Study of Release Kinetics of Kulekhara (Herbal Drug) from Transdermal Patch to Control Anaemia Disease

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in

Nanoscience & Technology

by

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of his "**Study of Release Kinetics of Kulekhara (Herbal Drug) from Transdermal Patch to Control Anaemia Disease**", studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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CERTIFICATE OF RECOMMENDATION

This is to certify that Alok Paine(Roll No. **M4NST24004** and Registration No. 137326 of 2016-17) has completed his entitled "**Study of Release Kinetics of Kulekhara (Herbal Drug) from Transdermal Patch to Control Anaemia Disease**", under the supervision and guidance of prof. Dr. Mahua Ghosh Chaudhuri, Jadavpur University, Kolkata. We are satisfied his work, which is being presented for the partial fulfillment of the degree of "**Master of Technology in Nanoscience**", Jadavpur University,Kolkata-700032.

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Chapter 1

Introduction

1.1 Introduction

Medicinal plants are of great importance to health of individuals and communities. The role of medicinal plants in disease prevention or control has been attributed largely to antimicrobial, anti-diabetic, hypolipidemic, anti-carcinogenic, anti-inflammatory as well as antioxidant activity. The plant products that are derived mainly from leaves, fruits, seeds, root or even stem barks are known to act as phytomedicine and influence various physiological functions in human body. All these plants products contain one or more natural bioactive substances like alkaloids, tannins, phenolic compounds, flavonoids and saponin. Among these substances the anti-oxidant property is largely associated with amphipathic compounds like polyphenols and in recent years reports of anti-oxidant property of plant products has been increased immensely . Reactive oxygen species (ROS) such as superoxide radical (O2 .-), hydroxyl radical (.OH), hydrogen peroxide (H_2O_2) as also nitric oxide (NO.), peroxynitrite anion (ONOO-) play an important role in oxidative stress and are related to pathogenesis of important diseases like cancer, Alzheimer's, diabetes, arthritis etc . ROS induced damage primarily targets biological macromolecules and causes reaction such as lipid peroxidation, protein carbonylation, protein nitrosylation, oxidative damage to DNA etc . In healthy individuals, there is a stoichiometric balance of free radicals and anti-oxidant systems (both enzymatic and non-enzymatic); however, oxidative stress is generated when the equilibrium is tilted towards free radicals either due to increased generation of free radicals or depletion of anti-oxidant levels . Anti-oxidant molecules act either by inhibiting the process of free radical generation or can directly scavenge the ROS or chelate the transition metals that catalyze the free radical generation5 . To overcome the oxidative stress, in recent years, synthetic anti-oxidants have been developed like gallic acid esters, tertiary butylated hydroquinone but they are suspected to have some adverse effects on health. Hence, their use are restricted and replaced with naturally occurring antioxidants as the later are safe, having minimum side effects compared to the synthetic ones . Hygrophilia spinosa, commonly known as kulekhara belonging to the family Acanthaceae and has been used in Ayurvedic literature from ancient times . This plant is commonly found in India, Srilanka, Myanmar and Nepal. Importantly, various part of this plant has been used for controlling diseases including anemia, leukorrhea, gonorrhoea, asthma, blood diseases, hyperdipsia, dysentery, cancer, gastric diseases, and inflammation etc . Phytochemically, the whole plant contains almost all bioactive substances such as tannins, phytosterols, alkaloids, polyphe-

nols, carbohydrates etc which entails for its multiple activities. The present study was carried out to explore the in vitro anti-oxidant activity and phytoconstituents of the leaf extract of Hygrophilia spinosa. leafy vegetables (GLV) some of which are locally grown and utilized. GLV are rich sources of vitamins such as β -carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as iron, calcium and phosphorous. They are also recognized for their characteristic colour, flavour and therapeutic values. Some of the commonly consumed leafy vegetables are amaranth, spinach, ipomoea, bottle gourd leaf, etc., the nutritive value of which has been reported in the book of "The Nutritive Value of Indian Food" by C. Gopalan. [12] Apart from these, there are various types of less utilized leafy vegetables, which are not seasonal and easily available throughout the year. Among these non unutilized green leafy vegetables, Kulekhara leaves are such type of leafy vegetable which has no nutritional and anti-nutritional information available in the above said book.. Kulekhara or, Hygrophila auriculata belongs to Acathaceae family. Hygrophilla auriculata has several common name in different language. In Bengali it is called kulekhara, In Sanskrit Ikshugandha, Kokilaksha and in Hindi Talimakhana.



Figure 1.1: Kulekhara Live Medicinal Plant

The root contains alkaloid , Phytosterol, Potassium Salt of Oxalic Acid, Diastase, Protease, Essential Oil [29] It is a spiny bush and is common throughout India. Though whole plant has been used medicinally but root and leaves are used more. Anciently it has been found effective in skin diseases, dropsy and sleeplessness. It is found effective in renal stones too, where in lower potencies have been proved more beneficial. The plant is used as demulcent, aphrodisiac, diuretic, urinary tonic and hepato protective substance. The aerial parts and the roots are used in herbal preparations. [18] The people in India believes in use of different types of plants as medicine to prevent different types of diseases since early stage of life. There is a myth that use of Kulekhara Leaves can prevent aneamia successfully. Micronutrient malnutrition is of major and serious concern for many tropical developing countries. [3, 19] Micronutrient deficiency affects over two billion people worldwide, resulting in poor health, low worker productivity, high rate of mortality and morbidity [10] Iron deficiency anemia, for example, is one of the important worldwide health problems affecting nearly thirty percent of the world's population [16].The prevalence of anaemia in all the groups is higher in India as compared to other developing countries [18]. In India, anaemia affects an estimated 50% of the population and among them women are severely affected than men. It is estimated that about 20-40 of maternal deaths in India are due to anaemia and one in every two Indian women (56%) suffers from any form of

anaemia [22]. National Nutrition Monitoring Bureau (NNMB) Indian Council of Medical Research (ICMR) [23] and District Level Household Survey (DLHS) [28] have shown that prevalence of anaemia is very high (ranging between 80% $\rightarrow$ 90%) in preschool children, pregnant and lactating women and adolescent girls. [13] A.Gomes et all. studied the effect of ethanolic extract of kukekhara leaves on male albino rats. The result of this study showed the significantly increased of heamoglobin, haematocrit, RBC and total WBC counts and the Total Iron Binding Capacity in male albino anaemic rats. [11] As because there is no nutritional information available and very less scientifically proved medicinal properties are present regarding kulekhara leaves, people are unaware about this leaves. In spite of easy availability and less cost, people are very much reluctant to purchase it. So in our research work we tried to focus on this underutilized kukelkhara leaves which were believed to be nutritious and might help in achieving nutritional security, specially in the prevention of micronutrient deficiency. trace elements India, being blessed with a variety of natural surroundings and varying climates and seasons, has a number of edible green leafy vegetables (GLV) some of which are locally grown and utilized. GLV are rich sources of vitamins such as -carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as iron, calcium and phosphorous. Among them, kulekhara (Hygrophila auriculata) is very promising owing to the content of various groups of phyto-constituents viz. phytosterols, polyphenols, proanthocyanins, mucilage, alkaloids, flavonoids, terpenoids, glycosides etc. and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhoea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea etc. Therefore, proper morphological characteristics and uses should be explored for its popularization among farming community as well as researchers. Ecological and Geographical Distribution : Habitat : It is native to India and commonly grows in most wetlands, fresh as well as brackish water. Occurrence : It is widely distributed in India, Sri Lanka, Myanmar, China, Malaysia, Burma, Bangladesh, Nepal, Pakistan and Tropical Africa. In West Bengal, it is commonly found in moist places on the banks of paddy fields, tanks, ditches etc. Ethnobotany : Kulekhara is an annual, robust and erect herb, belonging to the family Acanthaceae, with subquadrangular thickened nodes, oblanceolate leaves with yellow spines in axils, flowers pale to purple blue, densely clustered in axils, fruits oblong, glabrous capsules 4- to 8-seeded. In India, it is commonly used as vegetable in some states like Odisha, Chhattisgarh and West Bengal, and is employed for the treatment of cancer, arthritis, hepatotoxicity, inflammation, blood diseases, diabetes, fever, constipation, bacterial infection etc. Details of morphological features (botany) along with utilities of different plant parts (ethnobotany) are given hereunder. Root : It is used for the treatment of diuretics, dropsy, rheumatism, jaundice and also to kill abdominal insects. Stem : The plant is a robust herb or undershrub, growing up to 80 cm tall with sparsely sub-hispidly hairy stems and whorled spines at the nodes; spines 1.3 cm long, often in a whorl. Leaves : Leaves are sessile, pseudo-whorl from the shortened axillary branchlets; leaves often in a whorl, the two outer one often larger; oblong or linear oblong, 5-15 cm x 1.3-2.5 cm, entire or mi-nutely dentate, acute and sub-hispidly hairy. The decoctions of the young leaves are taken orally for two consecutive weeks in empty stomach to treat anemia. Flower : Flowers are purple blue, in axillary whorled fascicles, 8 at each node, 3.0 -3.7 cm long, bracts lanceolate, bracteoles linear; calyx 4-lobed, silky, posterior lobes 1.8 cm, lateral, smaller, anterior two-toothed; corolla tube 1.2-1.5 cm, cylindric below, enlarged above; upper lip 2-lobed, the lower trilobed with 2 crested folds on the paltate; stamens 4, didynamous, all fertile, anther

oblong; ovule oblong, 4-ovuled and style filiform. Flowering and fruiting occurs during the months of August-March. Seed : Capsule is linear oblong 7.5 mm long, shorter than calyx, 4-8 seeds on hard retinacula, flat and white hairy when wet. Seeds are orbicular and 0.3 mm across. Seed powder mixed with raw cow milk is taken in the morning for treating impotency. Dry seed powder mixed with milk and sugar is taken to cure spermatorrhoea. It is also used for removal of gall stones as aphrodisiac.



Figure 1.2: kulekhara a perfect medicine of anemia

Nanotechnology is the science and technology at nanoscale, which ranges about 1-100 nanometer. The term nanotechnology is derived from the Greek word nano, meaning dwarf and applies the principles of engineering and manufacturing at a molecular level. Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale or one billionth of a meter. At these scales, consideration of individual molecules and interacting groups of molecules in relation to the bulk macroscopic properties of the material or device becomes important, since it is control over the fundamental molecular structure that allows control over the macroscopic chemical and physical properties.

Kulekhara, scientifically known as Hygrophila auriculata, is a plant commonly found in tropical regions like India and Sri Lanka. In traditional medicine systems such as Ayurveda and Unani, Kulekhara leaves are valued for their medicinal properties. Here are some of the purported medical purposes of using Kulekhara leaves:

- **Diuretic Properties:** Kulekhara leaves are believed to have diuretic properties, meaning they can promote urination. This property is often used to alleviate conditions like urinary tract infections (UTIs) and kidney stones.
- **Anti-inflammatory Effects:** The leaves are also thought to possess anti-inflammatory properties, which could be beneficial in managing conditions associated with inflammation, such as arthritis and gout.

- **Antioxidant Activity:** Kulekhara leaves contain antioxidants, which can help neutralize harmful free radicals in the body. This antioxidant activity may contribute to overall health and wellbeing and may have anti-aging effects.

- **Potential Aphrodisiac:** In traditional medicine, Kulekhara is sometimes considered to have aphrodisiac properties. It is believed to enhance sexual health and improve libido.

- **Hepatoprotective:** Some traditional medicine practices suggest that Kulekhara leaves may have hepatoprotective effects, meaning they could help protect the liver from damage.

It's important to note that while Kulekhara leaves have been used traditionally for various medicinal purposes, scientific research validating these claims is still limited. As with any herbal remedy, it's essential to consult with a healthcare professional before using Kulekhara leaves, especially if you have any underlying health conditions or are taking medications.



Figure 1.3: Kulekhara Chutney

1.1.1 CORRELATION OF KULEKHARA JUICE WITH BIOTECHNOLOGY

The correlation of Kulekhara juice with biotechnology could involve several aspects:

- **Bioprospecting for Active Compounds:** Biotechnological methods can be employed to analyze the chemical composition of Kulekhara juice to identify bioactive compounds. This involves techniques such as chromatography, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy to isolate and characterize compounds with potential medicinal properties.

- **Bioprocessing for Enhanced Production:** Biotechnological approaches can be used to optimize the cultivation and extraction of Kulekhara plants to enhance the yield of bioactive compounds. This might involve techniques such as tissue culture, genetic engineering, and fermentation to improve the production of desired metabolites.

- **Biomedical Applications:** Biotechnology can facilitate the development of novel formulations or delivery systems for Kulekhara-derived products, such as nanoparticles or liposomes for targeted drug delivery. This can enhance the bioavailability and efficacy of bioactive compounds present in Kulekhara juice.
- **Pharmacological Studies:** Biotechnological methods can aid in conducting pharmacological studies to investigate the therapeutic potential and mechanisms of action of Kulekhara juice or its constituents. This includes in vitro and in vivo studies to evaluate its effects on various cellular and physiological processes.
- **Quality Control and Standardization:** Biotechnology can contribute to the development of standardized protocols for the quality control and authentication of Kulekhara-derived products. This involves the use of molecular techniques, such as DNA barcoding, to ensure the authenticity and purity of raw materials and finished products.



Figure 1.4: : Kulekhara: The Wonder Herb

1.1.2 Pain and pain management

Pain is a distressing feeling, a warning sign from our body for some underlying condition. Pain influences the body to withdraw itself from damaging situations, protects itself while it heals and restrain itself from similar situation in future. As our body grows, it installs pain detectors almost everywhere in it. These detectors are specialized nerve cells, called nociceptors that stretch from our spinal cord to skin, muscles, joints, teeth and some of internal organs. Nociceptors usually remain silent but it gets activated due to any noxious stimulus. Noxious stimulus stimulates the nociceptor nerves by some chemical tissue events and electrical events in the nerve. This events lower the threshold of nociceptor nerves to such a level that even a touch can produce painful sensation. Inflammation is a natural biological response produced by the tissues within our body as a reaction to the harmful stimuli in order to eradicate the necrotic cells (dead cells) and initiate the tissue repairing process. Neutrophils are usually the first respondents of an inflammatory response and gather at the site of injury via the bloodstream, followed by the release of other chemical mediators. The cardinal signs of inflammation include the hot inflamed site due to increase in blood flow towards the region, redness, and swelling due to vascular permeability pain caused by the activation and sensitization of primary afferent neurons

and lasting loss of function. The localized inflammatory response then induce the release of free arachidonic acid (AA) from the phospholipids, which are converted into prostaglandins (PG) via the cyclooxygenase (COX) pathways [30]. Preventing the formation of prostaglandins will eventually lead to an anti-inflammatory effect. Cyclooxygenase (COX), also known as prostaglandin-endoperoxide synthase (PTGS), is an enzyme (specifically, a family of isozymes) that is responsible for formation of prostanoids, including thromboxane and prostaglandins such as prostacyclin, from arachidonic acid. Two isozymes which are found in the body are COX-1 and COX-2. COX-1 is responsible for many normal bodily functions such as secretion of protective gastric mucus, regulation of gastric acid, protection of the stomach mucosa from the acidic environment, promotion of platelet aggregation and maintenance of renal blood flow whereas COX-2 which is found in inflammatory sites, produces prostaglandins. Many non-steroidal anti-inflammatory (NSAIDs) drugs are non-selective COX-1 and COX-2 inhibitors, usually inhibits the inflammatory response by blocking both COX-1 and COX-2 and block the production of inflammatory prostaglandins and thromboxane. By blocking COX-1, which also normally acts to protect the gastrointestinal mucosa, non-selective NSAIDs can significantly damage the gastric tissue. Some selective COX-2 inhibitor which is another type of NSAIDs may lead to increased risk of thrombotic cardiovascular and cerebrovascular events. Because of these significant side effects of NSAIDs, there is an increased interest in herbal drugs which has very less or no side effects in reducing pain and inflammation [4].

1.1.3 Kulekhara Drug

Kulekhara, scientifically known as Hygrophila auriculata, is a herbaceous plant native to India, commonly found in wetlands and marshy areas. In traditional Ayurvedic medicine, Kulekhara has been used for its medicinal properties. Its roots and leaves are particularly valued for their potential health benefits.

Kulekhara is believed to have several therapeutic properties, including anti-inflammatory, diuretic, and aphrodisiac effects. It has been traditionally used to treat various conditions such as inflammation, urinary disorders, sexual weakness, and rheumatism.

However, it's important to note that while Kulekhara has a long history of traditional use in Ayurvedic medicine, scientific evidence supporting its efficacy and safety for various medical conditions is still limited. As with any herbal remedy, it's essential to consult with a qualified healthcare professional before using Kulekhara or any other herbal supplement, especially if you have any underlying health conditions or are taking medications.

1.1.4 Origin

Kulekhara has been traditionally used in Ayurvedic medicine for various purposes, including its purported aphrodisiac, diuretic, anti-inflammatory, and immune-boosting properties. The origin of the use of Kulekhara in traditional medicine can be traced back centuries in India, where it has been a part of Ayurvedic practices. It's often used in various Ayurvedic formulations and remedies to treat conditions like urinary disorders, male sexual disorders, inflammation, and more. The plant has gained attention in recent years for its potential health benefits, and there's ongoing research to explore its pharmacological properties and medicinal applications. However, it's important to note that while traditional uses and anecdotal evidence may suggest

its efficacy, scientific studies are still needed to fully understand its mechanisms of action and potential side effects.



Figure 1.5: kulekhara pakora recipe

1.1.5 Importance of Sustained Release

Most conventional (immediate release) oral drug products, such as tablets and capsules, are formulated to release the active drug immediately after oral administration. In the formulation of conventional drug products, no deliberate effort is made to modify the drug release rate. Immediate-release products generally result in relatively rapid drug absorption and onset of accompanying pharmacodynamic effects. In the case of conventional oral products containing prodrugs, the pharmacodynamic activity may be slow due to conversion to the active drug by hepatic or intestinal metabolism or by chemical hydrolysis. Alternatively, conventional oral products containing poorly soluble (lipophilic drugs), drug absorption may be gradual due to slow dissolution in or selective absorption across the GI tract, also resulting in a delayed onset time. The dosing interval, in case of conventional release system, is relatively short that means in order to maintain the amount of drug in the blood for long duration of time, it is so important that every three, four or six hours, depending upon the type of dosage form we have to take second dose. Therefore, a presence of large bands and valleys in the drug level are observed for which the drug then shows the side effects. Hence to overcome these problems such as to maintain relatively constant therapeutic level of drugs instead of unpredictable fluctuations in the bloodstream and to enhance patient compliance conceptualization of sustained release dosage form occurred. Sustained release system is a form of modified drug release system which provides an immediate release of drug for normal therapeutic response, followed by the gradual release of drug in amount sufficient to maintain therapeutic response for a specific extended period of time. The sustained release drug delivery system [2] optimizes the biopharmaceutical, pharmacokinetic and pharmacodynamics properties of a drug in such a way that its utility is maximized, side-effects are reduced and cure of the disease is achieved. There are several advantages of sustained release drug delivery over conventional dosage forms [15] : Improved patient compliance due to less frequent drug administration Reduction of fluctuation in steady-state drug levels Maximum utilization of the drug Increased safety margin of drug Reduction in healthcare costs through improved therapy Shorter treatment period. In spite of all these advan-

tages there are certain disadvantage of sustained release dosage form which cannot be avoided: Decreased systemic availability in comparison to immediate release conventional dosage forms due to incomplete release, increased first pass metabolism, increased instability, site specific absorption, pH dependent stability etc. Poor retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions. Poor in vitro-in vivo correlation.

1.1.6 Use of nanocarriers in drug delivery:

Nanocarriers play a crucial role in various fields within nanotechnology, particularly in medicine and drug delivery. Here are some of their key uses:

1.1.6.1 Drug Delivery :

Nanocarriers, such as liposomes, micelles, dendrimers, and nanoparticles, can encapsulate drugs, protecting them from degradation and enabling targeted delivery to specific tissues or cells. This targeted delivery reduces side effects and enhances the therapeutic efficacy of Nanocarriers can be designed to carry imaging agents, such as fluorescent dyes or magnetic nanoparticles, allowing for precise imaging of tissues or cells. This is particularly useful in diagnostics and monitoring disease progression.

1.1.6.2 Therapeutics

Besides delivering drugs, nanocarriers themselves can possess therapeutic properties. For example, nanoparticles can be engineered to have inherent anti-cancer or antimicrobial properties, enhancing their therapeutic potential.

1.1.6.3 Gene Delivery:

Nanocarriers can be used to deliver nucleic acids, such as DNA or RNA, into cells for gene therapy purposes. This can involve the use of viral vectors or non-viral carriers like lipid nanoparticles or polymer-based nanoparticles.

1.1.6.4 Vaccine Delivery

Nanocarriers can improve vaccine efficacy by enhancing antigen stability, promoting targeted delivery to immune cells, and facilitating controlled release of antigens. This can lead to improved immune responses and longer-lasting immunity.

1.1.6.5 Environmental Remediation

Nanocarriers can be employed for environmental applications, such as the removal of pollutants or toxins from water or soil. Functionalized nanoparticles can selectively bind to contaminants, facilitating their removal or degradation.

Overall, nanocarriers offer a versatile platform for targeted delivery, imaging, and therapy across various fields, contributing to advancements in medicine, biotechnology, and environmental science. Certain drugs are selected for full-scale development based on their safety and

efficacy but fail to reach clinical development because of poor biopharmacological properties. Some of these properties are poor solubility, poor permeability across the intestinal epithelium, poor bioavailability and undesirable pharmacokinetic properties. The new drug delivery methods utilizing nanotechnology can be formulated in order to deal with these drawbacks.

1.1.7 Sol gel process and incorporation of drug into mesoporous silica gel network

The sol gel process is a wet chemical technique which is used for preparing various types of nanomaterials such a thin films, fine powders, ceramic fibers, porous ceramic materials etc. The sol gel process involves a series of simultaneously occurrence of hydrolysis and condensation reaction implicate the fabrication of inorganic matrix through the formation of colloidal solution (sol) leading to gelation followed by the formation of a wet gel with globally connected solid matrix, which in turn forms the dry gel. This is a co-gelation process. The condensation process consists of two part alcohol condensation and water condensation. The reaction can be given as follows:

1.1.7.1 Hydrolysis

Si-$(OR)_4$ + $nH_2O \rightarrow$ $(RO)_3$-n-Si-(OH)n + nROH

1.1.7.2 Condensation:

-Si-OR + -Si-OH $\rightarrow$ -Si-O-Si- + ROH

-Si-OH + -Si-OH $\rightarrow$ -Si-O-Si- + H_2O A three dimensional network is formed due the polymerization reaction. Drying of alcohol and water gives rise to capillary action and thus there is shrinkage of gel. The product is known as xerogel. Mesoporous silica gel is found to be biocompatible, that is, it does not have any adverse effect to the surrounding tissue and organs. The sol gel produced silica gel act as a carrier for sustained delivery of drug. Incorporation of drug into silica gel is very simple. During the co-gelation process the drug molecules are introduced into the sol state during the formation of gel would be positioned within porous silica xerogel network [9, 17].

1.1.8 Transdermal drug delivery

Transdermal drug delivery is an alternative to orally administrated drug delivery and hypodermic injection. For thousands of year people have placed substances for getting desired therapeutic effects. Even turmeric and slaked lime mixture was also used to be placed over the skin to get relieve of pain and inflammation. The first transdermal patch of scopolamine which was used to treat motion sickness was approved in United States in 1979.

Transdermal patch of nicotine being a success, raised the profile for transdermal patch in medicine and for general public use.

Transdermal drug delivery has a variety of advantages over injection and oral routes. Hypodermic injection are painful, develop a huge amount of dangerous medical wastes and pose the risk of disease transmission by needle re-use, especially in developing country.

Conventional oral release for is known to provide immediate release of drug, that is one cannot control the release of drug and effective concentration cannot be maintained at the targeted site. In particular, transdermal route is used when there is a significant first-pass effect of the liver that can prematurely metabolize drugs [1, 14] .

The advantages of using transdermal drug delivery system can be summed up in few important points [21] : It offers controlled release of drug

It offers steady blood level profile Using this drug delivery route can reduce systemic side effects It can be self-administered Sometimes there is an improved efficacy over other release forms

1.1.9 Kinetic study

Mathematical models play a vital role in the interpretation of mechanism of drug release from a dosage form. It is an important tool to understand the drug release kinetics of a dosage form thus helping researchers to develop highly effective drug formulation and more accurate dosing regimens saving time and money. It allow the measurement of some important parameters (e.g. drug diffusion coefficient) and resort to model fitting on experiment release data. The amount and type of active agent, polymer and adjuvants as well as the size and shape of the system designed to achieve a certain drug release profile can be predicted theoretically. Mathematical equations enable the quantitative interpretation of the values obtained from a dissolution or drug release assay. The equation needs to have the ability to transform the release curve in function of some other parameter related to the dosage form under analysis. Therefore, the models have been used mainly to predict the release of the encapsulated molecules in function of time. Therefore, mathematical evaluation of drug release kinetics adds value, ensuring optimal design of pharmaceutical formulations as well as understanding release mechanisms through experimental verification. However, model-dependent methods, based on different mathematical functions, are very useful to describe the release profile. Once a suitable function has been selected, the dissolution profiles are evaluated depending on the derived model parameters. Some important release kinetic models are zero order, first order, Higuchi, Korsmeyer–Peppas [24]. In this study we will encapsulate kulekhara juice into the nano pores of silica gel and study their release kinetics in stimulated body fluid (SBF). We will then plot the release data against time and try to figure out the kinetic model, best suited for the release of the herbal drug in in-vitro release study like zero-order (cumulative amount of drug release versus time), first-order (log cumulative percentage of drug remaining versus time), Higuchi (cumulative percentage of release versus square root of time), and Korsmeyer–Peppas (log cumulative percentage of drug released versus log time) equation models. Also we will be making a transdermal patch out of the nanocomposite containing the herbal drug .

1.1.10 Materials and Methods

1.1.10.1 Materials:

Tetraethyl orthosilicate (TEOS), Hygrophila Spinosa(Kulekhara Juice), sodium chloride(Nacl) , and Ammonia (NH_3) were purchased from Sigma-Aldrich. Polyvinyl alcohol (PVA) was purchased from Hi Media labs. Di-n-butyl phthalate was purchased from Ranbaxy Laborato-

ries Limited. Ethylcellulose (EC) and polyvinylpyrrolidone (PVP) were purchased from Loba Chemie Pvt. Ltd. All reagents were of A.R. grade. Deionized water was used for Sol-gel experiment.

1.1.10.2 Synthesis of SNPs

SNPs were synthesized by acid and base-catalyzed sol-gel method at room temperature (37 degree celcious) [25]. This method involves a series of hydrolysis of the silica precursor, tetraethyl orthosilicate (TEOS), followed by poly condensation of the hydrolyzed products. Deionized water, ethanol, and TEOS were taken in a volume ratio of 1:2:1. The mixture of a catalyst (0.1 M), deionized water, and ethanol was magnetically stirred at 350 rpm followed by the dropwise addition of TEOS for 1 h. Then, the resulting colloidal solution was dried at 40 degree celcious. After the gel formation, it was calcined at 550 degree celcious for 3 h and ground to white silica powder form by mortar and pestle.

1.1.10.3 Drug Loading Procedure into SNPs

Five milligrams of SNPs were added to 5 mL of ethanolic solution of the drug. After that, the suspension was gently stirred for 24 h to achieve equilibrium. The rest was centrifuged at 7000 rpm for 30 min after loading, and the amount of drug that remained in the supernatant phase was measured by a UV-Visible spectrophotometer at its maximum wavelength (λmax). Drug loading capacity and loading efficiency were determined using the following formula: % Loading Capacity = x 100 % Loading efficieny = 100

1.1.10.4 Preparation of Transdermal Patches

The transdermal patches of Kulekhara were prepared with a bit of modifcation in methodology published in an article [20] using the polymers ethylcellulose (EC) and polyvinylpyrrolidone (PVP), as mentioned in Table 1. First, the bottom portion of both sides opened cylindrical glass mould was wrapped with aluminium foil. After that, 5 mL of 4% (w/v) aqueous solution of polyvinyl alcohol (PVA) was poured on the foil, and then the mould with PVA solution was kept for drying at 50 degree celcious for 6 h to obtain the backing membrane of the patch. Next, the matrix was prepared by mixing EC-PVP (2:1) and 100mg Kulekhara-loaded SNPs in 15 mL ethanol. Ten per cent (v/v) of Di-n-butyl phthalate was used as a plasticizer. Eventually, the uniform dispersion was cast on the PVA backing membrane. The resulting solution in the mould was allowed to dry again at 50 degree celcious for 6 h. The dried patch was taken out from the mould and stored in a desiccator for further studies. Few patches were prepared without nanoparticles keeping the other constituents and amount the same Amount of materials used to fabricate transdermal patches Formulations Drug-loaded SNPs Amount of polymers F_1 Absent. (Free drug present) 1. 4% aqueous solution of PVA for backing membrane 2. Weight ratio of EC: PVP=2:1 for the matrix 3. 10% (v/v) Di-n-butyl phthalate F_2 Drug-loaded P1 SNPs $F-3$ Drug-loaded P_2 SNPs

1.1.10.5 Swelling Index

A strip of (1 x 1) cm^2 was cut out from the patch and weighed. Then, it was kept in a Petri dish, and phosphate bufer solution (PBS) of pH 7.4 was added until the patch was completely immersed. After every 1 h, the strip was taken out, blotted to eliminate the excess water, and weighed again. This process was continued for up to 5 h, and the following formula calculated the value of the swelling index at diferent time intervals. % swelling index = x 100

1.1.10.6 Surface pH

A strip of the patch was swelled by keeping it in 5 ml distilled water, and after 4 h, the pH was measured by placing the electrode on the surface of the patch.

1.1.10.7 Folding Endurance

Taking a strip of the patch, it was folded repetitively in the same area until it was broken. The value of folding endurance is the number of times the patch can be folded until it breaks.

1.1.10.8 Weight Uniformity

A defnite area was cut from diferent parts of the dried transdermal patch and weighed using digital balance to determine the average weight and standard deviation values.

1.1.10.9 Thickness of the Patch

The thickness of the patch was measured using a Vernier caliper at diferent points of the patch. The average thickness, as well as standard deviation values, were calculated from the individual data

1.1.10.10 Percentage Elongation

Initially, the length of the patch was noted. After that, it was stretched by applying stress, and the increased size of the patch was again noted down to evaluate the percentage elongation by using the following formula: % elongation = x 100

1.1.11 Development of the Spectrophotometric Method

The stock solution was prepared by dissolving 5 ml Kulekhara juice in a mixture of 10 mL ethanol and 40 mL buffer solutions of diferent pH (5.5, and 7.4). Furthermore, the stock solution was diluted taking 0.5 mL of it into 9.5 mL of bufer solution before UV Visible spectrophotometric analysis. The analysis was repeated thrice and the λmax values obtained were expressed as mean $\pm$ S.D. The λmax of Kulekhara in pH 5.5, and 7.4 were observed at 243.15$\pm$ 0.005 nm, 243.24$\pm$ 0.015 nm, and 243.09$\pm$ 0.017 nm, respectively. Total six sets of standard solution were prepared through same dilution technique to obtain the calibration curve.

1.1.11.1 Drug Content

Another (1x1) cm^2 strip was cut out from the patch and placed in a beaker. Then, 100 mL of phosphate bufer solution (PBS) of pH 7.4 was added to the beaker and stirred with a magnetic bead for 24 h. The solution was filtered, and the drug content was determined by a UV-Visible spectrophotometer at its λmax.

1.1.12 Characterization

FTIR spectra were taken using an FTIR spectroscope (Model: Prestige 22, Shimadzu). XRD sample pattern was collected using Rigaku, Ultima IV, Japan.

1.1.12.1 In Vitro Drug Release Study for KulekharaLoaded SNPs

Five milligram of drug-loaded SNPs was exposed to sodium acetate bufer of pH 5.5, and phosphate bufer (PBS) of pH 7.4. All of the dissolutions were carried out in 300 mL of dissolution media using dialysis membrane-60 (Avg. fat width, 25.27 nm; Avg. diameter, 15.9 nm; capacity approx., 1.99 ml/cm). One millilitre of sample solution was withdrawn at various time intervals, and an equal volume of dissolution media was added to maintain the total volume. The samples were analyzed by UV Visible spectrophotometer at λmax of the drug. The plot of percentage cumulative release against time was drawn.

1.1.12.2 Ex Vivo Skin Permeation Study

The formulated patches (1x1) cm^2 were located individually on the stratum corneum side of the human skin previously soaked overnight in the phosphate bufer solution (PBS) of pH 7.4. Then, the skin with a patch was placed into Franz diffusion cell in such a manner that the dermis side of the skin remained towards the receptor part of the cell containing 100 mL of PBS at room temperature. The solution in the receptor compartment was continuously stirred using a magnetic bead at 40 rpm. One millilitre of solution was collected from the receptor part at diferent time intervals, and an equal volume of PBS was added to maintain the total volume. Finally, the samples were analyzed using a UV-Visible spectrophotometer to determine the kulekhara concentration permeated through the skin [20] . A plot of the cumulative amount of drug permeated per square centimetre of skin against the time was drawn for three formulations .

1.1.13 Release Kinetic Study

The data obtained from the abovementioned skin permeation study were used to plot various kinetic models such as zero order, first order, Higuchi model, and Korsmeyer Peppas model. Zero - order equation (C= k0t, where k0 is rate constant) depicts that the rate of drug release is not subjected to the change in initial concentration of drug, thus encouraging slow drug release from the matrix. Congruently, the first-order equation (log C=log Co - k1t/2.303; here, C0=the initial concentration of drug and k1=first order constant) signifies that the rate of drug release is directly proportional to the amount of drug left in matrix. Moreover, Higuchi model (Q =k $\sqrt{}$ t; where Q represents cumulative amount of drug released and k denotes the Higuchi constant)

can best explain the diffusion controlled release of the drug molecules from the matrix based on the Fick's law. The "n" values, called "release exponent", obtained from the Korsmeyer-Peppas model (Mt / = Ktn ; where Mt /M ∞ =fraction of drug released at time t, K =the rate constant) were used to characterize the drug release mechanism. The value of n $\leqslant$0.45 indicates Fickian difusion, 0.45¡n0.89 non-Fickian (super case II transport) [27].

Chapter 2

Literature Review

2.1 Literature review on the Kulekhara

Kulekhara, scientifically known as Hygrophila Auriculata, is a herbaceous plant belonging to the Acanthaceae family. This annual herb typically reaches up to 60 cm in height and thrives in marshy areas and tropical climates. Morphologically, it possesses a tetragonal stem, hairy and stiff at the nodes, with dark brown bark. The leaves are the most prominent feature, arranged in opposite pairs along the stem. These elliptic-lanceolate leaves boast a vibrant dark green color with a subtle bronze hue. During the flowering season, the plant produces small, delicate flowers, primarily in shades of purple or violet blue. Kulekhara's natural habitat encompasses tropical and subtropical regions of Asia and Africa. It flourishes in wet environments like marshlands, riverbanks, and low-lying areas with ample moisture. The plant's distribution is widespread, particularly in India, Sri Lanka, and parts of Southeast Asia. Medicinally, Kulekhara leaves have garnered significant attention in traditional medicine systems, particularly Ayurveda and Siddha. These systems recognize the leaves' potential benefits for various ailments. The presence of various phytochemicals, such as alkaloids, glycosides, and terpenoids, is believed to contribute to the plant's purported medicinal properties.

In India, the micronutrient deficiency is a major and serious concern, among them iron deficiency anemia is one of the most important health hazards during reproductive age group of women. In the present study, micro elements (Na, K, Ca) and trace elements (Fe, Zn, Ni, Cu) content of fresh Kulekhara leaves are examined. Kulekhara leaves are widely grown, neglected, nutritious green leafy vegetable which is not consumed due to lack of knowledge by common people. It can be investigated to improve nutritional scenario of our country. Quantitative estimation of micro-nutrients has showed that 100gm fresh kulekhara leaves contain 56.1mg of sodium, 266mg of potassium, 27.93 mg of calcium, o.44 mg of zinc, 4.37 mg of copper, 7.03 mg of iron, 50.08 mg of vitamin-c, 102 microgram of vitamin-B2 (riboflavin),1microgram of folic acid, 2.5mg of β carotene.



Figure 2.1: Ayurvedic Kulekhara Syrup for hemoglobin

2.1.1 Kulekhara: The Local Nutritional Herb that helps Increasing Hemoglobin and Reducing Anemia

In ancient Ayurveda ,we find a mention of a medicinal plant named ‘’Kokilasha” which means ‘eyes like’. This is the plant commonly known as Kulekhara , which is native to India and is typically grown in swamp regions .This plant contains many micronutrients and anti-bacterial elements which have all the essential ingredient for healthy growth of cells. Though the nutritional value of the Kulekhara leaves is very high , it is highly under utilised due to lack of knowledge and awareness by general people.

Table 2.1: A Study Report of Comparison of macronutrient content of conventional green leafy vegetables with Kulekhara leaves is given below:

Name of Leaves	Carbohydrate (gm)	Protein (gm)	Fiber (gm)	Moisture (%)
Spinach	2.9	2	0.6	92.1
Amaranth	2.0	3.0	1.0	90
Bottle gaurd leaves	6.1	2.3	1.3	87.9
Ipomoea	3.1	2.9	1.2	90.3
Fenugreel leaves	6.0	4.4	1.1	86.1
Mustard Leaves	3.3	4.0	0.8	89.8
Kulekhara leaves	12.2	4.69	1.80	83.37

2.1.2 Power to fight Anemia

Leaves and stem of Kulekhara prevents and fights Anemia by increasing haemoglobin level in the body.

Statistically significant $p< 0.001$. Research study reveals that the way Kulekhara helps in increasing hemoglobin count is very interesting one. The chlorophyll molecule of Kulekhara is found to be similar to the molecular structure found in hemoglobin.The function of both is the electron transfer to maintain the dynamics to be formed as chemical energy. The water molecule plays a key role in this .However the exact mechanism is subject to further in –depth research Latter Gomez et al. showed in an experimental animal study that the ethanolic and aqueous extract of Kulekhara leaves have a very effective heamatinic effect and that can increase the

Table 2.2: EFFECT OF KULEKHARA ON HEMOGLOBIN, TOTAL RBC COUNT AND PCV

Parameters	Before Treatment (0 day)	After Treatment (30 days)	percent
Hb g/dl	8.37±0.04	11.22±0.07	34
Total RBC	3.78±0.16	4.34± 0.12	14.8
PCV %	31.11±1.43	37.83±0.88	21.6

total RBC count, WBC count and hemoglobin level effectively.

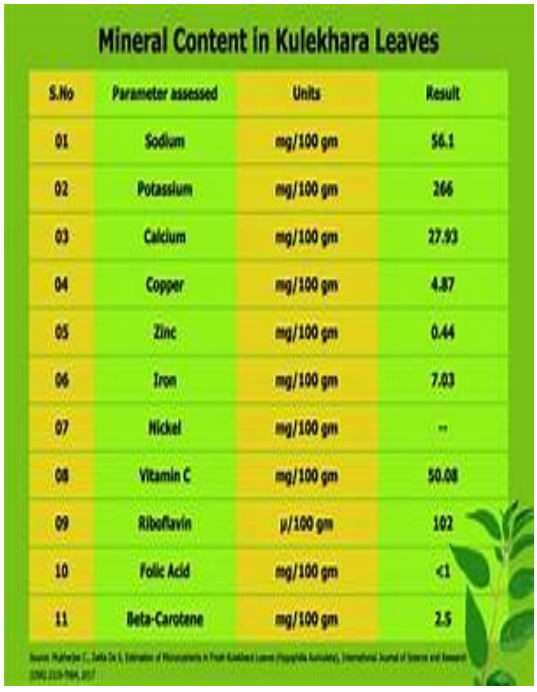


Figure 2.2: Kulekhara: The Wonder Herb — Branolia

2.1.3 KUKERRON – Anemic medicine to fight Anemia and increase Hemoglobin

Kulerron is an anemia reducing Ayurvedic tonic powered by Kulekhara. Kulekhara , which is the best-known antidote in Ayurveda against iron deficiency in the blood, acts as hemoglobin enhancer in Kulerron and serves as an effective anaemia reducing ayurvedic tonic .This anemic medicine does not lead to any adverse side effects unlike most iron supplements. Kulerron can be consumed by people of all ages but it is especially useful for women and girls in their growing up stage. Kulerron is serving thousands in the country suffering from Iron Deficiency Anemia by increasing their hemoglobin count and reducing Anemia in Ayurvedic natural way. Kulerron is manufactured by one of the most reputed, century old Ayurvedic Company “Branolia” in West Bengal,in a GMP certified facility . Branolia remain committed to the national fight against Anaemia and have extended the availability of Kulerron across the country through online sale(https://www.branoliachemicals.com/buy-now/)

This paper briefly describes the ecology and geographical distribution and provides details of morphological features along with uses of different plant parts (ethnobotany) of H. auriculata. This plant encompasses various phytoconstituents, which are responsible for its various medicinal properties. Though the plant is investigated by many workers for its medicinal properties, still further research for studying in detail is needed. Moreover, purification and identification of active principles and standardization of dosage forms of H. auriculata will have an immense therapeutic implication in human and animal health care system. Kulekhara leaves have shown potential in increasing RBC counts in humans. Studies have demonstrated that the extract of Hygrophila spinosa, known as kulekhara, significantly increased RBC counts in male albino rats, indicating a haematinic effect [1]. Additionally, the use of Moringa leaves, which are rich in iron, has been associated with increased hemoglobin levels, suggesting a positive impact on RBC counts in adolescent girls and pregnant women [2] [3]. Furthermore, the synthesis of silver-iron oxide nanoparticles using kulekhara leaves has shown promising results in degrading cationic dyes, indicating the potential health benefits of kulekhara leaves due to their iron-rich composition [4]. Overall, the data suggests that kulekhara leaves may indeed contribute to increasing RBC counts in humans, potentially aiding in the management of conditions like anemia. Medicinal herbs form the foundation of the traditional medical system. The use of herbal medications in the treatment of chronic illnesses is seen to be healthy, risk-free, and beneficial. Also, medicinal plants are the only source of affordable, sustainable, and nature-friendly medications for the expanding world population. Hygrophila auriculata belongs to the Acanthaceae family and is cultivated throughout India, Sri Lanka, Burma, Nepal, and Malaysia. It is traditionally used to prepare ancient Indian ayurvedic medicines for treating chronic disorders. Clinical studies reveal that the plant has various bioactive components with therapeutic applications. The plant contains a variety of bioactive components, including fatty acids, minerals, polyphenols, proanthocyanins, alkaloids, enzymes, amino acids, terpenoids, vitamins, glycosides, phytosterols, etc. Moreover, parts of the plant (seeds, roots, leaves, and flowers) are useful in treating a range of chronic diseases. Due to its high nutritional content and bioactive components, this plant is also eaten as a functional food in many parts of India. H.auriculata possesses various Pharmacological properties (Antioxidant, Cardioprotective, Neuroprotective, Diuretic, etc). Future research should focus on developing bio-packaging, isolating bioactive compounds from different parts of plants, and analyzing the mechanisms of action of such compounds. Also, various functional food and other natural products can be developed and utilized to treat chronic diseases. This review focused on the overview of the biological, bioactive components, and medicinal properties of H. auriculata, emphasizing the mechanism of action.

2.1.4 Kulekhara: This Local Plant helps in Increasing Haemoglobin; Know How to Use it

In English Khulekhara is called swamp weed, in Hindi Talimakhana it is called a flowering plant with medicinal qualities. Botanically, it is named as Hygrophila T spinose. In Ayurveda it is stated that the plant Kulekhara, which means eyes like, is derived from Kokilasha. This plant is considered to be native to India and is typically grown under tropical or subtropical climatic conditions in swamp regions. The flowers of this plant are typically purple and used

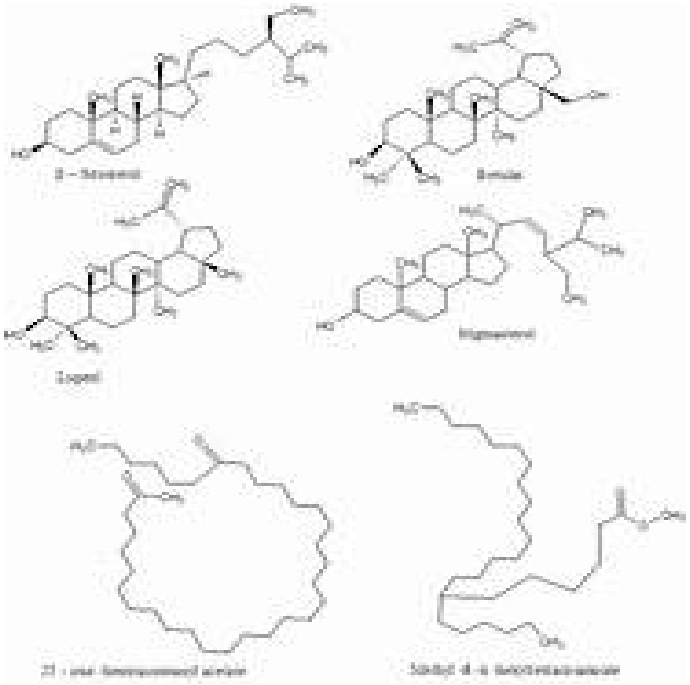


Figure 2.3: Hygrophila spinose T / Swampweeds / Talimakhana / Kulekhara

for medicinal use. Since the "Atmanirbhar" campaign started, there has been a lot of importance given to the local business and products. Now as the new campaign started, we should also try and learn about the local agriculture crops which are of utmost importance. In this 21st century as we are moving ahead with this pandemic, there are other health related issues which are never coming to an end. If we start counting there are lakhs of people who are suffering with many such diseases which are very common but tiring enough for those who are suffering with it. Anemia is a one such disease which has been a common problem among the younger children more due to the fact of malnutrition that has prevailed in this nation for a long time. This disease is where one lacks sufficiently healthy red blood cells to provide ample oxygen to the tissues of the body. The person may feel drained and weak with anemia. There are many causes of anemia, all of which have their own origin. Anemia may be acute or long-term, with moderate to extreme. Anemia therapies vary from supplementation to surgical care. Any forms of anemia could be avoided by a balanced and different diet too. It's actually better to find a solution which is more natural and has zero side effects. So there are some local plants which are so good for this disease that it has almost cured people from it permanently. Let's know more about it:

2.1.5 Beetroot Benefits: Eat This Red Vegetable to Fight against Diabetes, Blood pressure and Wintery Diseases

Wintery days are on the corner and a few days left to welcome those heart-chilling foggy days. So it's the...

Taxonomy	Synonyms	Names
Kingdom : Plantae	*Astercantha longifolia* (L.) Nees;	English : Long leaved barleria
Clade : Angiosperms	*Barleria auriculata* Schumach.;	Hindi : Talimakhana
Clade : Eudicots	*Barleria longifolia* L.;	Odiya : Koilekha
Clade : Asterids	*Hygrophilaschulli* M. R. Almeida	Telugu : Nerrugubbi
Order : Lamiales	& S. M.	Bengali : Kuliakhara
Family : Acanthaceae	Almeida;*Hygrophilaspinosa* T.	Sanskrit :*gokanta*
Genus : *Hygrophila*	Anderson	
Species : *auriculata*		

Figure 2.4: Taxonomy and vernacular names of kulekhara

2.1.6 Khulekhara:

In English Khulekhara is called swamp weed, in Hindi Talimakhana it is called a flowering plant with medicinal qualities. Botanically, it is named as Hygrophila T spinose. In Ayurveda it is stated that the plant Kulekhara, which means eyes like, is derived from Kokilasha. This plant is considered to be native to India and is typically grown under tropical or subtropical climatic conditions in swamp regions. The flowers of this plant are typically purple and used for medicinal use. Kulerron was researched and prepared by a popular pharmaceutical company Branolia Chemicals that exploits Kulekhara's goodness to combat iron deficiency in the blood. Kulekhara has Kulerron in its primary component and is one of the most effective iron deficiency cure antidotes.

2.1.7 Kulekhara's common uses:

The leaves of the plant are frequently used in the everyday food of East India in particular. The leaves are often used as a daily saag on with small fish and mustard. There is a lot of anti-oil derived from the whole plant. The Khulekhara leaf extract is also used for treating diarrhoea, inflammation, stomach pain and anaemia. The seeds of this plant are also medicinal and used for many blood diseases and urinary problems.

2.1.8 Kulekhara's Leaf's effects:

Information have been investigated and researched on the different positive behaviour of the Kulekhara plant. Two of the main advantages are: Anti-inflammatory, analgesic and antipyretic effects – The anti-inflammatory and antipyretic effect of H Spinosas/Kulekhara were supported

by studies in animal and isolated cell. The most effective chloroform extract in decreasing body temperature at doses of 400 mg/kg body weight was given to Brewster's yeast-induced pyrexia rats.



Figure 2.5: Kulekhara (Hygrophila auriculata) Juice

2.1.9 Hematopoietic influence

The plant's leaves and stalks is used to cure anaemia, which is indicative of iron deficiency of the blood as a disorder that affects people of all ages worldwide. Haemoglobin in the blood is known to rise. A rodent research showed that their red blood cell level and WBC count were increased. Antibacterial and anthelmintic activity – the herb is strongly anti-bacterial in nature especially in the leaves and other extracts. Astringent-meaning herbal blood cleansers are also classified as antifungal plants and have been found in this plant. A list of prescription anti microbiologic products intended for destroying dangerous bacteria is referred to in the phrase non- (contra)-biotic (life). They help shield the body from such infectious bacteria and strengthen the immune system. This plant has the above mentioned compounds too

2.1.10 Kulekahara origins and where is it found:

As mentioned earlier this leafy vegetable is originally found in the parts of West Bengal and have their roots from the northern parts of the state from where it got its local name and was made famous among the Bengali's by the Tribal people. These grow maximum as a wild plant near the damp areas of wells or any water bodies. Freshly it is harvested by the localites and is extremely cost effective. Therefore, instead of looking for chemical supplements why not try out this plant, all we need to do is boil the leaves and drink the strained water or else cook it as a normal saag and eat it with Chapatti or rice. The results are wonders that no one could have ever imagined for.

Chapter 3

Statement of Problem

Iron deficiency anemia is a common type of anemia — a condition in which blood lacks adequate healthy red blood cells. Red blood cells carry oxygen to the body's tissues. As the name implies, iron deficiency anemia is due to insufficient iron. Without enough iron, your body can't produce enough of a substance in red blood cells that enables them to carry oxygen (hemoglobin). As a result, iron deficiency anemia may leave you tired and short of breath. Hemoglobin is the protein in red blood cells that carries oxygen throughout the body. Anemia often occurs due to iron deficiency, so increasing iron intake through herbs like Kulekhara may help address this deficiency.
The objective of the thisis are

- To synthesise the mesocorous silica.
- To characterise silican nano particle(SNP)using SRD.
- To extract Kulekhara juice from kulekhara leaves to entrap the extracted values into silica nano carrier .
- To characterise the herbal drug loaded nano carrier using FTIR and XRD.
- To formation of transdermal patch using verbal drug loaded silican nano particle(SNP).

Chapter 4

Methodology

4.1 methodology

The following methodology is maintained for the fulfilment of the research objectives stated previously:

4.1.1 Synthesis of bare silica gel

Silica gel was synthesized using sol-gel technique at room temperature. Tetraethyl orthosilicate (TEOS) acts as a precursor of silica in sol-gel process. TEOS, ethanol and deionized water was taken in a molar ratio of 1:2:1. First, solution containing 5 ml of TEOS and 5 ml of ethanol were stirred for 20 min at 350 rpm. Then mixture of 5 ml of ethanol and 5 ml of deionized water was added dropwise into the above solution and stirred till the solution becomes clear and the homogeneity was ensured. The solution was then kept at room temperature to form gel. After cogelation, it was dried at room temperature till the weight of the gel becomes constant with time.At this stage the gel was collected.

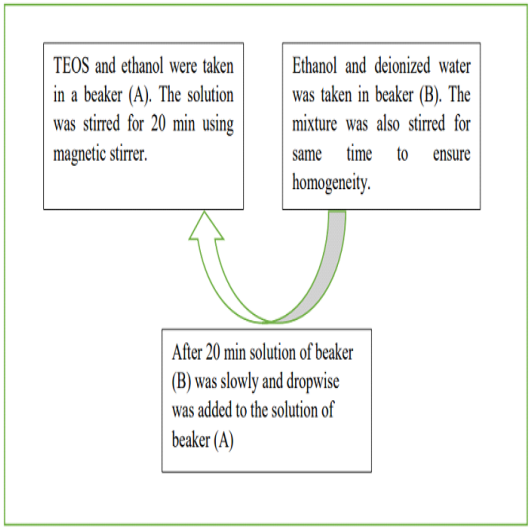


Figure 4.1: selica gel graph

4.1.2 Synthesis of drug containing silica gel

- Silica gel entrapping Kulekhara drug is to be synthesized with the help of sol-gel technique.
- The gel, after drying is to be characterized using Fourier Transform Infrared Spectroscopy (FTIR).
- Comparative study of FTIR of bare silica gel, kulekhara drug containing silica gel is to be done, to ensure if the drug has been entrapped in silica gel matrix.
- Invitro release study is to be done in a suitable release medium. The amount of drug release is evaluated with the help of UV-Visible spectroscopy.
- Determination of release kinetics of the released drug in a medium is to be done. The data from invitro release study is fitted against a suitable kinetic model to recognize the kinetic model.

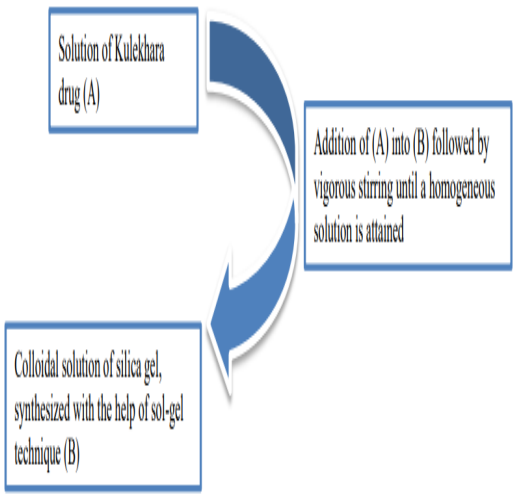


Figure 4.2: selica gel graph

4.1.3 Making of transdermal patch

The polymers EC, PVA, and PVP were utilized to fabricate the transdermal patches. First, aluminum foil was wrapped over the bottom portion of two side-opened cylindrical glass molds. Then, 5 mL of 4% (w/v) aqueous PVA solution was poured onto the foil, and the mold with PVA solution was kept at 50 degree celcius for 6 h to produce a very thin layer of the backing membrane. This was followed by the formation of the matrix in which EC-PVP (weight ratio of 2:1), CH/Na-MMT composite (5 mg of CH was loaded), and 10% (v/v) di n-butyl phthalate

(acted as a plasticizer) were dispersed homogeneously. The freshly prepared patches were stored in a desiccator for further experiments. A few patches were produced with free drug molecules keeping the amounts of other constituents the same.

- Transdermal patch containing silica gel entrapping drug mixture is to be synthesized.
- Invitro release of the drug mixture from transdermal patch is to be done in a suitable release medium. The amount of drug release is evaluated using UV-Vis spectroscopy.
- Determination of release kinetics of the released drug in a medium is to be done. The data from invitro release study is fitted against a suitable kinetic model to recognize the kinetic model.

4.1.4 Ex Vivo Skin Permeation Study

The polymers EC, PVA, and PVP were utilized to fabricate the transdermal patches. First, aluminum foil was wrapped over the bottom portion of two side-opened cylindrical glass molds. Then, 5 mL of 4% (w/v) aqueous PVA solution was poured onto the foil, and the mold with PVA solution was kept at 50 degree celcius for 6 h to produce a very thin layer of the backing membrane. This was followed by the formation of the matrix in which EC-PVP (weight ratio of 2:1), CH/Na-MMT composite (5 mg of CH was loaded), and 10% (v/v) di n-butyl phthalate (acted as a plasticizer) were dispersed homogeneously. The freshly prepared patches were stored in a desiccator for further experiments. A few patches were produced with free drug molecules keeping the amounts of other constituents the same.

Chapter 5

Result and Discussion

5.1 Implementation

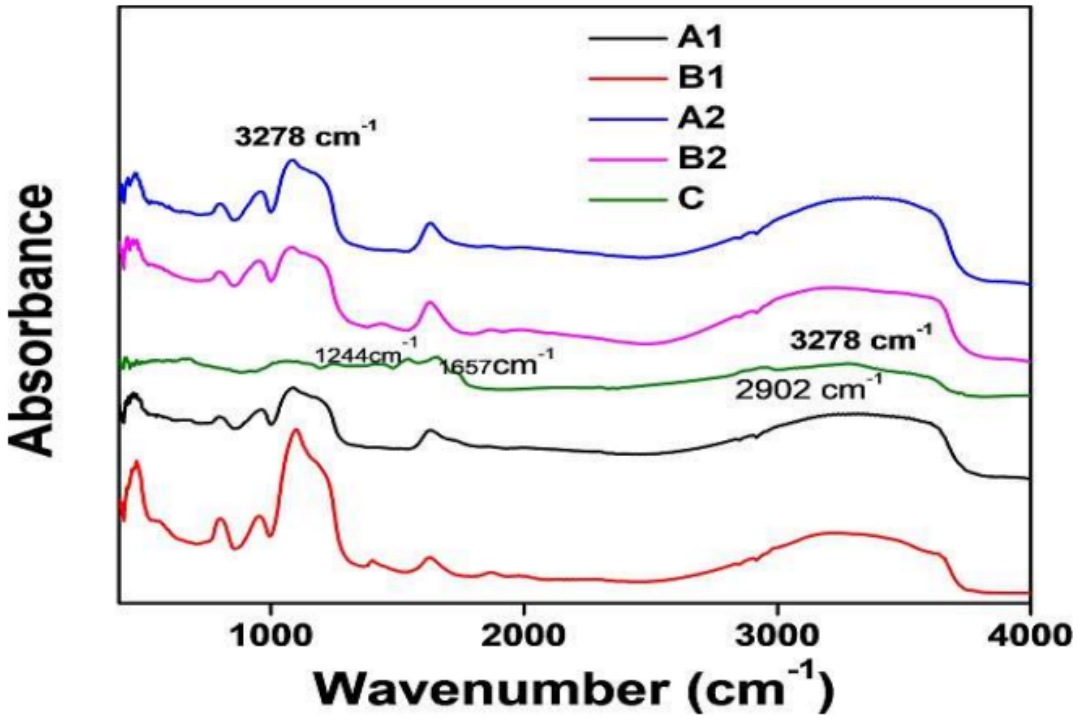


Figure 5.1: FTIR analysis for Kulekhara drug : pure kulekhara powder(C), Base catalyst SNPs(B2), Acid catalyst SNPs(A2), Drug loaded Base Catalyst(B1), Drug loaded acid catalyst(A1)

FTIR spectra of pure kulekhara powder(C), Base catalyst SNPs(B2), Acid catalyst SNPs(A2), Drug loaded Base Catalyst(B1), Drug loaded acid catalyst(A1). The FT-IR spectra reveasl the presence of different functional groups like C–N, C–O–C, amide linkages and –COO– , C-H, N-H, C=C , Alkyl group, Halo formyl group etc. .The peak at 1041 was associated with

stretch vibration of –C–O–C– [8], while other peaks at 1357, 1384, 1450 and 1587 cm À 1 were aroused for C–N stretching vibrations of aromatic amines [8], residual –NO 3 [25] [8], symmetric stretching vibrations of –COO– present in carboxylic acid present in amino acids and aromatic C–C skeletal vibrations, respectively [25] [8], which are normally present and associated with protein biomolecules. The peak at 1657 cm-1 was associated with stretch C =C and is assigned to the aldehydes, ketones, carboxylic acids and ester compounds. The peak value 1244 cm-1 attributes to OH bending vibrations. The peak at 2902 cm-1 indicates the presence of C-H stretching. The peak value at 2346 cm-1 indicates presence of C-N stretching. There is no absorbance in region 2220-2260 cm-1 . it indicates there is no cyanide group in the extract.

FTIR spectra of pure kulekhara powder(C), Base catalyst SNPs(B2), Acid catalyst SNPs(A2), Drug loaded Base Catalyst(B1), Drug loaded acid catalyst(A1)

5.2 XRD Analysis of Drug:

5.2.1 XRD Analysis of bare silica gel

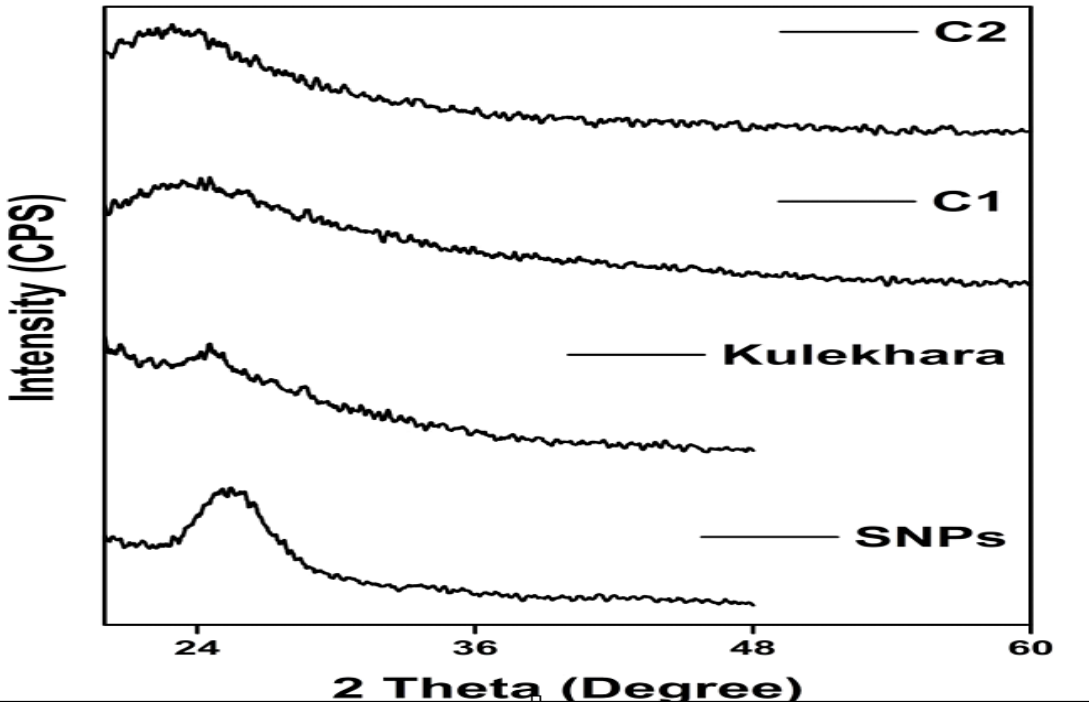


Figure 5.2: XRD analysis of pure SNPs, Pure kulekhara powder, Drug loaded acid catalyst (C_1) ,Drug loaded base catalyst (C_2)

A crystal is composed of periodically arranged in a three dimensional space. On the other hand, amorphous materials do not possess that periodicity and atoms are randomly distributed in the three dimensional space. When there is periodic arrangement of atoms, the X-rays will

be scattered only in certain directions which will cause high intensity bands. For the amorphous phase X-rays will be scattered in many directions leading to a large hump distributed in a wide range (2 Theta) instead of high intensity narrower bands. From the figure 6.3 it is seen that a hump exists in between 15-30 degree of two theta values, which represents the amorphous nature of silica gel [7]. This Amorphous nature of silica gel is not toxic for human body [5]. The absence of high-intensity difraction peaks for SNPs implied its amorphous nature. Highly intense narrower difraction peaks were the indications of the crystalline characteristic of the drugs. But after the SNPs were incorporated, a small ammount of crystalline drug was detected in the XRD pattern of drug-loaded SNPs.

5.2.2 Invitro release study of kulekhara from silica gel matrix:

The invitro release study of the drug from silica gel matrix was performed in PBS. The amount of drug released in PBS from silica gel matrix was measure by UV-vis spectroscopy at a maximum wavelength (λmax) of 262 nm. The concentration of released kulekhara at different interval of time was calculated from their absorbance values using standard curve and then the result was plotted against time (Figure 6.5). Initially the release was very rapid in first 4hrs then it was gradually slowed down at around 96 hours. Further release of Kulekhara could not be seen due to possible degradation of the Kulekhara molecule. UV–Visible spectroscopic analysis showed cumulative release percentages of 59.9% for Kulekhara in a period of 96 hours [6, 26]. To find out the concentrations of the unknown solutions from their absorbances obtained from the UV- Vis spectroscopy, we first need to plot a calibration curve of few standard solutions, whose concentrations are known to us. So, six sets of standard solutions having concentrations 5, 10, 15, 20, 25, 30 μg/ml was prepared. The stock solution of Ku8lekhara was prepared by dissolving 5 mg of Kulekhara in the mixture of 10 ml of ethanol and 40 ml of PBS. Then the six sets were prepared by dilution process using above stock solution.

After that, the absorbance values of six sets of standard solutions were taken from UV-Vis spectroscopy and a calibration curve was plotted taking X-axis as concentration and Y-axis as Absorbance

From release profile it was known that the release mechanism was biphasic. The drug was released rapidly from the silica matrix for the first six hours after which the release rate slowed down up to 96 hours. The initial four hours show a characteristic burst release due to the drug adsorbed at the surface of the silica gel matrix. Also it can be said that there was a higher concentration gradient of the kulekhara between the silica matrices and the dissolution medium initially which was the driving force for higher dissolution of the drug in first phase. The later stage is characterized by a slower rate of release due to the slow diffusion of drug from within the pores of the silica matrix. The drug release at this stage occurs when the solvent medium diffuses into the dense network of pores, cracks and interparticular spaces present in the matrix.

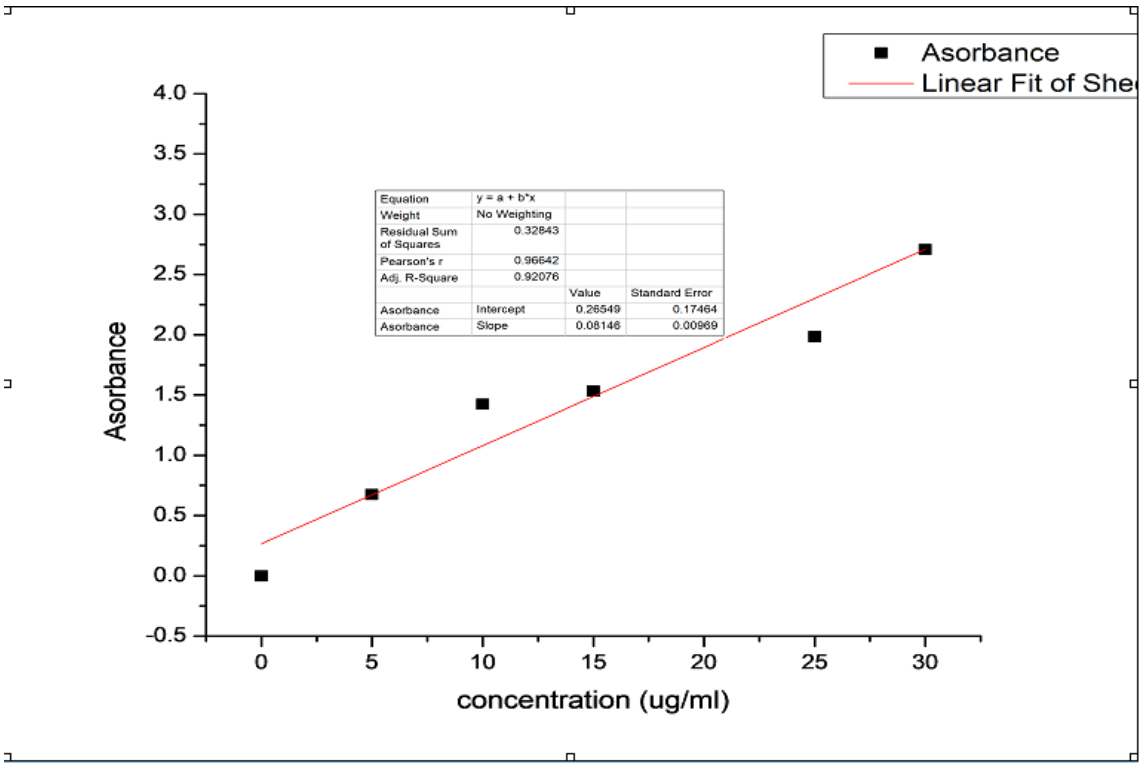


Figure 5.3: Calibration curve for Kulekhara drug

Table 5.1: A Study Report of Comparison of macronutrient content of conventional green leafy vegetables with Kulekhara leaves is given below:

Formulations	Zero Order (R2)	First Order (R2)	Higuchi model (R2)	Korsmeyer Peppas model (n2)
pH 7.4 Drug loaded acid	0.6615	0.8328	0.7976	0.7697
pH 7.4 Drug loaded base	0.8054	0.1058	0.9033	0.8029
pH 5.5 Drug loaded acid	0.7012	0.0234	0.8339	0.8324
pH 5.5 Drug loaded Base	0.8033	0.0171	0.9008	0.8617

Table 5.2: Ex vivo Release Data of kulekhara patch through huma skiin PBS during time period 15 min to 168 hr:

Formulations	Zero Order (R2)	First Order (R2)	Higuchi model (R2)	Korsmeyer Peppas model (n2)
Kulekhara Patch	0.6615	0.017	0.9236	0.9518
Drug Loaded Acid Patch	0.9621	0.0136	0.9509	0.9071
Drug loaded ase Patch	0.9071	0.3269	0.661	0.7199

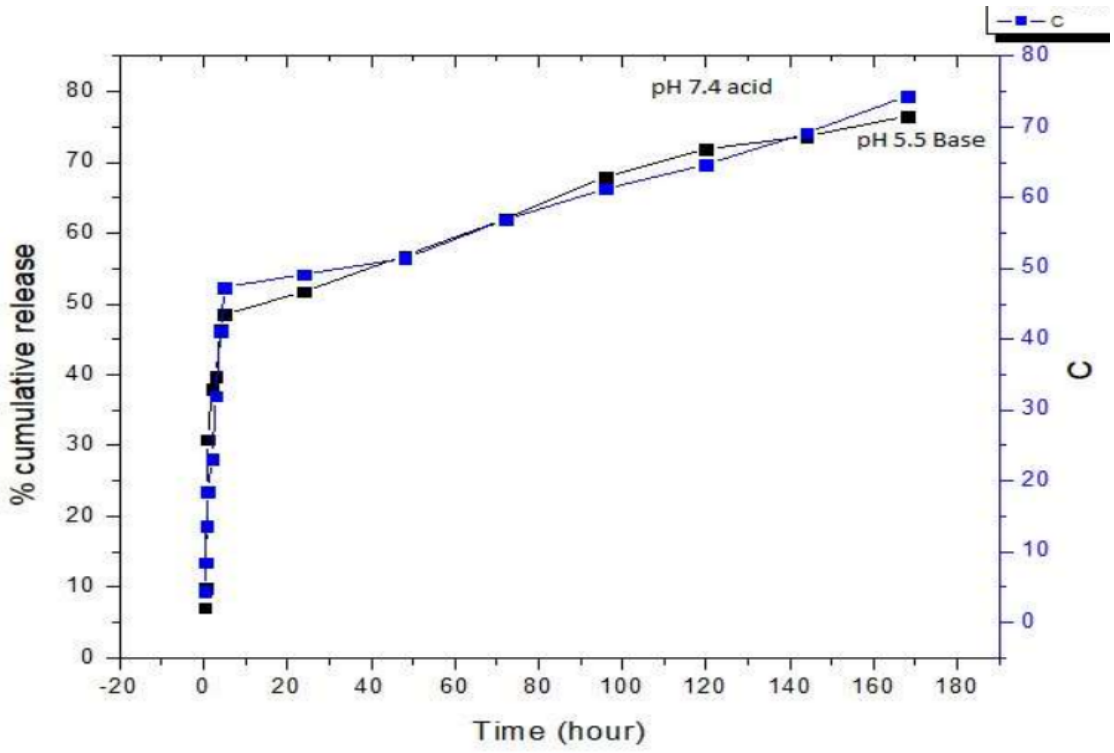


Figure 5.4: Time vs % cumulative release

Table 5.3: Ex vivo Release Data of kulekhara patch through huma skiin PBS during time period 15 min to 168 hr:

Formulations	Zero Order (R2)	First Order (R2)	Higuchi model (R2)	Korsmeyer Peppas model (n2)
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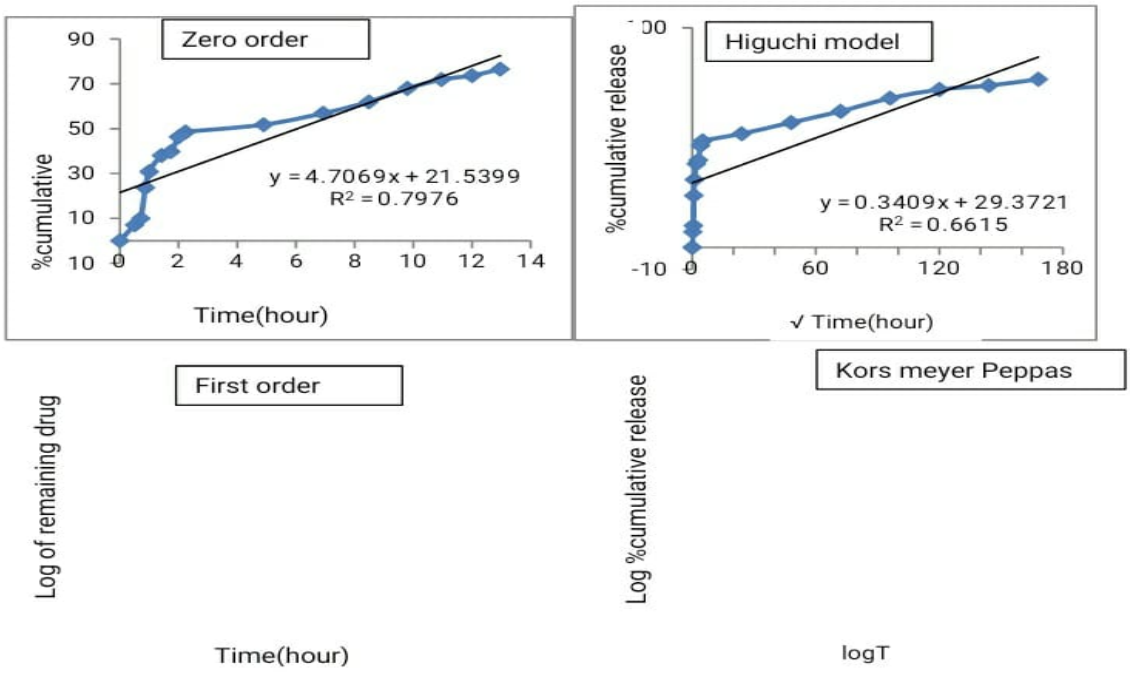


Figure 5.5: Calibration curve for Kulekhara drug

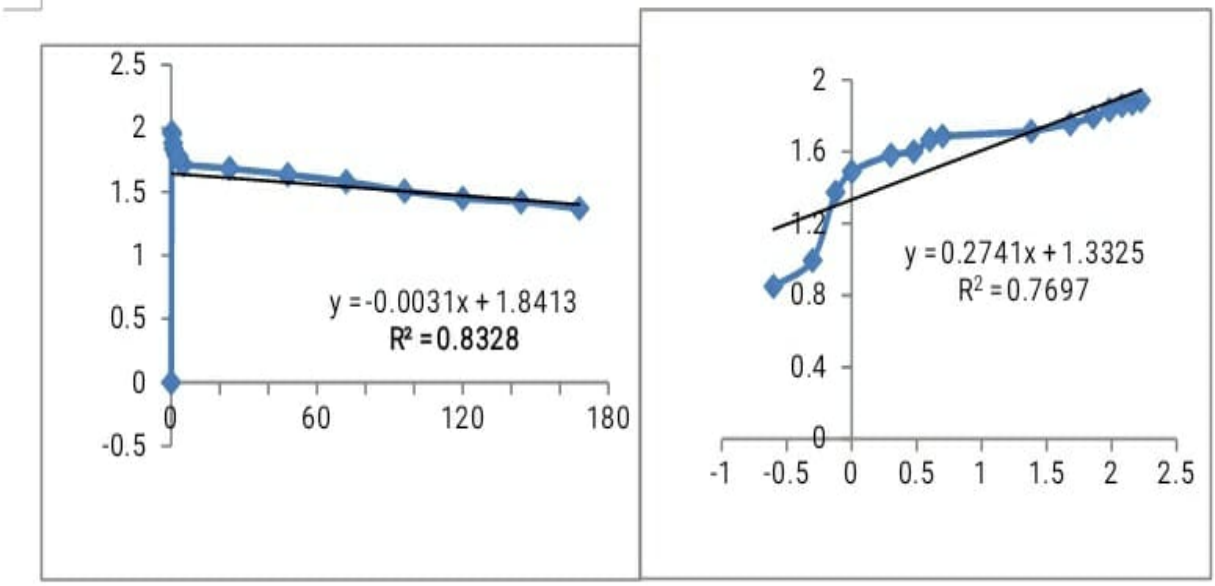


Figure 5.6: Calibration curve for Kulekhara drug

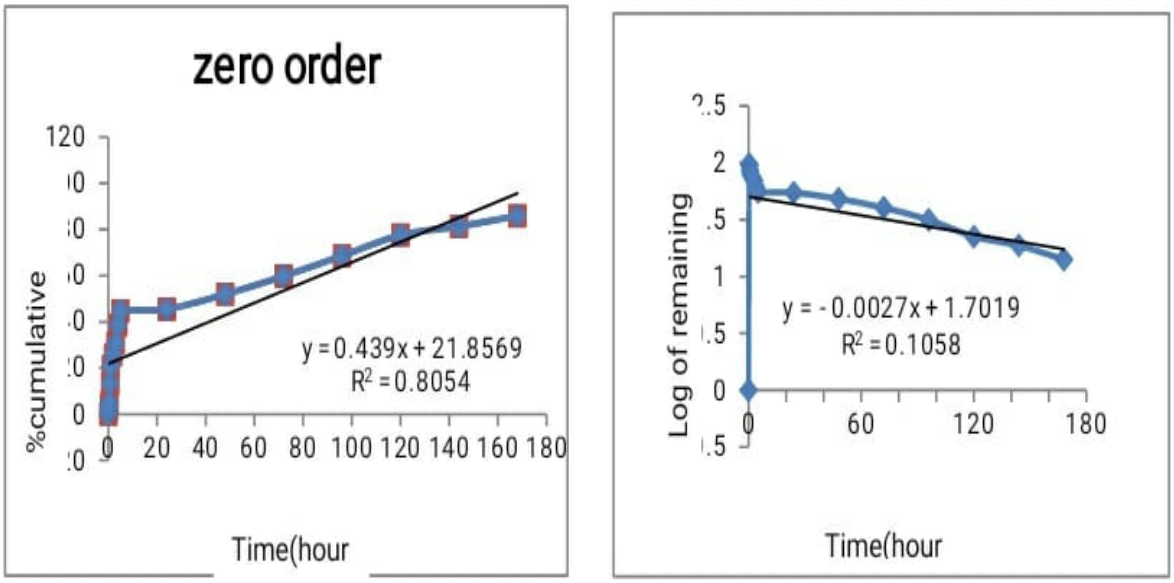


Figure 5.7: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

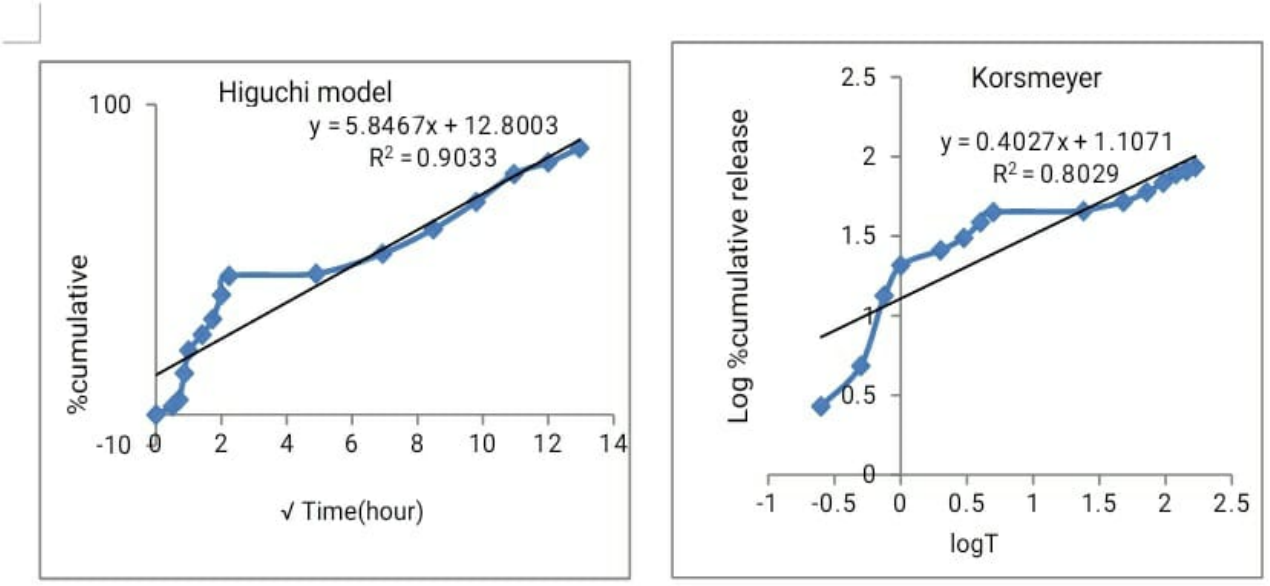


Figure 5.8: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

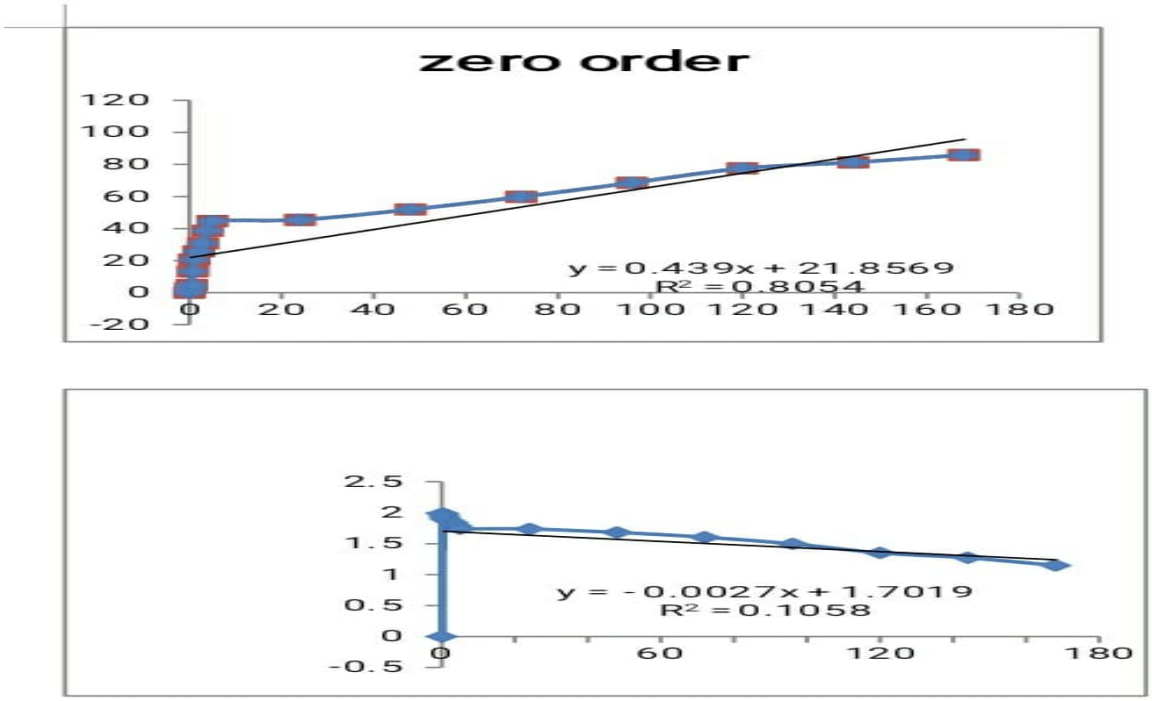


Figure 5.9: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

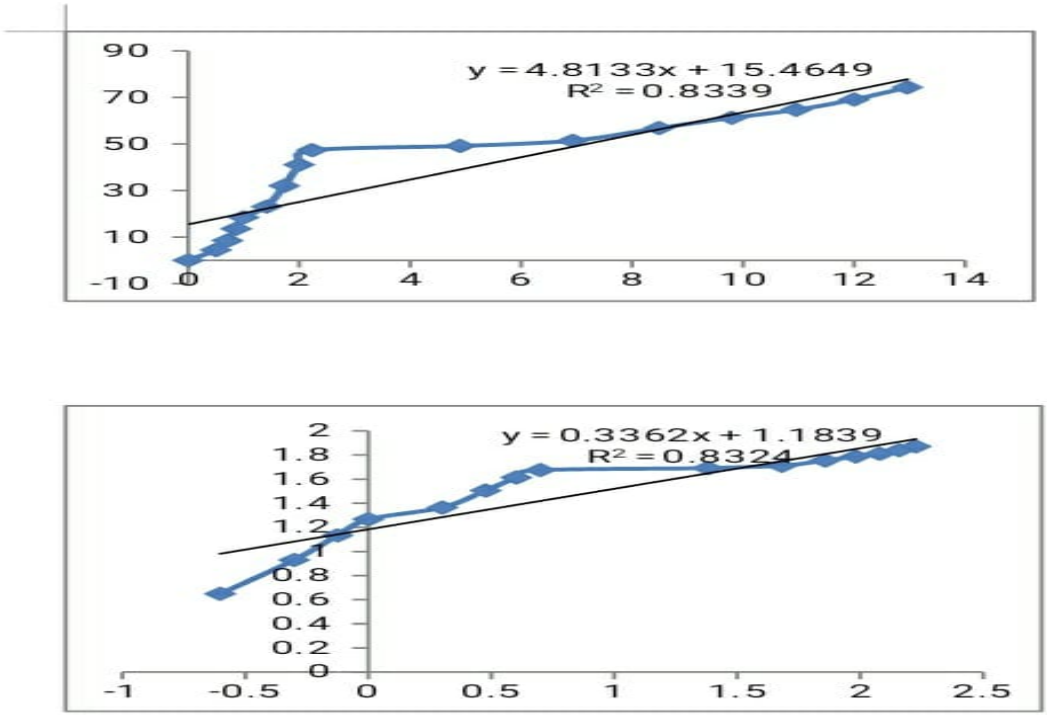


Figure 5.10: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

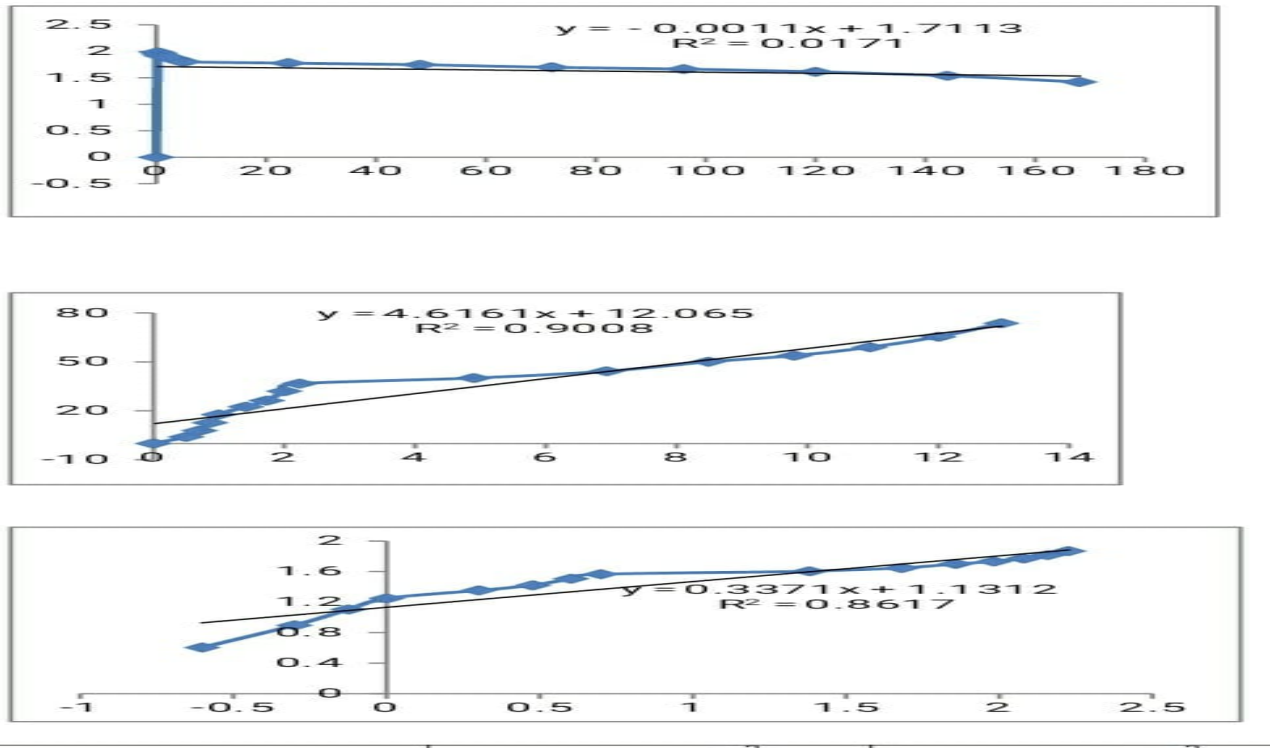


Figure 5.11: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

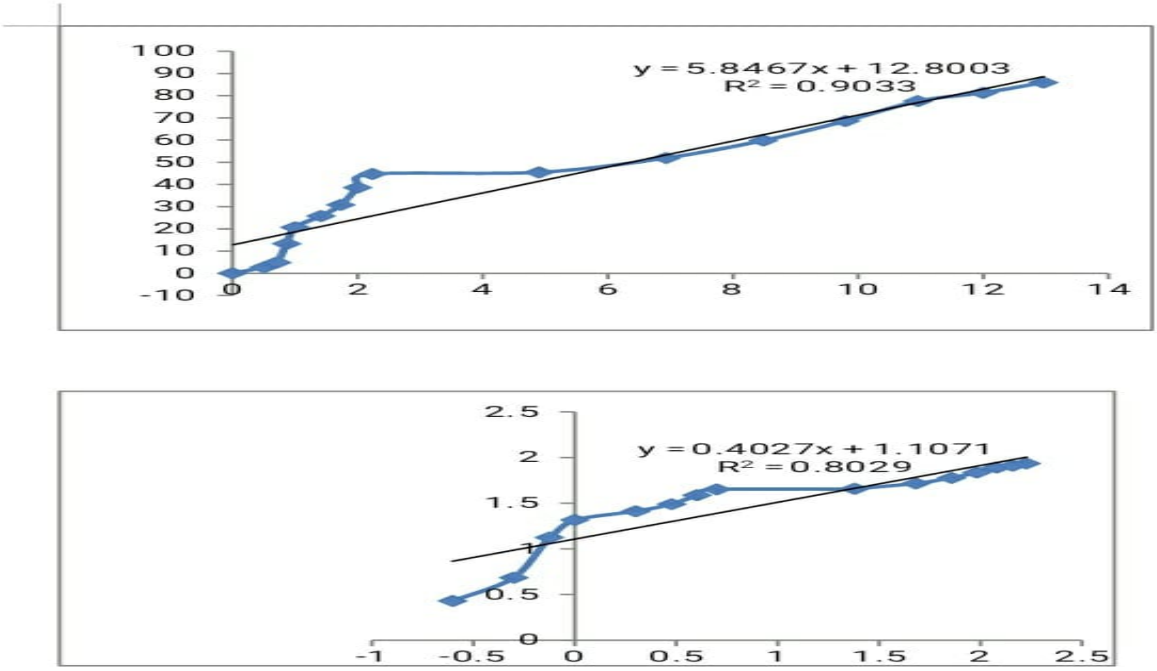


Figure 5.12: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

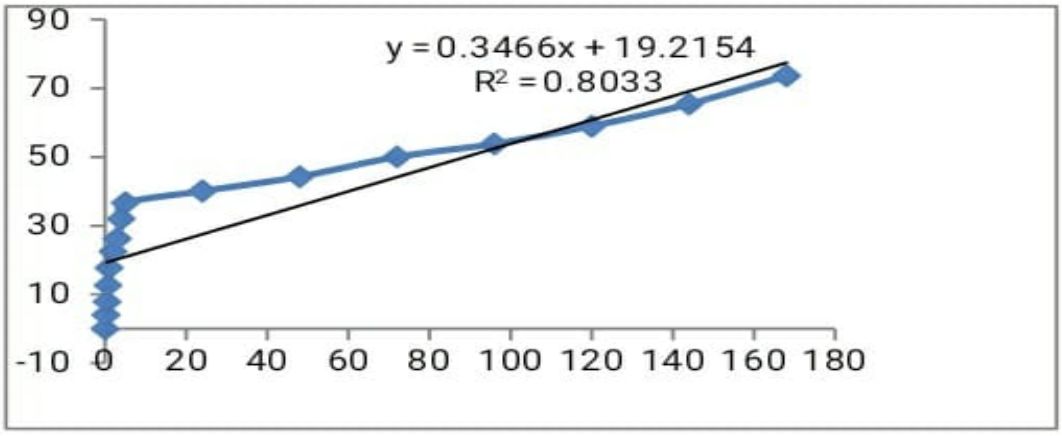


Figure 5.13: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

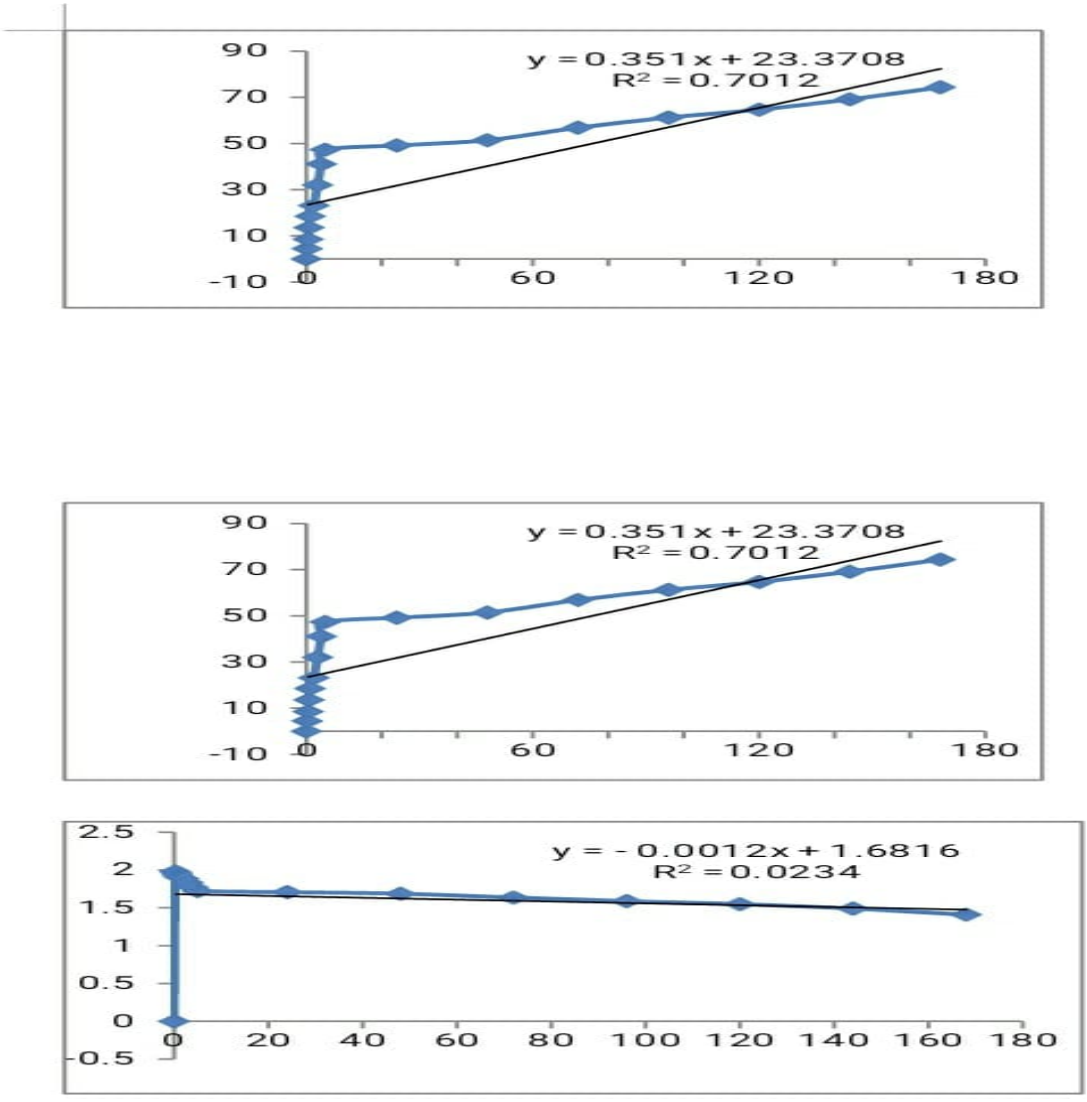


Figure 5.14: Zero order,First order, Higuchi model,Krosmeyer-Peppas for pH 5.5 Drug loaded ase

Chapter 6

Conclusion

6.1 Conclusion

- Herbal drug was successfully entrapped into silica nano carrier.
- Herbal drug loaded nano career was characterised using FTIR and XRD.
- Transdermal patch using herbal drug loaded SNP, s was formed. .
- Initially, the release was very rapid in first 4 hours and gradually slowed down around 96 hours.
- UV- Visible spectroscopic analysis showed that the cumulative release percentages was 61% in SBF in a period of 96 hours.
- FTIR analysis confirmed the successfull entrapment of curcumin in the mesoporous silica gel.

Chapter 7

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