
Tablet and capsule diluent	5.0-30.0

- **Description**

Talc is an extremely fine, crystalline, Odourless, impalpable, unctuous, white to grayish-white powder. It is gentle to the touch, easily clings to the skin, and doesn't feel gritty.

- **Properties**

  - **Moisture content**

At 25 °C and relative humidity levels of up to 90%, talc absorbs just a little quantity of water.

  - **Solubility**

essentially insoluble in water, organic solvents, and weak acids and alkalis.

  - **Stability and storage conditions**

Since talc is a stable substance, it may be sterilized by being heated at 160° C for at least one hour. Gamma radiation or ethylene oxide exposure can also be used to sterilize it.

Talc has to be kept dry and cold in a container that is tightly closed.

- **Incompatibilities**

incompatible with compounds made of quaternary ammonium.

- **Safety**

Most tablet and capsule formulations use talc. Talc is considered to be a mostly harmless substance since it is not absorbed into the body after oral consumption. However, excessive usage of talc-containing products intranasally or intravenously can result in granulomas in bodily tissues, notably the lungs. Talc shouldn't be used to dust surgical gloves since it can lead to granulomas if it gets into wounds or bodily cavities. Talc inhalation irritates the throat and may cause babies to have acute respiratory distress.

The data is equivocal despite the fact that talc has been thoroughly examined for its ability to cause cancer and that it has been hypothesized that women who use talc have an increased risk of ovarian cancer. But since asbestos-contaminated talc has been shown to cause cancer in people, pharmaceutical items should only employ grades devoid of asbestos.

Additionally, newborns who were unintentionally exposed to talc tainted with significant amounts of hexachlorophene experienced severe, permanent neurotoxicity.

9.2.3. Magnesium stearate [9]

- **Empirical formula**

  $C_{36}H_{70}MgO_4$

  According to the USPNF 20, magnesium stearate is a combination of solid organic acids and magnesium that primarily contains different amounts of magnesium stearate and magnesium palmitate. According to the PhEur 2002, magnesium stearate is a combination of magnesium salts of several fatty acids, mostly stearic acid and palmitic acid with trace amounts of other fatty acids.

- **Functional category**

  Lubricant for tablets and capsules.

- **Application in Pharmaceutical Formulation or Technology**

  Magnesium stearate is often used in food, pharmaceutical, and cosmetic compositions. It is principally employed as a lubricant in the production of capsules and tablets at concentrations ranging from 0.25% to 5.0% w/w. Additionally, barrier creams employ it.

- **Description**

  Magnesium stearate is a white, impalpable, precipitated or milled powder with a low bulk density, a little stearic acid odor, and a distinctive flavor. When touched, the powder feels oily and immediately sticks to the skin.

- **Properties**

  - **Crystalline forms**

Three different forms of high purity magnesium stearate—a trihydrate, a dihydrate, and an anhydrate—have been discovered.

  - **Flowability**

clingy, poorly flowing powder

  - **Solubility**

Slightly soluble in warm benzene and warm ethanol (95%), but practically insoluble in ethanol, ethanol (95%), ether, and water.

  - **Stability and storage conditions**

Magnesium stearate should be kept in a tightly covered container in a cold, dry location since it is stable.

  - **Incompatibilities**

Strong acids, alkalis, and iron salts are incompatible with it. Keep strong oxidizing materials away from your mixtures. In goods containing aspirin, several vitamins, and the majority of alkaloidal salts, magnesium stearate cannot be utilized.

  - **Safety**

Magnesium stearate is frequently used as a medicinal excipient and is typically thought to be harmless when taken orally. Large doses taken orally, however, might have a laxative effect or irritate the mucous membranes.

Magnesium stearate has not been shown to be hazardous when taken orally or breathed, according to rat toxicology studies.

When inserted into the bladder of mice, magnesium stearate has not been proven to be carcinogenic.

LD_{50} (rat, inhalation): >2 mg/ L

LD_{50} (rat, oral): >10 g/kg

9.2.4. Carbomer [9, 10]

- **Empirical formula**

  $(C_3H_4O_2)_n$

  Poly (acrylic acid) (**PAA**; trade name **Carbomer**) is a polymer with the chemical composition (CH2-CHCO2H)n. It is an acetate acid (CH2=CHCO2H) derivative. A wide range of copolymers, crosslinked polymers, and their partly deprotonated derivatives are known and have economic utility in addition to homopolymers. PAA is an anionic polymer in a water solution with a pH of 7, meaning that many of its side chains lose their protons and pick up a negative charge. Polyelectrolytes, which can absorb and hold water and grow to several times their initial volume, are partially or completely deprotonated PAAs. Numerous applications are built on these two characteristics: acid-base and water-attracting. Aryl Sucrose or allyl ethers of pentaerythritol are used to crosslink synthetic high molecular weight polymers of acrylic acid known as carbomers. On a dry basis, they have between 56% and 68% of carboxylic acid groups. Numerous monographs defining specific carbomer grades with

varying aqueous viscosities and labels for oral or non-oral use may be found in the USP NF 20.

- **Functional Category**

  Bioadhesive, emulsifying, release-modifying, suspending, tablet-binding, and viscosity-increasing substances.

- **Application in Pharmaceutical Formulation or Technology**

  Carbomers are mostly utilized as suspending or viscosity-increasing agents in liquid or semisolid pharmaceutical formulation. For topical, rectal, and ocular treatments, formulas include creams, gels, and ointments. Carbomers are employed in tablet formulations as dry or wet binders as well as rate-regulating excipients. Water or an alcohol-water mixture is utilized as the granulating fluid in wet granulation procedures. A polymeric binder has also been employed in conjunction with anhydrous organic solvents. With the addition of certain cationic species to the granulating fluid or, in the case of water with talc in the formulation, talc, the tackiness of the wet mass can be minimized. Additionally, carbomer resins have been studied for their potential as intestinal protease enzyme inhibitors in peptide dosage forms, bioadhesives for cervical patches and intranasally administered microspheres, and magnetic granules for site-specific drug delivery to the esophagus. In the creation of oil-in-water emulsions for external application, carbomers are also used as emulsifying agents. The carbomer is partially neutralized for this purpose using sodium hydroxide and partially using a long chain amine like stearylamine. In order to increase viscosity while creating numerous emulsion microspheres, carbomer 951 has been studied. Cosmetics also make use of carbomers.

- **Uses of carbomers**

Use	Concentration (%)
Emulsifying agent	0.1-0.5
Gelling agent	0.5-2.0
Suspending agent	0.5-1.0
Tablet binder	5.0-10.0

- **Description**

Carbomers are powders that are white in color, fluffy, acidic, hygroscopic, and have a faint distinctive smell.

- **Properties**

  - **Melting point**

At 260° C, decomposition starts after 30 minutes.

  - **Moisture content**

Up to 2% w/w water is considered normal. Carbomers are hygroscopic, nevertheless, and have an average equilibrium moisture content of 8–10% w/w at 25 °C and 50% relative humidity. A carbomer's moisture content has no bearing on how well it thickens, but a higher moisture content makes the carbomer harder to handle since it disperses less easily.

- **Solubility**

soluble in water, ethanol (95%) after neutralization, and glycerin.

Since there are three dimensionally crosslinked microgels, carbomers do not dissolve while being labeled as soluble; instead, they just expand to an impressive level.

- **Viscosity**

Carbomers dissolve in water to create low-viscosity acidic colloidal dispersions, which when neutralized result in very viscous gels. In order to neutralize carbomer powders, bases must first be added after carbomer powders have been thoroughly mixed into water and forcefully swirled to prevent the formation of dispersible lumps.

- **Stability and storage conditions**

Carbomers are hygroscopic, stable compounds that can be heated for up to two hours at temperatures below 104 °C without losing their ability to thicken the solution. On the other hand, exposure to high temperatures might cause discoloration and decreased stability. Heating at 260°C for 30 minutes results in complete breakdown. Cloudiness and a decrease in viscosity of carbomer dispersions can be brought on by the addition of certain antimicrobials at high concentrations (0.1% w/v), such as sodium benzoate or benzalkonium chloride.

Carbomer powder has to be kept in a cold, dry location in an airtight, corrosion-resistant container. For the storage of formulations containing carbomer, the use of glass, plastic, or resin-lined containers is advised. To extend carbomer stability, packing in aluminum tubes often required a formulation with a pH lower than 6.5, whereas packaging in other metallic tubes or containers usually requires a formulation with a pH higher than 7.7.

❖ **Incompatibilities**

Resorcinol discolors carbomers, and they are incompatible with phenol, cationic polymers, strong acids, and high electrolyte concentrations. Additionally, some antibacterial adjuvants are to be avoided or used sparingly. Iron and other transition metal traces can catalyze the degradation of carbomer dispersions. If a carbomer comes into contact with a highly basic substance like ammonia, potassium or sodium hydroxide, or strongly basic amines, intense heat may be produced.

Certain amino functional actives combine with carbomer to generate complexes that are insoluble in water; frequently, this may be avoided by modifying the fluid phase's solubility parameter with the proper alcohols and polyols.

Additionally, carbomers and certain polymeric excipients can create pH-dependent interactions. In this case, modifying the solubility settings may be effective.

❖ **Safety**

In nonparenteral medications, notably topical liquid and semisolid treatments, carbomers are widely employed. They can also be utilized in oral preparations, but only in specific grades. Studies on acute oral toxicity in animals show that carbomer 934P has a low oral toxicity; dosages of up to 8 g/kg have been given to dogs without any deaths. There is no indication that carbomers applied topically in people cause hypersensitive responses; they are typically thought of as largely nontoxic and nonirritating compounds. Carbomer has been administered orally to people in amounts of 1-3 gm as a bulk laxative.

❖ **Handling precautions**

Follow standard safety procedures relevant to the situation and amount of material handled. The risk of an explosion should be reduced by minimizing excessive dust formation. The respiratory system, mucous membranes, and eyes are all irritated by carbomer dust. Because of the gelatinous film that develops when carbomer dust comes into contact with the eye, it is difficult to remove with water; saline should be used instead. During handling, gloves, eye protection, and a dust respirator are advised.

9.2.5. Hypromellose [9, 11, 12]

- **Empirical Formula: $C_{56}H_{108}O_{30}$**

Hypromellose (INN), short for **hydroxypropyl methylcellulose** (**HPMC**), is a semi-synthetic, inert, viscoelastic polymer that may be found in many commercial goods. It is utilized in eye drops and as an excipient and controlled-delivery component in oral medications. By adding a four-digit number to the nonproprietary name of hypromellose, as defined in USP 25, the substitution type is indicated. For example, hypromellose 1828. The methoxy group (OCH3)'s approximate % content is shown by the first two numbers. The hydroxypropoxy group's approximate percentage content (OCH2CH(OH)CH3), measured on a dry basis, is indicated by the second two numbers.

- **Chemical Structure:**

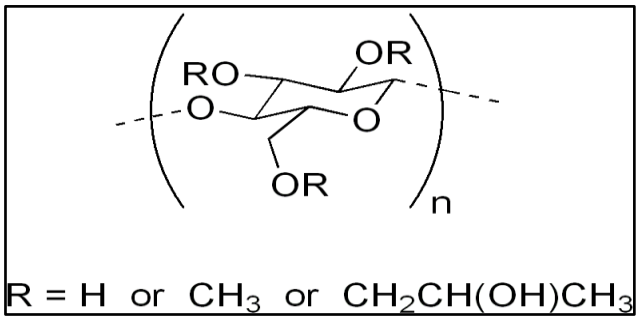


Figure 9.3. Structure of Hypromellose

- **Functional category**

Coating agent, film-former, sustained-release rate-controlling polymer, stabilizing agent, suspending agent, tablet binder, and viscosity-increasing agent.

- **Application in Pharmaceutical Formulation or Technology**

Pharmaceutical formulations for use topically and orally frequently utilize hypromellose.

In oral goods, hypromellose is typically utilized as a film coating, tablet matrix, and tablet binder. In either wet or dry granulation procedures, a binder may be utilized at concentrations of 2% to 5% w/w. When utilized in tablets and capsules, high viscosity grades may be employed to delay the release of medicines from a matrix at levels of 10–80% w/w.

For the film forming solutions to film coat tablets, concentrations of 2-20% w/w are employed, depending on the viscosity grade. Higher viscosity grades are employed with organic solvents, whereas lower viscosity grades are used in aqueous film coating solutions.

Hypromellose is also utilized in topical formulations, notably ophthalmic treatments, as a thickening and suspending ingredient. In formulations for ocular use, hypromellose is favored over methylcellulose because it generates solutions that are clearer and contain fewer undispersed fibers. Hypromellose can be used as a thickening agent in eye drop and artificial tear solutions at concentrations of 0.45 to 1.0% w/w. Hypromellose is also employed in topical gels and ointments as an emulsifier, a suspending agent, and a stabilizing agent. It can prevent droplets and particles from congregating or agglomerating as a protective colloid, preventing the development of sediments.

Additionally, hypromellose serves as a wetting agent for hard contact lenses, an adhesive in plastic bandages, and is utilized in the production of capsules. Additionally, it is commonly utilised in culinary and cosmetic items.

- **Description**

Hypromellose is a fibrous or granular powder that is white or creamy white, odorless, and tasteless.

- **Properties**

  - **Melting point**

chars at 225–230° C; browns at 190–200° C. 170-180° C is the glass transition temperature.

  - **Moisture content**

No more than 5% w/w. Hypromellose is able to absorb moisture from the air, but how much depends on the starting moisture content, the surrounding air's temperature, and its relative humidity.

❖ **Solubility**

Practically insoluble in chloroform, ethanol (95%) and ether, but soluble in mixes of ethanol and dichloromethane, methanol and dichloromethane, and combinations of water and alcohol. Soluble in cold water, creating a thick colloidal solution. Aqueous acetone solutions, dichloromethane and propan-2-ol combinations, and other organic solvents can dissolve some types of hypromellose.

❖ **Viscosity**

There are several varieties of viscosity that may be purchased commercially. The most typical preparation method is aqueous solutions, however hypromellose may also be dissolved in aqueous alcohols such ethanol and propan-2-ol as long as the alcohol level is less than 50% w/w. Viscous hypromellose solutions may also be made using mixes of dichloromethane and ethanol. In addition to increasing concentration, solutions made using organic solvents also tend to be more viscous.

It is advised that hypromellose be completely hydrated and distributed in around 20–30% of the necessary quantity of water before creating an aqueous solution. The remainder of the hypromellose should be added after the water has been rapidly agitated and heated to 80–90° C. The necessary amount of cold water should then be added.

The hypromellose should first be dispersed into the organic solvent in a ratio of 5-8 parts of solvent to 1 part of hypromellose when using an organic solvent that is water miscible, such as ethanol, glycol, or mixes of ethanol and dichloromethane. After that, cold water is added to provide the necessary volume.

❖ **Stability and storage conditions**

Although it becomes hygroscopic after drying, hypromellose powder is a stable substance.

At pH 3-11, solutions are stable. Temperature increases cause a decrease in solution viscosity. On heating and chilling, respectively, hypromellose transforms into a reversible sol-gel. Depending on the grade and concentration of the substance, the gel point ranges from 50 to 90° C.

Aqueous solutions have high viscosity stability during long-term storage because they are relatively enzyme-resistant. However, aqueous solutions are susceptible to microbial deterioration and should be kept with an antimicrobial preservative; benzalkonium chloride is typically employed as the preservative when hypromellose is utilized as a viscosity-increasing agent in ocular solution. Aqueous solutions can also be sterilized using an autoclave, however after cooling, the coagulated polymer needs to be shaken again to re-disperse it.

Hypromellose powder has to be kept in a tightly covered container in a dry, cold environment.

❖ **Incompatibilities**

Some oxidizing agents are incompatible with hypromellose. Hypromellose won't combine with metallic salts or ionic organics to generate insoluble precipitates since it is nonionic.

❖ **Safety**

Pharmaceutical formulations for topical application and oral administration frequently utilize hypromellose as an excipient. Additionally, it is widely utilised in culinary and cosmetic items.

Although excessive oral ingestion of hypromellose may have a laxative effect, it is often regarded as a harmless and nonirritating substance. Since the quantities ingested were not deemed to pose a risk to health, the WHO has not established a recommended daily intake for hypromellose.

❖ **Handling precautions**

Follow standard safety procedures relevant to the situation and amount of material handled. Eye protection is advised since hypromellose dust has the potential to irritate the eyes. The risk of explosion should be reduced by avoiding excessive dust formation. Hypromellose can catch fire.

9.2.6. Ceric Ammonium Nitrate: [4, 13]

- **Common Synonyms:** Ammonium ceric nitrate; cerate (2-), hexakis (nitrato-O)-, diammonium (OC-6-11)
- **Molecular structure:**

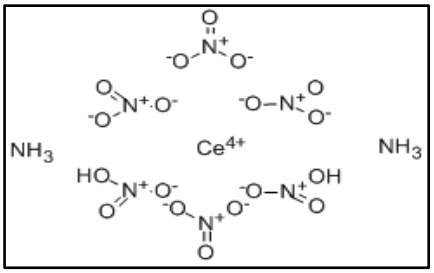


Figure 9.4. Structure of Ceric Ammonium Nitrate

- **Molecular formula: (**$(NH_4)_2Ce\ (NO_3)_6$

- **Molecular weight:** 548.23
- **CAS No:** 16774-21-3
- **Physical and chemical properties:**

❖ **Appearance**: Orange-red, monoclinic crystals that are small.

❖ **Odor:** little odor that is distinctive.

❖ **Solubility:** 141g in 100 ml of water at 77 °F (25 °C).

❖ **Density:** 1.10g/ml at 20°C.

❖ **Percent volatiles by volume:** 0 (at 21°C or 7^0F).

❖ **Boiling Point:** No information found.

❖ **Melting Point:** 107-108°C.

❖ **Stability and reactivity:** stable under typical usage and storage circumstances.

❖ **Hazardous Decomposition Products:** Nitrogen oxides.

❖ **Hazardous Polymerization:** won't happen.

❖ **Conditions to Avoid:** Heat, shock, friction, incompatibles.

❖ **Aggravation of Pre-existing Conditions:** No information found.

❖ **Incompatibilities:** Organic and flammable substances, reducing agents, aluminium powder, boron phosphide, cyanides, esters, phosphate, phosphorus, sodium cyanide, sodium hypophosphite, stannous chloride, and thiocyanates are among the substances that can ignite.

- **Potential Health Effects:**

❖ **Inhalation:** causes the respiratory system to become irritated. Shortness of breath and coughing are possible symptoms.

- **Ingestion:** Nitrates can be toxic in high amounts and can result in nausea, vomiting, diarrhea that is bloody, weakness, seizures, and collapse.
- **Skin Contact:** causes skin inflammation. Pain, itching, and redness are some of the symptoms.
- **Eye Contact:** causes discomfort, redness, and irritation.
- **Chronic Exposure:** In rare cases, the conversion of nitrate to nitrite by bacteria in the stomach results in methemoglobinemia in people. If this conversion occurs, symptoms including nausea, vomiting, dizziness, a fast pulse, uneven breathing, convulsions, coma, and death may appear.

9.3. References:

[1] B.S. Williams, A. Buvanendran, Nonopioid analgesics: NSAIDs, COX-2 inhibitors, and acetaminophen, Essentials of pain medicine, Elsevier2011, pp. 130-139.

[2] I. Pharmacopoeia, The Indian Pharmacopoeia Commission Ghaziabad Vol, II, 2010.

[3] L.L. Brunton, R. Hilal-Dandan, B.C. Knollmann, As Bases Farmacológicas da Terapêutica de Goodman e Gilman-13, Artmed Editora2018.

[4] S. Budavari, M.J. O'Neil, P.E. Heckelman, The merck index an encyclopedia of chemical, drugs, and biologicals, The merck index an encyclopedia of chemical, drugs, and biologicals, Merck & Co1989.

[5] W.-H. Kang, H. Van Nguyen, C. Park, Y.-W. Choi, B.-J. Lee, Modulation of microenvironmental pH for dual release and reduced in vivo gastrointestinal bleeding of aceclofenac using hydroxypropyl methylcellulose-based bilayered matrix tablet, European Journal of Pharmaceutical Sciences 102 (2017) 85-93.

[6] J. Martindale, W. Westcoot, The extra pharmacopoeia, the complete drug reference, London: Pharmaceutical Press, 2007.

[7] D. Smiljanić, B. de Gennaro, F. Izzo, A. Langella, A. Daković, C. Germinario, G.E. Rottinghaus, M. Spasojević, M. Mercurio, Removal of emerging contaminants from water by zeolite-rich composites: A first approach aiming at diclofenac and ketoprofen, Microporous and Mesoporous Materials 298 (2020) 110057.

[8] V. Shah, G. Hathi, M. Misra, M. Nivsarkar, Design, development and QbD based optimization of double coated spheronized aceclofenac pellets for effective palliative care in rheumatoid arthritis, Journal of Drug Delivery Science and Technology 67 (2022) 102920.

[9] R.C. Rowe, P. Sheskey, M. Quinn, Handbook of pharmaceutical excipients, Libros Digitales-Pharmaceutical Press2009.

[10] T. Ohara, T. Sato, N. Shimizu, G. Prescher, H. Schwind, O. Weiberg, K. Marten, H. Greim, Acrylic acid and derivatives, Ullmann's encyclopedia of industrial chemistry (2000).

[11] D.J. de Silva, J.M. Olver, Hydroxypropyl methylcellulose (HPMC) lubricant facilitates insertion of porous spherical orbital implants, Ophthalmic Plastic & Reconstructive Surgery 21(4) (2005) 301-302.

[12] R.O. Williams, M.A. Sykora, V. Mahaguna, Method to recover a lipophilic drug from hydroxypropyl methylcellulose matrix tablets, AAPS PharmSciTech 2 (2001) 29-37.

[13] V. Nair, A. Deepthi, Cerium (IV) ammonium nitrate a versatile single-electron oxidant, Chemical reviews 107(5) (2007) 1862-1891.

CHAPTER - X

EXPERIMENTAL WORKS: MATERIALS & METHODS

10. Experimental works: Materials & Methods

10.1. Drug used: Diclofenac sodium, purchased from TCI, India.

Aceclofenac, gifted from East India Pharmaceutical Works Ltd, Kolkata, India.

10.2. Chemicals used: Following chemicals are used during this project work:

Table 10.1. List of various chemicals used in project work

Sl. No	Chemical Used	Manufacturer
1	Cassia fistula seeds	Collected from tree in the campus of Jadavpur University, Kolkata, India
2	Acrylic acid	Merck, India
3	Cerric Ammonium Nitrate	SD Fine chemicals, India
4	Ethanol	Sigma aldrich
5	Methanol	Merck India limited, Mumbai
6	Acetone	Sd Fine Chem Limited Mumbai
7	Polyvinyl pyrrolidone K-30	Central Drug House(P) Ltd.,New Delhi
8	Talc	Qualikems Fine Chem Pvt. Ltd., India
9	Magnesium stearate	Sisco Research Laboratories Pvt. Ltd, India
10	D-mannose	SRL, India
11	D-galactose	Himedia Laboratories Pvt. Ltd
12	Trifluoroacetic acid	Merck, India
13	1-phenyl-3-methyl-5-pyrazolone	Sigma-Aldrich
14	Lactose monohydrate	SD FineChemicals, India
15	Gum acacia	SD FineChemicals, India
16	HPMC K15M	Colorcon Asia pvt. Ltd., India

10.3. Instrument handled: Following instruments are handled during my project:

Table 10.2. List of various instruments used in experimental work

Sl. No	Instrument	Model no	Manufacturer
1.	Electronic Balance	ML204/A01	Mettler Toledo, Switzerland
2.	Digital pH meter	MK- V1	Systronics, Ahmadabad, India
3.	Sieves	18 and 100 mesh size	Excel Enterprises, Kolkata
4.	Digital Slide Caliper	CD-P15 MW	DigimaticCaliper Mitutoyo Products, Japan
5.	Hot air oven	-	Indian Instruments Manufacturing Co., India
6.	Magnetic Stirrer	2MLH	Remi, India
7.	UV-Visible Spectroscopy	Pharmaspec-1900i,	Shimadzu, Japan
8.	Scanning Electron Microscope	JSM-IT 100	Jeol, Japan
9.	FTIR spectroscopy	Alpha II, ECO-ATR	Bruker, Germany
10.	X-Ray Diffractometer	SmartLab 9kW	Rigaku, Japan
11.	Hardness Tester	TH-1050M	Lab India, India
12	Friabilator	FT 1020	Lab India,India
13	Disintegration test apparatus	DT 1000	Lab India, India
14.	Differential Scanning Calorimetry	Q-10	TA Instruments, USA

15.	TGA and DTA thermograms	DTG-60	Shimadzu, Japan
16.	Tablet Compression Machine	10 station tablet press	Harsiddh, India
17.	USP dissolution apparatus II	DS 8000 (6+2)	Lab India Analytical Instrument Pvt. Ltd.
18.	CHN Analyser	Vario EL III	Elementar, Germany,
19.	Digital Viscometer	DV-1	AmetekBrookfield, India
20	Centrifuge machine	Z32HK	Hermle,Germany
21	Particle size analyser	Litesizer 500	Anton Paar
22	Microwave oven	32BC4, 230 V; 50 Hz	IFB, India
23	HPLC	600E	Water, USA
24	Column	ZORBAX Eclipse XDB-C18 analytical column (4.6 x250mm; 5-micron	Agilent, USA
25	NMR spectroscopy	ECX400	JEOL, Japan
26	Stability Chamber	GMP model 600Lt	Thermolab scientific equipment, Mumbai, India

PART - I

10.4. Evaluation of *Cassia fistula* seed gum (CFSG) as tablet binder

10.4.1. Determination of λ_{max} of Diclofenac sodium in phosphate buffer pH 7.5

20.0 mg of diclofenac sodium (assay value- 100.0%) was accurately weighed. Then it was transferred into a 100 ml volumetric flask and volume was made up to 100 ml by 7.5 pH phosphate buffer medium (stock solution). The concentration of the stock solution is 0.2 mg/ml. different aliquots of the drug solution was taken in 50 ml volumetric flasks and volume was made up to the mark with the same solvent. Thus, different concentrations of diclofenac sodium ranging from 8 to 24µg/ml were obtained.The resultant solution was scanned in Perkin Elmer UV/MS spectrophotometer (Lambda 20) in the UV region of 200-400 nm against the phosphate buffer pH 7.5[1]. The λ_{max} was found to be 276 nm in concordance with the reference standard. The UV spectrum was shown in **Figure 10.1**.

10.4.2. Preparation of Standard calibration curve of Diclofenac sodium in phosphate buffer pH 7.5

20.0 mg of diclofenac sodium (assay value- 100.0%) was accurately weighed. Then it was transferred into a 100 ml volumetric flask and volume was made up to 100 ml by 7.5 pH phosphate buffer medium (stock solution). The concentration of the stock solution is 0.2 mg/ml. different aliquots of the drug solution was taken in 50 ml volumetric flasks and volume was made up to the mark with the same solvent. Thus, different concentrations of diclofenac sodium ranging from 8 to 24µg/ml were obtained. The absorbance was recorded of those solutions in the Perkin Elmer UV-Visible spectrophotometer (Lambda 20). This was repeated three times. Then the average concentration versus absorbance curve was plotted and the equation and R^2 value of the curve was obtained.

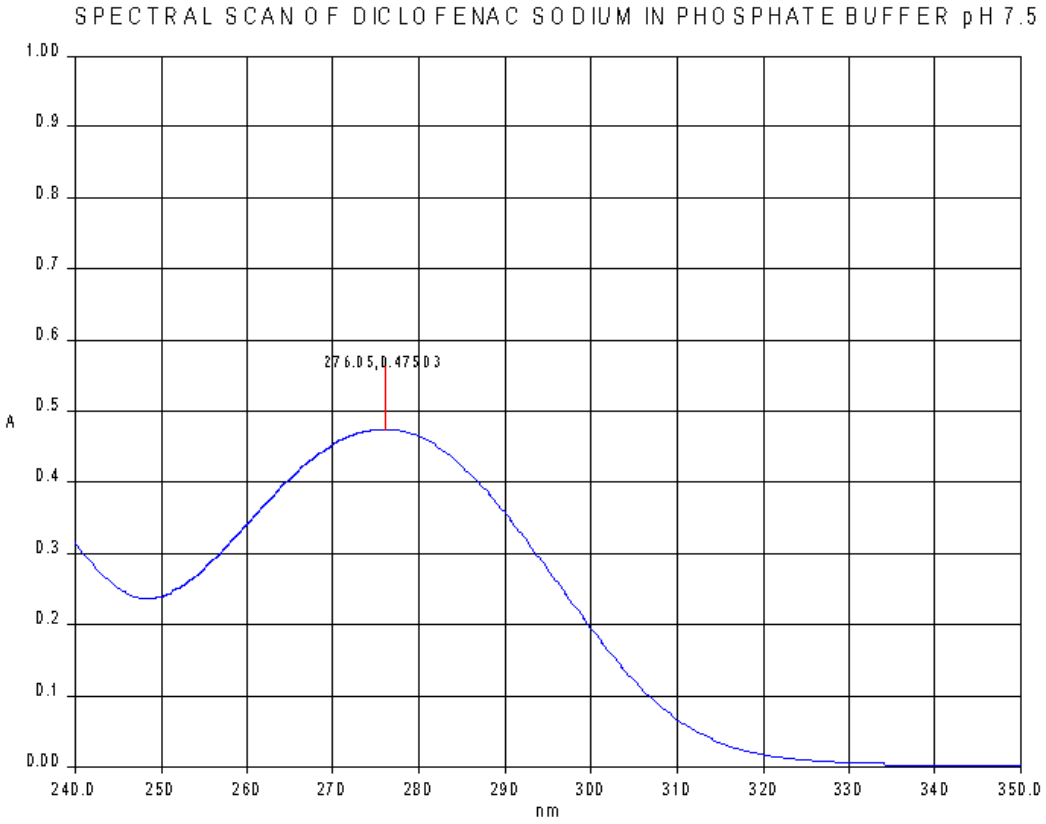


Figure 10.1. Wavelength scanning of Diclofenac sodium

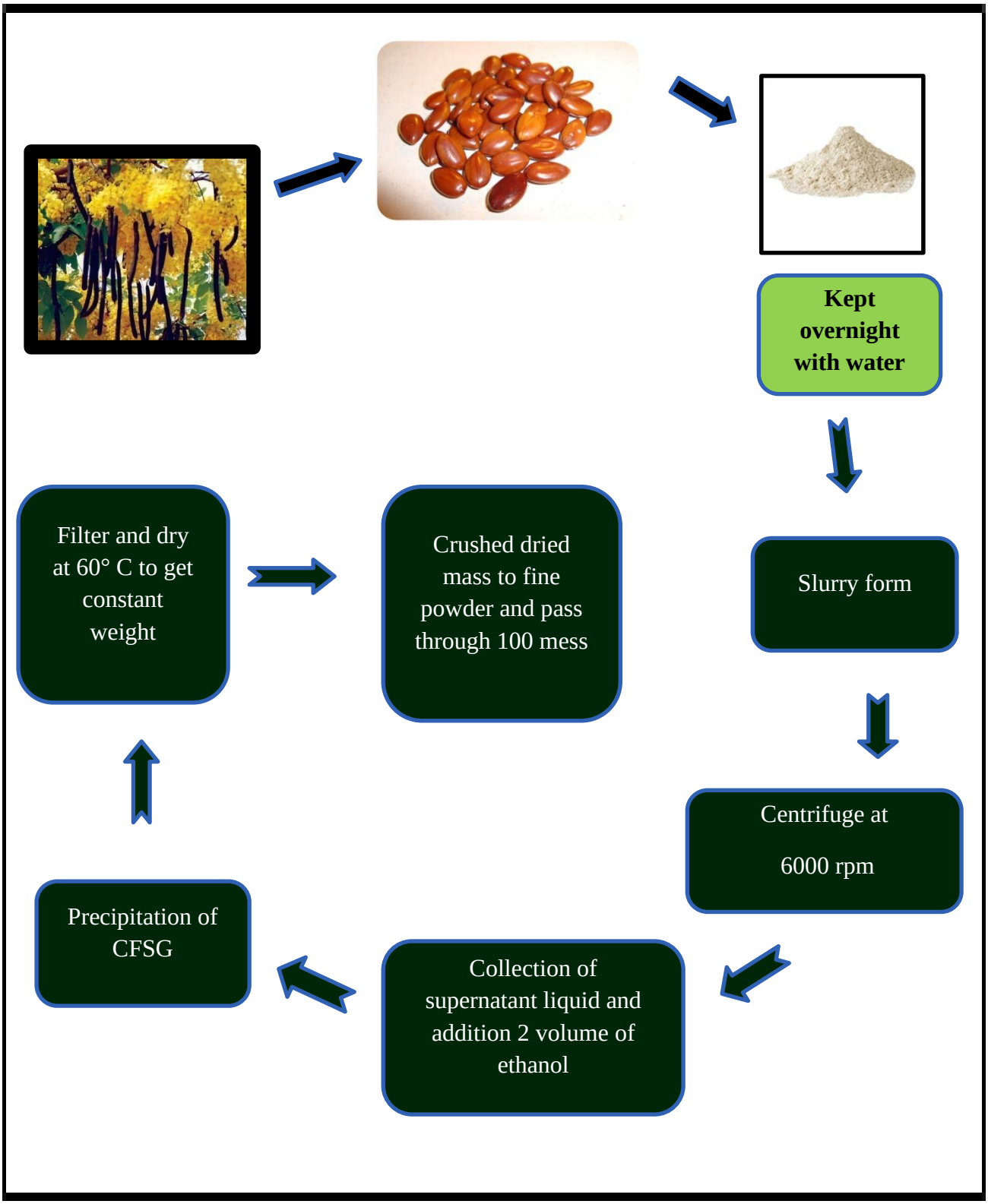


Figure 10.2. Graphical representation of extraction and purification of *Cassia fistula* seed gum

10.4.3. Extraction and purification of CFSG

CFSG was isolated and consequently purified employing the method reported in our earlier work with slight modification [2]. Seeds were collected from the ripened fruits of the tree *Cassia fistula* grown in the campus of Jadavpur University, Kolkata, India, washed, soaked in water overnight and crushed in a domestic grinder prior to extraction. Approximately 100 g of crushed seeds were boiled in 1 litre distilled water for 1 h followed by stirring for another 5 h. The slurry was then allowed to stand for 2 h to promote the precipitation of insoluble components and coarse seed particles. The upper fluid portion was then separated, diluted to 1000 ml with double distilled water, mixed and centrifuged at a speed of 5000 rpm for 20 mins (Hermle, Z32HK, Germany). CFSG was precipitated from the supernatant obtained from centrifugation by addition of absolute ethanol (purity 99.9% v/v; supernatant: ethanol 1:2). The precipitate was then collected and dried until constant weight by hot air oven at 60^0C (Indian Instruments Manufacturing Co., India). The dry flakes were grinded to a fine powder and the fraction passed through #100 mesh was stored in a vacuum desiccator until used. Total process was graphically represented in **figure 10.2**

10.4.4. Characterization of CFSG

10.4.4.1. Phytochemical investigation

Presence of different phytochemicals in the extracted gum was investigated by performing various identification tests such as Molisch's test for carbohydrate, Ruthenium red test for mucilage, Iodine test for starch, Mayer's and Dragendroff's test for alkaloid, Legal's, Keller-Killani and Borntrager's test for glycosides, Ferric chloride and lead acetate test for tannin, Libermann-Burchard's test for steroid, Ninhydrin test for proteins and amino acids [3].

10.4.4.2. Polysaccharide content

Glucose was taken as standard for estimation of total polysaccharide content in the extracted gum. A standard curve for glucose was prepared with five different standard solutions of glucose (50, 60, 70, 80 and 90 µg/ml). 1 ml of 5% w/v phenol solution was mixed with 1 ml of aqueous solution of the extracted gum (100µg/ml) and 5 ml of concentrated H_2SO_4 was then added to it. The final mixture was allowed to stand for 10 mins and then its absorbance was measured at 488 nm using a double beam UV-Visible spectrophotometer (UV-1900i, Shimadzu, Japan) against the blank solution prepared similarly without gum [3, 4]. The estimation was repeated in triplicate.

10.4.4.3. Analysis of monosaccharide composition by HPLC

HPLC method with precolumn derivatization was employed to investigate the composition of the monosaccharides present in the extracted gum [5]. 1-phenyl-3-methyl-5-pyrazolone (PMP) was used for precolumn derivatization. The polysaccharide was hydrolysed into monosaccharides by dispersing approximately 10 mg fine powder of the extracted gum in 8 ml of 4M trifluoroacetic acid (TFA) and subsequently heating at 110°C for 5 h. Excess TFA was eliminated by adding methanol and consequently evaporating under nitrogen-atmosphere. The residue was dried and dissolved in 1 ml triple-distilled water. 1 ml of 0.3M NaOH and 1 ml of 0.5M methanolic solution of PMP were added to the previous solution of the residue (hydrolysed gum) and heated for 2 h at 65ºC in order to complete the derivatization reaction. The mixture was allowed to cool to ambient temperature and then neutralized with 1 ml of 0.3 M HCl. Finally, 5 ml chloroform was added to it, stirred for 5 mins and allowed to stand for another 5 mins. The aqueous-phase was filtered through 0.45 µm membrane and 20 µl of the filtrate was injected into a ZORBAX Eclipse XDB-C18 analytical column (4.6 x250mm; 5-micron; Agilent, USA) in a HPLC system (Water, USA with quaternary gradients pump 600E) with UV detector (Tunable absorbance detector 486) and software Millennium 32. The mobile

phase composed of acetonitrile and ammonium acetate solution (pH 5.5) in 4:1 ratio was run with a flow rate of 1 ml/min. The retention times were compared to that from standard galactose, mannose, glucose, rhamnose, xylose, arabinose and galacturonic acid treated similarly as test.

10.4.4.4. Elemental analysis (C, H, O, N)

Percent content of carbon, hydrogen, oxygen and nitrogen in the extracted CFSG was analysed using Elemental analyzer (Elementar, Germany, Vario EL III).

10.4.4.5. FTIR study

FTIR spectra of CFSG was obtained from FTIR (Bruker, Alpha II, ECO-ATR, Germany) to identify the functional groups present in its polysaccharide backbone. KBr pellet was prepared using a hydraulic compressor and scanned in the range from 500 to 4000 cm^{-1}.

10.4.4.6. Solid-state ^{13}C NMR study

^{13}C NMR spectra (solid-state) of CFSG was obtained from a solid-state NMR spectrometer (JEOL, ECX400, proton frequency 400MHz) operated at 400 MHz.

10.4.4.7. Molecular weight, zeta potential, viscosity, pH and surface tension

Molecular weight, zeta potential, refractive index and turbidity of CFSG were determined using particle analyzer (Litesizer 500, Anton Paar, Austria). For molecular weight, three different aqueous solution of CFSG (0.01, 0.02, and 0.05% w/v) were prepared. The constant parameters were: solvent- water: dn/dc 1.0 ml/g; solvent refractive index 1.3303; Reference- toluene: reference refractive index 1.4925; reference Rayleigh ratio 0.0000115 cm^{-1} and the temperature was maintained at 25°C. For zeta potential, the constant parameters were as follows: solvent - water; solvent refractive index, viscosity and relative permittivity: 1.3297, 0.7973 mPa.s, and 76.6, respectively; adjusted voltage 200.0 V; filter optical density 2.2557; temperature 30°C. The viscosity of 1% w/v aqueous solution of CFSG was measured using a programmable digital Brookfield Viscometer (LV, Ametek, USA) with spindle no. CPA-40Z at 31.2ºC. The

spindle was rotated at 0.5 rpm (Torque-27%; Shear stress-6.21 dyne/cm^2; shear rate-0.375 s^{-1}). pH and surface tension of the same solution were measured at 29.2°C using a pH meter (pH 700, Eutech Instrument, Singapore) and K9 optical tensiometer (Kruss GmbH, Hamburg, Germany), respectively.

10.4.4.8. Rheology study

Rheogram (shear stress versus rate of shear) of a 5%w/v aqueous dispersion of native CFSG was obtained from MCR-302e Rheometer (Rheoplus, Anton Paar, Germany) with measuring system CP50-1 (SN45793) in order to investigate the rheological behavior of CFSG aqueous dispersion. Change in viscosity with increase in shear-rate and change in storage modulus (G') and loss modulus (G'') with shear strain and stress were also studied [6]. The study temperature was set at 25˚C. The dispersion was kept covered with a very tinny layer of light liquid paraffin to prevent evaporation of water from the surface till the study. The rate of shear was varied from 0 to 100 s^{-1} for the rheogram and the dynamic viscosity (η, mPa.S) was determined at different rates of shear (γ) ranging from 0.1 to 100.0 s^{-1} at constant shear-stress. In amplitude-sweep study, storage modulus (G', Pa) and loss modulus (G'', Pa) were obtained at different % shear-strain ranging from 0.1 to 1000% and shear-stress (τ, Pa) ranging from 0.1 to 1000 Pa at a constant angular-frequency (ω) of 10 rad/s.

10.4.4.9. DSC, TGA, and DTA study

DSC, TGA and DTA spectra of the extracted CFSG were obtained from a Differential Scanning Calorimeter (Q10, TA Instruments, USA) under nitrogen-flow of 150 ml per min using a platinum crucible for heating of sample at a heating rate of 10˚C per min from 30˚C to 400˚C and alpha alumina powder as reference. TGA- and DTA-thermograms were also obtained from a Thermogravimetric Analyzer (DTG-60, Shimadzu, Japan).

10.4.4.10. Powder X-ray Diffraction (PXRD) study

PXRD diffractograms of native CFSG was obtained from X-Ray-Diffractometer (SmartLab 9kW, Rigaku, Japan) to investigate its crystalline nature. The diffractometer was operated at 25˚C with a 40 kV voltage, 30 mA current and nickel filtered CuK alpha1 radiation (1.54060A˚). The range of 2θ scattering angle was set at 0 to 90˚ with minimum step size of 0.001˚.

10.4.4.11. Scanning Electron Microscopy

The surface topography of the CFSG-particles was studied by Scanning Electron Microscope (Jeol, JSM-IT 100, Japan) after gold-coating by mounting the sample on a brass-stub with a double-sided adhesive tape in an ion-sputter under vacuum for enhancing the conductivity of the electron-beam. The voltage was maintained at 2.0 kV and the working distance at 7 mm.

10.4.4.12. Acute oral toxicity and histological study

Safety profile of CFSG as tablet excipient for oral consumption was checked by carrying out oral acute toxicity study following OECD guideline (Organisation of Economic Co-operation and Development) for the test of chemicals 425, adopted 17 December 2001. As per guideline, 5 no eight weeks old, healthy, nulliparous and non-pregnant female mice of *Swiss albino species*, were taken for the study. The study was conducted after getting approval from the Institutional Animal Ethics Committee of University of North Bengal (Establishment Registration no. 840/GO/Re/S/04/CPCSEA dated 16/08/2018). The animals were housed in polycarbonate cage and provided sufficient food and demineralized water ad libitum. The temperature and humidity of the room were maintained at 22 ± 3˚C and 50-60%, respectively. After acclimatization, a single dose of native CFSG powder (calculated on the basis 2g/kg body weight) in suspension form was administered to the first animal using a stomach tube by gavages. After survival of the first animal, remaining four test animals were administered and all were observed at predetermined intervals up to 14 days following the protocol reported in

our previous work [7]. After toxicity study, histological study was carried out following the method reported in our previous work [8, 9]. The photomicrographs of the permanent slides of each organ (control and test) were obtained from an optical-microscope (AX10, Lab A1, Zeiss, Germany) fitted with a camera (Carl-Zeiss-Promenade-10, Zeiss, Germany). The test-micrographs were compared to that of controls in order to detect any significant histological changes.

10.4.5. Preformulation studies

In preformulation study, drug-excipient compatibility was checked by FTIR and DSC following the same procedures mentioned in earlier sections. Powder X-ray diffraction was also performed to check the crystalline nature of pure drug as well as in tablets. The studies were done with pure diclofenac sodium, drug-loaded tablet and blank tablet.

Table 10.3. Formula of each tablet of diclofenac sodium composed of CFSG, gum acacia or PVP-K30.

Batch no.	Diclofenac sodium (mg)	Lactose (mg)	CFSG (mg)	Gum acacia (mg)	PVP-K30 (mg)	Magnesium stearate (mg)	Purified talc (mg)	Total weight (mg)
B1	100	89	2.5% (5)	-	-	4	2	200
B2	100	84	5% (10)	-	-	4	2	200
B3	100	79	7.5% (15)	-	-	4	2	200
B4	100	74	10% (20)	-	-	4	2	200
B5	100	89	-	2.5% (5)	-	4	2	200
B6	100	84	-	5% (10)	-	4	2	200
B7	100	79	-	7.5% (15)	-	4	2	200
B8	100	74	-	10% (20)	-	4	2	200
B9	100	89	-	-	2.5% (5)	4	2	200
B10	100	84	-	-	5% (10)	4	2	200

B11	100	79	-	-	7.5% (15)	4	2	200
B12	100	74	-	-	10% (20)	4	2	200

CFSG: *Cassia fistula* seed gum; PVP: polyvinylpyrrolidone.

10.4.6. Preparation and characterization of drug loaded granules

10.4.6.1. Preparation of granules by wet granulation method

Diclofenac-loaded granules were prepared employing wet granulation method before tablet compression. The formulations of the granules were same as the tablets given in **Table 10.3**. Amount of granules equivalent to 100 number tablets for each batch were prepared. Briefly, diclofenac sodium was intimately mixed with lactose using a large pestle and mortar. The mixture was then moistened with respective binder solution (CFSG/gum acacia/PVP K-30) and passed through sieve no. 18 in order to form granules. The resultant granules were then dried at 60ºC for 20 min in a hot air oven and stored in desiccator for evaluation.

10.4.6.2. Micromeritic and derived properties of the granules

Size distribution by weight was studied by sieving method using sieve shaker (Lab Solution, Kolkata, India). Nine sieves with different aperture size (2000 µm, 1700 µm, 1400 µm, 710 µm, 600 µm, 355 µm, 212 µm, 180 µm and 150 µm) were arranged in ascending order according to their aperture size from bottom to top. The granules (10 g) were placed on the top and sieved at 60 rpm for 1 min. The fractions were collected from each separate sieve and weighed individually. Percent weight frequency was calculated using the Eq. 1.

$$\%f_w = \frac{W_i \times 100}{W_0} \quad \text{(Eq.1)}$$

Where, W_i and W_0 were powder weight at individual sieve and whole powder weight, respectively. Finally, average granule-size was obtained by extrapolating the peak point to X-axis of $\%f_w$ versus granule-size (µm) curve, where, the average of the aperture sizes of the retain-sieve and passing-sieve was considered as the granule-size of each division of granules.

The shape and surface texture were studied by SEM following the same method mentioned in the previous section. The tapped bulk volume (V_b) was measured by tapping the granules in a 25 ml measuring cylinder until constant volume in a bulk density tester (TD-1025, LabIndia, India). Bulk density was obtained by dividing mass of the granules by tapped bulk volume. True volume (V_t) of the granules was determined by liquid (non-solvent) displacement method using acetone as non-solvent liquid and pycnometer. True density was obtained by dividing mass of the granules by true volume. Total porosity ($\in_T$) was calculated using Eq. 2.

$$\in_T = \frac{(V_b - V_t) \times 100}{V_b} \quad \text{(Eq. 2)}$$

Where, V_b and V_t were tapped bulk volume and true volume, respectively. The flowability of the granules were assessed by determining angle of repose, Carr's compressibility index and Hausner ratio. Angle of repose (θ) was determined by a fixed funnel and cone method (Angle of Repose apparatus, EFT-01, Electrolab, India) using Eq.3.

$$\theta = \frac{2H}{D} \quad \text{(Eq. 3)}$$

Where, *H* and *D* were the height and base-diameter of the resultant cone, respectively. Carr's compressibility index was calculated by (Initial bulk volume – Tapped bulk volume) ×100/Initial bulk volume [10]. The Hausner ratio was determined by Loose bulk volume/tapped bulk volume [11]. All determinations were done in triplicate (n = 3).

10.4.7. Compression and evaluation of diclofenac sodium loaded tablets

10.4.7.1. Tablet compression from prepared granules

The tablets of diclofenac sodium with CFSG (as binder) and lactose (diluent) were prepared by wet granulation method. Some batches were prepared with standard binder gum acacia and PVP K-30 to compare with CFSG. The compositions of the tablets were shown in **Table 10.3**. The granules were prepared as mentioned in the previous section and then sieved through 18/22 sieve. The granules passed through sieve no. 18 but retained on sieve no.22 were collected and used for compression. After lubrication with magnesium stearate and purified talc, the granules

were compressed in a rotary 10-stations tablet press (Harsiddh, India) using 6 mm die and flat-faced punch. Some batches were prepared with HPMC K15M separately to compare the drug-release-sustaining capacity of CFSG.

10.4.7.2. Evaluation of binding capacity of CFSG and other tablet-properties

Tablet-volume was measured using the formula ($V = \pi D^2 H/4$ where D, diameter and H, height of tablet). Diameter and height were measured using digital slide callipers (CD-P15MW, Mitutoyo, Japan). Apparent tablet-density was determined by weight/volume. Total percent porosity of the tablet was determined using Eq. 2 and packing fraction or relative density (P_f) was calculated using Eq. 4 [12].

$$P_f = \frac{W_T}{V_T \times D_g} \quad (Eq.4)$$

Where, W_T, D_g and V_T were tablet-weight, granular density and tablet-volume, respectively. Percent Elastic Recovery (PER) was determined using Eq. 5 [10].

$$PER = \frac{(H_R - H_0) \times 100}{H_0} \quad (Eq.5)$$

Where, H_0 and H_R were tablet-height after ejection and at recovery after 24 h, respectively. Weight uniformity, hardness, drug content, percent friability and disintegration-time were assessed as per USP, 2009. Hardness, percent friability and disintegration time were measured using Monsanto hardness tester (TH-1050M, Lab India, India), Roche-type friabilator (FT1020, Lab India, India), and Disintegration test apparatus (DT1000, Lab India, India), respectively. Percent friability was calculated using Eq. 6.

$$\%\, Friability = \frac{W_0 - W_f}{W_0} \times 100 \quad (Eq.6)$$

Where, W_0 and W_f were the initial and final weight, respectively. Disintegration test was performed in a phosphate buffer (0.05 M; pH 7.5) maintained at 37ºC. The surface texture of the tablet and the xerogel obtained from the hydrated tablet was analyzed by the Scanning Electron Microscope (Jeol, JSM-IT 100, Japan).

10.4.7.3. Hydration kinetic study

Equilibrium water absorption and hydration kineticity were studied following our previously reported method with slight modification [13]. For equilibrium hydration, one tablet from each batch was placed in a wire basket after taking weight and fully immersed for 24 h in a 100 ml 0.05M phosphate buffer (pH 7.5) maintained at 37ºC. The weight of the hydrated tablet plus basket was noted at the end of the 24 h period after removing the adhered water from the surface with tissue paper. Equilibrium water absorption (%) was calculated using Eq.7:

$$W_E = \frac{(W_1 - W_0) \times 100}{W_0} \ (Eq.\ 7)$$

Where, W_E, W_0 and W_1 were the equilibrium water absorption (%), dry tablet-weight and hydrated tablet-weight, respectively. Hydration kineticity of the tablets was studied by determining the increase in amount of water absorbed per gram of dry tablet-matrix (W) as a function of time, t. Hydration isotherm was obtained by plotting W against time, t in minutes. The horizontal part of the curve indicated hydration equilibrium. *Initial hydration rate* ($dW/dt = 1/A$) when $t \rightarrow 0$ and *equilibrium hydration* ($W_\infty = 1/B$) at t_∞ were obtained from the intercept (A) and slope (B) of t/W versus t curve according to following expressions (Eq. 8 and 9).

$$\frac{t}{W} = A + Bt \ (Eq.\ 8) \quad \frac{dW}{dt} = \frac{A}{(A+Bt)^2} \ (Eq.\ 9)$$

The *Matrix Hydration* (H) was determined using Eq. 10.

$$H = \frac{(W_S - W_0)}{W_S} \ (Eq.\ 10)$$

Where, W_S and W_0 were the weight of hydrated tablet at equilibrium and initial dry tablet. The water penetration velocity (V) into the tablet-matrix was calculated from the hydration-kinetic data using Eq. 11.

$$V = \frac{1}{2\rho A} \times \frac{dW}{dt} \quad (Eq.\ 11)$$

where dW/dt, ρ and A were the slope of W versus time curve, density of phosphate buffer at 37ºC and tablet-surface area, respectively.

10.4.7.4. Drug-release study

In vitro drug-release was studied following the method mentioned in the monograph for diclofenac sodium tablet in USP-2009 using USP-type-II dissolution test apparatus (DS-8000; 6+2; SC/TR, LABINDIA, Navi Mumbai, India). Briefly, the tablet was placed in a 900 ml phosphate buffer (pH 6.8) maintained at 37°C and stirred at 50±5 rpm. 5 ml aliquot was withdrawn at 45 min to analyze drug concentration by UV-Visible spectrophotometer (UV-1780, Shimazdu, Japan) at 276 nm. Finally, cumulative percent drug-release (CPR) at 45 min was calculated. The study was performed in triplicate (n = 3) for each batch.

10.4.7.5. Accelerated stability study

The tablets from the batch having optimum characteristic were packed in a tightly-closed amber-colored glass-bottle and stored in a stability chamber in a challenged environment (40 °C ± 0.5 °C and 75 ± 5% relative humidity) for a period of 3 months. At the end, the aged tablets were inspected for any probable change in physical appearance, evaluated for all tableting properties and characterized with FTIR, DSC and XRD studies.

10.4.8. Statistical analysis

The results were expressed as average ± standard deviation and calculated using MedCalc software, version 11.6.1.0.3.

PART II

10.5. Chemical modifications of *Cassia fistula* seed gum through graft copolymerization with poly (sodium acrylate) and used as gastroretentive drug delivery excipients

10.5.1. Determination of λ_{max} of Aceclofenac in 0.1 (N) Hydrochloric Acid with 0.2% sodium lauryl sulphate (SLS) Medium

20.0 mg of aceclofenac (assay value- 99.62%) was accurately weighed. Then it was transferred into a 50 ml volumetric flask and volume was made up to 50 ml by 0.1 (N) hydrochloric acid with 0.2% SLS (pH 1.2) medium (stock solution). The concentration of the stock solution is 0.398 mg/ml. different aliquots of the drug solution was taken in 25 ml and 20 ml volumetric flasks and volume was made up to the mark with the same solvent. Thus, different concentrations of aceclofenac ranging from 0.00797 to 0.03188 mg/ml were obtained. The resultant solution was scanned in Perkin Elmer UV/MS spectrophotometer (Lambda 20) in the UV region of 200-400 nm against the 0.1 (N) Hydrochloric Acid (0.2%SLS) Medium [1]. The λ_{max} was found to be 275 nm in concordance with the reference standard. The UV spectrum was shown in **Figure 10.3**.

10.5.2. Preparation of Standard Curve of Aceclofenac in 0.1 (N) Hydrochloric Acid with 0.2%SLS Medium

20.0 mg of aceclofenac (assay value- 99.62%) was accurately weighed. Then it was transferred into a 50 ml volumetric flask and volume was made up to 50 ml by 0.1 (N)hydrochloric acid with 0.2% SLS, pH 1.2) medium (stock solution). The concentration of the stock solution is 0.398 mg/ml. different aliquots of the drug solution was taken in 25 ml and 20 ml volumetric flasks and volume was made up to the mark with the same solvent. Thus, different concentrations of aceclofenac ranging from 0.00797 to 0.03188 mg/ml were obtained. The absorbance was recorded of those solutions in the Perkin Elmer UV/MS spectrophotometer (Lambda 20). This was repeated three times. Then the average concentration versus absorbance curve was plotted and equation and R^2value of the curve was obtained.

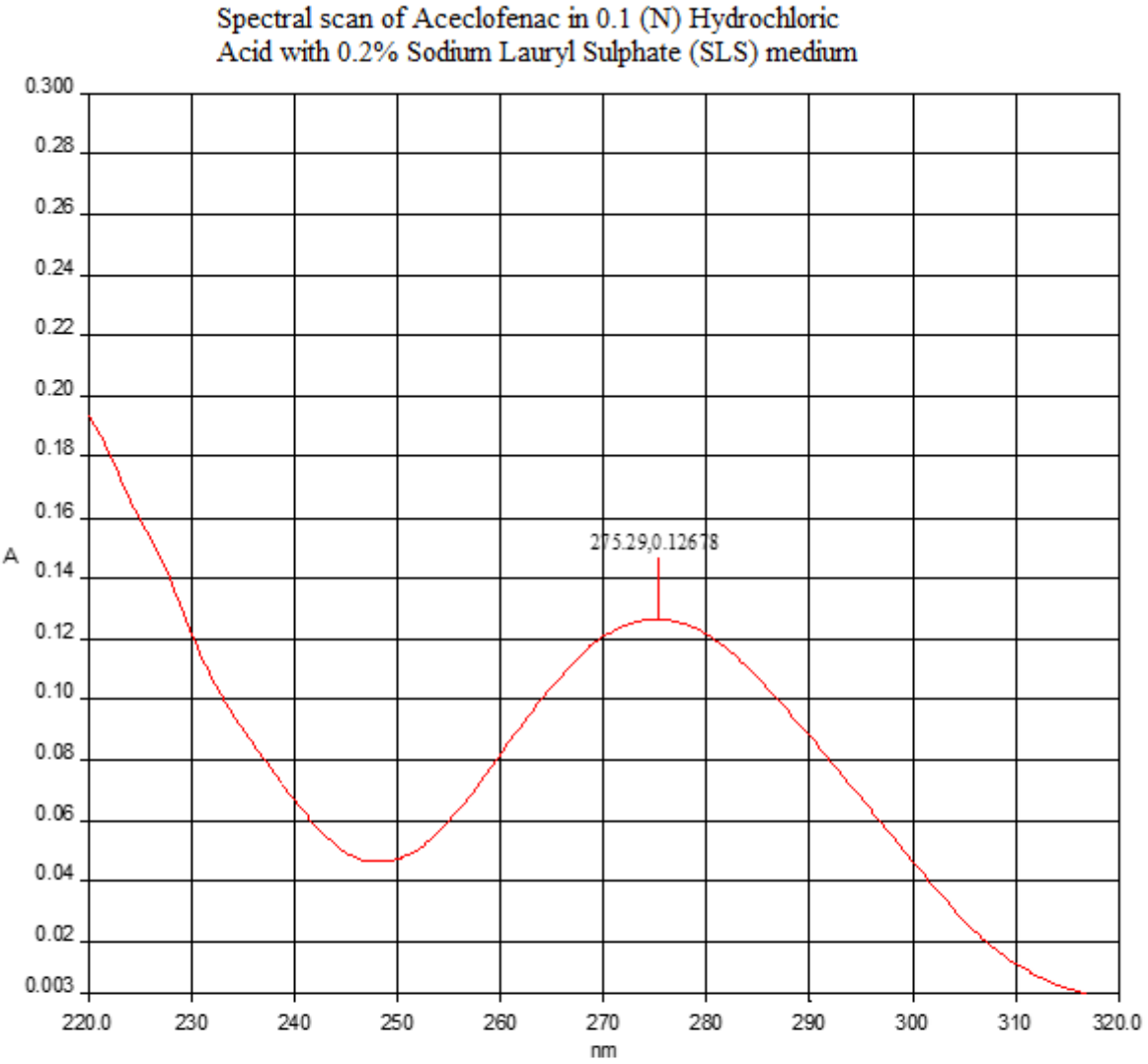


Figure 10.3.: Wavelength scanning of Aceclofenac

10.5.3. Synthesis of CFSG-g-PSA

CFSG-g-PSA was synthesized employing microwave-assisted free-radical initiation method reported in our earlier works with little change [7, 8, 14]. Ceric ammonium nitrate (CAN) was used as a free-radical initiator in the synthesis. Variable amounts of monomer acrylic acid (AA) and CAN along with variable microwave-irradiation time were used in the study and presented in **Table 10.4** (S1 – S8). Specified amount of acrylic acid was mixed in 30 ml distilled water and then mixed to 150 ml 0.67% w/v aqueous solution of CFSG with continuous stirring by magnetic stirrer (Remi, 2MLH, India) for 10 min at ambient temperature. A freshly prepared aqueous solution of CAN (30 ml) was mixed with the previous mixture with stirring for 1 min. The resultant mixture was immediately exposed to the microwave in a domestic microwave oven (IFB, 23BC4, India; 230 V; 50 Hz) at 900 W for specified time-period following 1 min irradiation and 1 min cooling cycle. Ice-cold water was used for cooling. After keeping overnight, double volume acetone was added to the mixture for precipitation of the grafted CFSG. The precipitate was separated by straining, added in 100 ml methanol-water mixture (4:1), stirred for 5 min magnetically to promote the leaching of homopolymer and free reactants from the grafted mass and then allowed to stand for further precipitation. The precipitate was separated, washed with water, dried in a hot air oven at 60°C until constant weight and powdered. The powder was dissolved completely in a minimum volume of 0.1N NaOH solution to convert acrylic acid units of grafted side chains of poly (acrylic acid) into sodium acrylate units. This promotes gelling of the copolymer by increasing hydration capacity. The modified copolymer, CFSG-g-poly (sodium acrylate) (CFSG-g-PSA) was precipitated, washed, dried and powdered in a similar way. The fine fraction of the powder (passed through #100 sieve) was stored in a vacuum desiccator for experiments. Some batches (Table 1; H1 – H4) were prepared similarly without a microwave by heating at 60°C using a magnetic stirrer with a thermostatic hot-plate. Various grafting parameters such as % grafting (*%G*), % grafting

efficiency (*%GE*) and % conversion *(%C_n*) were calculated to evaluate the synthetic method using Eq. 12 to 14[7].

$$\%G = \frac{(W_1 - W_0) \times 100}{W_0} \quad \text{(Eq. 12)}$$

$$\%GE = \frac{(W_1 - W_0) \times 100}{W_2} \quad \text{(Eq. 13)}$$

$$\%Cn = \frac{W_1 \times 100}{W_2} \quad \text{(Eq. 14)}$$

Where, W_0, W_1 and W_2 were the amount of CFSG, CFSG-g-PSA and acrylic acid in grams, respectively. The influences of independent synthetic variables such as amount of acrylic acid (AA), CAN and microwave irradiation time (MW) on various grafting parameters were studied by 2^3 full factorial designs using Design-Expert software (version 12). Independent variables, their levels and response-variables were presented in **Table 10.5** and **Table 10.6**. Numerical optimization was also performed using the same software with targets at the maximum level of%G, %GE and %Cn in order to obtain the best synthetic condition.

Table10.4. Various synthetic conditions for the synthesis of CFSG-g-PSA and various characterization parameters

Batch No.	Acrylic acid (g)	CAN (g)	MW (min)	%G	%GE	%C_n
CFSG	-	-	-	-	-	-
H1	5	0.25	-	6.6	1.3	21.3
H2	5	0.50	-	11.2	2.2	22.2
H3	10	0.25	-	8.5	0.85	10.9
H4	10	0.50	-	23.8	2.4	12.4
S1	10	0.50	1	790	79.0	89.0
S2	5	0.50	1	353	70.6	90.6
S3	5	0.25	5	63	12.6	32.6
S4	5	0.25	1	125	25.0	45.0
S5	10	0.25	1	165	16.5	26.5
S6	10	0.50	5	570	57.0	67.0
S7	10	0.25	5	139	13.9	23.9
S8	5	0.50	5	286	57.2	77.2

Table 10.5. Different independent synthetic variables along with their two levels in the 2^3 full factorial design.

Independent factors	**Levels**	
	+1 (high)	-1 (low)
Acrylic acid (AA)	10 g	5 g
Ceric ammonium nitrate (CAN)	0.5 g	0.25 g
Microwave irradiation time (MW)	5 min	1 min

Table 10.6. Numerical optimization of synthetic independent variables by 2^3 full factorial designs.

Sl. No.	Independent variables		Response variables				
	Parameters	Optimized value	Parameters	Target value	Predicted value	Observed value	% Error
1.	Acrylic acid (AA)	10 g	% Grafting (%G)	maximum	1172.1	1181.5	0.80
2.	Ceric ammonium nitrate (CAN)	0.5 g	% Grafting efficiency (%GE)	maximum	117.2	118.15	0.81
3.	Microwave irradiation time (MW)	1 min	% Conversion ($\%C_n$)	maximum	127.2	128.15	0.75

10.5.4. Characterizations of CFSG-g-PSA

10.5.4.1. Elemental analysis

Percent content of the elements (carbon, hydrogen and oxygen) of the native CFSG and another twelve different batches of CFSG-g-PSA were estimated by an Elemental analyzer (Elementar, Germany, Vario EL III).

10.5.4.2. FTIR

FTIR spectra of native CFSG, CFSG-g-PSA (S1 batch), pristine aceclofenac, tablet powder (MT10) and blank tablet (MT10; drug free) were generated using FTIR (Bruker, Alpha II, ECO-ATR, Germany) in order to detect the possible changes of functional groups of native CFSG due to grafting reaction as well as any interaction between aceclofenac and CFSG-g-PSA. KBr pellets were prepared with a sample: KBr ratio of 1:10. The selected scanning range was from 500 to 4000 cm^{-1}.

10.5.4.3. ^{13}C Solid state NMR spectroscopy

^{13}C solid state NMR analysis of native CFSG and CFSG-g-PSA (S1 batch) was done using a solid-state NMR spectrometer (JEOL, ECX400, proton frequency 400MHz) operated at 400 MHz.

10.5.4.4. Determination of molar mass and zeta potential

Molar mass and zeta potential of native CFSG and all synthetic batches were determined using particle analyzer (Litesizer 500, Anton Paar). Three different concentrations (0.01, 0.02 and 0.05% w/v) in water were prepared for determination of molar mass. The temperature was set at 25°C. Other parameters were: dn/dc 1.0 ml/g; solvent-water; solvent refractive index 1.3303; reference-toluene; reference refractive index 1.4925; reference Rayleigh ratio 0.0000115 cm^{-1}. The temperature was set at 30°C for zeta potential and other parameters were: adjusted voltage 200.0 V; filter optical density 2.2557; solvent-water; solvent refractive index 1.3297; solvent viscosity 0. 7973mPa.s; relative permittivity 76.6.

10.5.4.5. Thermal study (DSC, TGA and DTA)

DSC, TGA and DTA studies were done for investigation of thermal changes of the native and grafted CFSG along with drug decomposition or any other incompatibility between drug and excipients. DSC thermograms of native CFSG, CFSG-g-PSA (S1), pristine aceclofenac, tablet (MT10) and blank tablet were obtained from TA Instruments, USA, Q10 under N_2 flow of 150 mL/min. each sample was heated in a platinum crucible with a heating rate of 10˚C/min in the temperature range from 30˚C to 400˚C and alpha alumina powder was used as reference. TGA and DTA thermograms were also obtained from Thermogravimetric analyser (Shimadzu, Japan, DTG-60).

10.5.4.6. Powder XRD

Powder X-ray diffractograms of native CFSG, CFSG-g-PSA (S1 batch), pristine aceclofenac, tablet (MT10 batch) and blank tablet were obtained from XRD-Diffractometer (Rigaku, Japan, SmartLab 9kW) with Nickel filtered CuK alpha1 radiation (1.54060A˚) at a 40 kV voltage,30 mA current and 25˚C. The range of the scattering angle (2θ) was 0 to 90˚ with minimum step size of 0.001˚. This study was done to check the crystallinity of the polymers and drug as well as their changes and compatibility between drug and excipients.

10.5.4.7. SEM study

The morphology of the particle-surface of CFSG and CFSG-g-PSA (S1 batch) was studied by SEM (Jeol, JSM-IT 100, Japan). The morphology of the tablet surface before and after drug-release study was also studied. The tablet-residue was collected at the end of drug-release study and freeze-dried. Each sample was gold-coated by mounting on a brass stub using a double-sided adhesive tape in an ion sputter under vacuum in order to enhance the conductivity of the electron beam. The accelerating voltage and working distance were maintained at 2.0 kV and 7 mm, respectively.

10.5.4.8. Viscosity and pH measurement

The viscosity of CFSG and different batches of CFSG-g-PSA (0.1%, w/v aqueous solution) were measured by a programmable and digital viscometer (Brookfield, DV-1, Ametek) with spindle no. RV-06 at 30. 8ºC.The rotation-speed of the spindle was selected at 1 rpm. pH of 1%w/v aqueous solution of native CFSG and CFSG-g-PSA (S1 batch) was measured at 29.6°C using a pH meter (pH 700, Eutech Instrument, Singapore).

10.5.4.9. Water uptake study

Equilibrium water uptake capacity of native CFSG and its various grafted batches were determined by immersing the blank tablets of each batch in 0.1 N HCl acid using basket of dissolution test apparatus at 37°C [9]. Initial dry weights and weights of hydrated tablets at 24

h were noted. The blank tablets were prepared with only polymer by wet granulation and subsequent compression with average weight of 200 mg, average diameter of 7 mm and thickness of 4 mm using rotary tablet press (Harsiddh, India). The equilibrium water uptake was calculated using the following formula (Eq. 15):

$$W_E(\%) = \frac{(T_H - T_0)\times 100}{T_0} \qquad \text{(Eq. 15)}$$

Where, W_E, T_H and T_0 were % equilibrium water uptake, weight of hydrated tablet at 24 h and initial weight of dry tablet, respectively.

10.5.4.10. Biodegradation study

Biodegradable nature of CFSG-g-PSA was investigated following the method reported in our earlier work [14]. Various simulated gastrointestinal fluids such as simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF) were taken as medium for the study. Colonic probiotics such as *Streptococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus* and *Lactobacillus sporogenes* were mixed with SCF. The tablets prepared with S1 batch for water uptake study were used in this study. At first, three tablets of CFSG-g-PSA (S1batch) were weighed, dipped completely in 50 ml of distilled water, SGF and SIF separately in three beakers and incubated at 37ºC for 24 h. Another two tablets weredipped in 50 ml SCF [15] in another two beakers separately with and without probiotics commonly found in colon with composition of Streptococcus faecalis T-110^{TM} JPC (30 million), Clostridium butyricum TO-A^{TM} (2 million), Bacillus mesentericus TO-A^{TM} JPC (1 million) and Lactobacillus sporogenes (50 million) (BIFILAC, hard gelatin capsule I. P., Tablets (India) Ltd., India). 1% peptone was also added to the beaker with probiotics to support their growth. The beakers were kept in an incubator at 37ºC for 14 days. At the end of the specified intervals the tablets were taken out from the medium, dried and weighed. Then % weight loss was calculated using the following formula (Eq.16):

$$W_L(\%) = \frac{(W_i - W_t) \times 100}{W_i} \text{(Eq. 16)}$$

where W_L *(%)*, *Wi* and W_t are the percent weight loss, initial dry weight and dry weight at the end of the specified interval, respectively. The study was repeated three times (n = 3).

10.5.5. Preparation of sustained-release mucoadhesive tablets of aceclofenac with CFSG-g-PSA

The tablets of aceclofenac with native CFSG and its different grades of grafted forms were prepared employing wet granulation methods. The compositions of the tablets of different batches are shown in **Table 10.7**. 100 number tablets were prepared for each batch. Briefly, aceclofenac and corresponding polymer were mixed intimately using large pestle-mortar and the mixture was then moistened with a minimum amount of water. The mass was passed through sieve no. 18 to obtain granules. The granules were dried at 50ºC for 30 min in a hot air oven and screened through sieve no. 18. The fraction of the granules retained on the sieve was lubricated with magnesium stearate and purified talc and compressed in a rotary 10-stations tablet press (Harsiddh, India) using 10 mm die. Similar tablets were also prepared with HPMC K15M and carbopol 974P separately.

Table 10.7. Formula per tablet of different batches of mucoadhesive sustained release tablets of aceclofenac with CFSG-g-PSA

Batch No.	Aceclofenac (mg)	Polymer (mg)	Purified talc (mg)	Magnesium stearate (mg)	Total weight (mg)
MT1	200	150 (CFSG)	5	5	360
MT2	200	150 (S1)	5	5	360
MT3	200	150 (S2)	5	5	360
MT4	200	150 (S3)	5	5	360
MT5	200	150 (S4)	5	5	360
MT6	200	150 (S5)	5	5	360
MT7	200	150 (S6)	5	5	360
MT8	200	150 (S7)	5	5	360
MT9	200	150(S8)	5	5	360
MT10	200	100 (S1)	5	5	310
MT11	200	200(S1)	5	5	410
MT12	200	150 (HPMCK15M)	5	5	360
MT13	200	150 (Carbopol 974P)	5	5	360
Marketed tablet	200	Hifenac SR (200mg)	-	-	-

S1, S2, S3, S4, S5, S6, S7 and S8 are different synthetic batches of CFSG-g-PSA; HPMC, hydroxypropyl methyl cellulose.

10.5.6. Evaluations of tablets

Different evaluating parameters such as thickness, diameter, weight uniformity, drug content, hardness, friability and disintegration time of tablets of each batch were determined following official compendium (Indian Pharmacopoeia, 2018). Dimension, hardness, friability and disintegration time were measured using digital slide caliper (Digmaticcaliper, CD-P15MW,

Mitutoyo, Japan), hardness tester (Lab India, TH-1050M, India), friabilator (Lab India, FT1020, India), Disintegration test apparatus (Lab India, DT 1000, India), respectively. Friability was calculated using Eq. 17.

$$\%\, Friability = \frac{W_1 - W_2}{W_1} \times 100 \qquad \text{(Eq. 17)}$$

Where, W_1 and $W_2$are the initial and final weight, respectively. 0.1N HCl acid (pH 1.2) was taken as medium for disintegration test and the temperature was maintained at 37ºC.

10.5.7. Ex-vivo mucoadhesion study

Mucoadhesion time: Mucoadhesion time (MT) was determined following the method reported elsewhere [16]. Briefly, a fresh goat-gastric-mucosa was collected from a local slaughter shop in Jadavpur area (Kolkata, India). The mucosa was immersed in cold normal saline (0.9%w/v sodium chloride in water) immediately after excision, taken in the laboratory within 1 h, washed with distilled water followed by normal saline and soaked in simulated gastric fluid (SGF, pH 1.2) for 5 min. It was then fixed to the inner-side-wall of a 250 ml beaker with cyanoacrylate glue. A tablet was soaked in SGF for 1 min and fixed onto the mucosal surface with light force by finger-tip for 1 min. 200 ml of SGF (pH 1.2) was added to the beaker to submerge the tablet. The content of the beaker was maintained at 37ºC and stirred at 50 rpm for a period of 10 h. The time taken by the tablet for complete detachment or erosion form the mucosal surface was noted as mucoadhesion time.

Mucoadhesive strength: Mucoadhesive strengths of the tablets were determined following the method described by Singh et al. [17] and Priyadarshini et al. [18] with little modification. The gastric mucosa of goat was collected and treated as before in determination of mucoadhesion time. It was then equilibrated at 37 ±1°C for30 min in 0.1N HCl acid (pH 1.2) prior to the study. The tablet was soaked in 0.1N HCl acid for 1 min and then lowered onto the mucosal surface under a constant weight of 5 g over a total period of 60 s. The counter weight was gradually increased with 1 g increment until the detachment of the tablet from the mucosal

surface. The counter weight in grams at the point of detachment was noted as mucoadhesive strength (*F*) of the corresponding tablet. The determination was repeated in triplicate for each tablet batch.

10.5.8. In-vitro drug-release study

In vitro drug-release rate of the tablets of different batches was studied in 900 ml 0.1 N HCl with 0.2% SLS (pH 1.2) medium at 37°C with paddle-rotation speed of 50±5 rpm using USP dissolution test apparatus type-II (DS-8000; 6+2; SC/TR, LABINDIA analytical Instruments Pvt. Ltd., Navi Mumbai, India). Cumulative percent drug-release (CPR) at different time-points was measured by withdrawing 5 ml aliquot each time from the release-media, consequent dilution and spectrophotometric analysis at λ_{max} of 275.5 nm (UV–Vis double beam spectrophotometer, Pharmaspec-1900i, Shimadzu, Japan). The volume of the release media was maintained at constant level by adding 5 ml media each time after withdrawal of aliquot. The Study was repeated three times for each batch (n=3).

Release difference (f_1) and similarity factor (f_2) were determined to evaluate the similarity between the release profile of each batch and marketed product (Hifenac SR 200mg, Intas Pharmaceuticals Ltd., India) using Eq. (18) and (19), respectively.

$$f_1 = \frac{\sum_{t=1}^{n} [R_t - T_t]}{\sum R_t} \times 100 \qquad (Eq.18)$$

$$f_2 = 50 \log \log \left[\left\{ 1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} (R_t - T_t)^2 \right\}^{-0.5} \times 100 \right] \qquad (Eq.19)$$

where, R_t, T_t and t are designated as the CPR obtained from marketed product, experimental batch and number of replicates, respectively [19]. The time for 50% and 90% drug-release in an hour ($T_{50\%}$ and $T_{90\%}$, respectively) were calculated from the best fitting kinetic model equation for each batch.

10.5.9. Kinetic modeling of drug release profile

The cumulative % drug-release at different intervals were plotted following the integrated equations of different release-kinetic models (zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas) in order to investigate the best-fitting-model and probable drug-release mechanism. Best-fitting-model equations are also required for calculation of release-rate constant, $T_{50\%}$ and $T_{90\%}$.

Zero order kinetic: $CPR_t = K_0 t$ (CPR_t is the cumulative % drug-release in time, t, K_0 is zero order rate-constant), which demonstrates the drug-release of different modified-release devices such as transdermal matrices, matrix-tablets of low soluble drugs, coated particles, osmotic pumps, etc. [20].

First order kinetic: $log\ CPR_t = log\ Q_0 + K_1 t/2.303$ (Q_0 is the quantity of drug in release-media at time 0), which describes drug-release from porous-matrices loaded with water-soluble drugs in such a way that drug-release is proportional to the residual drug present in the matrix and release-rate lessens with time [21].

Higuchi model: $CPR_t = K_H t^{1/2}$, which explains drug-release as a diffusion process based on fick's law and square-root time dependent found in transdermal systems [22], matrix tablets of water-soluble drugs and matrices having pores and continuous channels [23].

Hixson-Crowell: $(1-f_t)^{1/3} = 1 - K_\beta t$ ($f_t$is the fraction of drug released at time, t), which illustrates drug-release from tablets where tablet-dimension diminishes proportionally but retains its initial geometric shape [24].

Korsmeyer-Peppas: $\frac{M_t}{M_\infty} = at^n$ (M_t/M_∞ is fractional drug-release at time, t, a is the rate-constant demonstrating structural as well as geometric features of the dosage forms, n is the release-exponent explaining the drug-release mechanism) [25].

10.5.10. Accelerated stability study

The tablets of batch with best release similarity (MT10, 100 no.) were packed in a glass bottle and kept in a stability chamber (Thermolab scientific equipment, GMP model 600Lt, Mumbai, India) for 90 days, where 40°C ± 0.5°C and 75 ± 5% relative humidity were maintained. At the end, the aged tablets were inspected for any change in appearance and evaluated for thickness, diameter, weight uniformity, drug content, hardness, friability, disintegration time, mucoadhesion and in vitro drug release. FTIR and DSC studies were also done to investigate whether any drug-additives interaction developed or not during storage [26].

10.6. References:

[1] I. Pharmacopoeia, The Indian Pharmacopoeia Commission Ghaziabad Vol, II, 2010.

[2] D. Jain, A. Sodani, S. Ray, P. Ghosh, G. Nandi, Formulation of Extended-Release Beads of Lamotrigine Based on Alginate and Cassia fistula Seed Gum by QbD Approach, Current drug delivery 17(5) (2020) 422-437.

[3] U.V. Deore, H.S. Mahajan, Isolation and characterization of natural polysaccharide from Cassia Obtustifolia seed mucilage as film forming material for drug delivery, International journal of biological macromolecules 115 (2018) 1071-1078.

[4] T. Kaewmanee, L. Bagnasco, S. Benjakul, S. Lanteri, C.F. Morelli, G. Speranza, M.E. Cosulich, Characterisation of mucilages extracted from seven Italian cultivars of flax, Food Chemistry 148 (2014) 60-69.

[5] S. Karimi, B. Ghanbarzadeh, L. Roufegarinejad, P.M. Falcone, Polysaccharide extracted from Althaea officinalis L. root: New studies of structural, rheological and antioxidant properties, Carbohydrate Research 510 (2021) 108438.

[6] M.C. García, L.A. Trujillo, J.A. Carmona, J. Muñoz, M.C. Alfaro, Flow, dynamic viscoelastic and creep properties of a biological polymer produced by Sphingomonas sp. as affected by concentration, International journal of biological macromolecules 125 (2019) 1242-1247.

[7] G. Nandi, A. Changder, L.K. Ghosh, Graft-copolymer of polyacrylamide-tamarind seed gum: Synthesis, characterization and evaluation of flocculating potential in peroral paracetamol suspension, Carbohydrate polymers 215 (2019) 213-225.

[8] G. Nandi, P. Patra, R. Priyadarshini, S. Kaity, L.K. Ghosh, Synthesis, characterization and evaluation of methacrylamide grafted gellan as sustained release tablet matrix, International journal of biological macromolecules 72 (2015) 965-974.

[9] S. Patra, N.N. Bala, G. Nandi, Synthesis, characterization and fabrication of sodium carboxymethyl-okra-gum-grafted-polymethacrylamide into sustained release tablet matrix, International Journal of Biological Macromolecules 164 (2020) 3885-3900.

[10] K. Gowthamarajan, G.K.P. Kumar, N.B. Gaikwad, B. Suresh, Preliminary study of Anacardium occidentale gum as binder in formulation of paracetamol tablets, Carbohydrate Polymers 83(2) (2011) 506-511.

[11] H.H. Hausner, Friction conditions in a mass of metal powder, Polytechnic Inst. of Brooklyn. Univ. of California, Los Angeles, 1967.

[12] O. Odeku, O. Itiola, Evaluation of the effects of khaya gum on the mechanical and release properties of paracetamol tablets, Drug Development and industrial pharmacy 29(3) (2003) 311-320.

[13] G. Nandi, A.K. Nandi, N.S. Khan, S. Pal, S. Dey, Tamarind seed gum-hydrolyzed polymethacrylamide-g–gellan beads for extended release of diclofenac sodium using 32 full factorial design, International journal of biological macromolecules 114 (2018) 214-225.

[14] D. Sarkar, G. Nandi, A. Changder, P. Hudati, S. Sarkar, L.K. Ghosh, Sustained release gastroretentive tablet of metformin hydrochloride based on poly (acrylic acid)-grafted-gellan, International journal of biological macromolecules 96 (2017) 137-148.

[15] M.R. Marques, R. Loebenberg, M. Almukainzi, Simulated biological fluids with possible application in dissolution testing, Dissolution Technol 18(3) (2011) 15-28.

[16] S. Malik, A. Kumar, M. Ahuja, Synthesis of gum kondagogu-g-poly (N-vinyl-2-pyrrolidone) and its evaluation as a mucoadhesive polymer, International journal of biological macromolecules 51(5) (2012) 756-762.

[17] B. Singh, S.K. Chakkal, N. Ahuja, Formulation and optimization of controlled release mucoadhesive tablets of atenolol using response surface methodology, AAPS PharmSciTech 7(1) (2006) E19-E28.

[18] R. Priyadarshini, G. Nandi, A. Changder, S. Chowdhury, S. Chakraborty, L.K. Ghosh, Gastroretentive extended release of metformin from methacrylamide-g-gellan and tamarind seed gum composite matrix, Carbohydrate polymers 137 (2016) 100-110.

[19] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, European journal of pharmaceutical sciences 13(2) (2001) 123-133.

[20] C.G. Varelas, D.G. Dixon, C.A. Steiner, Zero-order release from biphasic polymer hydrogels, Journal of controlled release 34(3) (1995) 185-192.

[21] N. Mulye, S. Turco, A simple model based on first order kinetics to explain release of highly water soluble drugs from porous dicalcium phosphate dihydrate matrices, Drug development and industrial pharmacy 21(8) (1995) 943-953.

[22] P. Costa, D. Ferreira, J. Sousa Lobo, Nitroglicerina em sistemas de libertação transdérmicadeterminação da velocidade de libertação, Rev. Port. Farm 46 (1996) 4-8.

[23] S.J. Desai, P. Singh, A.P. Simonelli, W.I. Higuchi, Investigation of factors influencing release of solid drug dispersed in inert matrices III. Quantitative studies involving the polyethylene plastic matrix, Journal of pharmaceutical sciences 55(11) (1966) 1230-1234.

[24] P. Niebergall, G. Milosovich, J. Goyan, Dissolution Rate Studies II: Dissolution of Particles Under Conditions of Rapid Agitation, Journal of pharmaceutical sciences 52(3) (1963) 236-241.

[25] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, Mechanisms of solute release from porous hydrophilic polymers, International journal of pharmaceutics 15(1) (1983) 25-35.

[26] S. Das, A. Ghosh, A. Changder, G. Nandi, L.K. Ghosh, Quality-by-design approach for development of sustained-release multiple-unit beads of lamotrigine based on ion-cross-linked composite of pectin and okra mucilage: An in vitro appraisal, International Journal of Biological Macromolecules 163 (2020) 842-853.

CHAPTER - XI

RESULTS AND DISCUSSION

11. Results and Discussion

PART I

11.1. Evaluation of *Cassia fistula* seed gum (CFSG) as tablet binder

11.1.1. Determination of λ_{max} and preparation of standard calibration curve of diclofenac sodium in phosphate buffer pH 7.5

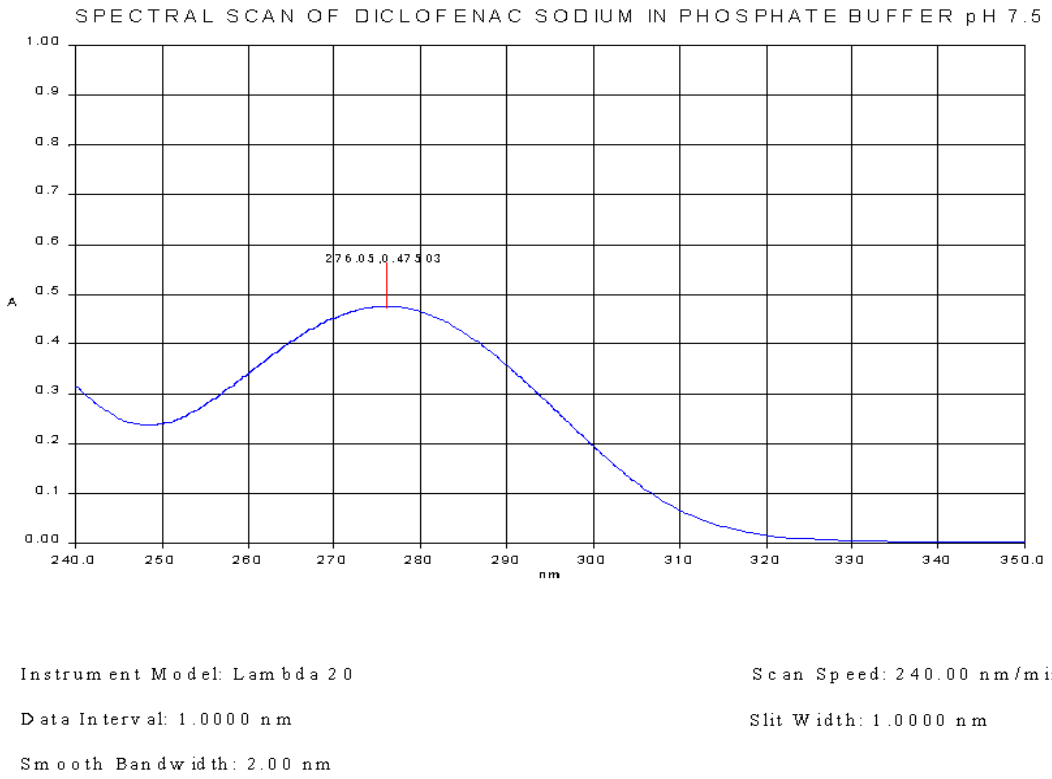


Figure 11.1. Wavelength scanning of Diclofenac sodium

Table 11.1. Standard curve data of Diclofenac sodium in phosphate buffer pH 7.5

Sl. No.	Concentration (µg/ml)	Absorbance (Average)	Equation	R^2 Value
1	8	0.258	y = 0.0262x + 0.05226	0.9993
2	12	0.369		
3	16	0.475		
4	20	0.581		
5	24	0.676		

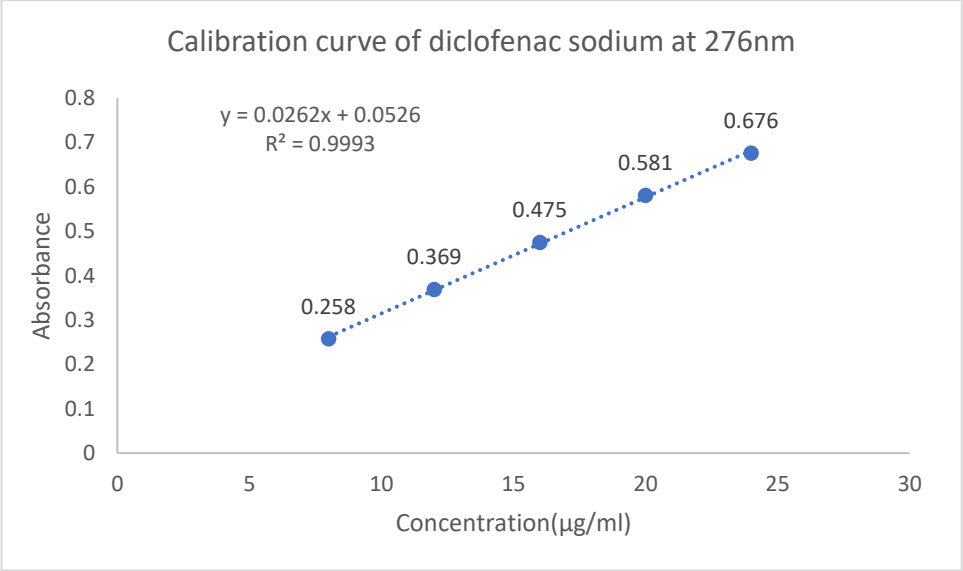


Figure 11.2: Standard Curve of Diclofenac sodium in Phosphate buffer medium at pH 7.5

Discussion:

The λ_{max} was found to be 276 nm in concordance with the reference standard. The UV spectrum was shown in **Figure 11.1**. Graph of absorbance versus concentration was plotted and found to be linear over the range of 8 to 24 µg/ml, (**Figure 11.2)** indicating its compliance with Beer's Lambert's Law. Standard curve equation was found be y = 0.0262x + 0.05226 and Correlation coefficient: R^2 =0.9993

11.1.2. Characterization of CFSG

11.1.2.1. Phytochemical investigation

The results of phytochemical screening are presented in **Table 11.2**. The study showed presence of only carbohydrate in the extracted gum. All other tests were negative.

Table 11.2. Phytochemical screening tests on isolated CFSG

Screening tests	Name of tests	Results
Test for carbohydrate	Molisch's test	positive
Test for starch	Iodine test	Negative
Test for mucilage	Ruthenium red test	Negative
Test for glycosides	Legal's test Keller-Killiani test Borntrager's test	Negative
Test for alkaloids	Mayer's test Dragendroff's test	Negative
Test for steroids and sterols	Libermann-Burchard's test	Negative
Test for tannins	Ferric chloride and lead acetate test	Negative
Test for proteins and amino acids	Ninhydrin test	Negative

11.1.2.2. Polysaccharide content

Total polysaccharide content was determined by phenol method and found to be 76.4% (**Table 11.3**).

Table 11.3. Different physicochemical characteristics of isolated CFSG

Physicochemical characteristics	Values
Colour	Brownish
Odour	Odourless
Taste	Bland
Solubility in water	Soluble in water at room temperature
Polysaccharide content	76.4%
Monosaccharides present and their ratio	Galactose and mannose (1.11:2)
Elemental analysis	C (39.19%), H (6.23%) and O (52.13%)
Molecular weight	1.93 (± 0.04) × 10^5 Da (n =3)
Zeta potential	-0.06±0.002 mV (n = 3)
Viscosity (1% w/v)	1655 cp
pH (1% w/v)	6.01
Surface tension (1% w/v)	62.7±1.27 mN/m

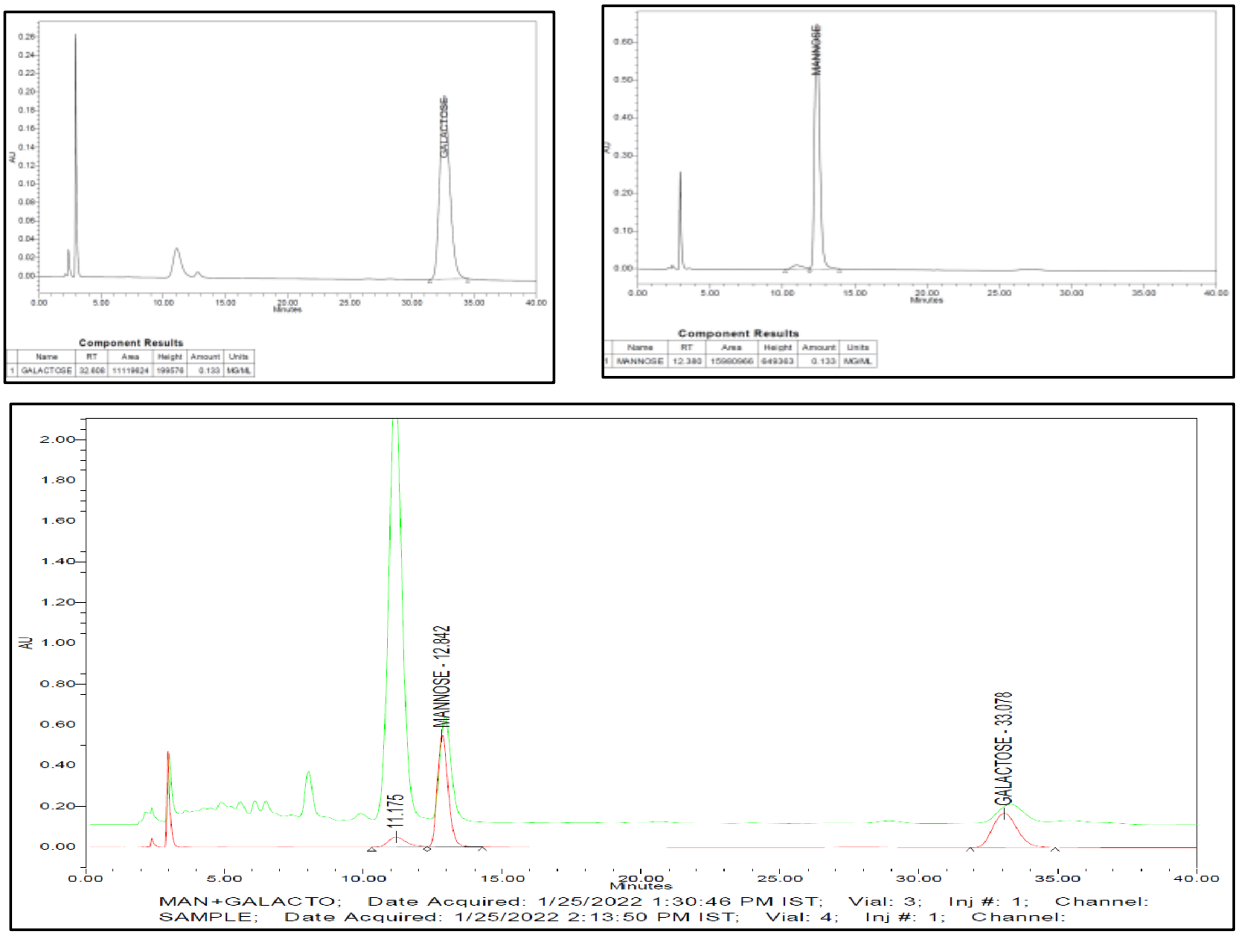


Fig 11.3. HPLC chromatograms of mixture of reference galactose, mannose and hydrolysed CFSG.

11.1.2.3. Analysis of monosaccharide composition by HPLC

Figure 11.3, showed that the chromatograms obtained from the samples were found to match with that obtained from reference galactose and mannose individually and their mixture. This indicated the presence of galactose and mannose. The ratio galactose: mannose was found to be 1.11:2 from the comparison between corresponding AUCs.

11.1.2.4. Elemental analysis (C, H, O, N)

Native CFSG was found to contain Carbon (39.19%), Hydrogen (6.23%) and Oxygen (52.13%). Nitrogen content was not observed in the analysis.

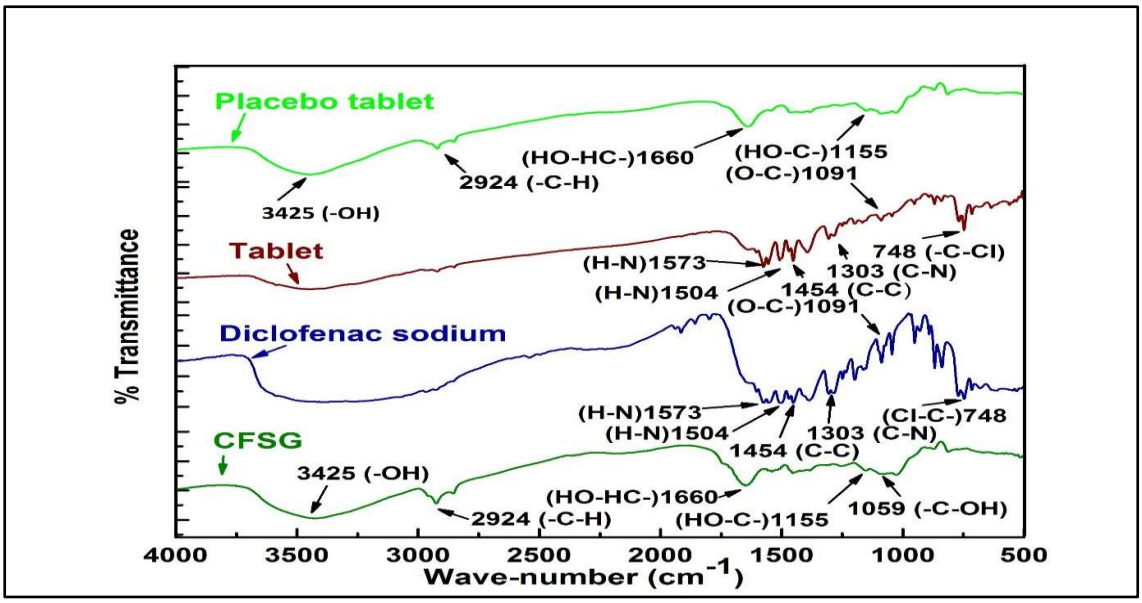


Fig 11.4. FT-IR spectra of native CFSG, pristine diclofenac sodium, tablet and placebo-tablet.

11.1.2.5. FTIR study

Figure 11.4 portrays the FTIR spectra of pristine CFSG, diclofenac sodium, tablet composed of CFSG and diclofenac sodium and placebo-tablet. Different characteristic peaks at 3425 cm^{-1}, 2924 cm^{-1}, 1660 cm^{-1}, 1155 cm^{-1}, 1059 cm^{-1} and 812 cm^{-1} observed in the spectrum of native CFSG may be assigned to –OH group stretching, symmetric C-H stretching, –CH-OH stretching, stretching vibration of –C-OH of pyranose and mannose ring, and anomeric deformation of α-D-galactopyranose, respectively [1] [1, 2]. The spectrum of pure diclofenac sodium represents different distinctive peaks at 1573 cm^{-1}, 1454 cm^{-1}, 1303 cm^{-1}, 1091 cm^{-1} and 748 cm^{-1} attributed to bending vibration of N-H, C-C stretching in aromatic ring, C-N stretching vibration, ester –C-O group and stretching vibration of –C-Cl, respectively. All these distinguishing peaks of pristine drug were also observed in the spectrum of tablet powder but not in that of drug-free placebo tablet, which ratifies the absence of drug-polymer or drug-additives incompatibility. The characteristic peaks of native CFSG were found in the spectrum of placebo tablet.

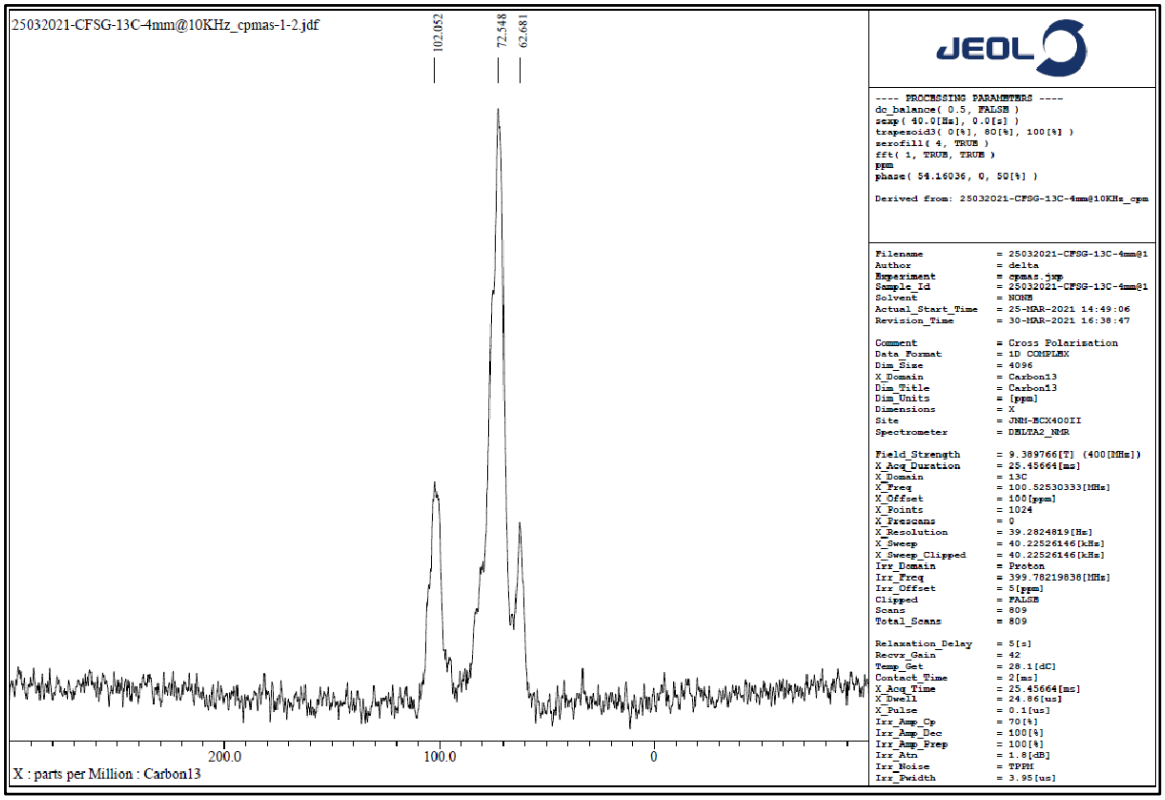


Figure 11.5. Solid-state ^{13}C NMR spectrum obtained from native CFSG.

11.1.2.6. Solid-state ^{13}C NMR study

Figure 11.5 depicts ^{13}C NMR spectra recorded from native CFSG in solid-state. Three discrete characteristic absorption peaks at δ = 62.8 ppm, δ = 72.7 ppm and δ = 101.8 ppm were observed, which could be assigned to the carbon atom of –CH_2OH of galactose unit [3], carbon-atom of the glycosidic-linkage (>C-O-C<) between sugar moieties, and the anomeric carbon atoms of β-D-mannose, respectively [1]. A good signal to noise ratio was found in all peaks.

11.1.2.7. Molecular weight, zeta potential, Viscosity, pH and surface tension

Molecular weight and zeta potential were found to be 1.93 (± 0.04) × 10^5 Da and -0.06±0.002 mV, respectively (**Table 11.3**). Negligible negative zeta potential value indicates non-ionic nature of CFSG. The viscosity, pH and surface tension of 1%w/v aqueous solution of native gum was found to be 1655 cp, 6.01 and 62.7±1.27 mN/m, respectively (Table 1). pH value

near to 7 substantiates its neutral nature which was observed in zeta potential value also indicates its suitability as oral drug delivery excipient. Reduced value of surface tension than purified water (solvent) indicates the surface tension lowering or wetting effect of the native gum, which promotes better penetration to inter-particular voids of drug powder and spreading over drug-particles during wet-massing in granulation step.

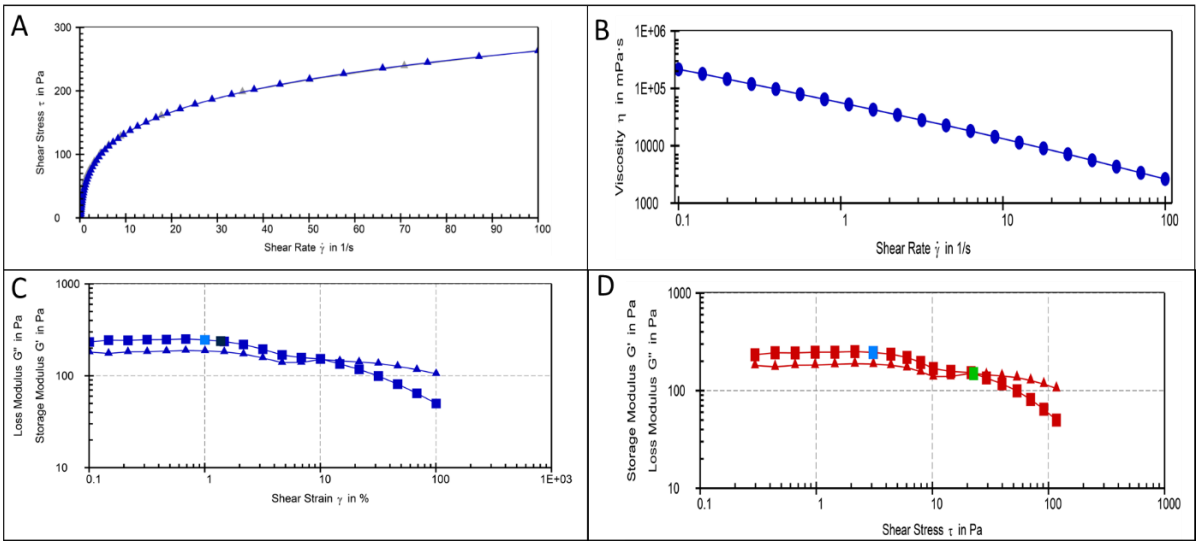


Figure 11.6 (A) Shear stress versus shear rate rheogram of CFSG aqueous dispersion, (B) Viscosity versus shear rate curve, (C) and (D) loss and storage modulus against shear strain and shear stress, respectively.

11.1.2.8. Rheology study

The rheograms and amplitude sweep curves are presented in **Figure 11.6**. Fig **11.6A** showed non-linear increase in rate of shear with increase in shear stress. The slope (1/η) at different points along the curve were found to increase gradually with increasing rate of shear, which indicated decrease in viscosity with increase in rate of shear. Further, the curve was also found to begin at origin without any yield value. Overall, the consistency curve resembled non-Newtonian pseudoplastic or shear-thinning flow behavior, which was also substantiated by decreasing viscosity with increasing rate of shear found in the curve **(Fig. 11.6B)**. This

rheological feature of CFSG aqueous dispersion might be due to shearing action on the linear and long-chain molecules of CFSG. The long-chain molecules of linear polysaccharides are usually found to remain in coiled form in dispersion resulting in higher flow-resistance and increasing shearing stress makes the long axes of disarranged molecules align towards the flow-direction. This change in molecular orientation decreases internal resistance and permits a higher rate of shear at each consecutive shearing stress. Furthermore, some amount of water may be released resulting in drop in concentration and subsequently apparent viscosity [4]. Viscoelastic nature of aqueous dispersion of CFSG was described by storage modulus (*G'*) and loss modulus (*G''*) using amplitude sweep approach [5]. Linear viscoelastic region (LVER) was obtained from the amplitude sweep curves (**Fig. 11.6C** and **11.6D**). The LVER was found to be at 10.73% strain and shear stress (τ) of 22.7 Pa with a crossover point at 149.6 Pa. The storage modulus was found to be slightly higher than loss modulus within LVER and the difference was very low, which indicated shear-thinning nature of CFSG dispersion. The shear-thinning property of CFSG may increase the penetration of CFSG into the pores of the drug particles and better spread over the surface of the drug particles due to application of shear during wet-massing in wet granulation technique and impart significant binding capacity.

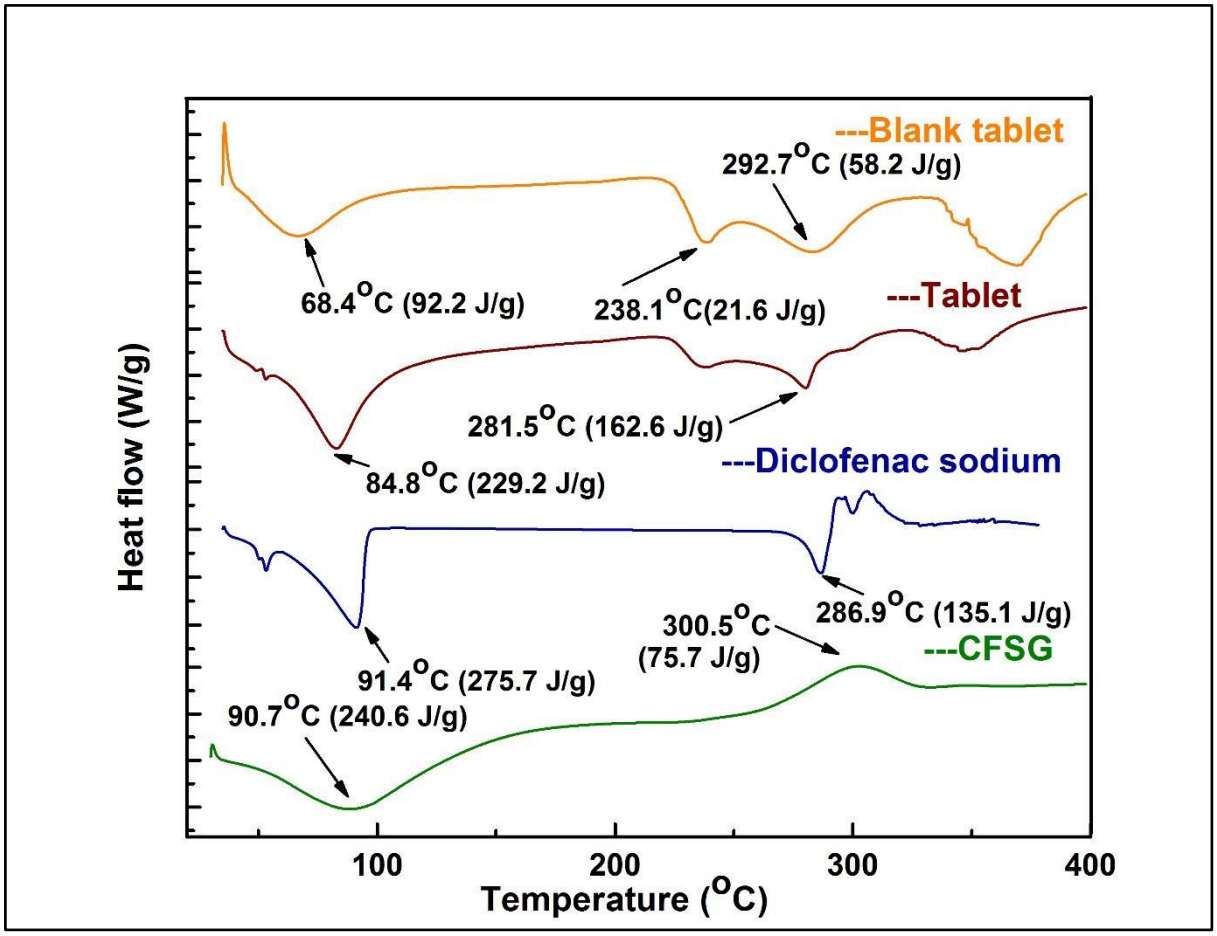


Figure 11.7 DSC thermograms of native CFSG, diclofenac sodium, tablet and blank formulation.

11.1.2.9. DSC, TGA, and DTA study

Figure 11.7 depicts the DSC-thermogram obtained from native CFSG. The DSC-thermogram shows an endothermic peak at 90.7°C (enthalpy 240.6 J/g), which might be due to loss of moisture present. Further, 7.8% weight-drop in the range 35°C to 200°C in TGA and endothermic peak at 70.3°C in DTA substantiate this moisture-loss. DSC, TGA and DTA curves also exhibit an exothermic peak at 300.5°C (enthalpy of 75.7 J/g), 57.6% weight-drop in the range 200°C - 350°C and exothermic peak at 301.8°C (enthalpy 323.2 J/g), respectively, which designate polysaccharide-decomposition into methane, carbon mono-oxide, and water [6].

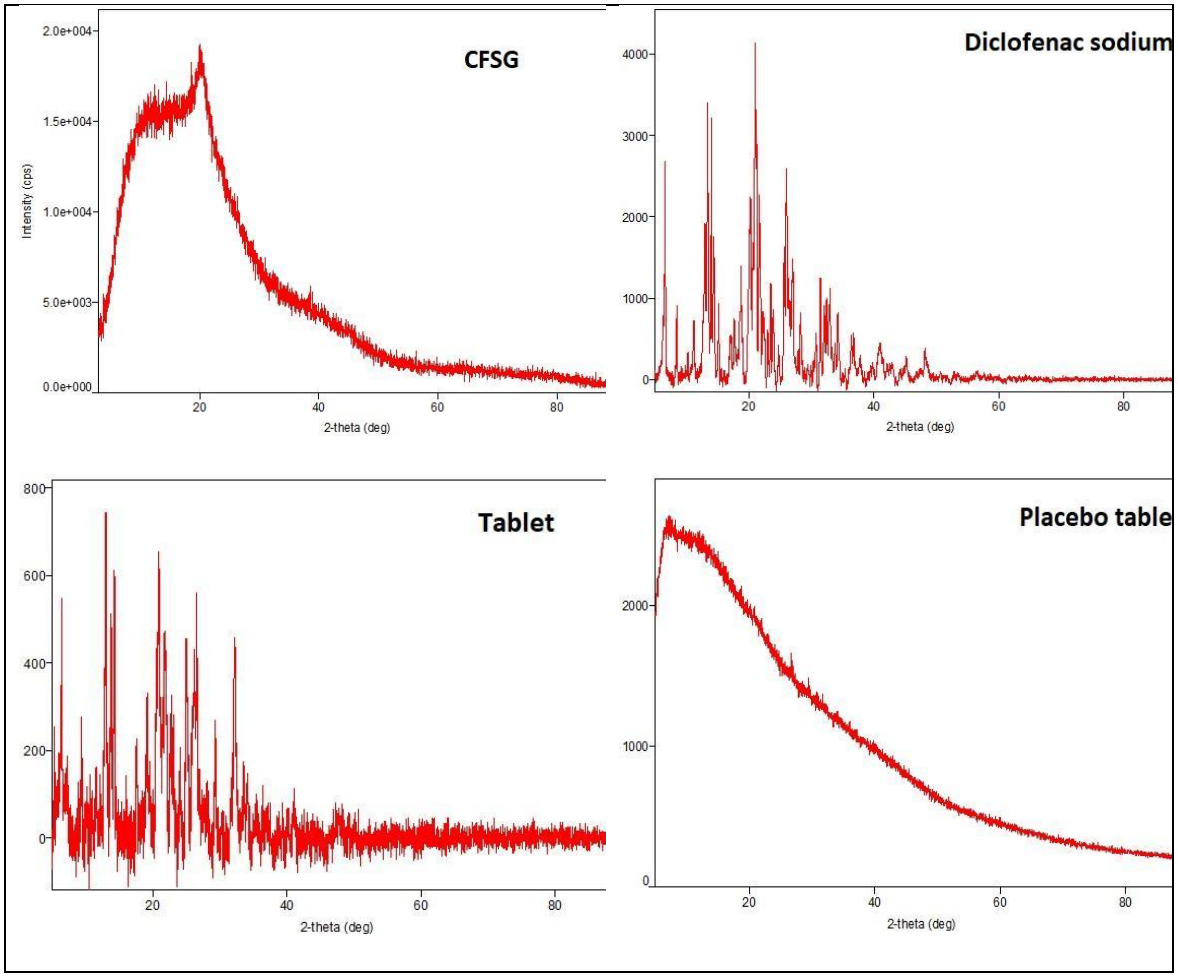


Figure 11.8. Powder-XRD diffractograms of native CFSG, pristine diclofenac sodium, tablet formulation and placebo-tablet.

11.1.2.10. Powder X-ray Diffraction (PXRD) study

Figure 11.8 portrays the PXRD diffractogram of native CFSG with no such intense peak except at 2θ of 14.5° and 20.1°, which implies the amorphous nature of the crude CFSG powder [7].

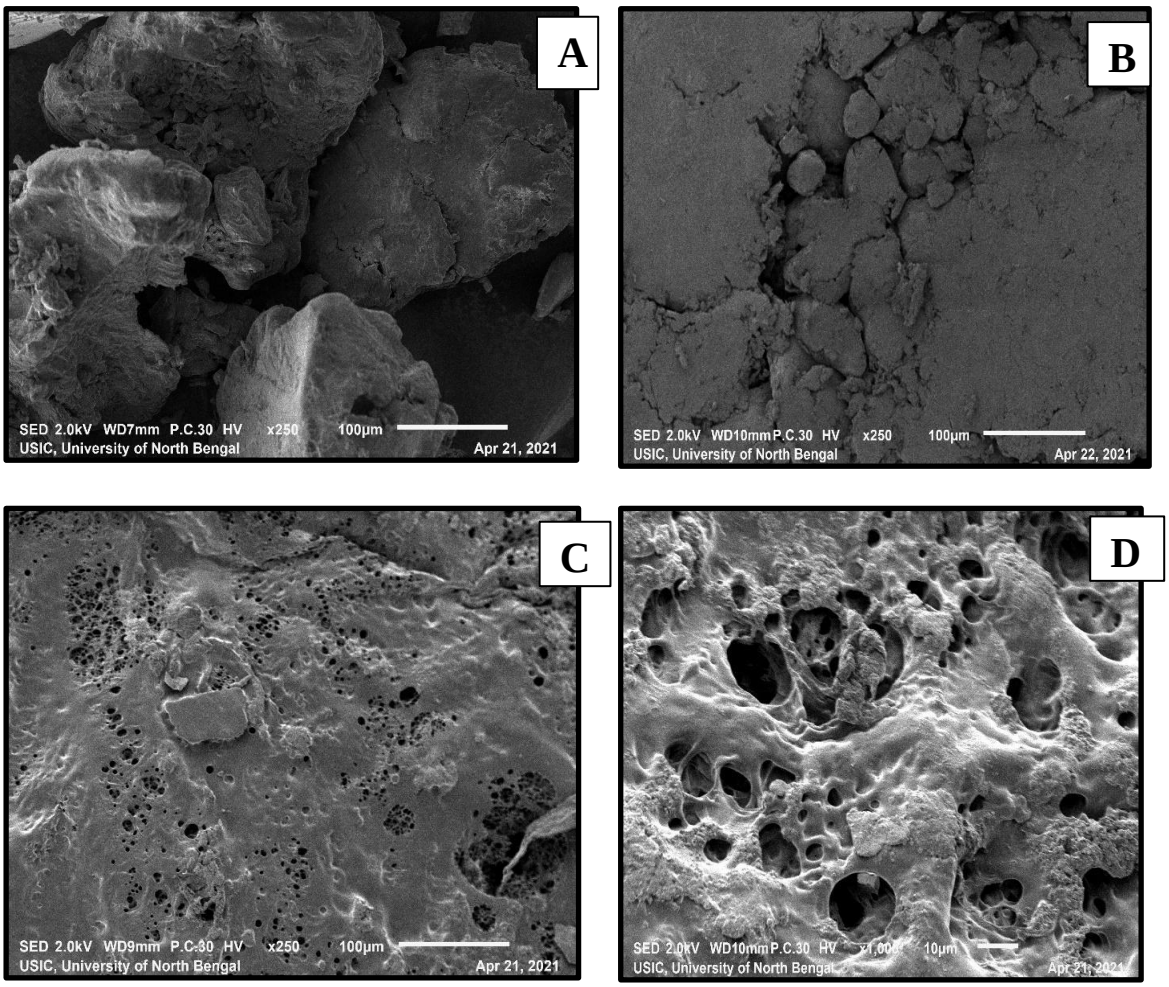


Figure 11.9 SEM micrographs of CFSG particles (A), tablet-surface (B), and xerogel surface obtained from hydrated tablet (C and D).

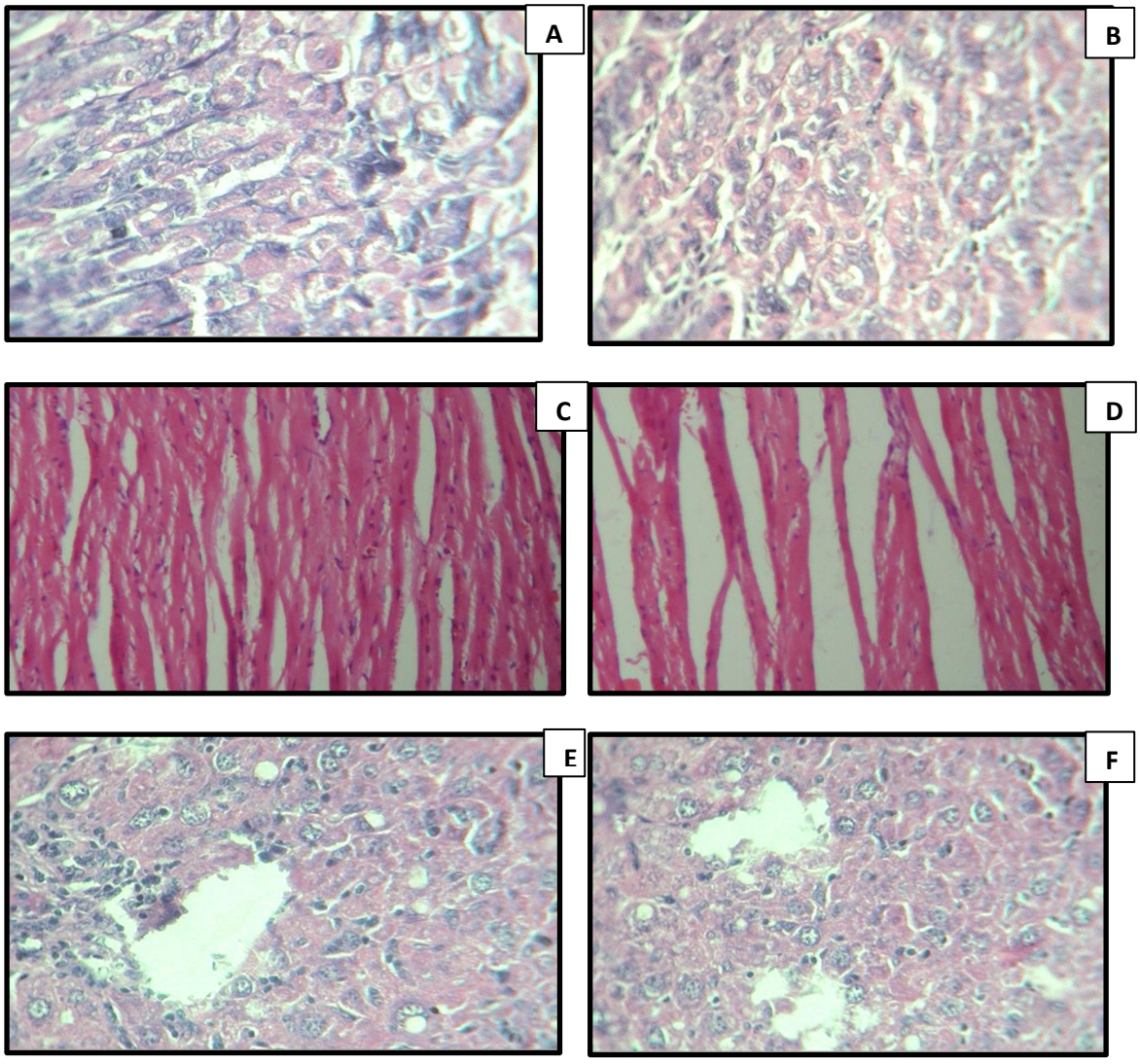
11.1.2.11. Scanning Electron Microscopy

SEM micrograph of CFSG dry powder depicted in **Figure 11.9A** shows discrete polydisperse particles having asymmetric pebble-like appearance with irregular shape and size. Presence of cracks and pores on the surface are observed. The overall texture of the surface shows a rough and lumpy appearance and evidence of brittle fracture upon size-reduction by grinding.

Table 11.4. Mortality rate of animals after a single dose of 2000 mg/kg body weight.

Observation time points	Mortality				
	Animal-1	Animal-2	Animal-3	Animal-4	Animal-5
0.5 h	0	0	0	0	0
3 h	0	0	0	0	0
6 h	0	0	0	0	0
12 h	0	0	0	0	0
3rd day	0	0	0	0	0
6th day	0	0	0	0	0
10th day	0	0	0	0	0
14th day	0	0	0	0	0

'0', survival and 'X', death.



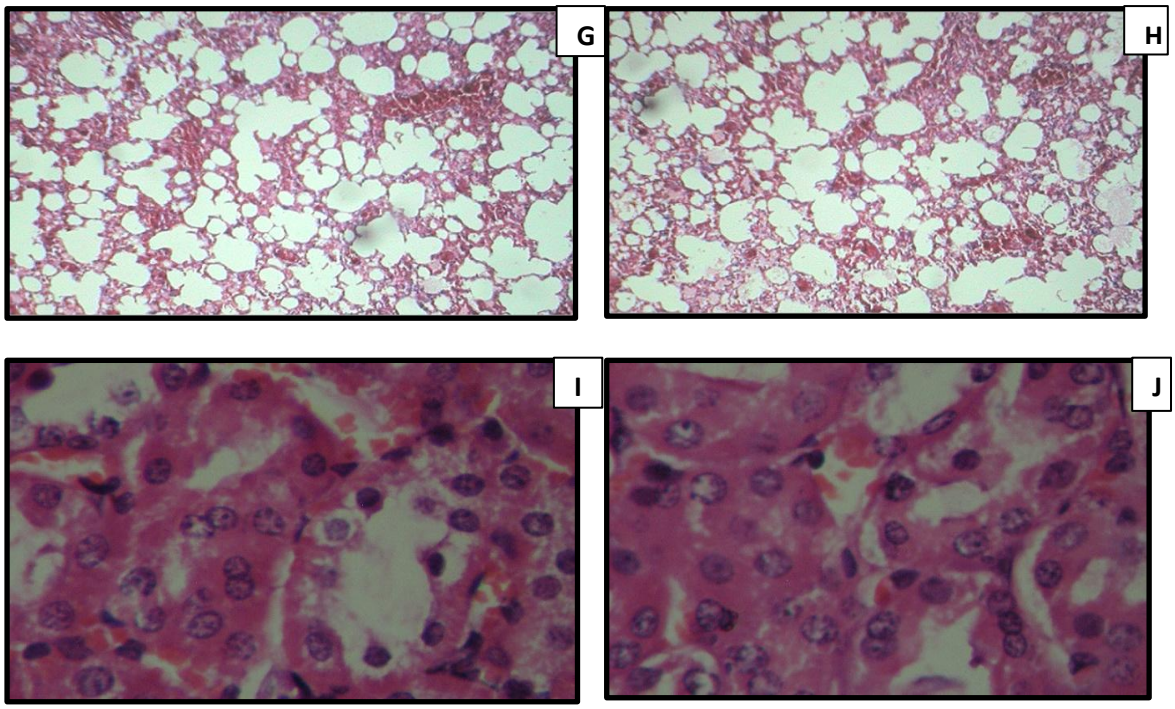


Figure 11.10. Microscopic views (40x) of the cross-sections (A) stomach-control (B) stomach-test (C) heart-control (D) heart-test (E) liver-control (F) liver-test (G) lung-control (H) lung-test (I) kidney-control (J) kidney-test.

11.1.2.12. Acute oral toxicity and histological study

Mortality rate in acute oral toxicity study and photomicrographs obtained from histological study are shown in **Table 11.4** and **Figure 11.10**, respectively. Zero mortality found in the study at a single oral dose of 2000 mg/kg body weight, designates that LD_{50} value (Lethal Dose for 50% population) would be greater than 2000 mg/kg body weight and therefore, CFSG may be considered as nontoxic (Category-5) as per OECD guideline (OECD guideline for the test of chemicals" 425, adopted “17 December 2001” Annexure-4). No such hypersensitive or allergic reaction, redness in buccal mucosa or eyes, and change in body weight, intake of food, fur colour or density were observed. Stool colour and form were also found to retain in the study period. No animal was found to suffer from any type of drowsiness, convulsion or over-salivation. In histological study, the morphology of the

stomach section including gastric glands, parietal cells, lamina propria and chief zymogenic cells were found normal and almost similar both in control and test (**Figure 11.10. A and B**). **Figure 11.10 (C) and (D)** both showed normal morphology of the heart-sections (control and test) including branched cardiac fibre, cross striations, intercalated discs, perinuclear sarcoplasm, central nucleus, binucleate fiber, endomysium and connective tissue. Both sections **(Figure 11.10. E and F**) of control and test liver were found to portray normal morphology of hepatocyte and endothelial cells, sinusoids, and Kupffer cells. The sections obtained from both control and test lung (**Figure11.10. G and H**) were also found to show normal morphology including alveoli, alveolar sac and duct, alveolar macrophages, alveolar septa, pneumocytes, and alveolar cells type I and II. Both the sections of the test and control kidney (**Figure11.10. I and J**) depicted normal features of bowman capsules, glomerulus, blood vessels, podocytes, macula densa, juxtaglomerular cells and capsular spaces. Overall, no significant changes in histology were found due to oral administration of CFSG to the animals, which corroborated the physiological compatibility of CFSG.

11.1.3. Preformulation studies

In pre-formulation study, drug-excipient compatibility was checked by FTIR, DSC and PXRD studies. FTIR spectra of pure diclofenac sodium, drug-loaded tablet and placebo-tablet were presented in **Figure 11.4**. The spectrum of diclofenac sodium depicted different characteristic peaks at 1573 cm^{-1}, 1504 cm^{-1}, 1454 cm^{-1}, 1303cm^{-1}, 1091 cm^{-1}, and 748 cm^{-1}, which might be attributed to N-H bending vibration, -C-C stretching in aromatic ring, C-N stretching vibration, ester –C-O group, and –C-Cl stretching vibration, respectively. Retention of all the characteristic peaks of pristine diclofenac sodium in the FTIR-spectrum obtained from tablet formulation and absence of such peaks in the spectrum of drug free placebo tablet indicated the physicochemical compatibility of the drug with CFSG. DSC thermograms of pristine diclofenac sodium, its tablet formulation and drug free blank tablet

are shown in **Figure 11.7**. The curve obtained from pure drug showed two characteristic endothermic peaks at 91.4°C with enthalpy of 275.7 J/g (66.9°C to 92.1°C) and 286.9°C with enthalpy of 135.1 J/g (277.8°C to 288.3°C), which indicated initial moisture loss from the drug-powder and melting of the drug-crystal, respectively. The narrow range of later endothermic peak suggested a narrow melting range with sharp melting point at 286.9°C [8] and crystalline nature of pristine diclofenac sodium. Similarly, two prominent endothermic peaks shown in a thermogram obtained from a tablet at 84.8°C with enthalpy of 229.2 J/g and 281.5°C with enthalpy of 162.6 J/g indicated moisture loss and melting, respectively. The little drop in melting point of the drug might be due to presence of additives in the formulation. No endothermic peak was found in the melting range of diclofenac sodium in the DSC-thermogram of blank tablet. The X-ray diffratogram of pristine diclofenac sodium depicted in **Figure 11.8** exhibited different characteristic peaks at 2θ of 3.1°, 12.8°, 14°, 17°, 18°, 20.3°, 21.2°, 22.5°, 24.7°, 26.5°, 27.8°, 28.4°, 32.1°, 32.8°, 36.6° and 37.4°, which indicated the crystalline nature of pure diclofenac sodium [9]. Retention of almost all peaks in the diffractogram of the tablet might be attributed to the presence of diclofenac sodium in crystalline form in the tablet-matrix. Presence of some noise in the tablet diffractogram might be due to presence of native CFSG. Overall, retention of almost all features of pristine diclofenac found in FTIR, DSC and XRD study in tablet formulation indicated required physicochemical compatibility between drug and CFSG and other excipients.

Table 11.5. Formula of each tablet of diclofenac sodium composed of CFSG, gum acacia or PVP-K30.

Batch no.	Diclofenac sodium (mg)	Lactose (mg)	CFSG (mg)	Gum acacia (mg)	PVP-K30 (mg)	Magnesium stearate (mg)	Purified talc (mg)	Total weight (mg)
B1	100	89	2.5% (5)	-	-	4	2	200
B2	100	84	5% (10)	-	-	4	2	200
B3	100	79	7.5% (15)	-	-	4	2	200
B4	100	74	10% (20)	-	-	4	2	200
B5	100	89	-	2.5% (5)	-	4	2	200
B6	100	84	-	5% (10)	-	4	2	200
B7	100	79	-	7.5% (15)	-	4	2	200
B8	100	74	-	10% (20)	-	4	2	200
B9	100	89	-	-	2.5% (5)	4	2	200
B10	100	84	-	-	5% (10)	4	2	200
B11	100	79	-	-	7.5% (15)	4	2	200
B12	100	74	-	-	10% (20)	4	2	200

CFSG: *Cassia fistula* seed gum; PVP: polyvinylpyrrolidone.

Table 11.6. Evaluation of diclofenac sodium loaded granules.

Name of binder	Binder conc. (Batch no)	Granule-size (µm)	Bulk density (g/ml)	True density (g/ml)	Total porosity (%)	Angle of Repose (°)	Hausner ratio	Carr's index (%)
CFSG	2.5% (B1)	875 ± 0.07	0.38±0.023	0.148±0.016	61.3±1.34	21.2±0.04	1.25±0.016	12.7±0.03
	5% (B2)	890 ± 0.06	0.39±0.020	0.146±0.019	64.1±0.76	22.6±0.07	1.29±0.012	14.5±0.02
	7.5% (B3)	955 ± 0.06	0.48±0.021	0.152±0.022	62.0±1.85	21.5±0.08	1.26±0.017	14.7±0.02
	10% (B4)	985 ± 0.05	0.47±0.018	0.141±0.011	66.1±2.52	26.7±0.12	1.27±0.018	13.1±0.01
Gum acacia	2.5% (B5)	880 ± 0.06	0.40±0.022	0.135±0.015	66.5±1.55	24.1±0.04	1.20±0.011	12.9±0.04
	5% (B6)	886 ± 0.08	0.37±0.014	0.137±0.020	69.7±2.15	28.4±0.06	1.17±0.021	10.4±0.07
	7.5% (B7)	960 ± 0.08	0.45±0.016	0.134±0.012	68.0±1.89	24.9±0.05	1.19±0.015	11.3±0.05
	10% (B8)	990 ± 0.04	0.46±0.024	0.141±0.018	72.7±3.21	29.7±0.08	1.14±0.010	15.6±0.03
PVP K-30	2.5% (B9)	875 ± 0.06	0.35±0.013	0.105±0.013	68.6±2.50	22.5±0.06	1.16±0.012	12.8±0.06
	5% (B10)	885 ± 0.08	0.40±0.022	0.107±0.010	70.1±3.02	27.5±0.14	1.11±0.010	9.7±0.08
	7.5% (B11)	955 ± 0.08	0.44±0.018	0.102±0.018	74.3±1.94	21.8±0.07	1.14±0.016	14.2±0.03
	10% (B12)	986 ± 0.04	0.47±0.012	0.104±0.011	73.8±1.32	24.4±0.08	1.12±0.017	11.5±0.04

11.1.4. Micromeritic and derived properties of the granules

Total twelve batches of granules were prepared with the compositions shown in **Table 11.5** by wet granulation technique, and evaluated for bulk density, true density, total porosity, angle of repose, Hausner ratio and Carr's index. The results of granule-evaluation are presented in **Table 11.6**. The average size of the granules composed of CFSG was within the range of 875 - 985µm, which was found to be almost similar with that of gum acacia and PVP K-30. Granule size was observed to increase minutely with increasing binder concentration in all cases, which may be due to increase in overall bulk-volume of the granular mass with increase in binder-concentration. The result of micromeritic study exhibited appreciable uniformity in granule-size which might promote good weight and content uniformity in the final tablet. Bulk density of CFSG-granules was within the range of 0.38 to 0.47 g/ml and no significant change in bulk-density was found with increasing binder-concentration and also binder-type. True-density is a qualitative parameter of a material and it was found to be within the range of 0.141 – 0.152 g/ml in case of CFSG-granules. True density of gum acacia granules was almost similar with CFSG, which may be due to their similar bio-polysaccharide nature. Granules of PVP K-30 (synthetic binder) showed slightly reduced true-density. Total porosity in CFSG granules was within the range of 61.3 – 66.1%, whereas granules obtained from gum acacia and PVP K-30 showed slightly higher porosity. This feature indicates comparatively better penetration and occupancy of voids by CFSG than that of gum acacia and PVP K-30. The granules should have optimum flow property for smooth and continuous transfer from hopper to die-cavity and proper and reproducible die-filling. Angle of repose, Hausner ratio and Carr's index were determined to assess the flowability of granules qualitatively. As per literature, angle of repose less than 30°, Hausner ratio less than 1.30 and Carr's index between 5 and 15% indicate free-flowing property of granules [10]. The values of angle of repose, Hausner ratio and Carr's index

were found to be 21.2 – 26.7°, 1.25 – 1.29, and 12.7 – 14.7%, respectively, which indicated the free-flowing property of CFSG granules. Similar results were also found in flowability assessment of granules composed of gum acacia and PVP K-30. Further, it was also found that the optimum granule-properties required for good tableting were obtained even at the minimum concentration of 2.5% CFSG as binder, which are comparable to that showed by 7.5% and 10% of gum acacia and PVP K-30 (batch B7, B8, B11 and B12).

Table 11.7. Evaluation of different tablet properties.

Evaluation Parameters	CFSG				Gum acacia				PVP K-30			
	2.5% (B1)	**5% (B2)**	**7.5% (B3)**	**10% (B4)**	**2.5% (B5)**	**5% (B6)**	**7.5% (B7)**	**10% (B8)**	**2.5% (B9)**	**5% (B10)**	**7.5% (B11)**	**10% (B12)**
Tablet-volume (cm^3) (n=10)	0.208± 0.0017	0.208± 0.0016	0.207± 0.0019	0.209± 0.0011	0.210±0. 0009	0.209± 0.0014	0.211± 0.0012	0.210± 0.0011	0.206± 0.0017	0.205± 0.0016	0.207± 0.0019	0.207± 0.0011
Apparent density (g/cm^3) (n=10)	1.212± 0.027	1.255± 0.042	1.264± 0.038	1.273± 0.121	1.238±0. 095	1.243± 0.074	1.260± 0.066	1.268± 0.105	1.188± 0.045	1.192± 0.107	1.187± 0.069	1.187± 0.085
Total porosity (%) (n=3)	3.87± 0.008	3.85± 0.011	3.85± 0.013	3.84± 0.007	3.92± 0.005	3.95± 0.009	3.92± 0.010	3.90± 0.013	3.81± 0.005	3.80± 0.009	3.80± 0.012	3.78± 0.004
Packing fraction (n=3)	0.186± 0.004	0.182± 0.006	0.171± 0.006	0.173± 0.005	0.163±0. 007	0.161± 0.003	0.162± 0.006	0.159± 0.008	0.148± 0.004	0.145± 0.006	0.144± 0.007	0.140± 0.005
Percent Elastic Recovery (n=3)	0.42± 0.002	0.38± 0.004	0.42± 0.003	0.41± 0.006	0.52± 0.003	0.48± 0.004	0.50± 0.005	0.47± 0.003	0.35± 0.004	0.32± 0.002	0.32± 0.001	0.34± 0.003
Weight uniformity (mg) (n=10)	200.5± 3.4	201.2± 2.1	198.2± 1.5	200.4± 4.4	202.3±1. 8	201.5± 2.3	198.7± 2.8	200.1± 3.7	199.2± 4.5	202.6± 1.8	202.5± 3.2	200.4± 2.6
Drug content (%) (n=20)	98.6± 0.12	99.5± 0.16	99.7± 0.23	101.2± 0.04	98.2± 0.13	99.3± 0.08	97.7± 0.15	99.4± 0.14	99.8± 0.22	102.3± 0.17	101.5± 0.25	102.6± 0.08
Hardness (Kg/cm^2) (n=10)	6.9±0.2	7.2±0.3	7.5±0.1	7.9±0.4	7.1±0.5	7.1±0.4	7.7±0.3	6.8± 0.2	7.0±0.3	7.0±0.5	7.7±0.2	7.5±0.5
Friability (%) (n=10)	0.32± 0.06	0.27± 0.04	0.25± 0.07	0.22± 0.04	0.45± 0.03	0.42± 0.07	0.39± 0.05	0.40± 0.06	0.30± 0.03	0.28± 0.07	0.28± 0.04	0.25± 0.02
Disintegration time (min) (n=6)	4.2± 0.03	4.5± 0.02	4.9± 0.05	5.9± 0.06	6.3± 0.05	6.7±0.0 3	6.9±0.0 2	7.5±0.0 5	4.2±0.04	3.9±0.04	3.5± 0.06	3.1± 0.05
CPR_{45min} (n=3)	93.3± 2.7	89.3± 1.6	89.1± 3.5	81.3± 2.9	95.5± 4.6	92.2±3. 5	88.4±1. 5	78.4±3. 3	90.4±3.2	92.7±2.5	94.3± 4.4	96.3± 2.8

11.1.5. Evaluation of tablets

11.1.5.1. Evaluation of binding capacity of CFSG and other tablet-properties

The results of tablet evaluations are presented in **Table 11.7**. Average volume of tablet was found to be 0.208 cm^3, which indicated better compaction of the granules into consolidated cohesive tablet. Apparent density also indicated the compactness of the finished tablet, which was identical to that found in case of standard binders, gum acacia and PVP K-30. The tablet-compactness was also substantiated by very low percent porosity of the tablet. The degree of initial packing in the die-cavity during die-filling is indicated by packing-fraction [11]. The tablets composed of CFSG (B1 – B4) were found to exhibit higher values of packing-fraction than that of the tablets composed of gum acacia (B5 – B8) and PVP K-30 (B9 – B12), which indicates better initial packing in the die by CFSG-batches than that of gum acacia and PVP K-30. Percent elastic recovery (PER) of tablets represents the binding efficacy as well as compressibility and tablet stability of the tableting mass. Lower value of elastic recovery indicates better binding efficacy and subsequent better compressibility with better consolidation. The tablets composed of CFSG (batch B1 to B4) showed comparatively lower percent of elastic recovery than that of acacia and PVP K-30, which substantiates the better binding capacity of CFSG than acacia and PVP K30. The variations in weight in all batches were within the USP-prescribed limit, which indicates the compliance of weight uniformity with official compendium. Similarly, drug contents in all batches were also found to be within official limits. Hardness and friability were determined to assess the binding capacity, cohesiveness and mechanical strength of the tablets. In the study, the tablets composed of CFSG (B1-B4 batches) were found to exhibit appreciable hardness (6.9 – 7.9 kg/m^2) identical to that of the batches composed of acacia and PVP K-30. Hardness was found to increase with increasing CFSG concentration. Percent friability less than 1% suggests excellent binding capacity of the binder and mechanical strength of

the tablet. The tablets composed of CFSG with minimum concentration even of 2.5% were found to show percent friability well below 1%, which ratifies excellent binding capacity of CFSG. Further, friability was observed to decrease with increasing CFSG concentration. The results were also found to be identical with standard binders. Friability assessment gives an indication towards the mechanical strength of the tablets to withstand the abrasion during production, packaging, transport and handling by patients during consumption. Disintegration time (DT) should be within 10 mins in case of conventional tablet as per official compendium (USP, 2017). In all batches of tablets composed of CFSG, DT was within 6 mins and the batch with 2.5% concentration showed lowest DT. DT was found to increase with increasing binder concentration in the batches B1 to B4 (CFSG), which may be due to extensive plastic deformation by CFSG, increase in inter-particular contact surface and subsequent reduction in fluid-penetration into inter-particular voids. Hardness×friability/DT has been considered as a better index of binding as well as mechanical strength of tablets than the previously used hardness: friability ratio. The index evaluates not only the mechanical strength (hardness) and weakness (friability) of the tablets but also their influence on disintegration time. In case of batch B1 (CFSG 2.5%w/w), the index was found to be better than gum acacia and PVP K-30, which was also identical to the results of other reports [11, 12]. **Figure 11.9 (B)**, **(C)** and **(D)** represent the SEM micrographs of the tablet surface at different states. **Figure 11.9 (B)** shows texture of the tablet surface after 24 hours of its compaction, i.e., after elastic recovery. The micrograph portrays signs of brittle fracture due to compaction force and simultaneous formation of new crystal lattice. Presence of some pores filled with plastically deformed granules is also depicted in the micrograph. **Figure 11.9 (C)** and **(D)** depict the dried tablet surface obtained from drying of the hydrated tablets at 1 h interval in hydration kinetic study. The micrographs exhibit the sign of formation of hydrogel on the surface of the tablet due to

hydration of CFSG and presence of macro and micropores extended to the interstitial channels which promote water penetration into the matrix and subsequent disintegration of the tablets into granules or primary particles. Overall, tablet evaluation results exhibited that 2.5% w/w CFSG (as binder) was sufficient to achieve optimum tablet properties and therefore, this concentration can be considered as optimum binder concentration when CFSG would be used as an alternative tablet binder.

Table 11.8. Equilibrium hydration and different hydration kinetic parameters of diclofenac sodium tablet formulated with CFSG as binder

Batch no.	**Equilibrium water absorption (%), W_E**	**Initial hydration rate (dW/dt), h^{-1}**	**Equilibrium hydration (W_∞), g/g**	**Matrix hydration (H), g/g**	**Water penetration velocity (V), cm/g.h**
B1	136.2±3.7	12.77±0.04	1.38±0.007	0.577±0.002	0.137±0.002
B2	182.2±4.9	8.13±0.02	1.98±0.003	0.646±0.004	0.172±0.004
B3	235.5±2.6	29.85±0.07	2.37±0.002	0.702±0.007	0.224±0.001
B4	252.2±3.5	79.37±0.06	2.53±0.005	0.716±0.005	0.223±0.005

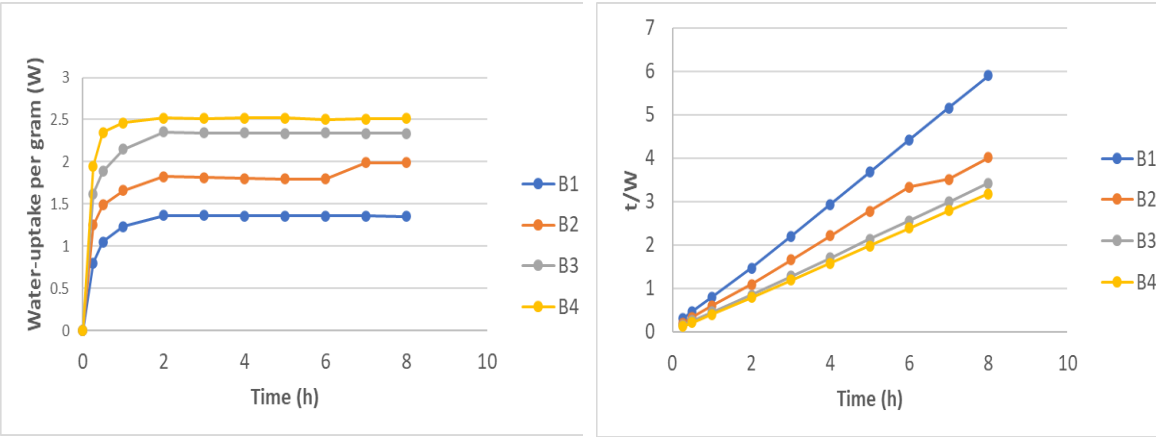


Figure 11.11. Hydration isotherms obtained from hydration kinetic study on the tablets composed of CFSG with varying concentration.

11.1.5.2. Hydration kinetic study

The results of equilibrium hydration and hydration kinetic study are presented in **Table 11.8** and the hydration isotherms are presented in **Figure 11.11**. The tablets composed of CFSG with varying concentration (2.5% to 10%; Batch B1-B4) were found to uptake water appreciably by 136.2 – 252.2%, which may be due to presence of hydrophilic CFSG in the tablet matrix. Presence of numerous -OH and -CH_2OH groups in the D-mannopyranose units of main polysaccharide-backbone and D-galactopyranose units in side chain imparts appreciable hydrophilicity and subsequent water-uptake capacity to CFSG moiety. The water-uptake capacity was found to increase with increasing CFSG concentration, which might be due to an increase in the number of hydrophilic groups with increasing concentration. The hydrophilic nature of CFSG allows use of water as granulating fluid and promotes the green granulation and tableting approach by wet granulation method without use of any harsh and organic solvents. The results of hydration kinetic shows that initial hydration rate increases with increase in binder concentration except B2 batch (5%w/w CFSG), which may contribute to higher concentration of hydrophilic CFSG in the tablet matrix. Initial hydration rate was within the range of 8.13 h^{-1} – 79.37 h^{-1}. The results also exhibited that the hydration equilibrium was attained at nearly 2 h in most cases. Matrix

hydration and water penetration velocity were within the range of 0.577 – 0.716 g/g and 0.137 – 0.224 cm/g.h, respectively. Both were found to increase with increasing binder concentration. On the other hand, increase in binder concentration resulted in delay in disintegration of the tablets, which may attribute to the fact that higher binder concentration would absorb more water and form a comparatively more viscous hydrogel in the inter-particular voids resulting in better cohesiveness and delay in disintegration of the tablet.

11.1.5.3. Drug-release study

Conventional tablet formulation requires immediate drug release after administration in order to achieve quick onset of drug action and the official monograph of such formulation prescribes a limit of drug release parameter which needs to be complied. In this study, in vitro drug release study was carried out to investigate the effect of binder on drug dissolution as well as check the compliance status to the official monograph (USP, 2017). As per USP-2017 monograph, cumulative percent drug release (CPR) at 45 mins should not be less than 75% for diclofenac sodium tablet. Different CPR obtained from different batches are given in **Table 11.7**. All batches showed CPR well above 75% at 45 mins, which indicated compliance to the official monograph. Further, drug release was found to decrease with increasing binder concentration in case of not only CFSG but also standard binders (gum acacia and PVP K-30), which may attribute to increase in matrix-cohesiveness with increase in binder concentration, subsequent delay in disintegration as well as in drug release.

11.1.5.4. Accelerated stability study

No substantial change was found in the appearance and colour of the tablets upon visual observation at the end of the stability study period (3 months). The height and diameter of the tablets were retained as almost the same as that at zero days. Drug content of the tablets under study was observed to decrease maximum by 0.42%. Hardness, friability, disintegration time (DT) and cumulative percent drug release at 45 mins (CPR_{45mins}) were found to deviate maximum by 0.36%, 0.57%, 0.82%, and 3.6%, respectively. No substantial change was observed in the FTIR spectrum, DSC-thermogram and XRD-diffractogram of the aged tablets when compared with the curves generated at zero days. Overall retention of the tablet properties was reflected in the study, which indicated an optimum stability of the tablets even in a challenged environment and predicted substantially lengthier shelf-life.

PART II

11.2. Chemical modification of cassia fistula seed gum through graft copolymerization with poly (sodium acrylate) and used as gastroretentive drug delivery excipients

11.2.1. Determination of λ_{max} and preparation of standard calibration curve of Aceclofenac in Hydrochloric Acid with sodium lauryl sulphate (SLS) medium

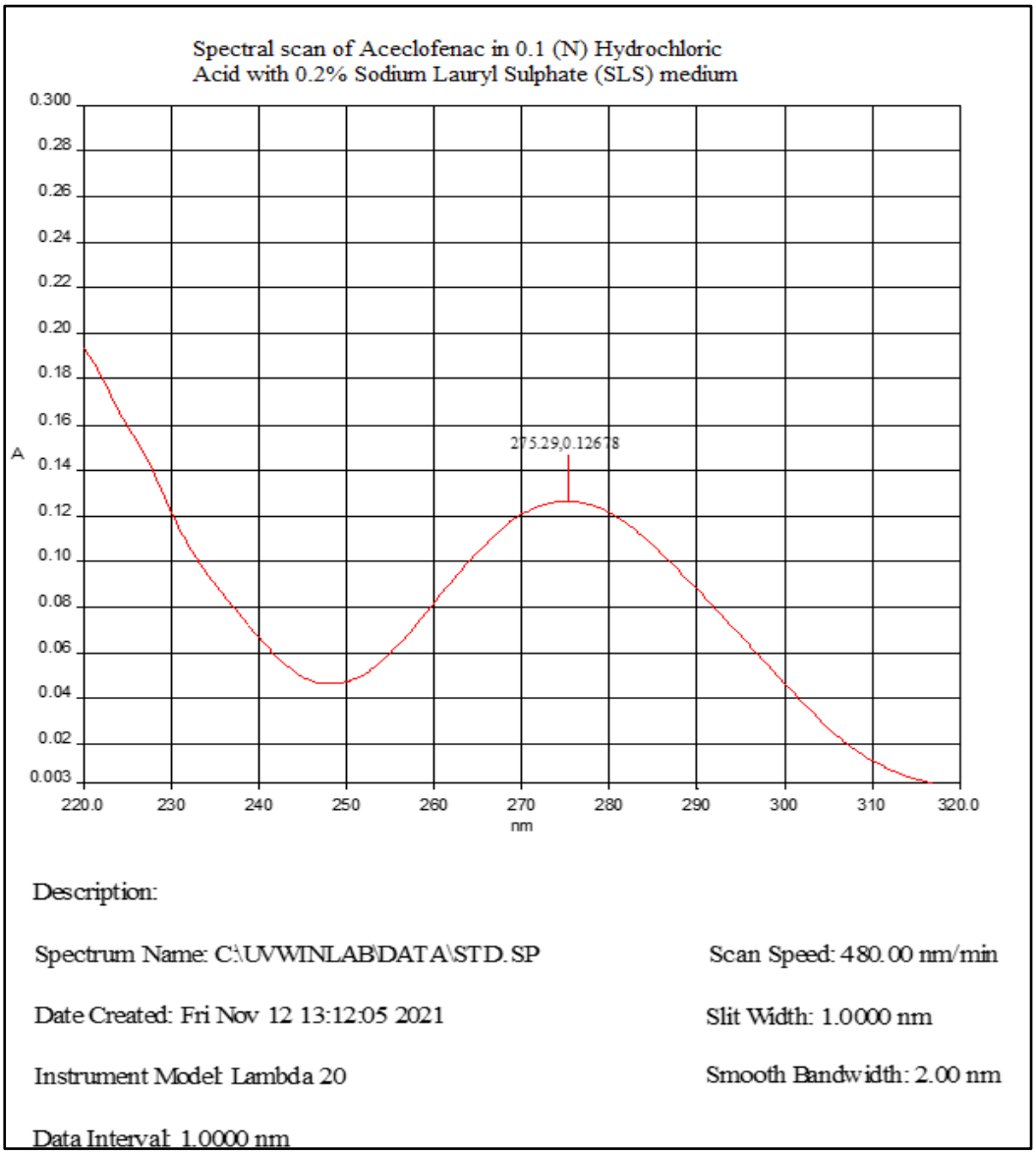


Figure 11.12. Wavelength scanning of Aceclofenac

Table 11.9. Standard curve data for Aceclofenac

Sl. No.	Concentration (mg/ml)	Absorbance (Average)	Equation	R^2 Value
1	0.00797	0.2979	y = 33.535x + 0.0281	0.9998
2	0.01594	0.5593		
3	0.01992	0.6915		
4	0.02391	0.8345		
5	0.02989	1.0352		
6	0.03188	1.0931		

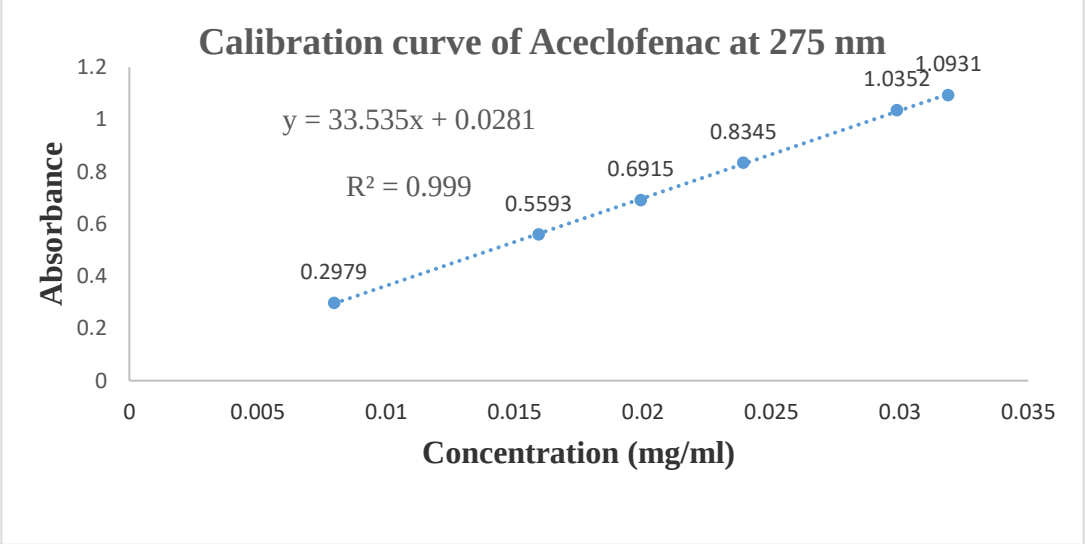


Figure 11.13: Standard Curve of Aceclofenac in 0.1 (N) Hydrochloric Acid with 0.2% SLS Medium

Discussion:

The λ_{max} was found to be 275 nm in concordance with the reference standard. The UV spectrum was shown in **Figure 11.12**. Graph of absorbance versus concentration was plotted and found to be linear over the range of 0.00797to 0.03188 mg/ml, (**Figure 11.13)** indicating its compliance with Beer's Lambert's Law. Standard curve equation was found be y = 33.535x + 0.0281 and Correlation coefficient: R^2 =0.9998

11.2.2. Synthesis of CFSG-g-PSA

Poly (acrylic acid) was grafted onto CFSG by microwave-assisted free-radical generation method using a potential redox-initiator, ceric ammonium nitrate. Application of microwave-irradiation along with redox-initiator in the synthesis of graft-copolymers has been reported as very beneficial for higher yield [13-16]. Different grafting parameters of each synthetic batch are presented in **Table 11.10**. In the batches H1 to H4 (without microwave-irradiation), % grafting was found to be very low (maximum value 23.8%), whereas, microwave batches (S1 to S8) showed significant yield (maximum value 790%) and ratify the synergistic effect of microwave on grafting reaction. Similar effect of MW was also reported by Makhado et al. ([16] The low degree of grafting in H1 to H4 at low and high level CAN may be due to lack of sufficient enthalpy required for reaction and the significantly higher degree of grafting in S1 to S8 may be attributed to the supply of sufficient energy through microwave-irradiation. In S1 and S2 batches, %G was found to increase by 2.2-fold with increase in monomer AA concentration at high level of CAN and low level of MW. In S3:S7 and S6:S8 batches, similar effects were shown at combinations of low-level of CAN with high-level of MW and also at high levels of CAN and MW. It may be attributed to the higher availability of AA molecules at the free-radical sites, which increases the rate as well as extent of grafting. But in S4 and S5, proportional effect of AA on %G at low levels of CAN and MW was little, which may be due to low concentration of CAN and therefore, less generation of free-radical sites. In S1 and S5, %G was found to increase by 4.8-fold with increase in [CAN] at high levels of AA and low levels of MW. In S2:S4, S3:S8 and S6:S7 also, %G increased very significantly with increase in [CAN] at all possible combinations of the levels of other two variables. This result may be attributed to higher-degree generation of free-radical-sites on CFSG backbone at higher concentration of CAN and subsequent extensive initiation of chain-elongation. Significant contributing effect

of MW on grafting reactions was also found in the study. Comparison between batch H4 and S1 shows that application of microwave-radiation for 1 min (S1) increased %G to 790% from 23.8%, which substantiates the greatly positive role of MW in graft-copolymerization. But batch S1 and S6 showed a decrease in %G by 27.8% with an increase in MW from 1 min to 5 min at high levels of both AA and CAN. Similar trend was also exhibited in batch S3:S4, S5:S7 and S2:S8 at all possible combinations of low and high levels of AA and CAN, which may be due to generation of free-radicals to a greater extent upon further irradiation above a threshold point and thereby premature termination and also breakage of propagating homopolymer side-chains. The overall effect of MW describes that an optimum threshold period of microwave-irradiation time is required for higher grafting-yield. Similar effects of AA, CAN and MW were also found in synthesis of poly (acrylic acid)-grafted-gellan gum ([15]. The log-transformed polynomial equation (Eq. 1) obtained from ANOVA also designates the same observations regarding the effects of the independent synthetic variables on %G.

$Log_{10}(\%G) = +1.23795+0.05568AA + 2.19258CAN – 0.06386MW+0.06943CAN.MW$ (Eq. 1).

$\%GE = -34.35 + 0.05\ AA + 226.4\ CAN + 0.675\ MW – 10.2\ CAN.\ MW$ (Eq. 2)

$\%C_n = -4.35 – 1.95AA + 226.4CAN + 0.675MW – 10.2\ CAN.MW$ (Eq. 3)

The equation also indicates a significantly positive combination effect of CAN and MW on %G.

The probable mechanism of the grafting reaction is based on the dissociation of CAN into Ce^{4+} ions in water, consequent binding of ceric ions to –OH groups of CFSG, subsequent abstraction of hydrogen atom from –OH groups and generation of oxygen free-radicals. These oxygen free-radicals of CFSG polymeric-backbone get coupled to the vinyl carbon atom of acrylic acid, which results in conversion of 1-carbon atom into free-radical and leads

to chain-elongation. Finally, termination of the grafting-reaction occurs through coupling between free-radical-points of two propagating-chains or between one propagating-chain and one AA-free-radical. S1-batch might be regarded as the best synthetic batch as it exhibits highest %G, %GE and %Cn of 790%, 79.0% and 89.0%, respectively. The scheme of the reaction along with the structures of native CFSG and CFSG-g-PSA has been shown in **Figure 11.14**. The statistically substantial relationships (with p-value < 0.05) between independent synthetic factors and grafting indicators were obtained from ANOVA study using the software. Various statistical parameters related to each selected log-transformed model for %G, %GE and %Cn have been presented in **Table 11.11**. F-value and p-value in each case indicate the significance of the model and adequate precision indicates adequate signal to noise ratio. Predicted R^2 was found to be in close agreement with adjusted R^2. Effects of independent variables on the response variables (%G, %GE and $\%C_n$) were depicted by contour and 3-D surface plots (**Figure 11.15**). The optimum synthetic condition was then obtained from numerical optimization for maximum %G, %GE and %Cn. The ranges of the independent synthetic variables were set within their initial individual high and low level selected in the design. The best solution having desirability of 0.940 was chosen from total 66 solutions given by the software and presented in **Table 12**, which are found to be similar to that of S1 batch. The predicted, observed values and relative errors are also given in **Table 11.12**. Very low relative errors substantiate the reliability of the software-based predictions.

The following is the reaction's suggested mechanism:

Initiation:

$Ce(NH_4)^2(NO_3)^6 \rightarrow Ce^{4+} + 2NH_4 + 6NO_3^-$

$Ce^{4+} + CFSG\text{-}OH \rightarrow complex \rightarrow CFSG\text{-}O^{\bullet} + Ce^{3+} + H^+$

$CFSG\text{-}O^{\bullet} + AA \rightarrow CFSG\text{-}OAA_1^{\bullet}$

$Ce^{4+} + AA \rightarrow AA^{\bullet} + Ce^{3+} + H^{+}$

$AA^{\bullet} + AA \rightarrow AA_2^{\bullet}$

Propagation:

$CFSG\text{-}OAA_1^{\bullet} + AA \rightarrow CFSG\text{-}OAA_2^{\bullet}$

$CFSG\text{-}OAA_{n-1}^{\bullet} + AA \rightarrow CFSG\text{-}OAA_n^{\bullet}$

$AA_2^{\bullet} + AA \rightarrow AA_3^{\bullet}$

$AA_{p-1}^{\bullet} + AA \rightarrow AA_p$

Termination:

$CFSG\text{-}OAA_n^{\bullet} + CFSG\text{-}OAA_m^{\bullet} \rightarrow CFSG\text{-}OAA_{(m+n)}$ (graftcopolymer)

$CFSG\text{-}OAA_n^{\bullet} + AA^{\bullet} \rightarrow CFSG\text{-}OAA_{(n+1)}$ (graftcopolymer)

$CFSG\text{-}OAA_n^{\bullet} + AA_p^{\bullet} \rightarrow CFSG\text{-}OAA_{(n+p)}$ (graftcopolymer)

$AA_p^{\bullet} + AA_q^{\bullet} \rightarrow AA_{(p+q)}$ (homopolymer)

(CFSG-OH = native cassia fistula seed gum; AA = acrylic acid)

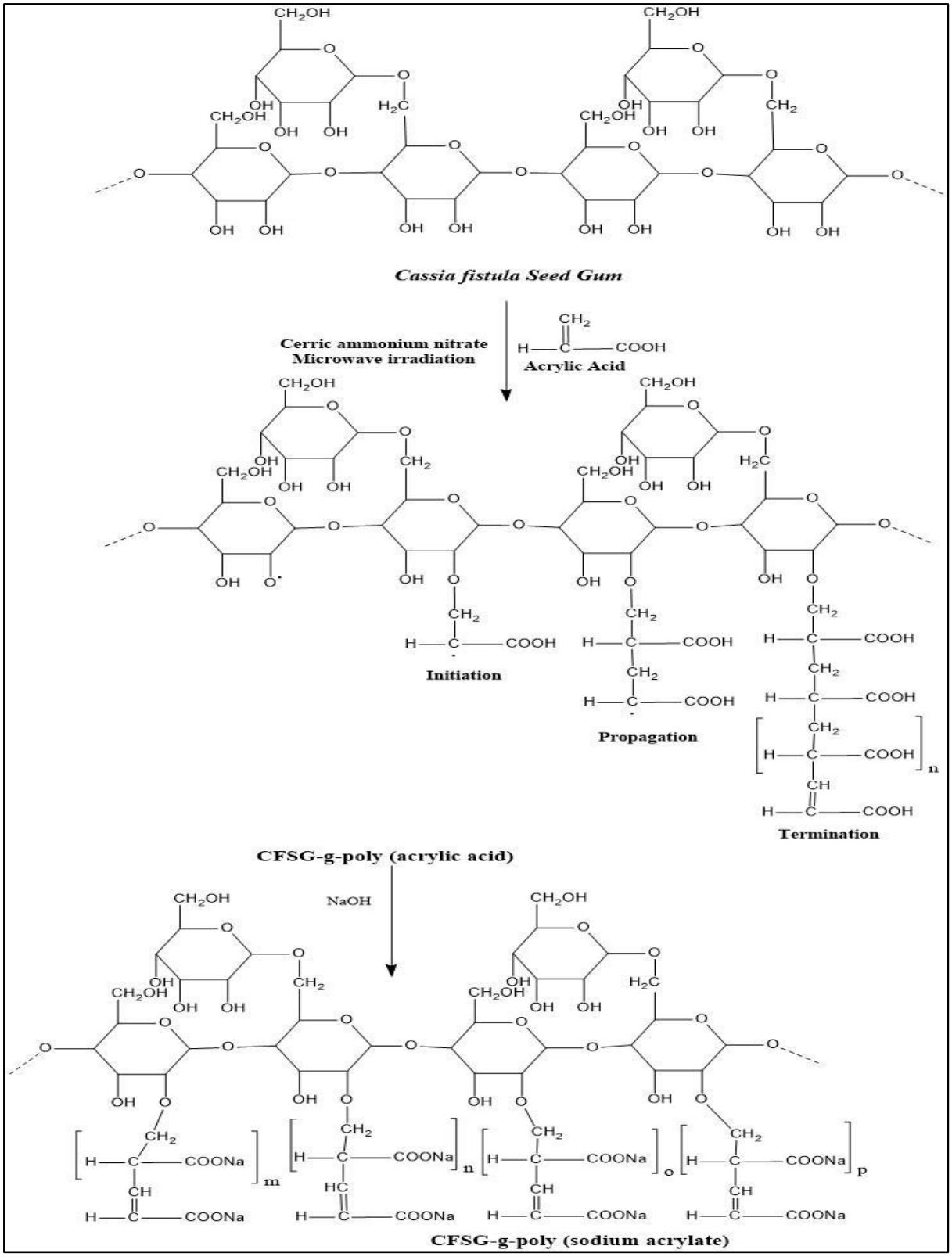


Figure 11.14. Schematic representation of the grafting of poly (sodium acrylate) onto the CFSG backbone.

Table 11.10. Various synthetic conditions for the synthesis of CFSG-g-PSA and various characterization parameters

Batch no.	Acrylic acid (g)	CAN (g)	MW (min)	%G	%GE	$\%C_n$	Elemental analysis			Viscosity (cP) @1.0 rpm	$\%W_E$	MM ($\times10^5$Da)	Zeta potential (mV)
							%C	%H	%O				
CFSG	-	-	-	-	-	-	39.21	6.25	52.09	755.6	136.8±3.2	1.96	-1.2±2.8
H1	5	0.25	-	6.6	1.3	21.3	39.50	6.43	52.52	770.1	138.1±3.5	1.97	-1.2±3.1
H2	5	0.50	-	11.2	2.2	22.2	40.05	6.72	53.71	772.5	139.0±2.7	2.02	-3.3±1.5
H3	10	0.25	-	8.5	0.85	10.9	39.59	6.53	52.63	771.3	139.3±2.9	1.98	-2.5±0.9
H4	10	0.50	-	23.8	2.4	12.4	40.11	6.85	52.88	793.6	156.5±1.3	2.05	-4.2±1.2
S1	10	0.50	1	790	79.0	89.0	43.84	7.45	66.30	1492.3	286.4±4.6	8.23	-64.2±3.3
S2	5	0.50	1	353	70.6	90.6	41.69	7.21	57.81	1057.7	215.1±5.3	3.22	-15.7±2.1
S3	5	0.25	5	63	12.6	32.6	40.44	6.95	53.53	814.8	164.0±2.6	1.82	-5.4±0.4
S4	5	0.25	1	125	25.0	45.0	40.85	7.05	54.43	855.7	173.9±4.5	2.15	-8.0±0.6
S5	10	0.25	1	165	16.5	26.5	41.13	7.09	54.75	871.2	182.5±3.3	2.22	-9.1±1.3
S6	10	0.50	5	570	57.0	67.0	41.57	7.25	62.70	1202.5	235.9±1.9	2.80	-26.0±4.9
S7	10	0.25	5	139	13.9	23.9	40.88	7.06	54.67	856.1	172.7±2.2	1.84	-8.5±1.9
S8	5	0.50	5	286	57.2	77.2	41.52	7.17	57.23	993.4	206.3±5.1	1.81	-15.2±0.9

$\%W_E$: Equilibrium water-uptake; MM: molar mass

Table 11.11. Different statistical parameters along with their values obtained from ANOVA analysis for the different response variables after a transformation to Base 10 log.

Response	Sum of square	Degrees of freedom	Mean square	F-value	Press	p-value	R^2	Predicted R^2	Adjusted R^2	Adequate precision
%G	0.924	4	0.2309	39.91	0.1235	0.0062	0.9816	0.8688	0.9570	17.1
%GE	5161.9	4	1290.5	53.66	513.03	0.0040	0.9862	0.9020	0.9678	15.9
%Cn	5351.9	4	1337.9	55.64	513.03	0.0038	0.9867	0.9054	0.9690	18.4

Table 11.12. Numerical optimization of synthetic independent variables by 2^3full factorial designs.

Sl. No.	**Independent variables**		**Response variables**				
	Parameters	Optimized value	Parameters	Target value	Predicted value	Observed value	% Error
1.	Acrylic acid (AA)	10 g	% Grafting (%G)	maximum	793.1	790.0	0.39
2.	Ceric ammonium nitrate (CAN)	0.5 g	% Grafting efficiency (%GE)	maximum	78.2	79.0	1.02
3.	Microwave irradiation time (MW)	1 min	% Conversion (%C_n)	maximum	88.3	89.0	0.79

% Error = (Predicted - Observed) ×100/Predicted.

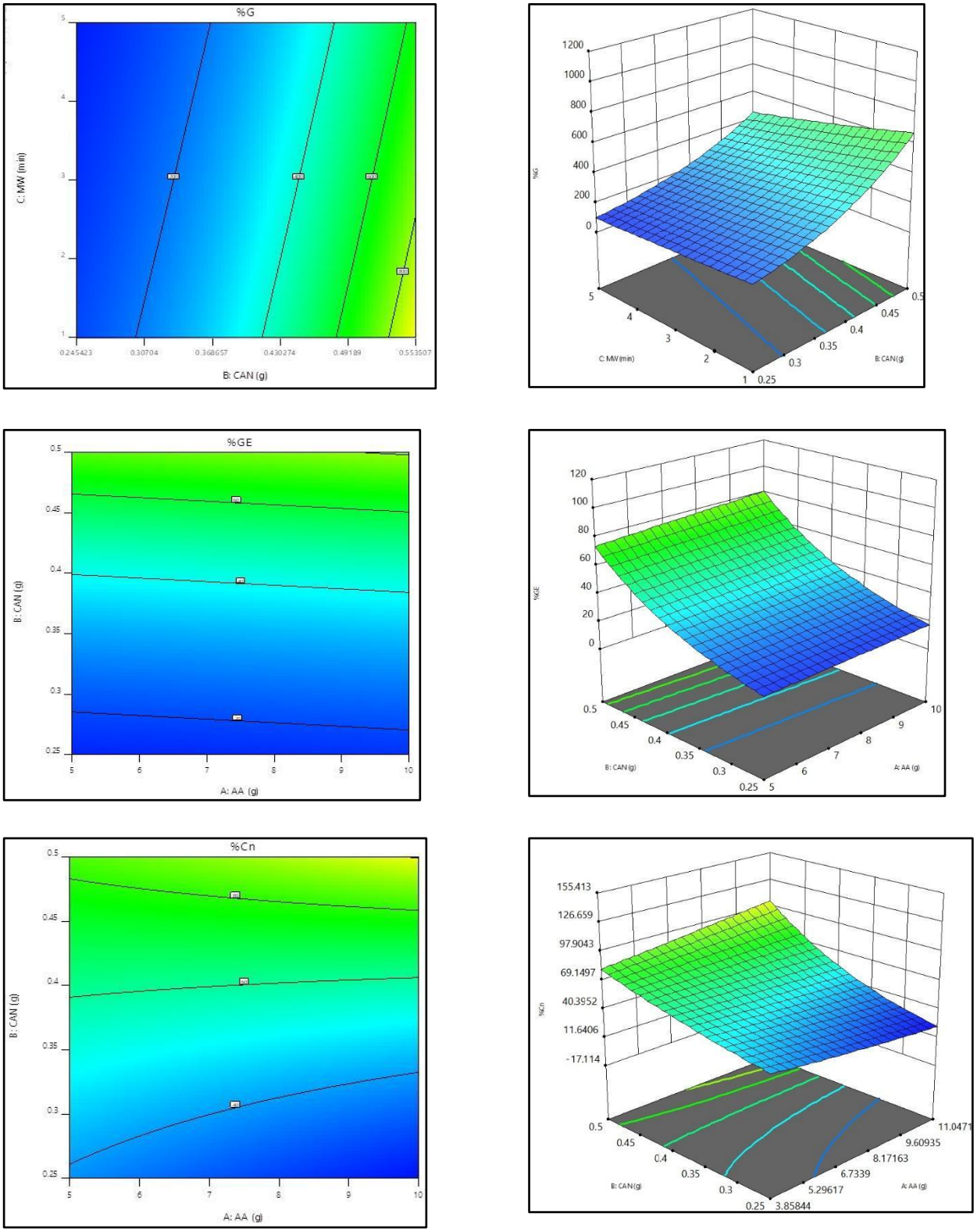


Figure 11.15. Contour and 3-D Surface plots for the dependent variables, %Grafting (%G), %Grafting efficiency (%GE) and % Conversion (%C_n).

11.2.3. Conversion of CFSG-g-poly (acrylic acid) into CFSG-g-poly (sodium acrylate)

Native CFSG was found to form a clear colloidal dispersion when mixed in water at ambient temperature but not the CFSG-g-poly (acrylic acid). To obtain a uniform aqueous colloidal dispersion required for ease of granule preparation by wet granulation technique, CFSG-g-poly (acrylic acid) was converted into CFSG-g-poly (sodium acrylate) by treating with NaOH. The copolymer becomes more hydrophilic and readily forms clear colloidal dispersion in water at ambient temperature upon gentle stirring with a glass rod. It may be attributed to the fact that NaOH reacts with –COOH groups of acrylic acids in grafted side chains resulting in formation of sodium acrylate which gets dissociated into hydrophilic anionic acrylate ions.

11.2.4. Characterizations of CFSG-g-PSA

11.2.4.1. Elemental analysis

The results of the elemental analysis are presented in **Table 11.10**. Increase in %C, %H and %O contents of CFSG-g-PSA compared to native CFSG indicates grafting. A further significant increase in CHO content from batch H4 to S1 substantiates the synergistic effect of MW on grafting yield. S1 and S6 batch showed decrease in CHO content and %G with increase in MW from 1 min to 5 min, which may attribute to breakage of propagated side-chains and premature termination after a threshold point of irradiation. Similar results were also found in synthesis of poly (acrylic acid)-g-gellan [15].

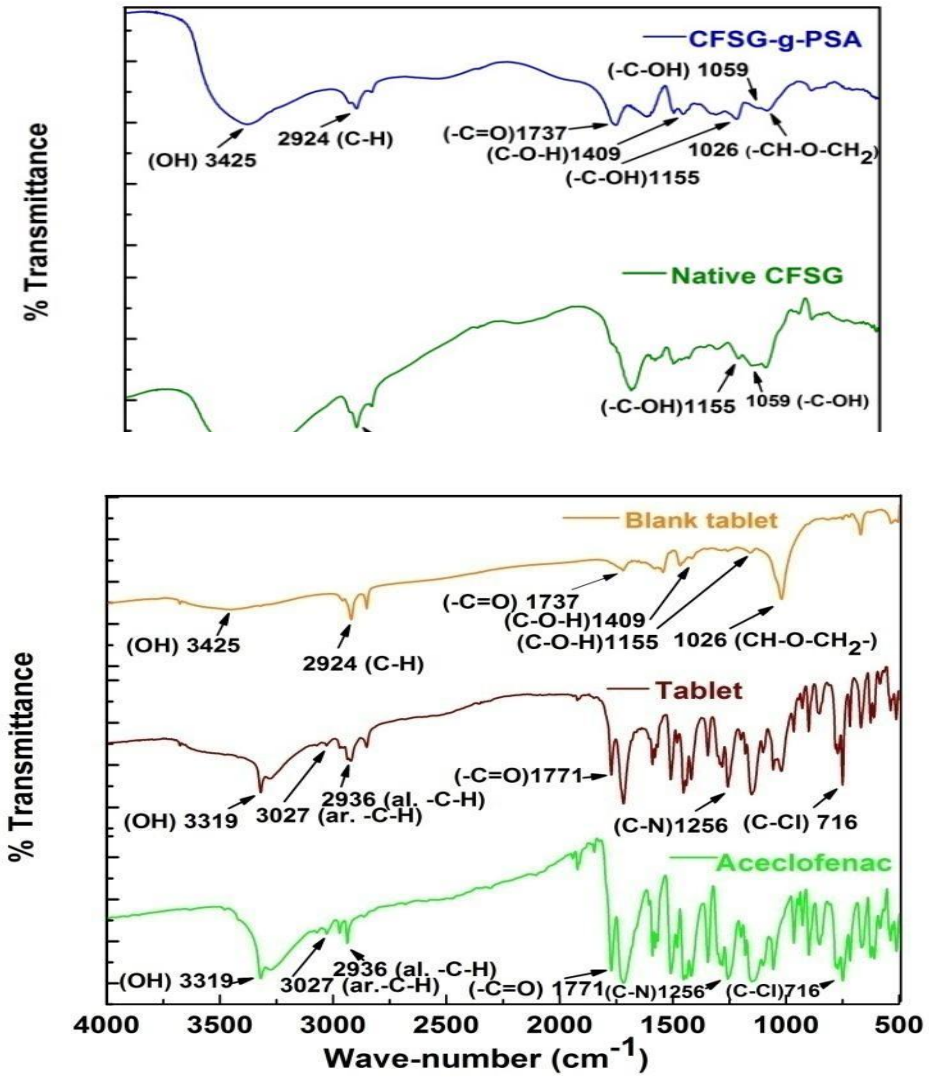


Figure 11.16. FTIR spectra of native CFSG, CFSG-g-PSA (S1), aceclofenac, tablet formulation (MT10) and blank tablet (drug free).

11.2.4.2. FTIR

Figure 11.16 depicts the FTIR spectrums of native CFSG, CFSG-g-PSA, pristine aceclofenac, tablet and drug-free blank tablet. The spectra obtained from native CFSG shows different characteristic bands and peaks at 3425 cm^{-1} for –OH group stretching, at 2924 cm^{-}

[1] for symmetric C-H stretching indicating polysaccharide, at 1660 cm^{-1} for –CH-OH stretching, at 1155 cm^{-1} and 1059 cm^{-1} for stretching vibration of –C-OH of pyranose and mannose ring, respectively, and at 812 cm^{-1} for anomeric deformation of α-D-galactopyranose [1, 2]. The spectrum from CFSG-g-PSA retains all the characteristic peaks of native CFSG and shows some additional peaks. It also shows the same band as CFSG at 3425 cm^{-1} for –OH stretching vibration but with less intensity compared to that of CFSG, which may attribute to decrease in the number of free –OH groups due to attachment of acrylic acid to a significant number of –OH groups. It also shows peaks at 2924 cm^{-1} for symmetric C-H stretching indicating polysaccharide, at 1660 cm^{-1} for –CH-OH stretching, at 1155 cm^{-1} and 1059 cm^{-1} for stretching vibration of –C-OH of pyranose and mannose ring, respectively, and at 812 cm^{-1} for anomeric deformation of α-D-galactopyranose. The peaks at 1737 cm^{-1} and 1409 cm^{-1} correspond to stretching vibration of –C=O and –C-O- present in –COONa group of sodium acrylate moiety, respectively. Another characteristic peak at 1026 cm^{-1} corresponds to stretching vibration of CH-O-CH_2 group, which indicates the covalent linkage between –OH group of CFSG and carbon atom of sodium acrylate at position-2 [15]. The last three peaks confirm the grafting of poly (sodium acrylate) onto CFSG backbone at –OH group sites.

The spectrum of aceclofenac shows characteristic peaks at 3319 cm^{-1} for stretching vibration of –OH of –COOH group, at 3027 cm^{-1} for aromatic –C-H, at 2936 cm^{-1} for –N-H stretching vibration, 1771 cm^{-1} for –C=O of –COOH group, at 1716 cm^{-1} for ester carbonyl (–C=O) group, at 1256 cm^{-1} for C-N stretching vibration of secondary aromatic amine, at 716 cm^{-1} for stretching vibration of 1,2 di-substituted C-Cl [17, 18]. All these peaks of aceclofenac were retained in the spectrum obtained from tablet formulation of aceclofenac with CFSG-g-PSA, but not observed in the spectrum of drug-free tablet formulation, which indicates absence of any incompatibility between drug and polymer.

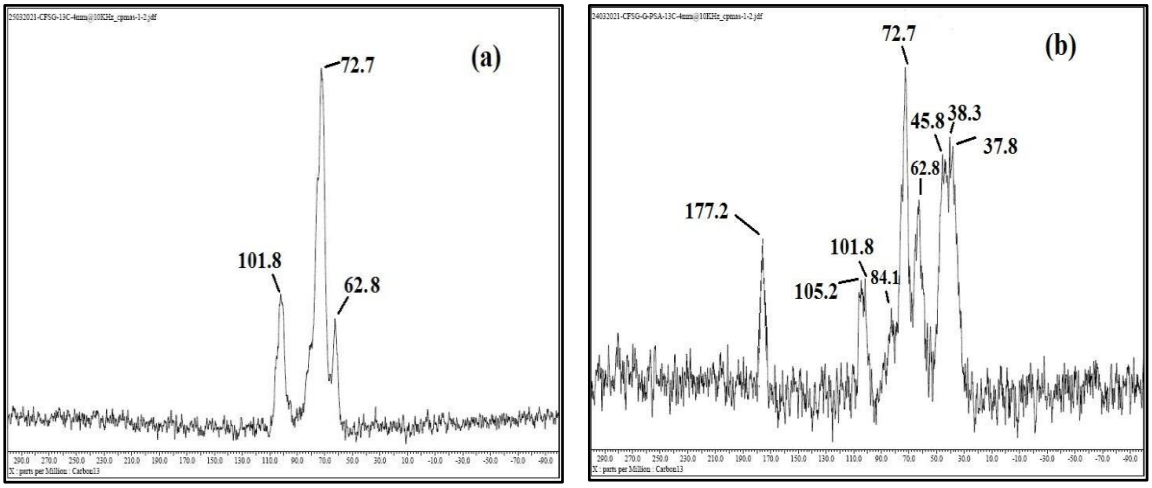


Figure 11.17. ^{13}C Solid state NMR spectra of (a) CFSG and (b) CFSG-g-PSA (S1).

11.2.4.3. ^{13}C Solid state NMR spectroscopy

The ^{13}C solid-state NMR spectra of native CFSG and CFSG-g-PSA are shown in **Figure 11.17 (a)** and **(b)**, respectively. The spectrum of CFSG (**Figure 11.17a**) showed three distinct absorption peaks with good signal to noise ratio at δ = 62.8 ppm for carbon atom of –CH_2OH of galactose unit [3], at δ = 72.7 ppm with very high intensity for carbon-atom of the glycosidic-linkage (>C-O-C<) between sugar moieties, and at δ = 101.8 ppm anomeric carbon atoms of β-D-mannose [1]. The ^{13}C NMR spectrum (**Figure 11.17b**) recorded from CFSG-g-PSA was found to retain all the peaks of native CFSG and also shows some different additional peaks. The peaks at δ = 177.2 ppm corresponds to carbonyl-carbon atom of –COONa groups of sodium acrylate units in grafted side chains [15] and another peak at δ = 45.8 ppm corresponds to the carbon atoms of grafted side chains (-CH_2-CH-CH_2-) [19]. These two additional peaks corroborate the grafting of acrylate homopolymer side-chain onto CFSG backbone. Fig. 2b also shows the peaks at δ = 62.8 ppm, 72.7 ppm and 101.8 ppm corresponding to –CH_2OH of galactose units, carbon-atom of the glycosidic-linkage (>C-O-C<) between sugar moieties, and at δ = 101.8 ppm anomeric carbon atoms of β-D-mannose, respectively. These were also found in the spectrum recorded from native form of CFSG.

11.2.4.4. Determination of molar mass and zeta potential

Molar mass and zeta potential of native CFSG and different batches of its grafted form were presented in **Table 11.10**. Native CFSG was found to have a molar mass of 1.96×10^5 Da, whereas its grafted forms obtained from microwave-assisted batches were found to have significantly higher molar mass. The molar mass of CFSG-g-PSA of S1 batch with 790%G was found to be 10.23×10^5 Da, which may attribute to increase in molecular size due to grafting of poly (sodium acrylate) homopolymer side-chains onto CFSG polysaccharide-backbone. The graft-copolymer from S2 batch (353%G) also shows significant increase in molar-mass compared to native form. In the microwave-assisted batches with 1 min MW (S1:S2 and S4:S5), molar-mass was found to increase proportionally with increase in %grafting, whereas, comparison between S1 (MW 1 min) and S6 (MW 5 min) showed that molar-mass increased but not proportionally with %G. In S3, S7 and S8 (MW 5 min), molar-mass of the copolymers was found to be lower than that of native CFSG in spite of having 63, 139 and 286%G, respectively. This result may be due to breakage of glycosidic linkage of polysaccharide backbone at different points by microwave-irradiation over a longer period [20]. Molar-mass of the graft-copolymers obtained from heating batches (H1 to H4; without ME) was not found to increase significantly, which may be due to very low degree of grafting and thereby very little increase in molecular size. Native CFSG showed to have very low negative zeta potential (-1.2±2.8 mV), which may attribute to only 2.08% (w/w) uronic acid content in CFSG as reported by da Silva et al. [1] and presence of anionic –COOH groups in uronic acid residue. CFSG-g-PSA with 790%G (S1 batch) shows a significantly higher negative zeta potential (-64.2±3.3 mV), which may be due to presence of numerous $–COO^-$ ions in acrylate units of grafted side-chains. The magnitude of zeta potential was also found to increase with increase in %grafting, which might be due to increase in number of anionic $–COO^-$ ions with increase in %grafting.

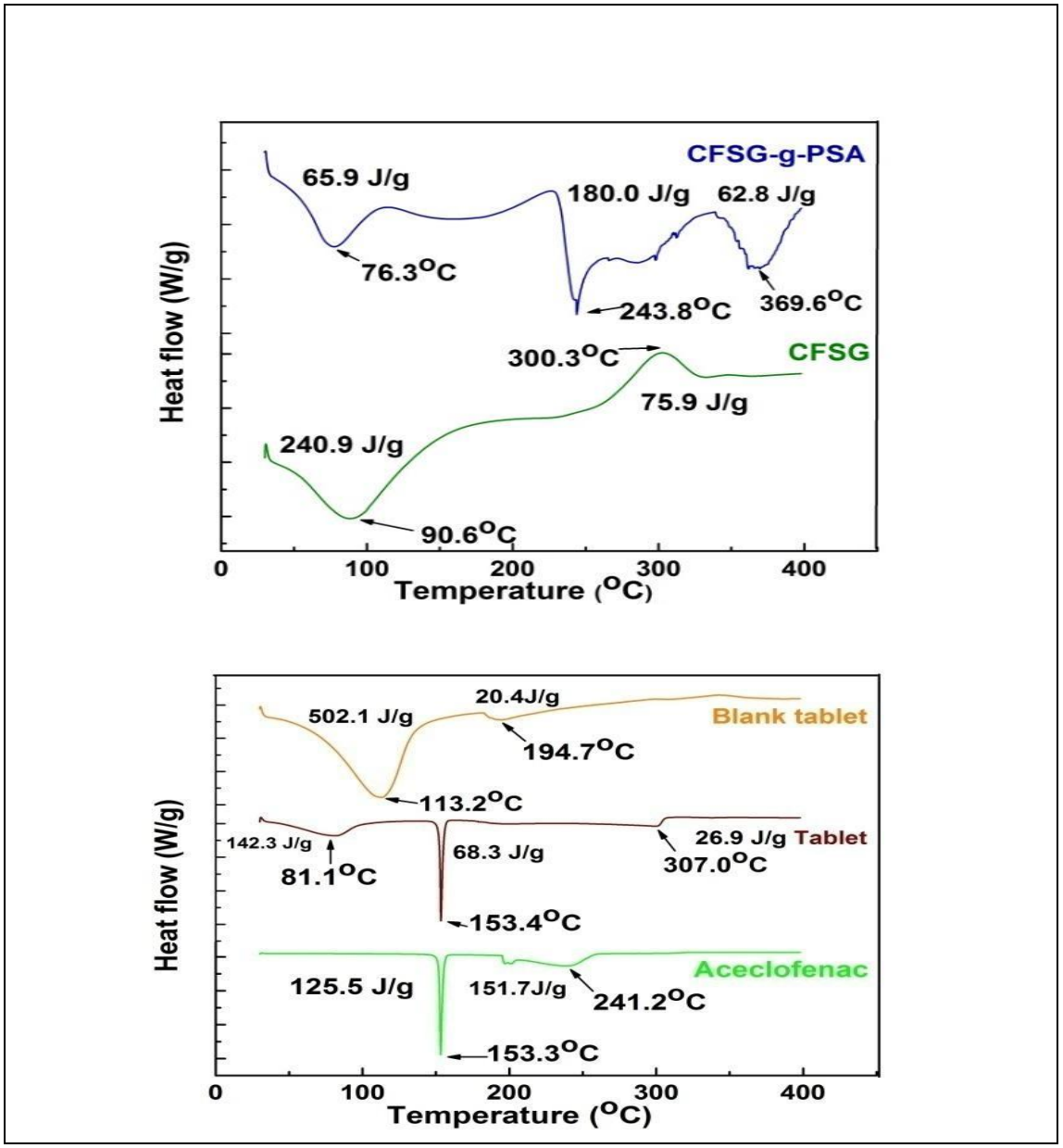


Figure 11.18. DSC thermograms of native CFSG, CFSG-g-PSA (S1), aceclofenac, tablet formulation (MT10) and blank tablet (drug free).

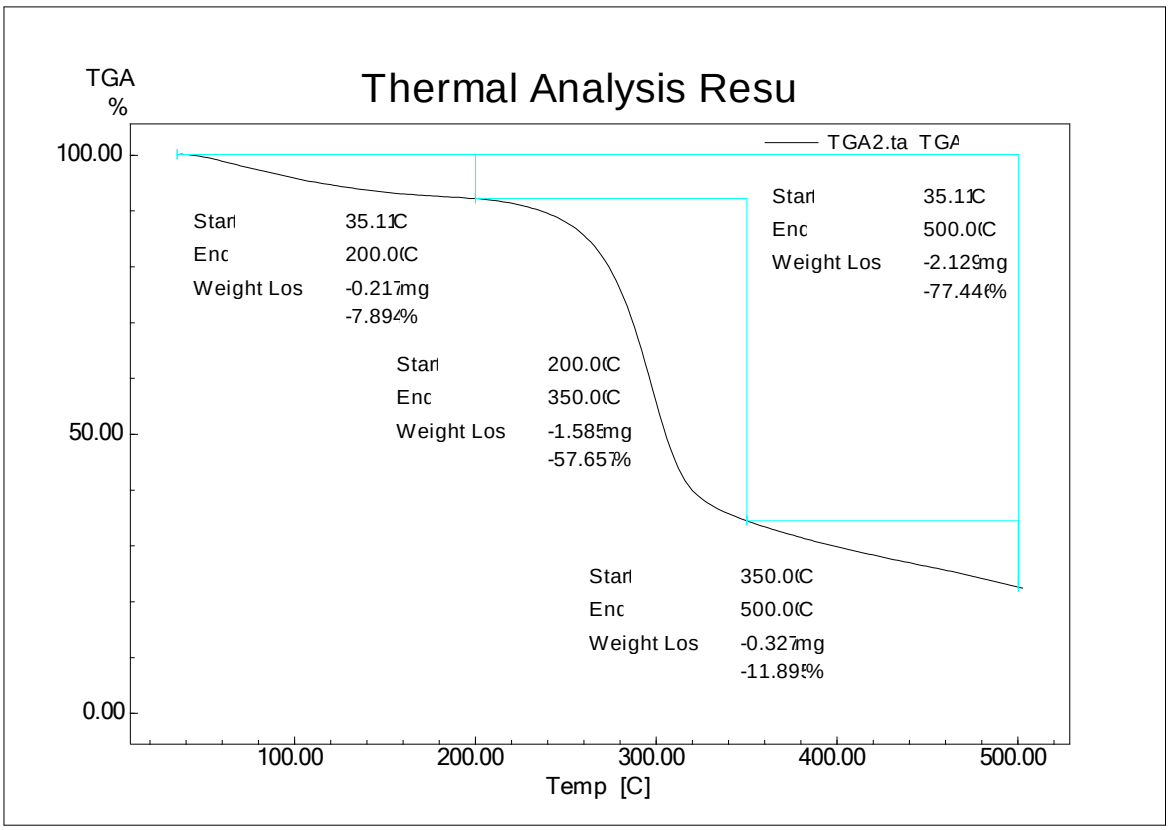


Figure 11.19. TGA thermogram of native CFSG

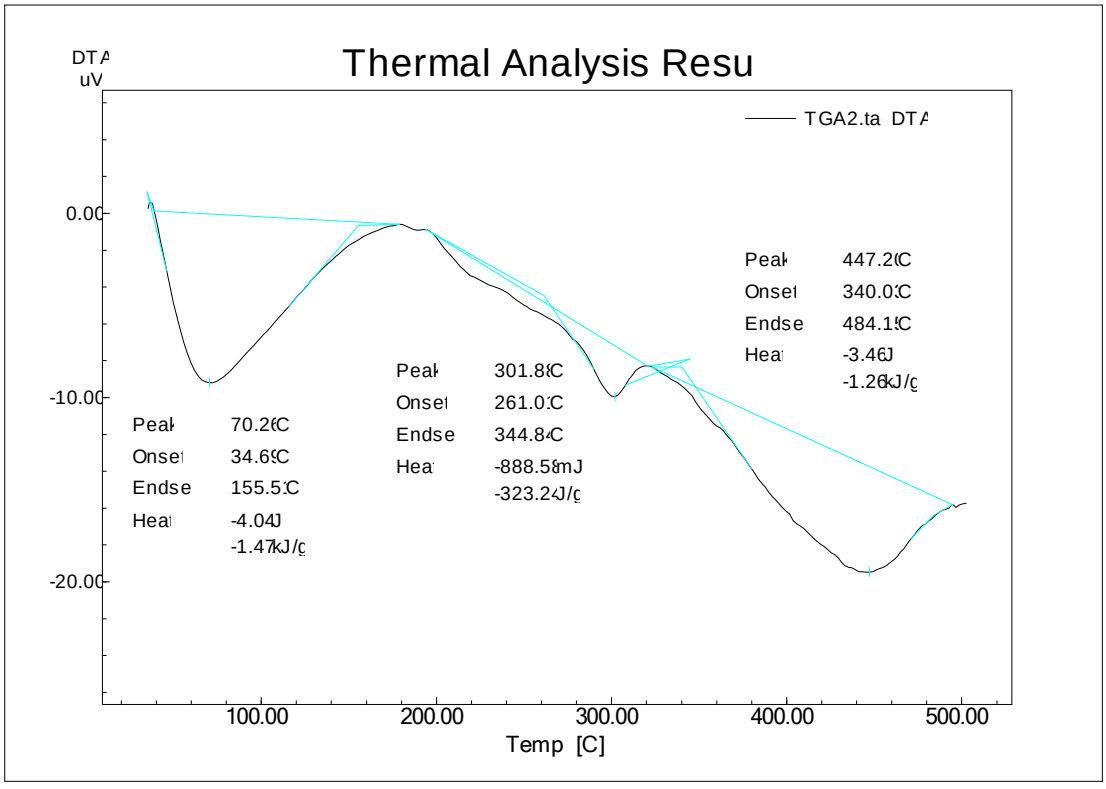


Figure 11.20. DTA thermogram of native CFSG

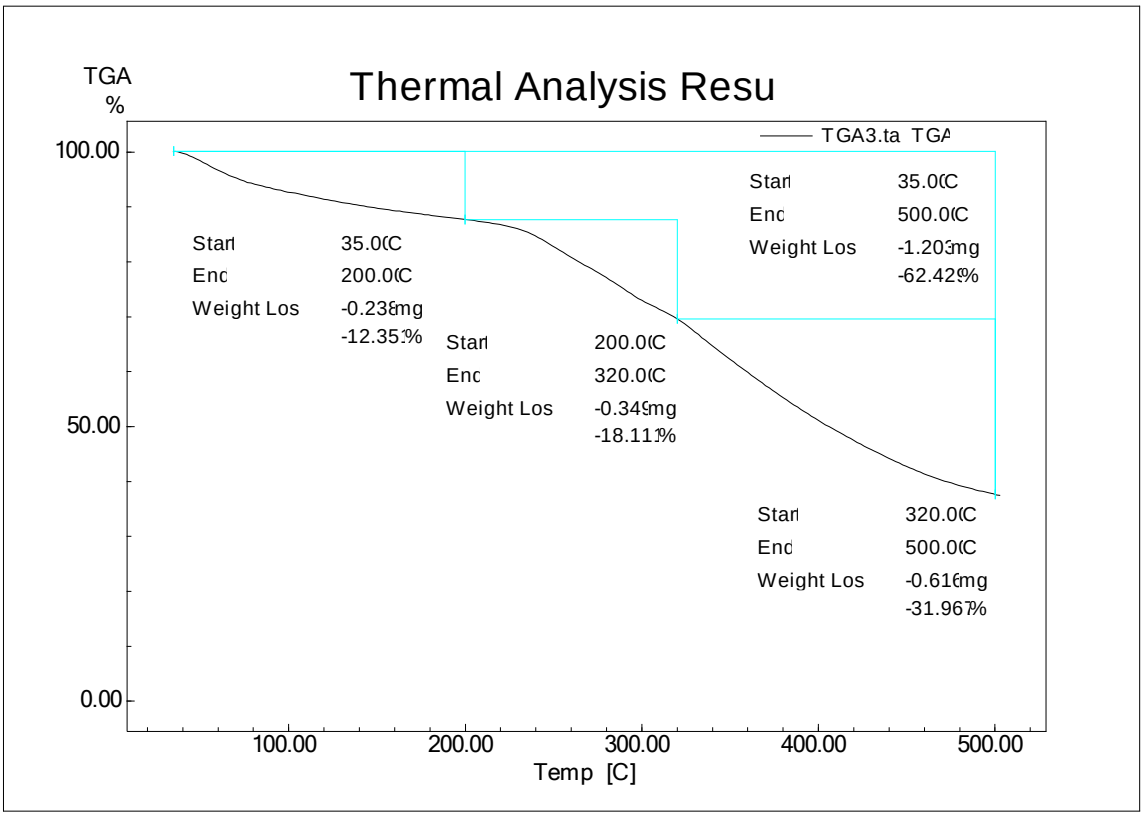


Figure 11.21:TGA thermogram of CFSG-g-PSA

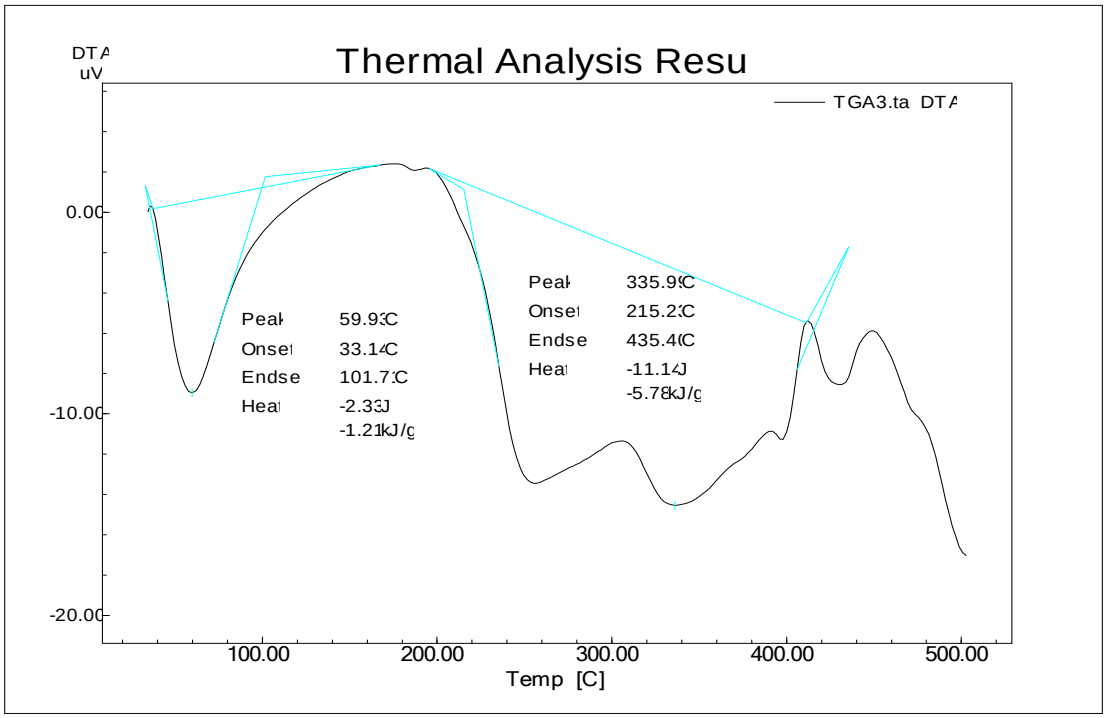


Figure 11.22: DTA thermogram of CFSG-g-PSA.

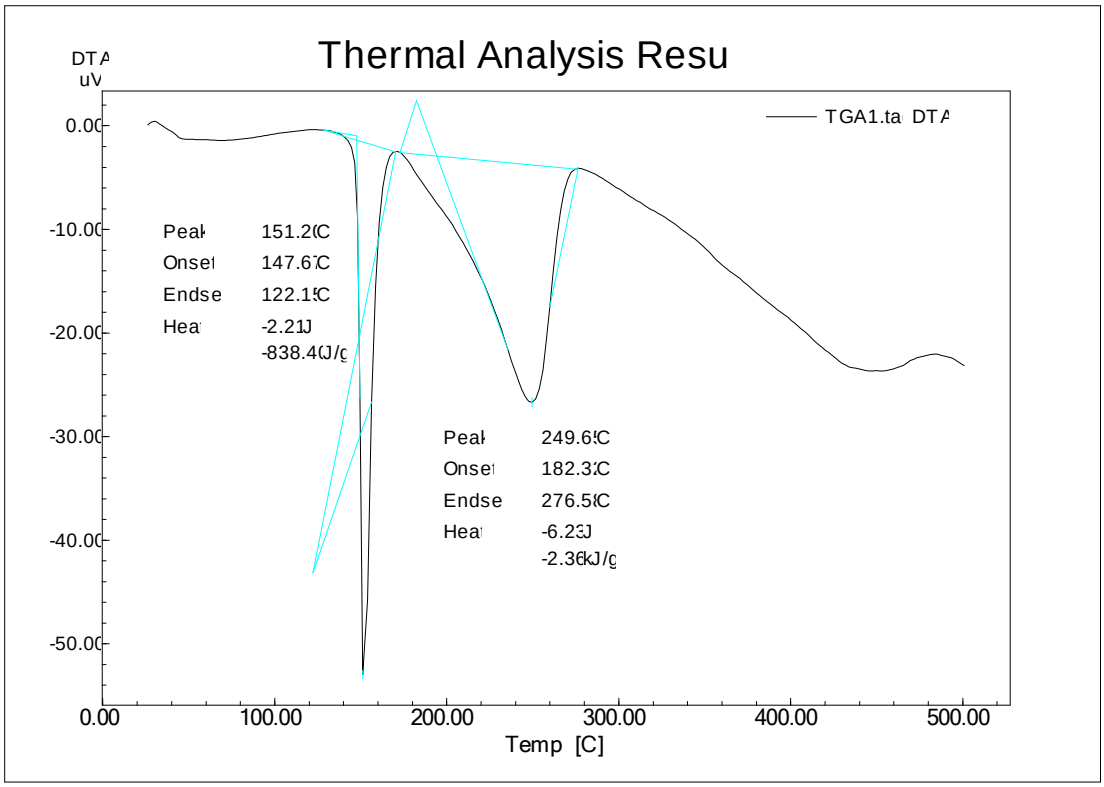


Figure 11.23: DTA thermogram of pristine aceclofenac

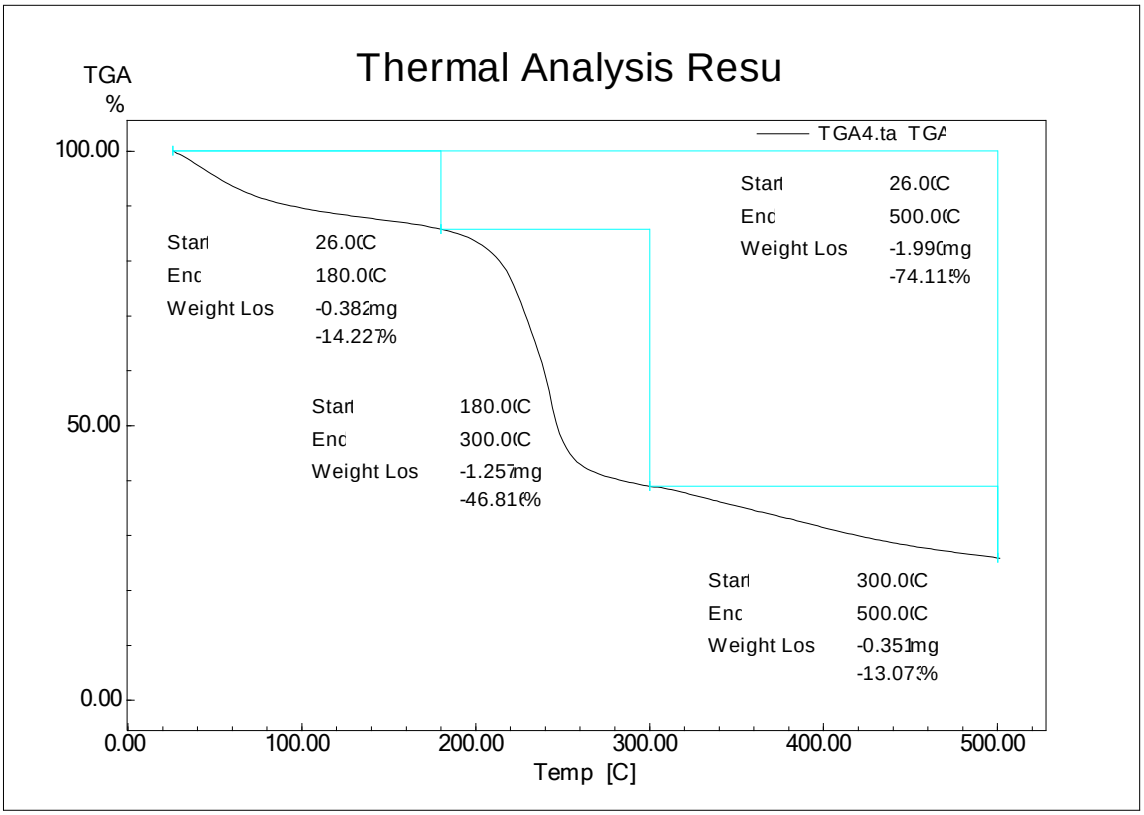


Figure 11.24: TGA thermogram of tablet (MT10 batch).

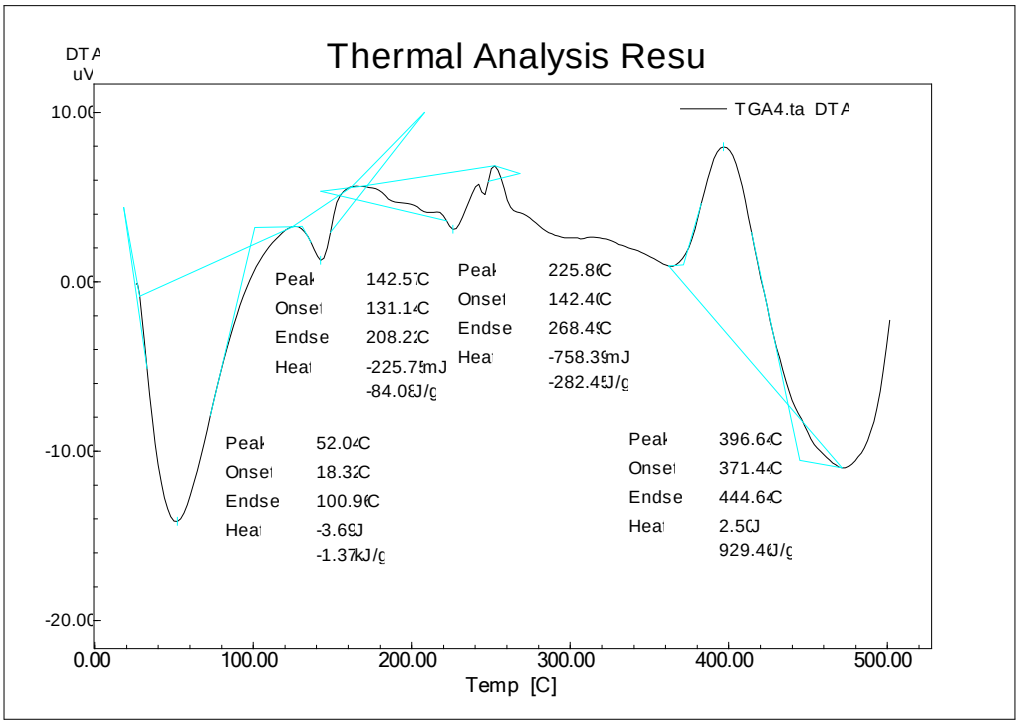


Figure 11.25: DTA thermogram of tablet (MT10 batch).

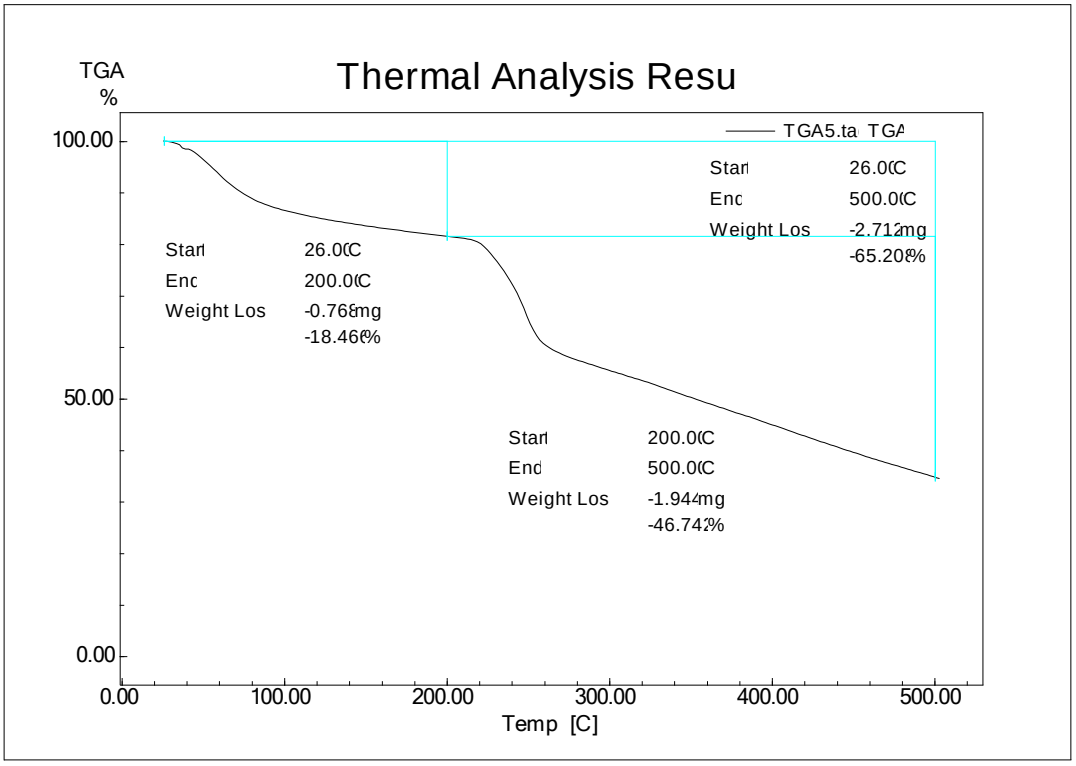


Figure 11.26: TGA thermogram of blank tablet (MT10 batch) without drug.

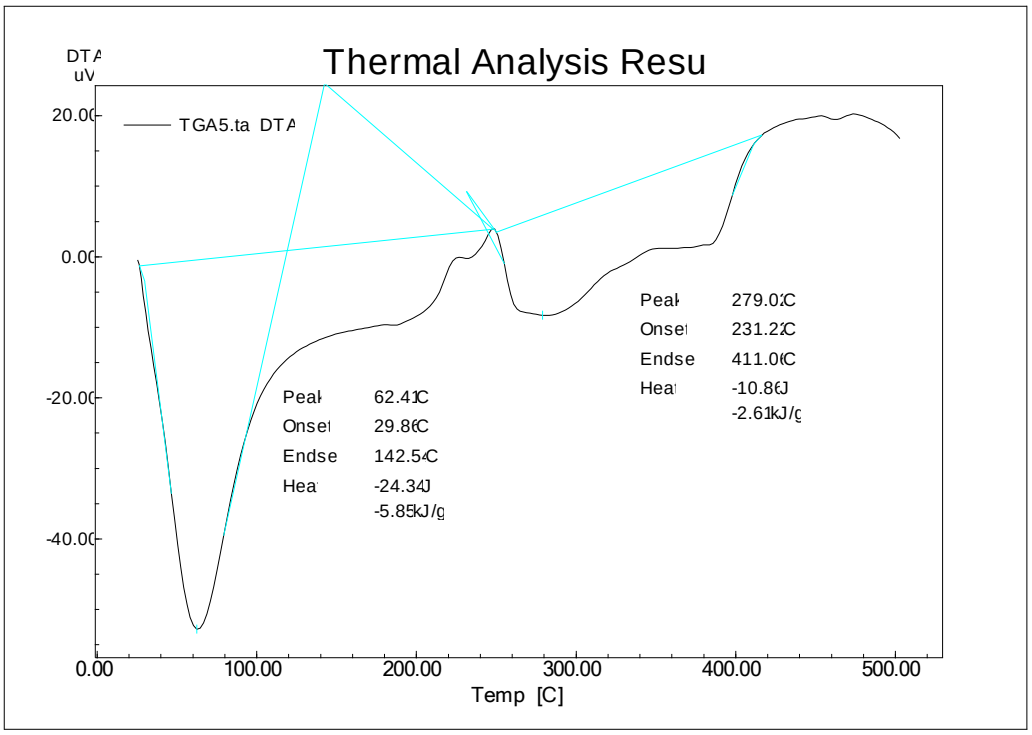


Figure 11.27: DTA thermogram of blank tablet (MT10 batch) without drug.

11.2.4.5. Thermal study (DSC, TGA and DTA)

DSC-thermograms of native CFSG, CFSG-g-PSA, pristine aceclofenac, tablet formulation and placebo tablet (drug-free) are presented in **Figure 11.18** and TGA and DTA curves are presented in **Figures** from **11.19 to 11.27**. DSC-thermogram of native CFSG shows an endothermic peak at 90.6°C with enthalpy of 240.9 J/g and corresponding TGA and DTA curves show 7.9% weight loss in the range from 35.1°C to 200.0°C and an endothermic peak at 70.2°C, respectively, which might be due to evaporation of water present in the powder as moisture. The exothermic peak at 300.3°C with enthalpy of 75.9 J/g exhibited in DSC-curve designates decomposition of polymer chains and consequent formation of carbon mono-oxide, methane and water [6], which is further substantiated by 57.7% weight-loss in the range 200°C - 350°C and another exothermic peak at 301.8°C found in TGA and DTA-curves, respectively. The endothermic peak at 76.3°C with enthalpy of 65.9 J/g exhibited by

DSC-curve of CFSG-g-PSA, 12.35% weight-loss in the range 35°C - 200°C (TGA-curve) and endothermic peak at 59.9°C (DTA-curve) indicate vaporization of moisture present in the powder of grafted-copolymer. The characteristic peaks at 243.8°C (enthalpy 180.0 J/g) and 369.6°C (enthalpy 62.8 J/g) shown in DSC-curve along with corresponding weight-loss by 18.1% in the range 200°C - 320°C and 31.9% weight-loss in the range 320°C - 500°C found in TGA-curve indicate the decomposition of grafted-copolymer at different points of polysaccharide-backbone as well as grafted side-chains. The peak at 335.9°C (onset at 215.2°C and end at 435.4°C) exhibited by DTA-curve substantiates the polymer-decomposition. The DSC-curve obtained from pristine aceclofenac shows a very sharp and narrow endothermic peak at 153.3°C with enthalpy of 125.5 J/g, which signifies the melting point of pure aceclofenac [21]. This is substantiated by the presence of another sharp endothermic peak at 151.2°C in the corresponding DTA-curve. The narrow and sharp peak also indicates the crystalline nature of aceclofenac in its pure form. The endothermic peak at 81.1°C exhibited in DSC-curve obtained from tablet formulation indicates moisture-loss, whereas, another sharp endothermic peak at 153.4°C designates the melting point of aceclofenac present in the tablet. Increase in the melting point of the drug in the tablet by only 0.1°C may be due to presence of additives. Absence of the characteristic melting point peak of the drug at 153.4°C or around in the DSC-curve of drug-free blank tablet confirms that the peak at 153.4°C in DSC-curve of tablet is only for melting of the drug. Overall, these features of DSC-curves obtained from tablet and drug-free tablet formulation indicate good compatibility between drug and additives.

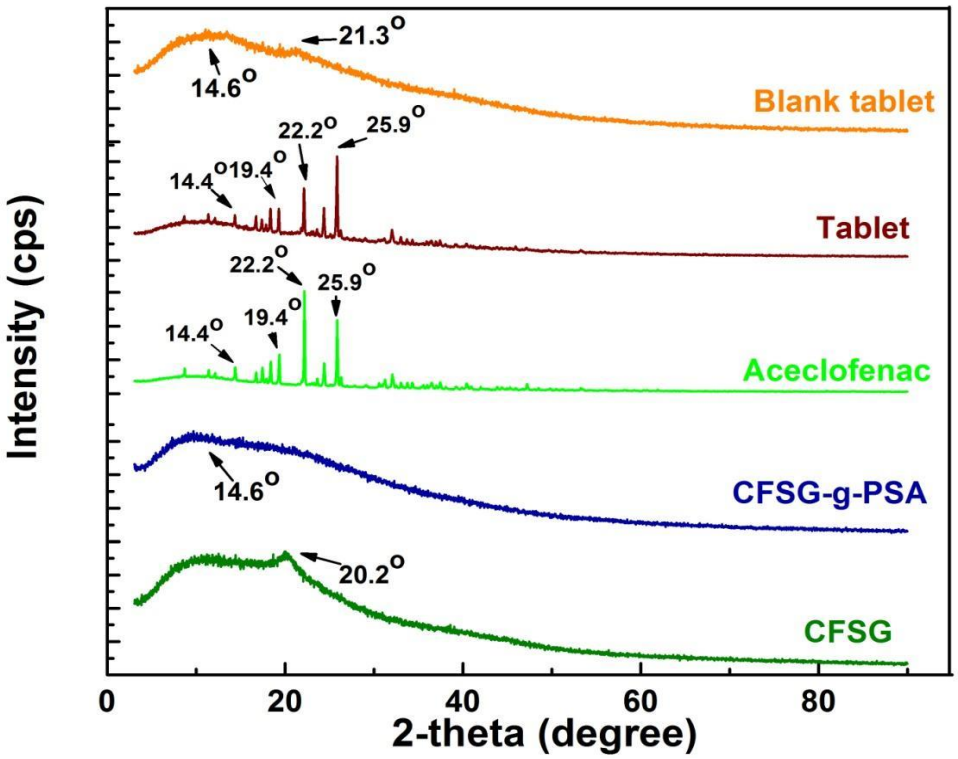


Figure 11.28. Powder X-Ray diffractograms of DSC thermograms of native CFSG, CFSG-g-PSA (S1), aceclofenac, tablet formulation (MT10) and blank tablet (drug free).

11.2.4.6. Powder XRD

X-ray diffractograms of CFSG, CFSG-g-PSA, pristine aceclofenac, tablet and blank tablet are presented in **Figure 11.28**. The diffractogram of native CFSG shows only two broad peaks at 2θ of 14.6° and 20.2° (relatively sharper), which signify the amorphous nature of the native CFSG powder [7]. The diffractogram of CFSG-g-PSA exhibits almost the same pattern with only one broad peak at 2θ of 14.6° and some noise, which corresponds to the withholding of structural integrity of the polysaccharide-backbone even after grafting. Although crystalline nature of grafted form to some extent was observed upon visual observation and rubbing on the hand surface with finger-tip, which was not reflected in the diffractogram. The diffratogram of aceclofenac shows several distinctive sharp and intensive peaks at 2θ of 8.7°, 11.4°, 12.1°, 14.4°, 16.8°, 17.5°, 17.8°, 18.4°, 19.4°, 22.2°, 23.6°, 24.4°,

25.9°, 26.3°, 31.2°, and 32.1°, which characterize the crystalline nature of pristine aceclofenac sodium [21]. The diffractogram obtained from the tablet was found to retain all the characteristic peaks of pristine aceclofenac, which indicates that the drug remains interspersed in crystalline form in tablet matrix and no significant drug-additive incompatibility is present. No distinctive peak of aceclofenac was found in the diffractogram of blank tablet except the pattern found in that of grafted form of CFSG.

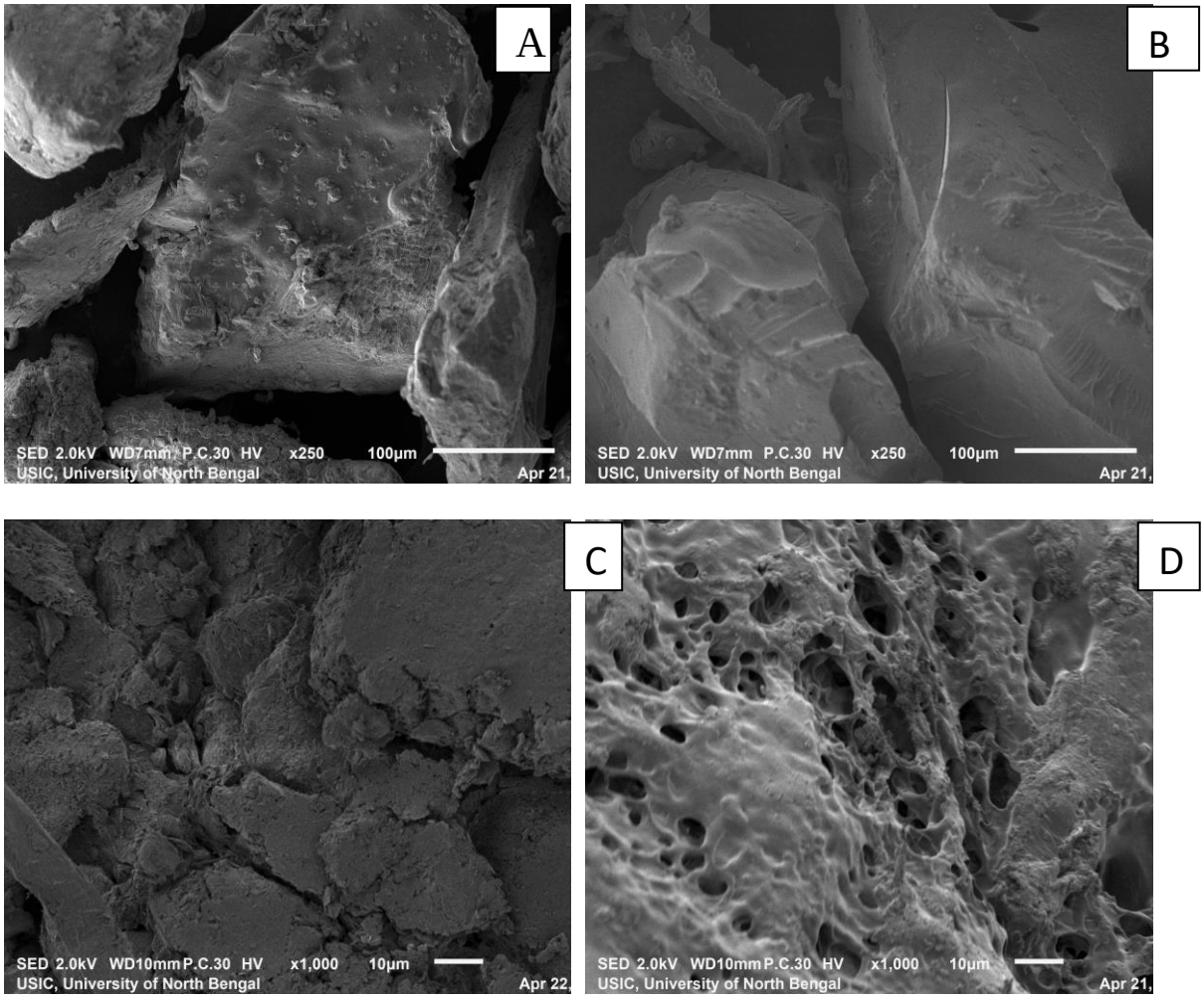


Figure 11.29. SEM photographs of (A) native CFSG, (B) CFSG-g-PSA (S1), (C) tablet surface before drug-release and (D) tablet surface after drug-release.

11.2.4.7. SEM study

Figure 11.29 depicts the SEM-micrographs of powders of native CFSG and CFSG-g-PSA copolymer along with pre- and post-dissolution tablet-surface. The micrograph obtained from the dry powder of native CFSG (**Figure 11.29A**) shows pebble-like asymmetric granular poly-disperse particles with irregular shape. The surface of the particles also shows partly smooth and partly rough appearance with presence of embedded smaller particles onto the surface. The micrograph of CFSG-g-PSA copolymer (**Figure 11.29B**) shows nearly similar type particles having sharper edge and the appearance of the surface is relatively smoother and like that of a xerogel. The micrograph of the pre-dissolution dry tablet surface

(**Figure 11.29C**) shows a rough surface texture with presence of compressed slugs, though visual observation finds a glossy smooth surface of the uncoated tablets. The micrograph of post-dissolution dry tablet surface (**Figure 11.29D**) portrays honeycomb-like appearance and presence of numerous pores running inwards to form interstitial channels having tortuosity. This image demonstrates the drug-release through these water-filled channels by diffusion process.

11.2.4.8. Viscosity and pH measurement

The expression relating viscosity of a polymeric colloidal dispersion with molecular weight of the polymer $[(\eta/\eta 0) - 1] = ckM^a$ has been derived from the so-called Mark-Houwink equation where, η, $\eta 0$, c, M, k and a are the viscosity of polymeric colloidal dispersion, viscosity of dispersion medium, concentration (%w/v), molecular weight of the polymer, and constants indicating the characteristics of the particular polymer-dispersion medium combination, respectively. This relation demonstrates that viscosity of the polymeric colloidal dispersion is proportional to the molecular weight of the polymer when concentration and viscosity of dispersion medium are kept constant [22]. Viscosities of native CFSG and its grafted copolymers of different batches have been presented in **Table 11.10**. Viscosity of 0.1% w/v aqueous dispersion of native CFSG was found to be 755.6 cp whereas S1 batch (790%G), S2 (353%G), S6 (570%G) and S8 (286%G) exhibited viscosity of 1492.3 cp, 1057.7 cp, 1202.5 cp and 993.4 cp, respectively. In microwave-batches with 1 min MW (S1, S2, S4 and S5), viscosity was found to increase with increase in molecular weight as well as %grafting almost proportionally, which may attribute to that increase in %grafting results in increase in molecular weight and subsequent increase in viscosity. The results of these low-microwave batches were found to correlate the Mark-Houwink equation. In higher-microwave-batches (S3, S6, S7 and S8), viscosity was found to increase with increase in %grafting proportionally, but the molecular weight was not found to

increase significantly because of breakage of polysaccharide backbone at different points by longer MW-irradiation above threshold-point. Increase in viscosity may be due to increase in molecular-entanglement as a result of grafting of side chains. Non-microwave batches (H1, H2, H3 and H4) showed nearly same viscosity as native CFSG because of very low degree grafting. The viscosity, i.e., resistance to flow of colloidal polymeric dispersion is function of the degree of molecular linearity, degree of branching emanating from the main polymeric-backbone and molecular-entanglement. Higher grafting yields higher branching → higher entanglement → higher viscosity. The pH of native CFSG was found to be 7.08 which indicates its neutral non-ionic nature. As per literature, CFSG is a nonionic galactomannan. The pH of CFSG-g-PSA (S1 batch) was found to be 5.8 which indicates its weakly acidic nature. This weakly acidic nature of CFSG-g-PSA may be due to dissociation of -COONa and $-COO^-$ in aqueous media. This weakly acidic nature may increase its affinity towards mucin molecules by forming hydrogen bonding and impart mucoadhesiveness. Being weakly acidic, it can be expected that this grafted copolymer would not be harmful to mucous membrane upon peroral administration.

11.2.4.9. Water uptake study

The sustained-release oral tablets composed of natural polysaccharide get hydrated in gastrointestinal fluids resulting in formation of a swollen gel layer around them, which governs the drug-release principally. Water-uptake study was performed in 0.1 N HCl acid in order to understand the influence of hydration on drug-release. The water uptake ($\%W_E$) by native CFSG and its grafted copolymer of different grades are presented in **Table 11.10**. Native form shows 136.8±3.2% equilibrium water uptake ($\%W_E$) designating its hydrophilic nature, which may attribute to the presence of numerous –OH groups of mannose and galactose residues and $–CH_2OH$ groups of galactose residues in its polysaccharide-

backbone. All grades of its grafted form show relatively higher water-uptake compared to native form, which may be due to the presence of numerous hydrophilic $–COO^-$ in grafted poly(acrylate) side-chains. CFSG-g-PSA of S1 batch with 790%G shows highest water-uptake of 286.4±4.6%, whereas, in other batches, water-uptake was found to increase with increase in %grafting, which might be due to increase in number of hydrophilic $–COO^-$ ions with increase in grafting. Furthermore, higher grafting may increase the intensity of the polymeric network and impart higher capacity to hold more water mechanically.

Table 11.13. % Weight loss of the CFSG-g-PSA (S1 batch) in different medium at different intervals in biodegradability study

Test period	**% Weight loss in different test medium (Average ± S.D.)**				
	Distilled water	Simulated gastric fluid	Simulated intestinal fluid	Simulated colonic fluid	Simulated colonic fluid with probiotics
24 h	0.8± 0.07	0.32 ± 0.05	6.2±0.4	-	-
7 days	-	-	-	8.3± 0.9	33.8±3.5
14 days	-	-	-	17.6±0.3	52.3±3.9

11.2.4.10. Biodegradation study

Percent weight-loss of CFSG-g-PSA at different time-points of biodegradation study is presented in **Table 11.13**. Weight-loss of the grafted copolymer in distilled-water and simulated gastric fluid in 24 h was found to be negligible, which may be due to swelling of the hydrophilic copolymer in distilled-water rather than surface-erosion as found in water-uptake study and low solubility of this acidic acrylic copolymer in acidic gastric fluid. Further, acid present in SGF with the concentration of only 0.1N is not sufficient for hydrolysis of polysaccharide and the proteolytic enzyme pepsin has no activity on the polysaccharide-backbone of CFSG-g-PSA. A weight-loss by 6.2% (±0.4) was found in presence of sodium hydroxide, potassium phosphate and the enzymes lipase, amylase and

trypsin in SIF, which may be due to partial hydrolysis of polysaccharide-chain into mono- or disaccharides by amylase, more ionization of –COONa groups in alkaline media, augmentation in aqueous solubility and subsequent surface-erosion. Weight-loss by 17.6% (±0.3) was observed in 14 days period in SCF in presence of sodium, potassium, chloride and phosphate ions, which may attribute to same effect of SIF except the effect of amylase. A very significant weight-loss by 52.3% (±3.9) in 14 days period was found in SCF with normal colonic flora, which might attribute to hydrolysis of polysaccharide-backbone of the grafted-copolymer by extracellular enzymes secreted from microbial cells into monosaccharide residues such as mannose and galactose and their subsequent microbial uptake and metabolic utilization as a source of carbon essential for microbial growth [23]. Thus, this study indicates the biodegradable nature of CFSG-g-PSA.

Table 11.14. Different evaluation parameters of mucoadhesive sustained release tablets of aceclofenac with CFSG-g-PSA

Batch	Thickness (mm) (n=10)	Diameter (mm) (n=10)	Average weight(mg) (n=10)	Hardness (kg/m^2) (n=10)	Drug content (%) (n=20)	Disintegration time (n=6)	Friability (%, w/w) (n=3)	Muco-adhesion time (h)	Muco-adhesive strength (g) (n =3)
MT1	2.80±0.03	10.05±0.02	362.8± 4.8	4.4±0.13	98.5±0.5	Large fragmentation at 5.5±4 h	0.45±0.12	3.8±0.08	19.3± 1.5
MT2	2.81±0.02	10.04±0.03	362.3± 3.0	4.4±0.18	98.9±0.3	No Disintegration	0.22±0.04	> 10	88.3±2.5
MT3	2.81±0.04	10.04±0.04	362.5± 4.5	4.2±0.24	99.2±0.7	No Disintegration	0.29±0.09	> 10	53.3±0.6
MT4	2.78±0.02	10.05±0.02	367.0± 3.4	4.5±0.34	98.2±0.4	No Disintegration	0.25±0.10	7.2±0.7	25.6±1.2
MT5	2.78±0.02	10.06±0.03	359.9± 4.0	4.7±0.16	99.8±0.5	No Disintegration	0.32±0.07	8.5±0.4	31.0±1.0
MT6	2.81±0.04	10.03±0.05	357.3± 1.6	4.1±0.22	98.4±0.3	No Disintegration	0.32±0.05	9.2±0.9	37.3±0.6
MT7	2.80±0.02	10.04±0.02	365.2± 2.6	4.8±0.30	101.7±0.7	No Disintegration	0.30±0.04	>10	67.3±0.6
MT8	2.79±0.04	10.05±0.05	357.7± 4.4	4.3±0.19	98.7±0.5	No Disintegration	0.38±0.05	8.7±0.4	35.0±1.0
MT9	2.82±0.01	10.00±0.03	367.7± 3.1	4.8±0.33	101.6±0.4	No Disintegration	0.43±0.07	>10	45.7±0.6
MT10	2.80±0.03	10.05±0.04	309.1± 3.5	4.6±0.42	98.7±0.3	No Disintegration	0.42±0.05	>10	80.3±1.5
MT11	2.77±0.02	10.05±0.02	416.0± 3.1	4.4±0.50	103.5±0.6	No Disintegration	0.20±0.06	>10	92.3±2.5
MT12	2.82±0.04	09.99±0.05	360.3± 2.0	4.6±0.20	100.7±0.5	No Disintegration	0.24±0.08	-	-
MT13	2.79±0.03	10.02±0.03	362.5± 3.6	4.6±0.29	99.1±0.4	Fragmentation at 6.5±0.7 h	0.47±0.05	>10	82.7±1.5

11.2.5. Evaluations of tablets

The results of physical characterization of the tablets of different batches are presented in **Table 11.14**. The variation in dimension (thickness and diameter) was found to be very less and within prescribed limit as per Indian Pharmacopoeia, 2018. Weight variation was also within ±5%. Hardness and friability of the tablets were within the range 4 – 5 kg/m^2 and well below 1%, which indicate their sufficient mechanical strength. Drug content was also found within the prescribed limit. Non-disintegration of the tablets composed of grafted-copolymer shows the structural integrity, cohesiveness and binding capacity of the polymeric matrix. Drug-release study also exhibits the retention of the intactness of the tablet-matrix throughout the whole period of the study.

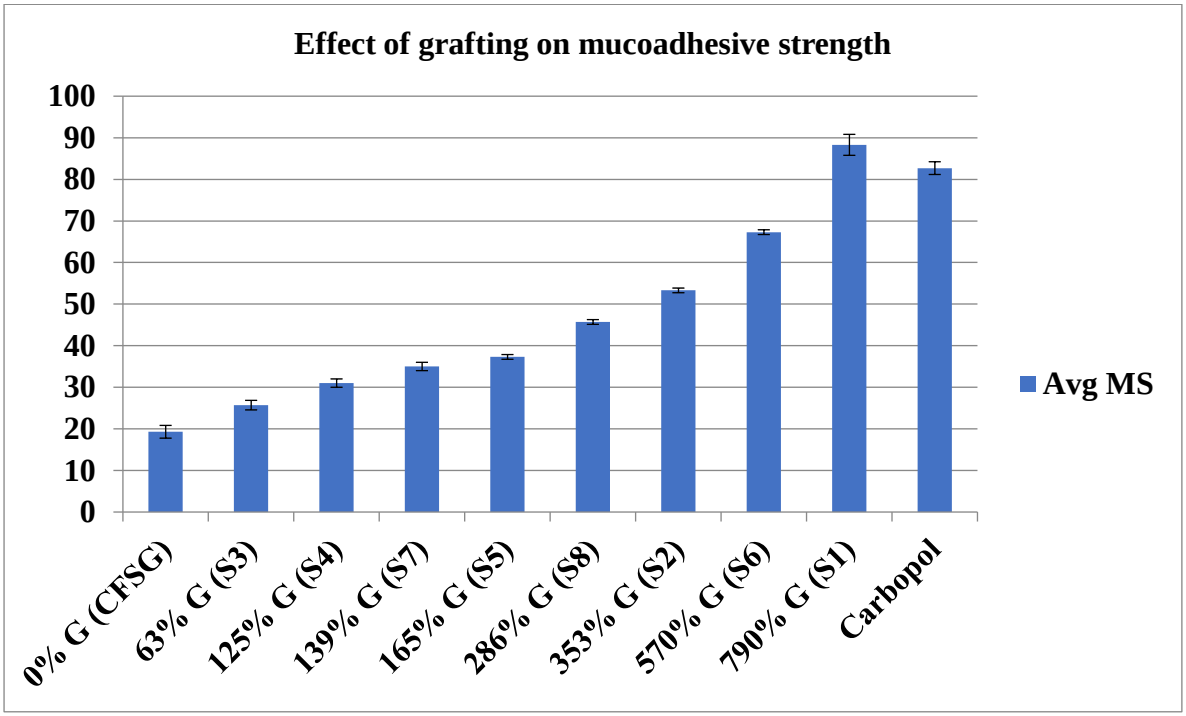


Figure 11.30. Effect of grafting on mucoadhesive strength

11.2.6. Ex-vivo mucoadhesion study

Table 11.14 presents different mucoadhesion-times and strengths exhibited by the tablets of various batches. The tablet composed of native CFSG (MT1) showed mucoadhesion to some extent (mucoadhesion-time 3.8±0.08 h and strength 19.3 ± 1.5 g), which may be due to

presence of –OH and –CH_2OH groups in mannose and galactose units. This extent of mucoadhesion is required to improve for sustained-release mucoadhesive devices. The tablets composed of different grades of CFSG-g-PSA showed significantly higher mucoadhesion time and strength indicating their strong adhesion to the goat- stomach-mucosa. The tablets were also found to retain the integrity of their matrices throughout the entire period with little surface-erosion. The improved mucoadhesion might attribute to the presence of numerous –COO^- ions in poly(acrylate) side-chains grafted onto CFSG-backbone, which get protonated in acidic gastric fluid and form numerous hydrogen bonds with mucin molecules. Further, acidic pH diminishes the degree of ionization of COOH groups, which results in increased affinity of COOH groups towards mucin molecules rather than water [15]. The effect of grafting on mucoadhesion-strength is depicted in **Figure 11.30**. The tablets of MT2, MT3, MT7 and MT9 batches composed of CFSG-g-PSA with higher %grafting exhibited excellent mucoadhesion over 10 h along with greater mucoadhesive strength, whereas, the tablets of MT4, MT5, MT6 and MT8 batches composed of the copolymer-grades with lower %grafting exhibited mucoadhesion for a comparatively shorter period of time and with lower strength. This may attribute to the presence of more acrylate ions in the grades of CFSG-g-PSA copolymer having higher degree grafting, more hydrogen-bond formation with mucin-molecules and thereby stronger attachment with mucus over a prolonged-period of time compared to copolymer-grades with lower %grafting. The tablets composed of CFSG-g-PSA copolymer with 790%G (S1 batch) were found to exhibit comparable mucoadhesion time and strength to that exhibited by the tablets composed of carbopol 974P considered as potential synthetic mucoadhesive polymer [24].

Table 11.15. Different drug-release kinetic parameters and release comparison factors

Batch	R^2 value						Rate constant (*K*)					$T_{50\%}$ (hr)	$T_{90\%}$ (hr)	f_1	f_2
	Zero order	First order	Higuchi kinetic	HC	KP		Zero order	First order	Higuchi kinetic	HC	KP				
					R^2	n									
MT1	0.987	0.903	0.996	0.748	0.995	0.685	0.120	0.205	0.447	0.152	0.267	2.4	5.9	108.4	19.8
MT2	0.998	0.919	0.968	0.982	0.997	1.054	0.069	0.244	0.292	0.032	0.059	7.5	13.3	12.1	66.9
MT3	0.998	0.916	0.980	0.964	0.998	0.829	0.081	0.191	0.345	0.048	0.126	5.4	10.3	28.1	48.9
MT4	0.975	0.857	0.995	0.846	0.988	0.707	0.098	0.173	0.404	0.113	0.224	2.9	7.3	89.7	23.9
MT5	0.979	0.868	0.991	0.763	0.991	0.731	0.091	0.164	0.392	0.102	0.195	3.4	8.1	*74.*9	27.7
MT6	0.996	0.901	0.989	0.950	0.998	0.770	0.086	0.177	0.366	0.059	0.159	4.4	9.4	48.9	36.9
MT7	0.998	0.929	0.968	0.964	0.997	0.907	0.079	0.212	0.336	0.043	0.098	6.1	11.1	12.3	65.8
MT8	0.983	0.847	0.989	0.889	0.982	0.779	0.089	0.175	0.379	0.075	0.169	3.7	8.9	62.1	31.9
MT9	0.996	0.903	0.982	0.949	0.997	0.813	0.084	0.187	0.356	0.053	0.137	4.9	10.1	37.5	42.8
MT10	0.998	0.924	0.971	0.979	0.998	0.975	0.076	0.226	0.320	0.038	0.079	6.6	11.9	4.5	83.4
MT11	0.987	0.926	0.937	0.969	0.997	1.258	0.061	0.292	0.255	0.026	0.031	9.1	14.7	35.0	44.1
MT12	0.995	0.932	0.958	0.981	0.988	1.124	0.067	0.263	0.281	0.030	0.049	8.1	14.0	21.3	55.2
Marketed tablet	0.995	0.960	0.956	0.969	0.985	0.875	0.072	0.207	0.303	0.036	0.092	6.8	12.3	-	-

HC: Hixon-Crowell; KP: Korsemeyer-Peppas

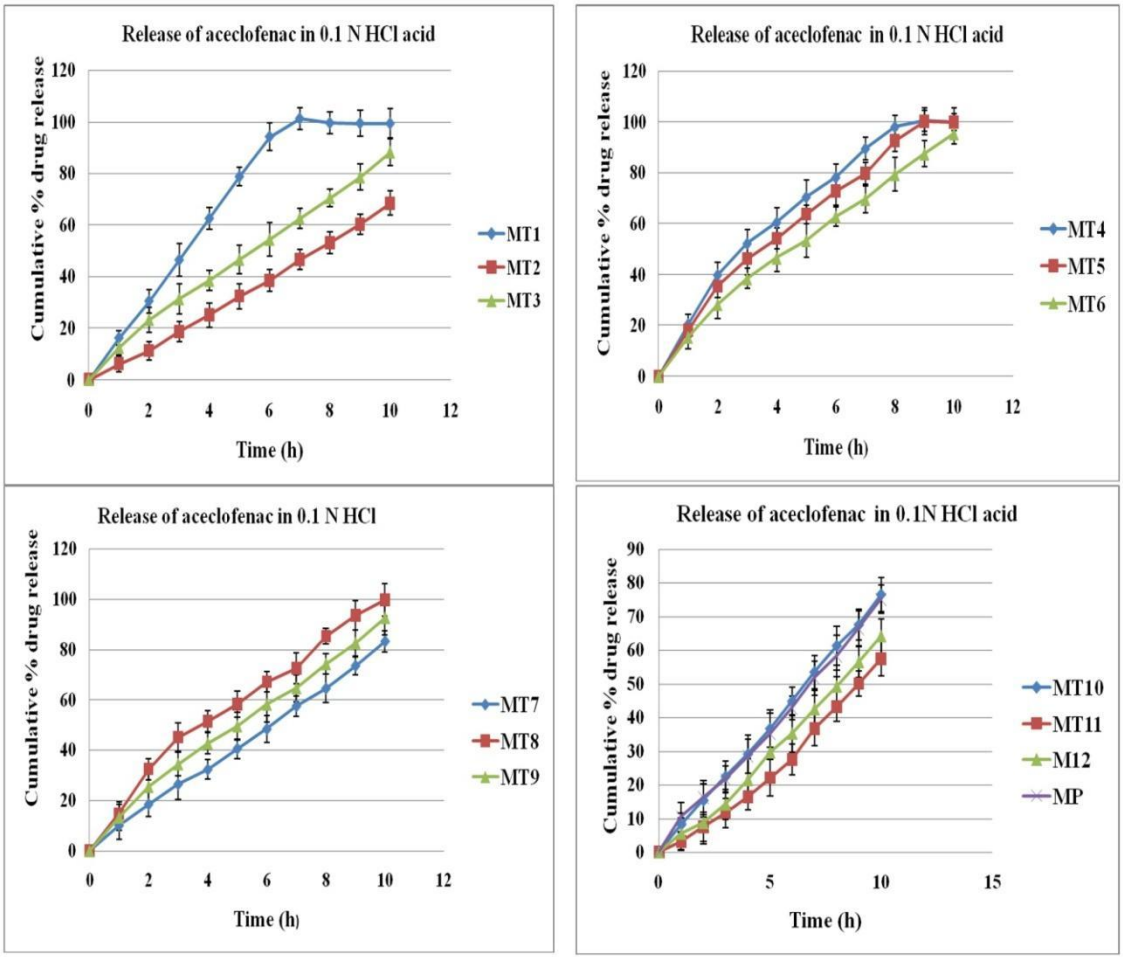


Figure 11.31. Zero order drug release curves for different batches of mucoadhesive tablets of aceclofenac (cumulative % drug-release versus time).

11.2.7. In-vitro drug-release study

Figure 11.31 portrays zero-order drug-release curves (cumulative percentage drug-release versus time) obtained from different tablet formulations. **Table 11.15** presents regression-coefficients of different kinetic models, release-exponents, rate-constants, $T_{50\%}$, $T_{90\%}$ and release similarity and difference factors.

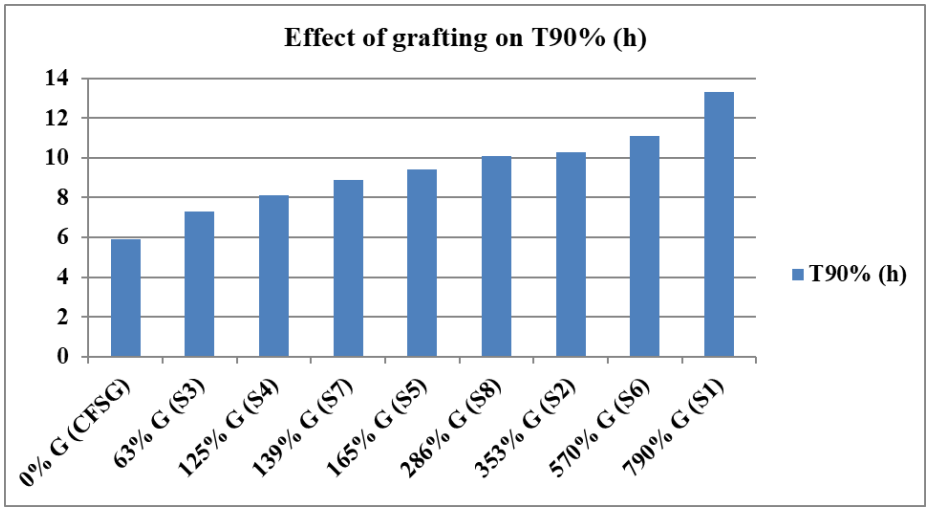


Figure 11.32. Effects of %grafting on drug release

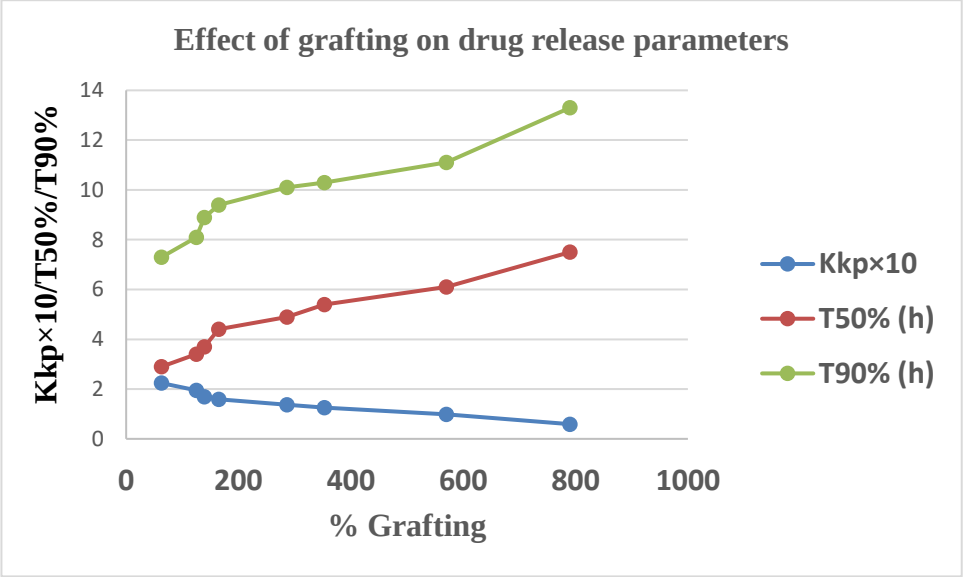


Figure 11.33. Effects of grafting on T50%, $T_{90\%}$ and Rate-constant (K_{kp}).

11.2.7.1. Effect of %grafting on drug-release parameters

The tablets (MT1) composed of native CFSG have shown release of aceclofenac at relatively rapid-rate (rate-constant K_{kp} 0.267 h^{-1}; $T_{50\%}$ 2.4 h; $T_{90\%}$ 5.9 h), whereas, the tablets composed of CFSG-g-PSA with lower %G (MT4, MT5, MT8, and MT6 batches) have shown drug-release at moderately-sustained rate (K_{kp} 0.159 – 0.224 h^{-1}; $T_{50\%}$ 2.9 – 4.4 h; $T_{90\%}$ 7.3 – 9.4 h) and the tablets composed of grafted-copolymer with higher %G (MT2, MT3, MT7 and

MT9 batches) have shown drug-release at significantly-sustained-rate (K_{kp} 0.059 – 0.137 h^{-1}; $T_{50\%}$ 4.9 – 7.5 h; $T_{90\%}$ 10.1 – 13.3 h). **Figure 11.32** represents the effect of %grafting on drug release and **Figure 11.33** represents the changes in release-rate-constant (K_{kp}), $T_{50\%}$ and $T_{90\%}$ with increase in % grafting. The curves exhibit that release-rate-constant reduces with increase in % grafting, whereas $T_{50\%}$ and $T_{90\%}$ both are found to increase accordingly, which portends augmentation in sustained-release-capacity of the matrix with increasing grafting. This effect of grafting on the release parameters is also found to reflect in the following relations between release parameters and % grafting expressed in Eqs. (4) – (6):

$K_{kp}\times10$ (h^{-1}) = -0.001 %G + 1.930 (R^2 = 0.830) (Eq.4)

$T_{50\%}$ (h) = 0.003 %G + 3.376 (R^2 = 0.891) (Eq.5)

$T_{90\%}$ (h) = 0.004 %G + 8.088 (R^2 = 0.888) (Eq.6)

The relatively higher drug-release-rate from the matrix composed of native CFSG may attribute to comparatively lesser hydration → formation of less-intensive polymeric front gel network with relatively lower thickness → advance chain relaxation→ relatively rapid erosion of front gel layer in drug-release medium due to shear. The tablets composed of grafted-copolymers show relatively more sustained-release profile compared to native CFSG, which may be due to the fact that grafting imparts molecular branching to the linear polysaccharide-backbone of CFSG resulting in enhancement in molecular-entanglement and augmented network-density and rigidity. Moreover, presence of numerous hydrophilic – COO^- ions in acrylate moieties of poly(acrylate) side-chains of grafted-CFSG increases the hydration capacity, which results in formation of denser and firmer outer (front) gel-layer with relatively more thickness when placed in release-medium. This front gel-layer around the tablet governs the drug-diffusion from the tablet-interior to the release-medium. The higher density and thickness of the front gel-layer diminish the diffusion rate and thereby resulting in sustained-drug-release. The enhanced firmness of the gel-network is also lesser

prone to erosion upon shear experienced by the front-layer of the tablet in release-medium. Increase in grafting of side-chains with hydrophilic monomeric units results in a polymeric matrix with enhanced molecular branching, entanglement, network-density, firmness and hydration, which slows down the rate of drug-release. The lowest values of rate-constant (K_{kp}; Korsemeyer-Peppas model) and highest values of $T_{50\%}$ and $T_{90\%}$ obtained from MT2 (S1; 790%G), MT3 (S2; 353%G), MT7 (S6; 570%G) and MT9 (S8; 286%G) portend their sustained-release potential.

11.2.7.2. Effect of water-uptake (swelling) on drug-release

The drug-release from hydrophilic polysaccharide-based matrix is greatly governed by its hydration-capacity, rate and extent of water-uptake, subsequent swelling and gel-formation. The results of water-uptake and drug-release study exhibit that rate-constant (K_{kp}) decreases and $T_{50\%}$ and $T_{90\%}$ increase with increase in water-uptake ($\%W_E$). The tablets (MT2) composed of CFSG-g-PSA of S1-batch with 790%G and highest water-uptake (286.4±4.6%) shows lowest rate-constant (0.059 h^{-1}) and highest values of $T_{50\%}$ (7.5 h) and $T_{90\%}$ (13.3 h), which portends highest sustained-release capacity. Overall, the result indicates the partially swelling controlled mechanism of sustained drug-release from hydrated-matrix, which is further supported by the values of release-exponent (n) in the range from 0.5 to 1.0 [25].

11.2.7.3. Release kinetic and mechanism

Korsemeyer-Peppas model was found to be best-fitting kinetic model as the regression-coefficients obtained from this model were found to be closest to unity in most batches (**Table 11.15**). The regression-coefficients of zero-order model were also found to be very closest to that of Korsemeyer-Peppas model. The value of the release-exponent, n, demonstrates different release-mechanisms (n = 0.5 for Fickian-diffusion; 0.5 <n < 1 for anomalous non-Fickian transport with combined diffusion and swelling controlled release

mechanism; n = 1 for Case-II transport and zero-order release; n > 1 for super case-II transport) [26]. The release-exponent value was within the range 0.5 – 1.05 in most batches (MT1 to MT10) except MT11, which indicates anomalous non-Fickian transport with combined diffusion and swelling controlled release mechanism. The overall sequence of drug-release includes formation of an outer barrier gel-layer around the tablet after getting hydrated in release-medium → dissolution of drug-particles in water in inner sub-layer of the outer gel layer → diffusion of drug-molecules through the exterior gel-layer to the release medium. The rate of diffusion is function of polymeric network density, molecular entanglement, hydration, degree of swelling, thickness of the barrier gel-layer and the presence of water-filled interstitial channels and their tortuosity. The viscosity of the front gel-layer adjacent to the release medium gradually decreases resulting in slow erosion upon shear from continuous stirring of release-medium and subsequent release of drug molecules [27]. The outer-gel-layer was observed to propagate towards the interior of the matrix with time by breaking the tablet at different intervals during the study. SEM-micrograph exhibits the presence of interstitial channels at the surface and indicates occurrence of partial drug-diffusion through them. Moreover, R^2 values obtained from zero-order model in most of the formulations were found to be very closer to unity, which portends the potential of CFSG-g-PSA copolymer as sustained-release polymer, since, zero-order release-kinetic is considered as ideal kinetic for sustained-release drug delivery systems.

11.2.7.4. Release-similarity and difference factors

The release-profiles of the tablets composed of native CFSG and its grafted-copolymer of various grades were compared to that of marketed product calculating difference, f_1 and similarity factors, f_2 (Table 4). f_1 values nearer to zero and f_2 value nearer to 100 are regarded for evaluation of similarity of the release profile and accepted by the Centre for Drug Evaluation and Research (FDA) and Human Medicines Evaluation Unit of The European

Agency for the Evaluation of the Medicinal Products (EMEA)[26]. Usually, f_1 values between 0 and 15 and f_2 values between 50 and 100 demonstrate the similarity. Only MT2, MT7 and MT10 batches were observed to accomplish the criteria, where the highest similarity was exhibited by the batch MT10 (composed of 100 mg S1 grade of CFSG-g-PSA 790%G) with the lowest value of f_1 (4.5) and the highest value of f_2 (83.4). In this study, MT10 may be considered as the best batch as it shows optimal sustained-release along with highest similarity standard. The results also showed that the sustained-release-profile obtained from the MT10 batch is parallel to that from the MT12 batch composed of HPMC K15M, which further substantiates the potential of CFSG-g-PSA as sustained-release polymer.

11.2.8. Accelerated stability study

At the end of 90 days period, no significant variation in the overall appearance and color of the tablets was found upon visual examination. Other physical characters such as thickness, diameter, weight, hardness, friability, disintegration time and mucoadhesion parameters were almost found to be retained by the tablets. Drug content was found to decrease only by 0.85%. No significant variation was found in zero-order drug-release profile (**Figure 11.34**). Sustained-release parameters such as T50% and T90% were found to reduce very slightly by only 1.34% and 1.52%, respectively. FTIR spectra and DSC thermogram of the aged tablets are presented in **Figure 11.35** and **Figure 11.36** respectively, which demonstrate that all the characteristic FTIR and DSC peaks of the drug are retained as found in that obtained from freshly prepared tablets. All these results designate an optimal stability of the tablets even in a challenging storage condition and a significantly longer shelf-life.

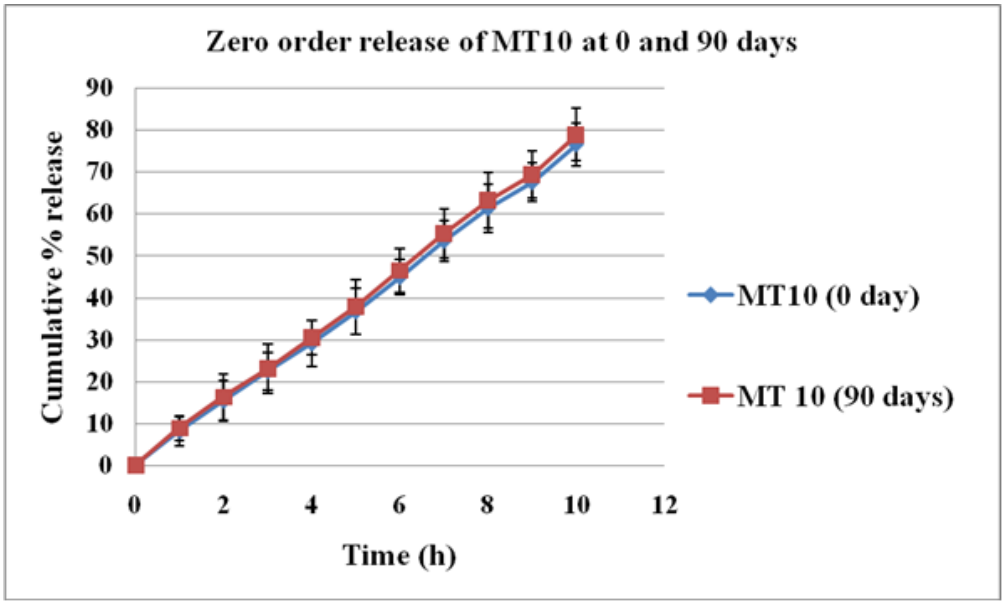


Figure 11.34. Cumulative % drug-release versus time profiles of tablet of MT10 batch at 0 and 90 days.

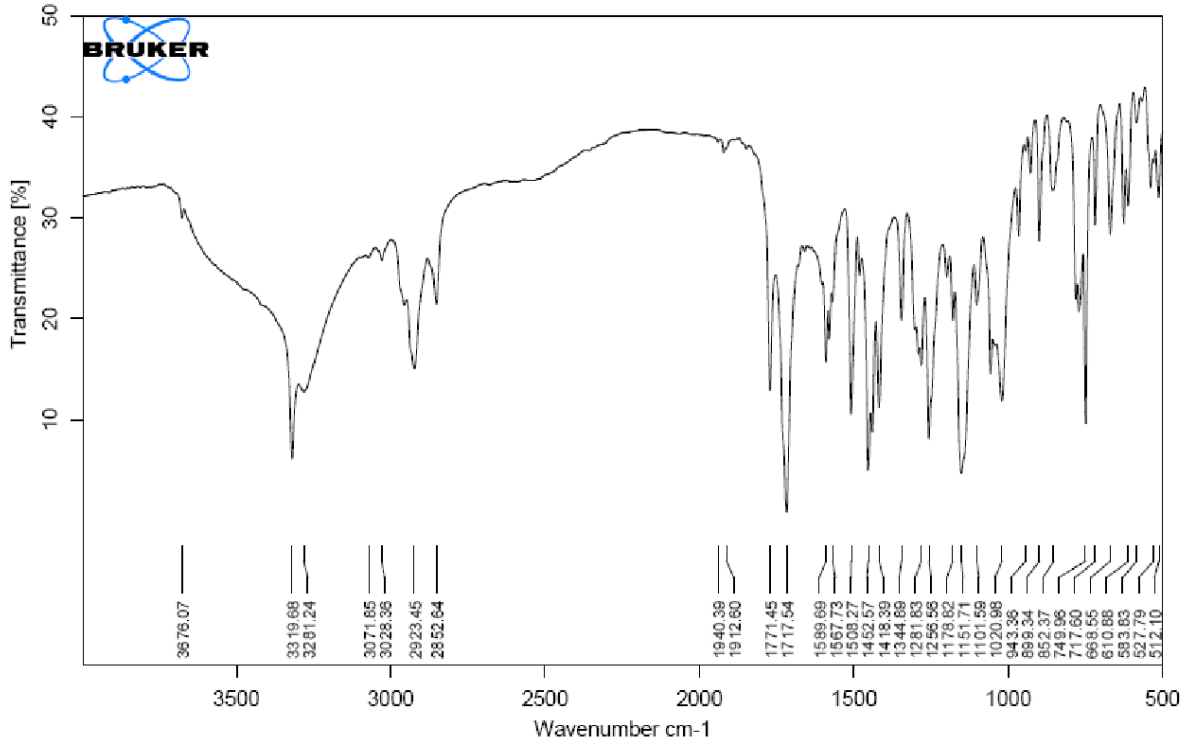


Figure 11.35. FTIR spectra of tablet (MT10 batch) at 90 days.

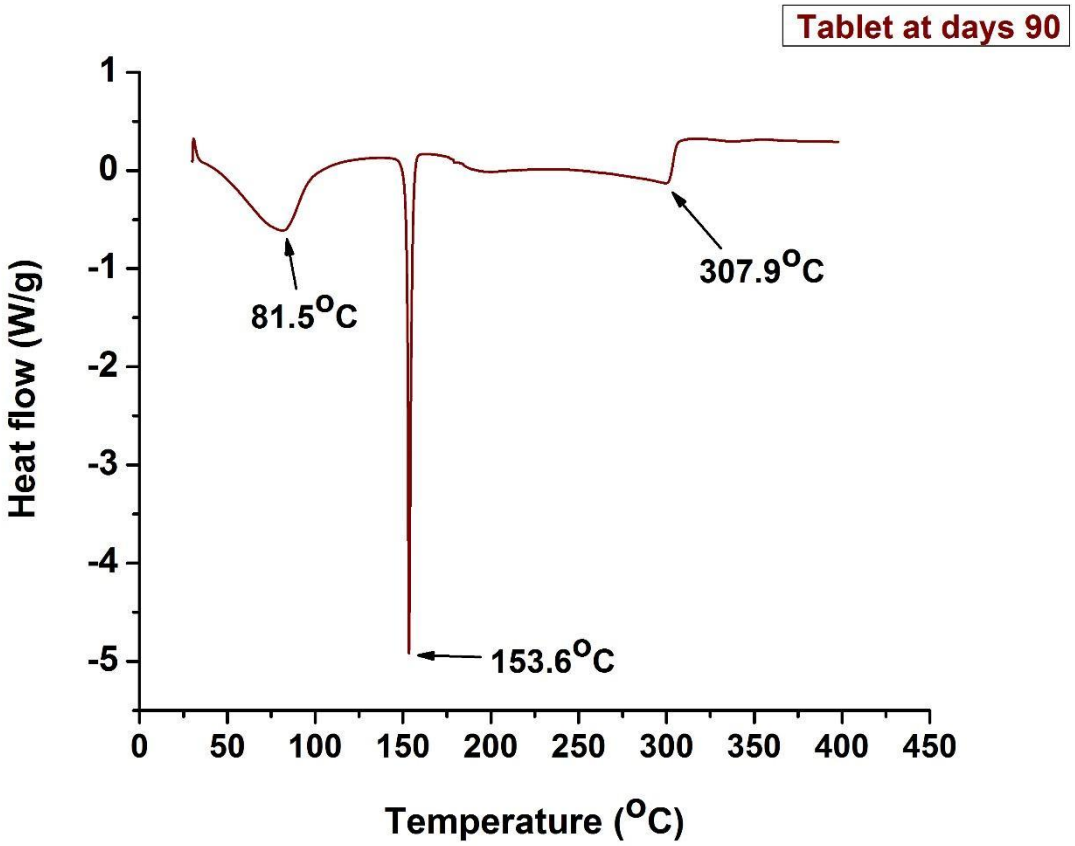


Figure 11.36. DSC thermogram of tablet (MT10 batch) at 90 days.

11.3. References

[1] L.M. da Silva, L.F.S. Araújo, R.C. Alvez, L. Ono, D.A.T. Sá, P.L. da Cunha, R.C.M. de Paula, J.S. Maciel, Promising alternative gum: Extraction, characterization, and oxidation of the galactomannan of Cassia fistula, International Journal of Biological Macromolecules 165 (2020) 436-444.

[2] K. Huanbutta, W. Sittikijyothin, Development and characterization of seed gums from Tamarindus indica and Cassia fistula as disintegrating agent for fast disintegrating Thai cordial tablet, Asian journal of pharmaceutical sciences 12(4) (2017) 370-377.

[3] W.S. York, L.K. Harvey, R. Guillen, P. Alberhseim, A.G. Darvill, Structural analysis of tamarind seed xyloglucan oligosaccharides using β-galactosidase digestion and spectroscopic methods, Carbohydrate Research 248 (1993) 285-301.

[4] P.J. Sinko, Martin's physical pharmacy and pharmaceutical sciences, Lippincott Williams & Wilkins2023.

[5] A. Singh, B. Mangla, S. Sethi, S. Kamboj, R. Sharma, V. Rana, QbD based synthesis and characterization of polyacrylamide grafted corn fibre gum, Carbohydrate polymers 156 (2017) 45-55.

[6] M. Zohuriaan, F. Shokrolahi, Thermal studies on natural and modified gums, Polymer Testing 23(5) (2004) 575-579.

[7] K. Huanbutta, W. Sittikijyothin, Use of seed gums from Tamarindus indica and Cassia fistula as controlled-release agents, Asian journal of pharmaceutical sciences 13(5) (2018) 398-408.

[8] G. Nandi, A.K. Nandi, N.S. Khan, S. Pal, S. Dey, Tamarind seed gum-hydrolyzed polymethacrylamide-g–gellan beads for extended release of diclofenac sodium using 32 full factorial design, International journal of biological macromolecules 114 (2018) 214-225.

[9] P. Sinha, U. Ubaidulla, M.S. Hasnain, A.K. Nayak, B. Rama, Alginate-okra gum blend beads of diclofenac sodium from aqueous template using ZnSO4 as a cross-linker, International journal of biological macromolecules 79 (2015) 555-563.

[10] L. Lachman, H.A. Lieberman, J.L. Kanig, The theory and practice of industrial pharmacy, Lea & Febiger Philadelphia1976.

[11] K. Gowthamarajan, G.K.P. Kumar, N.B. Gaikwad, B. Suresh, Preliminary study of Anacardium occidentale gum as binder in formulation of paracetamol tablets, Carbohydrate Polymers 83(2) (2011) 506-511.

[12] A. Adebayo, O. Itiola, Effects of breadfruit and cocoyam starch mucilage binders on disintegration and dissolution behaviors of paracetamol tablet formulations, Pharmaceutical technology 27(3) (2003) 78-78.

[13] G. Nandi, P. Patra, R. Priyadarshini, S. Kaity, L.K. Ghosh, Synthesis, characterization and evaluation of methacrylamide grafted gellan as sustained release tablet matrix, International journal of biological macromolecules 72 (2015) 965-974.

[14] G. Nandi, A. Changder, L.K. Ghosh, Graft-copolymer of polyacrylamide-tamarind seed gum: Synthesis, characterization and evaluation of flocculating potential in peroral paracetamol suspension, Carbohydrate polymers 215 (2019) 213-225.

[15] D. Sarkar, G. Nandi, A. Changder, P. Hudati, S. Sarkar, L.K. Ghosh, Sustained release gastroretentive tablet of metformin hydrochloride based on poly (acrylic acid)-grafted-gellan, International journal of biological macromolecules 96 (2017) 137-148.

[16] E. Makhado, S. Pandey, P.N. Nomngongo, J. Ramontja, Fast microwave-assisted green synthesis of xanthan gum grafted acrylic acid for enhanced methylene blue dye removal from aqueous solution, Carbohydrate polymers 176 (2017) 315-326.

[17] M. Ganesh, U.J. Jeon, U. Ubaidulla, P. Hemalatha, A. Saravanakumar, M.M. Peng, H.T. Jang, Chitosan cocrystals embedded alginate beads for enhancing the solubility and

bioavailability of aceclofenac, International journal of biological macromolecules 74 (2015) 310-317.

[18] S. Jana, A. Samanta, A.K. Nayak, K.K. Sen, S. Jana, Novel alginate hydrogel core–shell systems for combination delivery of ranitidine HCl and aceclofenac, International journal of biological macromolecules 74 (2015) 85-92.

[19] G. Sen, R. Kumar, S. Ghosh, S. Pal, A novel polymeric flocculant based on polyacrylamide grafted carboxymethylstarch, Carbohydrate Polymers 77(4) (2009) 822-831.

[20] S. Kaity, J. Isaac, P.M. Kumar, A. Bose, T.W. Wong, A. Ghosh, Microwave assisted synthesis of acrylamide grafted locust bean gum and its application in drug delivery, Carbohydrate polymers 98(1) (2013) 1083-1094.

[21] S. Jana, A. Das, A.K. Nayak, K.K. Sen, S.K. Basu, Aceclofenac-loaded unsaturated esterified alginate/gellan gum microspheres: in vitro and in vivo assessment, International journal of biological macromolecules 57 (2013) 129-137.

[22] P. Rani, S. Mishra, G. Sen, Microwave based synthesis of polymethyl methacrylate grafted sodium alginate: its application as flocculant, Carbohydrate polymers 91(2) (2013) 686-692.

[23] V. Rana, P. Rai, A.K. Tiwary, R.S. Singh, J.F. Kennedy, C.J. Knill, Modified gums: Approaches and applications in drug delivery, Carbohydrate polymers 83(3) (2011) 1031-1047.

[24] V. Belgamwar, V. Shah, S. Surana, Formulation and evaluation of oral mucoadhesive multiparticulate system containing metoprolol tartarate: an in vitro-ex vivo characterization, Current drug delivery 6(1) (2009) 113-121.

[25] R.V. Kulkarni, B.S. Mangond, S. Mutalik, B. Sa, Interpenetrating polymer network microcapsules of gellan gum and egg albumin entrapped with diltiazem–resin complex for controlled release application, Carbohydrate Polymers 83(2) (2011) 1001-1007.

[26] P. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, European journal of pharmaceutical sciences 13(2) (2001) 123-133.

[27] S. Patra, N.N. Bala, G. Nandi, Synthesis, characterization and fabrication of sodium carboxymethyl-okra-gum-grafted-polymethacrylamide into sustained release tablet matrix, International Journal of Biological Macromolecules 164 (2020) 3885-3900.

CHAPTER - XII

SUMMARY AND CONCLUSION

12.Summary and conclusion

The polysaccharides obtained from natural sources have been gaining popularity in fabrication of various pharmaceutical dosage forms such as immediate-release and controlled-release peroral tablets, beads, microspheres, microcapsules, nanoparticles, mucoadhesive gels, transdermal patches, etc. A significant number of natural polysaccharides such as gum acacia, sodium alginate, gelatin, guar gum, starch, etc. have been widely used as tablet binder from very beginning. Natural polysaccharides possess some advantages such as easy availability, low cost, physical and chemical compatibility with a wide range of drugs, biocompatibility, and biodegradability. Being hydrophilic they also allow green manufacturing without use of harsh organic solvents, environment friendliness make these natural polysaccharides preferable over synthetic polymers as vehicles for drug delivery and other biomedical applications such as wound healing, tissue engineering, etc.

Cassia fistula trees belonging to the fabaceae family are native to the Indian subcontinent and southeast countries. The endosperm of seeds of this plant is rich in galactomannan polysaccharide which is non-ionic in nature and chemically composed of β-(1→4) linked poly-D-mannopyranose backbone with a randomly distributed side chain of α-(1→6) linked D-galactopyranose unit. *Cassia fistula* seed galactomannan (CFSG) has been reported as film coating agent and its carboxymethylated form has been reported as disintegrating and controlled-release agent. Native form of CFSG has not been reported as tablet binder to date. One of the aims of the present investigation was to explore the capability of native CFSG as tablet binder and another one was to chemically functionalized CFSG towards its application as gastro-retentive mucoadhesive sustained release polymer.

In chapter I, the concept of excipients and categorization of excipients by its route of administration, origin and functionality have been briefly discussed. Ideal characterization and

use of excipients in solid dosages forms and liquid dosages forms as well as their purpose have also been discussed.

In chapter II, demonstration of the natural polysaccharide-based drug delivery excipients has been presented, where the isolation, purification, characterization and classification of polysaccharides (gum & mucilage) have been described along with their charge, origin, shape, chemical structure and semisynthetic nature. This chapter also covers the advantages and disadvantages as well as the application of polysaccharide.

Chapter III talks about chemically modified natural polysaccharide as drug delivery excipients. The process of carboxymethylation with the examples as well as with the schematic diagram has been briefed. This chapter also confers about the grafting process with the types or classification of grafting, techniques of grafting like chemical grafting or grafting initiated by chemical means, radiation grafting or free-radical grafting, grafting through living polymerization and photochemical grafting, cross linking of gums and their examples have also been discussed.

In chapter IV, existing information about *Cassia fistula* seed gum along with its origin, phytochemical composition and chemical structure have been discussed. This chapter also discusses different properties of *Cassia fistula* seed gum as well as the extraction process of the polymer from the seed.

In Chapter V, different gastroretentive mucoadhesive dosage forms have been presented along with the physiological aspects like gastric pH, gastrointestinal motility and transit time with the schematic representation of four successive phases of activity. The factors which influence gastric retention have been discussed. A list of gastro-retentive devices has also been presented. This chapter also talks about the mucoadhesive system as well as their advantage, stages of mucoadhesion, types of mucoadhesion, the factors affecting mucoadhesion, mucoadhesive

polymers and their classification based on their rheological properties, sources and ideal characteristics of mucoadhesive polymers.

In chapter VI, covers the sustained release dosage forms with their advantages, disadvantages and properties of drugs affecting the dosage forms like physicochemical and biological properties. This chapter also talks about the oral sustained release drug delivery design, where the approaches to sustain drug-release like dissolution-controlled release systems, diffusion-controlled release systems, both dissolution and diffusion-controlled release systems, ion exchange resin-drug complexes, pH dependent formulation, and osmotic pressure-controlled systems have been discussed.

In chapter VII, extensive literature surveys on binding properties of natural gums and their application in sustained release drug delivery systems have been performed. Different national & international journals have been reviewed. The current updated literature survey shows a wide spectrum of application of natural gum in conventional and sustained release drug delivery systems.

In chapter VIII, the aim and objective of the project have been described apparently.

Chapter IX presents the monograph information of diclofenac sodium which covers all the details regarding synonyms, chemical name, structure, molecular weight, category, dose, appearance, solubility, dissociation constant, partition coefficient, storage, standards, identification, clarity, colour, light absorption, related substances, heavy metals, loss on drying, assay, mechanism of action, as well as the pharmacokinetic details, indication, contraindication, interaction with other medicaments as well as the statement on usage during pregnancy and lactation and adverse or side effects. Beside the monograph of diclofenac sodium this chapter also discusses about the monograph of aceclofenac which also consists of chemical name, structure, formula, molecular weight, CAS number, BCS classification, half-

life, appearance, solubility, partition coefficient, melting point, dissociation constant as well as the preparation, description, pharmacodynamic description, mechanism of action, pharmacokinetics description, use, administration, adverse effects and drug interaction.

Apart from this the monograph of excipients like polyvinyl pyrrolidone, talc, magnesium stearate, Carbomer, Hypromellose and Ceric ammonium nitrate have been discussed, which include basically the formula, structure, functional category, application, description, physical and chemical properties.

In Chapter X, experimental work comprising of materials and methods has been described in detail, and in **Chapter XI**, results and discussion have been presented.

First part of the investigation deals with evaluation of binding properties of *Cassia fistula* seed gum (CFSG). This part describes the evaluation of the galactomannan obtained from the seeds of the plant *Cassia fistula* (CFSG) as tablet binder. Extracted and purified CFSG was characterized by HPLC based monosaccharide composition analysis, elemental analysis, FTIR, ^{13}C solid state NMR, molar mass, zeta potential, rheological behaviour, pH, surface tension, DSC, TGA, DTA, PXRD and SEM studies. Acute oral toxicity and histological study were also performed in order to check its safety as oral drug delivery excipient. Drug-galactomannan physicochemical compatibility was also checked by FTIR, DSC and PXRD study. Finally, granules and tablets prepared by wet granulation technique were examined to evaluate and compare the binding capacity of CFSG with standard binders such as gum acacia and PVP K-30. Phytochemical screening study revealed the carbohydrate nature of the extracted gum and monosaccharide composition analysis by HPLC method exhibited the presence of galactose and mannose units in the molecules of CFSG. The ratio of galactose and mannose and molar mass of the collected sample of CFSG were 1.11:2 and 1.93×10^5 Da, respectively. Presence of other elements other than carbon, hydrogen and oxygen were not observed in the elemental

analysis study. The value of zeta potential near to zero indicated the non-ionic nature of CFSG and value of pH of 6.01 indicates its neutral nature as well as its suitability as oral excipients. The wetting capability was also indicated by its lower surface tension than that of water. Rheology study revealed the pseudoplastic nature of the aqueous dispersion of CFSG. Safety profile of CFSG as oral excipient was exhibited in toxicity and histological study. The chemical structure and functional groups were identified by FTIR and solid state ^{13}C NMR study. Physicochemical compatibility of CFSG with diclofenac sodium was shown in preformulation study. The results indicated excellent flow property of the granules composed of CFSG and the tablets showed to have appreciable hardness, low friability and faster disintegration time. The cumulative percent drug release was within the limit prescribed by official compendium (USP, 2017). 2.5%w/w concentration of CFSG as tablet binder was found sufficient to achieve optimum tablet-properties. Overall results of the evaluation suggest that CFSG could be used as an alternative tablet binder in green wet granulation approach.

Second part of this investigation deals with Chemical modification of *Cassia fistula* seed gum through graft copolymerization with poly (sodium acrylate) and its application as gastroretentive drug delivery copolymer. The graft-copolymer of CFSG and poly (sodium acrylate) was synthesized employing microwave-assisted free-radical-initiation method using ceric ammonium nitrate as redox-initiator. Acrylic acid, CAN and microwave-irradiation-time were taken as synthetic independent variables with low and high levels and the study was designed by 2^3 factorial design in order to understand the effects of variables and optimize the synthetic conditions. Acrylic acid and CAN were found to exert a positive effect on the degree of grafting as well as grafting-efficiency, whereas a negative effect of MW was found above a threshold-point. A synergistic combination effect of CAN and MW was found on the yield of graft-copolymer. Software-based numerical optimization finds the S1-batch as the best synthetic batch (790%G) with 10 g AA, 0.5 g CAN and 1 min MW. Formation of graft-

copolymer was confirmed by elemental analysis, FTIR, ^{13}C NMR spectra and molar-mass determination. DSC, TGA and PXRD studies confirm the drug-polymer compatibility. The study also exhibits the biodegradation nature of the copolymer in a simulated colonic environment. The tablets composed of the graft-copolymer showed excellent stomach-specific mucoadhesion over 10 h in ex-vivo mucoadhesion study. In-vitro drug-release study exhibits excellent sustained-release profile of the tablets composed of CFSG-g-PSA, especially S1 batch (790%G) with rate-constant (K_{kp}) of 0.079 h^{-1}, $T_{50\%}$ of 6.6 h, $T_{90\%}$ of 11.9 h and release-similarity factor of 83.4 (respect to marketed product). The study demonstrates synthesis of graft-copolymer by simple, easy, rapid and one-pot method without use of any sophisticated instrument and nitrogen-environment. Thus, it can be concluded that CFSG-g-PSA can be applied as gastro-retentive, sustained-release mucoadhesive polymer in the fabrication of stomach-specific mucoadhesive sustained-release single-unit peroral delivery systems for NSAIDs.

The present investigation shows the possibilities of native *Cassia fistula* seed gum (CFSG) as well as its different chemically tailored forms of CFSG to be used in various types of functionalized drug delivery systems applications as polymeric excipients. Present research of selecting and identifying new plant and extraction of gum from its seed and subsequent chemical modification of gum and exploration of those native and modified gum as excipient for pharmaceutical dosage form as binder and as well as mucoadhesive-cum-gastroretentive sustained-release polymeric excipients respectively by well guided scientific approach helps to achieve the desired objective for which research has been undertaken. The research work from that angle is expected to show new light in the field of Pharmaceutics and Natural Polymeric excipients.

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