Fabrication and characterization of alginate-gelatin-nano silver composite

A Thesis submitted to the Faculty of Engineering and Technology of Jadavpur University for the partial fulfillment of the requirement for the degree of Master of Technology in Department of Food Technology and Biochemical Engineering.

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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 done by myself under the guidance of my guide, as a part of my Master of Technology in Food Technology and Biochemical Engineering studies.

I have conformed to the norms and guidelines given in the Ethical Code of Conduct and the academic rules of the Institution.

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The thesis entitled "Fabrication and characterization of alginate-gelatin-nano silver composite", prepared by Mr. Prithweejit Bhattacharyya (Registration No. 156629 of 2020-2021) at instance, is hereby approved as a creditable study of an Engineering subject carried out and presented in a manner satisfactory to warrant its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned does not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein, but approve this thesis for the purpose for which it is submitted.

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ABSTRACT

One of the essential components of every food product is the packaging material used. Common people and consumers generally discard off this packaging material (films/wraps/boxes etc.) and are not aware of the effects which these materials cause on the surrounding environment. This research work aims to design a packaging film which is biodegradable, inert to the food material packed inside it, easily disposable, has microbial barrier properties and can preserve the food material for a considerable period without any adverse effects.

For this, silver incorporated gelatin-alginate composite films are prepared using solely a green method of approach where the extracts of leaves of lamboo tree (*Khaya anthotheca*) have been used as the reducing agent. The polyphenol and antioxidant compounds present in the leaf extracts of lamboo tree reduced the molecular silver into silver nanoparticles.

For silver nanoparticles which were obtained by the reaction of lamboo tree leaf extract with silver nitrate, during their analysis in UV-visible spectroscopy the starting and ending wavelength range was kept between 200-700 nm respectively. The surface plasmon resonance band (SPR) was found at 437 nm. From the results obtained, it was seen that with increase in amount of leaf extract the absorbance increased as well as the sharpness and height of the peaks obtained. Gelatin and sodium alginate were mixed in double distilled water. Polyethylene glycol was used as a plasticizer. Different proportions of these two mixtures were combined in different ratios. The previously made silver nanoparticle was used in some of the proportion mixtures of Na-alginate and gelatin.

For each set of films analysis was done for hardness, solubility, shelf life of food packed in the films and microbial barrier properties of the films. The results showed that for composite films, hardness increased with the amount of silver nanoparticle being added to it whereas for films devoid of NPs, the hardness fell sharply after reaching a maximum value. Fresh tomatoes and grapes being packed in the NP incorporate d films showed better retention in its properties than tomatoes and grapes in gelatin-alginate films devoid of NPs. For antimicrobial properties, *Escherichia coli* and *Staphylococcus aureus* was inoculated on both the films along with nutrient agar plate as a reference model. After incubation for 24 hours at 38°C, no observable growth was observed on any of the composite films. Positive results were observed for solubility as well where 73.92% (almost 74%) solubility was found for NP incorporated films.

Therefore, the above composite films obtained with combination of silver nanoparticles along with combination of sodium alginate and gelatin can serve as suitable packaging films for food products.

Keywords: - Lamboo tree, leaf extract, silver nitrate, spectroscopy, sodium-alginate, gelatin, polyethylene glycol, nanoparticle, composite films.

CONTENTS

INTRODUCTION

Noble metal Nanoparticles and their production by using "Green synthesis": -

"Nanoparticles" as the term suggests itself defines it as certain materials whose identity is mainly thought of by their dimension meaning any material belonging to the nano range (1 to 100 nanometers). There are different types of materials that can be transformed into their nanomaterial formation. There are materials which being very much unstable in their nanoparticle formation are generally not obtained in the pure form, instead they are obtained as nanomaterials in the form of their oxides amongst which nanoparticle oxides of silicon, titanium and aluminum are prominent ones as shown by the works of **[**1**].** When study was done with certain noble metals was done it was found that the nanocomposites of these metals were much more stable in their nano formation and also portrayed better and enhanced functional properties compared to the large parent metal itself. Some of the notable noble metals in this context are gold, silver and platinum as observed by the works of [2**]**.
Now when discussing about the methods for the synthesis of nanomaterials different studies have shown different methods amongst which if summarized, there are three main approaches for the synthesis of nanomaterials which are divided into three categories such as physical methods, chemical methods and biological methods as showed by the works of **[**3**], [**4**]** and **[**5**]**.

In this work, biological method is used for the generation of silver nanoparticles form silver nitrate solution using the leaf extracts of lamboo tree (*Khaya anthotheca*). There are many positive aspects of the biological method or green synthesis of noble metal nanoparticles compared to the physical and chemical methods which are discussed in different research works. From the works of **[6]** the report obtained shows that biological methods are environment friendly as they are devoid of the application of any hazardous chemical components. The works done by **[**7**]** also state that the noble metal nanoparticles which are obtained by green synthesis are much more stable as they are produced using a single step method along with which the biological components that are used for the synthesis of the nanoparticles they themselves perform reducing action as a result of which the total cost of the nanoparticle generation procedure becomes economically more feasible as stated in the works of **[**8**]**. Although certain works and researches done show that the biological or green synthesis method has two major defects including the first one that biological species used whether microorganisms / plants / fungi etc. are dependent on the natural vegetation, overall climatic condition and the type of ecosystem prevailing in a particular area which is different and varies from one place to another. Therefore it is always not possible to obtain the desired biological agent to be used for nanoparticle synthesis everywhere in all parts of a particular area, **[**9**]**. As the green synthesis method uses reducing agents of organic origin therefore for strong type of metals at times it happens that the nanoparticles are not obtained, the reason being the components obtained are not very powerful reducing agents. But when compared with physical and chemical methods, in overall aspect biological method or method of green technique is a better and effective choice mainly due to three important points which are safety towards the environment, absence of using of any toxic and hazardous chemical components, no need of high amount of energy or pressure to be added separately and thus maintain and keeping the budget of the entire nanoparticle generation process within the desirable economic limit, **[**10**]**.

When discussing about the biological methods, then again there comes different ways for the production of nanoparticles which is a result of the presence of different agents that are used in this green synthesis method. The works done by [11] clearly mention that there are four main agents under the green synthesis techniques which are bacterial agent, fungal agent, and algal agent and plant agents. Some researchers also show that even agents such as agricultural wastes if properly utilized can be used for the green synthesis of nanomaterials, [12]. Certain works also show that enzyme oriented mechanism helps in the suitable production of nanomaterials as well, [13]. One of the main reasons include that green synthesis using plant sourced agent is a mono-step method, devoid of any pathogenic contamination and cost reducing because for certain species of microbial cultures their appropriate substrate is needed to be provided and proper growing parameters along with storage conditions which lead to an increase in the cost of the entire process. For enzymatic agents also, the requirement of appropriate storage of the enzymes and gradual depreciation in their functionality and the process is to be controlled very accurately in order to maintain the appropriate temperature so that the enzyme functions properly, [14].

There are different components of the plant part which are utilized for synthesis of nanoparticles such as roots, flowers, leaves, barks etc. Generally plant parts are enriched with certain components such as alkaloids, phenolic materials terpenoids which play the main role by performing the reducing function and thereby producing metal nanoparticles amongst which silver nanoparticles is a major one, [15]. Different works show different methods by which plants help in the generation of noble metal nanoparticles. The extracts obtained from plat sources perform the biotrimming function of the metal ions to form the nanoparticles and as a result certain benefits are obtained such as the shape and size of the nanoparticle can be easily monitored by proper monitoring of the pH of the reacting system and the temperature as well,[16]. The very important question that arises is whether every species of plants and their parts such as roots, leaves, stems, flowers etc. are capable of bioreduction of the metal ions to their nano formation? The answer is that not every plant species has this special property in them. There are certain species of plants with which many experiments and researches have been done, some of which have yielded excellent results of nanomaterial production whereas some of them have provided medium or moderate results and amongst them some provided results those are unsatisfactory and not considerable.

Researchers [17] through their works demonstrated that the extracts obtained from *Azadirachta indica* helps in the effective formation of gold and silver nanoparticles that generate very strong absorbance when observed in the visible range of wavelength. Research works showed that certain plant species which has abundance of phenolic components, alkaloids; terpenoids etc. are very much useful in the biological breakdown of large metal ions to their nanomaterial structures as reported by the works of [18].Many varieties of indian plants such as *C.camphora, Carica papaya, C. annum*, *Geranium indicum*, banana peel extract, orange peel extracts etc. have been reported as excellent plant originated agents for noble metal nanoparticle production in various research works. Therefore, plant species having an adequate amount of the organic components such as polyphenols, phenolic derivatives, terpenoids etc. can be used for the biosynthesis of noble metal nanomaterial production. Other type of plants may also be used for this purpose and nanomaterials may be obtained but the desired amount of yields of the nanomaterials, the appropriate shape and size, the required properties for which the synthesis is being done may not be fulfilled to the desired state as is obtained for plants with bright colored leaves and flowers

and dark colored stems and roots which are an indication of their rich phenolic and antioxidant content. Methods of preparation of nanomaterials are generally categorized mainly into two divisions based on the approach through which they are synthesized which are known as top-down approach and bottom up approach, **[**19**]**. The top-down approach is a particular method or approach which follows the sequence of preparation of the nanoparticles where the parent bulk materials are first broken down into smaller fragments and then gradually they are broken down into their nanoscale components, **[**20**]**, **[**21**]**.

Methods and approaches of Production of Nanoparticles: -

As discussed earlier, the three ways of nanomaterial production include physical, chemical and biological procedures based on the source agent that is being used. As shown by research works, in the method of attrition large macro particles are being continuously stroked and made to grind around themselves by the use of extensive force and pressure generally as it is observed in a ball milling process, as a result of which the parent particles which are massive in dimension, due to constant self striking are gradually broken down into fine particles which are later separated by different filtration methods and collected, **[**22**]**, although this should never be taken into consideration that method of top-down approach is only restricted to physical procedures, often it involves chemical methods as well but the basic difference lies that the starting area deals with a mammoth sized parent material which operates with huge amount of mechanical forces and the larger material is being fragmented out to its nanoparticle formation,**[**23**]**. In bottom-up approach as described by research works, the atoms / molecules are assembled among themselves in order to form the nanomaterial **[**24**]**, that is here the self generation starts from the nano-range dimension only and gradually it proceeds to obtain the desired nanoparticle in its actual required state. Bottom-up approach technique has certain benefits when it comes to the question of nanoparticle formation, as research works show that by using this approach, nanoparticles which are formed are found to have less defects, uniform chemical composition and small amount of contamination from any toxic or undesired components, **[**25**]**.

Certain methods come under the category of bottom-up approach such as nanoparticle formation by sol-gel technique, biosegregation or bioreduction, aerosol process etc. **[**26**]**. While using the top-down approach, a considerable quantity of waste material generation is present from the parent material due to application of high mechanical forces and pressure which are not utilized during the nanomaterial formation, which is absent in case of bottom-up approach as the process is self-assembly oriented as well as by bottom-up approach, nanoparticles generated can be obtained in accurate shapes devoid of any cracked or ununiform points and rough surfaces compared to top-down approach which results in nanomaterials with crooked and uneven pointed surfaces [27]. Although certain works show that in top-down approach the cost is comparatively lower as there is no investment of large funds required as that of bottom up approach [28] and in top-down approach the shape and size of the product can be monitored but when it comes to the question of better fabrication, uniformity and homogeneity of the nanoparticles produced with minimum number of imperfections , energy efficient and economically feasible in the long run then bottom-up approach is always to be chosen **[**29**]**.

Nanoparticle production by using plant extracts and selection of the appropriate plant species: -

Now when coming to the process of using the plant extracts for nanoparticle production the most common and effective method which comes forward is, that the extract is normally mixed with the metal solution at ambient room temperature condition and the reaction is completed within a couple of minutes, [30]. Certain noble components such as gold and silver are obtained by using this phenomenon, although for certain metal solution and extract mixtures it may require to be placed in a moderate warm water bath generally when the experiment is being carried out in monsoons or winter seasons as the room temperature being lower the reaction time takes comparatively more time. The previous agents mentioned are extreme rich sources of polyphenols and antioxidants, which play the main role in the simple monostep and ecofriendly process of forming nanomaterials from bulky noble materials, [31]. The important question is of availability of the parent material in a large geographical scale and throughout the year irrespective of all adverse climatic and environmental conditions. The previously discussed materials used are although cheap and easily available at certain parts of a particular locality but when considering a larger area then many parameters are to be considered such as for using tea as a material for obtaining the extract, the problem comes that a considerable time is required for the proper cultivation, growth and harvesting of tea after which it can be obtained as a green source for nanoparticle production, different climatic conditions are observed in the country which play a vital role in the cultivation of good quality tea products which is a very essential factor, because poor quality parent plant sources are devoid of sufficient levels of strong polyphenol concentration as a result which cannot be utilized for obtaining nanoparticles as per the requirement, [32], [33] and in India, the north-east parts of the country are the major producers of tea [34], therefore obtaining of good quality tea products from north-eastern parts to other distant parts of the country for nanoparticle production will involve a considerable hike in the transport costs which will increase the overall cost of the process.

Selection of "*Khaya anthotheca*" (Lamboo tree) leaf extracts for green synthesis of AgNPs: -

Therefore considering the above factors it is essential to select a suitable plant species which is devoid of all the above mentioned defects and the disadvantages therefore lamboo tree, scientifically called "*Khaya anthotheca*" is selected for the following research work which belongs to the family of Mahogany trees, a species of plants growing almost in all the major parts of India such as Maharashtra, Kerala, West Bengal, Tamil Nadu, Bangalore, Karnataka etc. [35] and this particular plant has ability to grow effectively in places having an average yearly temperature of 28°C and places as high as 1500m above sea level, [36]. Along with these properties, it is also observed from various research reports that this species of plants can grow nicely in a vivid and wide range of soil textures ranging from lateritic soil, metamorphic soils, alluvial soils, soils developed from volcanic eruptions and limestone as well and the very significant property is that, plants belonging to the mahogany family do not degrade or hamper the quality of the agricultural land in which they are being cultivated, instead these plants are very much useful and are recommended to be used for the successful restoration of the fertility and the quality of the soil, [37]. Along with this, mahogany species showed equally appreciable amount of polyphenol content which were measured using Folin-Ciocalteu method, vanillic assay and by using acid/butanol methods. It was observed that components such as gallocatechin,

fisetindin and trihydroxyflavan monomers were present as well in Khaya ivorensis species belonging to the mahogany family, [38]. Mahogany family is generally represented by seventy two of its species among which twelve of them are strictly restricted in the Indian subcontinent as a result of which there is very less requirement of importing of raw materials from outside and the overall cost can be kept within the limit making the process economically feasible, [39].
Different research works suggest that the amount of phytochemical and phenolic components that are obtained as a yield from the extraction process during solvent extraction is dependent on the procedure of extraction that is implemented along with greatly affected by the type of solvent that is being used for extraction purpose, [40] and [41].

Selection of appropriate solvent for extraction of plant components: -

Methanol, ethanol, hexane and water are the typically used solvents to be used in solvent extraction procedures for obtaining phenolic components from plant materials. Research works show that the yield percentage of the phenolic components obtained showed a severe and drastic increase in their amount when the solvent medium was gradually changed from only water to ethanol in the proportions of 25% (1 : 3 parts of ethanol : water), 50% (1: 1 ratio of ethanol : water), 75% (3 : 1 parts of ethanol : water) gradually in this manner, indicating the better yield with the increase in ethanol composition of the solvent, which indicates that a certain appreciable amount of non-polar components are present in the plant parts, [42]. The final selection of solvent to be used is dependent on the nature and characteristics of the phenolic components present in the plant because until it is known that whether the majority of the phenolic components present are polar or non polar in nature or semi polar accordingly to which the selection of a polar solvent (water), non-polar solvent (ethanol, petroleum ether) or mixed solvent (water: ethanol) cannot be made, [43].

Major polyphenol components whose abundance is observed by researchers in leaves of plants belonging to the mahogany species has been reported which include many polar water soluble components such as flavonols, quercetin, myricetins [44], [45], glycoside derivatives such as glycone [46] from which it can be seen that for extraction purpose water can be used as an effective solvent. But when green methodologies are to be used then research works suggest water as efficient solvent to be used for extraction because the alterations that occur in the physiochemical properties of water from ambient to near about critical helps to carry out certain extraction processes such as PHWE (pressurized hot water extraction) which is effective and beneficial as well [47]. On carrying out the UV-visible spectroscopy, it was seen that the highest absorption peak was obtained at near about 430 nm and the spectral range was seen at 200-800nm range, [48].

Spectroscopic analysis of AgNPs formed using green-synthesis technique: -

The absorption spectra zone for the silver nanoparticles being produced were generally analyzed against water as a reason to control the formation and the firmness of the silver nanoparticles and the surface plasmon resonance was obtained at the range of 400-450nm when using *Megaphyrium macrostachyum* as the parent plant material, [49]. The spectral data obtained through UV-visible method when carob leaf extract was used, showed that formation of nanoparticles took place within a very small span of time, approximately two minutes indicating

that carob leaf extract speeded up the bioreduction process as well, [50]. Research work shows that the maximum amount of absorbance was noticed at range of 460nm and the silver nanoparticles formed as a result of green synthesis when analyzed through UV-visible spectroscopy showed that firstly the surface plasmon resonance occurs at 430nm and slowly as the reaction proceeds the wavelength shifts slightly and gets its stability at 434nm [51].
It was also observed that when samples were analyzed using UV-visible spectroscopy, no proof of absorption was obtained when only plant extract samples were observed in the wavelength range of 400-800nm but when the same plant extract was combined with silver nitrate, then very prominent absorption was observed in the wavelength range of 434 nm for plant extracts obtained from cannonball leaf extracts which was in accordance with the other research works that showed generally the surface plasmon resonance of silver nanoparticles usually occur at wavelength range of 420nm [52]. Silver nanoparticles produced by the method of green synthesis was found to maintain their stable nature for a considerable time span of six months without any change in the range of wavelength for surface plasmon resonance indicating that the phytochemical components present in the plant material acts as bio-reducing agents [53] [54].
In the following research work, the silver nanoparticles are mainly produced to be used as effective packaging material. Different research works show that in everyday life the usage of materials made with plastics has drastically increased which is being observed in the last three decades because a major portion of the trade goods and consumer goods are being packed using plastic material, where it is found that approximately 40% of the production of plastics are used for packaging purpose and out of this 40% half of it is involved in food and beverage industries where extensively they are used for the production of trays, cups, films, sheets, wraps, bottles, covers, cups, tubs etc. [55]

Detrimental effects of use synthetic packaging materials on environment and mankind: -

Several factors are present for which the use of plastics have become an integral part of different industrial and urban sectors which include its firmness in the long run, production costs are much lower, good wrapping ability etc. for which companies are still involved in the use of plastics for packaging purposes, [56]. Researchers report that soft and light weight plastics, economically cheap and having good longevity are the prominent materials which are chosen to be used for packaging purposes but there is undoubtedly evidences which show that pollution created by plastics severely affect the marine and costal environments, poses serious harm to wildlife, a major cause of water pollution and degradation of surface water quality, degrades soil quality and releases toxic materials in it, a major cause of floods as it clogs the public water canals and runaways and ultimately harms human health and the major problem for which the strict prohibition on plastic use is to be took from every sectors of the society is hat irrespective of knowing these severe detrimental and ill effects of plastic which is a fact of global concern the consumption of plastic continues to inflate and reaches higher levels every year [57] [58].

Role of packaging films synthesized by using organic polymers in the packaging sectors: -

Here comes into action the role of packaging films designed by the use of organic components which have proved to be suitable alternatives to the synthetically manufactured plastic packaging items. There is a huge variety of the sustainable biopolymers used for single layer packaging purpose, among which the most used ones are starch, polyhydroxyalkanoates and its derivatives,

polylactic acid etc. [59]. As to make the properties of the biopolymers better, they can be formed into their composite formations or can be encapsulated as well as can be made edible in nature so as to reduce the wastage chances and also the antimicrobial properties of the biopolymers can be made better, [60]. With the advantage of being ecofriendly materials, these biopolymers can be produced from biologically synthesized monomers, can be chemically obtained from polymers of bio-origin or directly through extraction process they can be obtained from industrial wastes or left over biomass which is the most used way as it leads to the use of waste materials for the production of biopolymers and encourages sustainable production methods, [61].

A significant quantity of synthetic polymers are manufactured and processed from certain non-renewable resources such as coal and petroleum and therefore their production cannot be considered as a natural recycling system instead they pose a threat to the non-renewable resources and thus are not environment friendly. Research works have showed the advantages in terms of different aspects which biopolymers have over synthetic polymers. Biopolymers synthesized by using chitosan showed better properties such as economic feasibility, non-toxic in nature, safe towards the environment compared to petroleum based synthetically produced polymers, [62]. A major concern these days which is always to be thought of reducing is the generation of green house gasses, where certain research works show that 50% depreciation in the green house gas generations by the year 2050 is very much needed so that the entire global temperature can be avoided to be increased by 2°C [63]. The approach of switching towards the use of biodegradable polymers or bioplastics for packaging and other purposes is a very appropriate and suitable step which can be taken to cut down the chances of global warming and reduction of generation of green house gasses [64], [65] and [66].

Challenges faced by the packaging films produced using biopolymers: -

Irrespective of having many authentic advantages in the field of packaging compared to synthetic plastics and polymers, certain research works have shown many deficits in the property of biopolymers which put a question mark on the long and uninterrupted use of the biopolymers such as water vapor permeability and oxygen permeability, both of which are two very important parameters to be strictly controlled when the packaged product is a food item, not all but certain biopolymers designed using materials like Chitosan and starch show very weak mechanical properties and materials such as gelatin and cellulose show excess hardness and poor bendable nature showing that required all parameters of hardness, bending ability, tensile strength and flexibility together are not achieved by using one particular or blends of two polymers, being of organic origin their chance to self degradation and microbial attack always remains high and thus their use in food packaging for long span of time becomes a challenge [67] [68] and [69]. At present the formation of polymeric nano particles which are actually colloidal particles obtained from polymers have increased because their production is less complex and they are present in the form of uniform particle sizes, they have high stable nature due to polymeric matrix rigidity and they are biocompatible in nature along with optimized drug release properties [70]. In research works which were done to study the antimicrobial action showed by chitosan nanoparticles then it was seen that when chitosan nanoparticles where combined with polyethylene furanoate (PEF), then the combined action of PEF and chitosan nanoparticles together showed a 30% improvement in the antimicrobial activity against *Staphylococcus aureus*

bacterium compared to the antimicrobial effect shown by chitosan nanoparticles individually [71].

Improvement of packaging films made by biopolymers by implementation of NPs and other biopolymers: -

Researchers nowadays are more striving towards the implementation of biopolymer food packaging instead of using the synthetically manufactured ones, but the main challenge faced by the biopolymers are their irregular and varying mechanical properties. This major defect is being improved and enhanced by the addition of appropriate nanoparticles in the films resulting in formation of bio-nanocomposites which show advances in their mechanical, barrier and antimicrobial properties. It was seen that the tensile strength of chitosan-silicon nanocomposite films were drastically developed compared to the tensile strength of pure gelatin films and water vapor barrier properties of only gelatin films was 25.21 g mm/k Pa m^2 day which was seen to fall off at 3.30 g mm/k Pa m^2 day after the addition of 10% silicon nanoparticle to it [72]. Certain works show that the incorporation of cupper sulfide nanoparticles appreciably improved the mechanical strength, the water vapor barrier and the ultraviolet blocking properties of the gelatin film as well as made the copper sulfide-gelatin nanocomposite to gain and show antimicrobial properties to certain species of bacteria such as *Escherichia coli* and *Listeria monocytogenes* [73]. Another research work showed that with the addition of silicon dioxide nanoparticles to sodium alginate/hydrolyzed collagen composite films, the elongation at break point showed much better and higher results compared with the sodium alginate/hydrolyzed collagen composite films devoid of any nanoparticle added to them [74]. It was also seen in other research works that the tensile strength showed a considerable improvement for those sodium alginate/hydrolyzed collagen composite films which were incorporated with 10 % silicon dioxide nanoparticles by weight whose reason might be due to the strong hydrogen bonds which were generated between the carboxylic group of the polymer matrix and the hydroxyl group of the silicon dioxide nanoparticles, [75].

Studies were also done on other biopolymers made with carboxymethylcellulose (CMC) where it was observed that the films for packaging which were blended with silver nanoparticles showed better thermal stability and tensile properties along with developed antimicrobial properties [76]. By inclusion of silver nanoparticles which were produced by green synthesis using honey as a reducing agent into the matrix of CMC films, efficient inhibition property of carboxymethylcellulose was achieved [77]. Researchers also observed that when alginate and silver nanoparticles were implemented together then those bionanocomposites showed greater tensile strength, 6.4 times more than only individual alginate films [78]. The rise in the mechanical strength, mainly in the tensile properties of the nanocomposite films were mainly due to the physical attraction which was created between the nanoparticle which acted as filler and the polymer matrix [79]. Works also showed that when bionanocomposite films were made for packaging purposes, they showed an increase in their mechanical properties (tensile strength), excellent ultraviolet barrier properties and thermal stability which showed that these bionanocomposite polymers can be used for suitable food packaging by their effective water vapor and ultraviolet barrier properties [80]. Research works also showed that by adding a very mild concentration of CMC nanoparticle to the films which were made by sago starch greatly improved the physiochemical and heat sealability property of the films compared to the mono

sago starch films which clearly indicated that the use of composite films enhances certain required properties for packaging than a films which are generated by using a single biopolymer [81].

It was also seen by different research works, where the silver nanoparticles were being produced by plasma technique and incorporated into sodium alginate films and their mechanical properties on being analyzed showed that alginate silver nanocomposite films (alginate films having 1 mM of silver nanoparticles) showed a 40 times more tensile strength than pure alginate films [82]. The main explanation of the improvement in the mechanical properties of the biopolymers when they are being converted to bionanocomposites with inclusion of noble metal nanoparticles is actually due to the interaction which takes place between the base polymer and the nanofiller material, which when becomes more the mechanical properties increase [83]. Research works showed the enhanced water barrier properties, better thermal stability and good antibacterial action against certain gram –ve bacterial species such as Escherichia coli was noticed for composite films made by combining flaxseed protein and alginate with silver nanoparticles [84]. It was also observed by research works that when along with silver nanoparticles other organic antimicrobial agents were incorporated then they showed a combined better effect as observed when lemongrass essential oil (LGO) and silver nanoparticles were included in alginate films designed for food packaging. It was seen that a synergistic antimicrobial effect was observed compared to lemongrass essential oil and silver nanoparticles individually towards two gram +ve strains, *Bacillus cereus* and *Staphylococcus aureus* and two gram –ve strains such as *Escherichia coli* and *Salmonella typhi* [85].

It has been found in research works that when gelatin is included and blended with starch in the ratio of gelatin : starch as 1:4 then excellent barrier properties were observed and scanning electron microscopy showed good harmony with the composite film showing that compared to individual starch film when gelatin was combined the results were better [86]. Biopolymers which are made only with starch based materials although posses good barrier and optical properties but they lack the enhancement in their mechanical properties as well and to overcome these deficits methods such as incorporation of nanoparticles, other copolymers and additives are being used to improve the tensile and mechanical properties of starch films [87]. Early research works had showed that when films were produced with a combination of two types of biopolymers the tensile properties got improved. It was noticed that the elongation break percentage increased for starch-gelatin composite films with the increase in gelatin concentration of approximately more than 20% by weight [88]. Researches also showed that with an increase in the percentage of the starch added (obtained from potato) to gelatin films (which was synthesized using chicken liver), a 13.9 times decrease was noticed in the percentage of elongation break when the starch content was increased from zero to 6% by weight added [89] whereas when composite films were made using alginate and gelatin elongation break percentage was observed near about 19% with 50% by weight of gelatin and 50% by weight of alginate [90].

Preparation of Silver nanoparticles by using Lamboo tree leaf extract and silver nitrate: -

Reagents-

Double distilled water (d.d.w), 2mM Silver nitrate ($AgNO_3$) solution, freshly plucked lamboo tree leaves.

Equipments and Instruments-

250 ml borosil conical flask, borosil measuring cylinder (100 ml capacity), borosil test tubes, borosil beakers, Perkin Elmer Lambda 25 UV-Vis Spectrophotometer, Remi magnetic stirrer, heating mantle, Mettler Toledo ML 203E Digital Weighing balance, scissors, cotton gauge.

Green synthesis of silver nanoparticles, preparation of Lamboo tree leaf extract-

Lamboo tree leaves were obtained from Lamboo trees planted in the banks of Ganges at Panihati on the day the experiment to be done. 2.02 gm of lamboo tree leaves were weighed and cut into small pieces. 250 ml of d.d.w was measured and poured in a 250 ml conical flask to which the small pieces of the lamboo tree leaves were added and was magnetically stirred by keeping on a heating mantle at 55°C for 15 minutes and r.p.m was set at 300 r.p.m. After this, the extract along with the leaves were sieved out and was collected in a beaker.

Preparation of Silver Nitrate Solution-

$1.9 * 10^{-3}$ M $AgNO_3$ solution was prepared by dissolving 0.084 gm of silver nitrate into 250 ml d.d.w in a 250 ml volumetric flask.

Preparation of Silver Nanoparticles-

The $AgNO_3$ solution prepared was poured in four test tubes and in each test tubes the previously prepared leaf extracted was added as per the proportions given in the table below: -

Four samples were prepared as follows: -

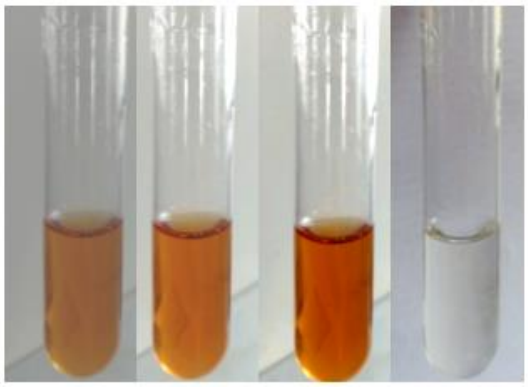
Table 01: -

Serial No.	**Volume of Silver nitrate added (ml)**	**Volume of double distilled water added (ml)**	**Volume of Leaf extract added (ml)**	**Total Volume (ml)**
1. Sample 1	10	0	1	11
2.Sample 2	10	0	2	12
3. Sample 3	10	0	3	13
4. Sample 4	0	10	1	11

After this the four test tubes were kept to stand still with cotton plugging for 15 minutes at room temperature.

Observations: -

Image 1- The below given image shows the changes observed in the four test tubes after they were kept undisturbed at room temperature for 15 minutes.



Sample1 Sample 2 Sample 3 Sample 4

Detection of Silver nanoparticles by using UV-visible spectroscopy method: -

The previous samples prepared were taken for UV-visible spectrophotometric analysis. The starting wavelength range and the ending wavelength limit were set between 200nm and 700nm respectively. Here Sample 4 (sample having only silver nitrate and lamboo tree leaf extract) is used as a control setup.

Results and Discussion: -

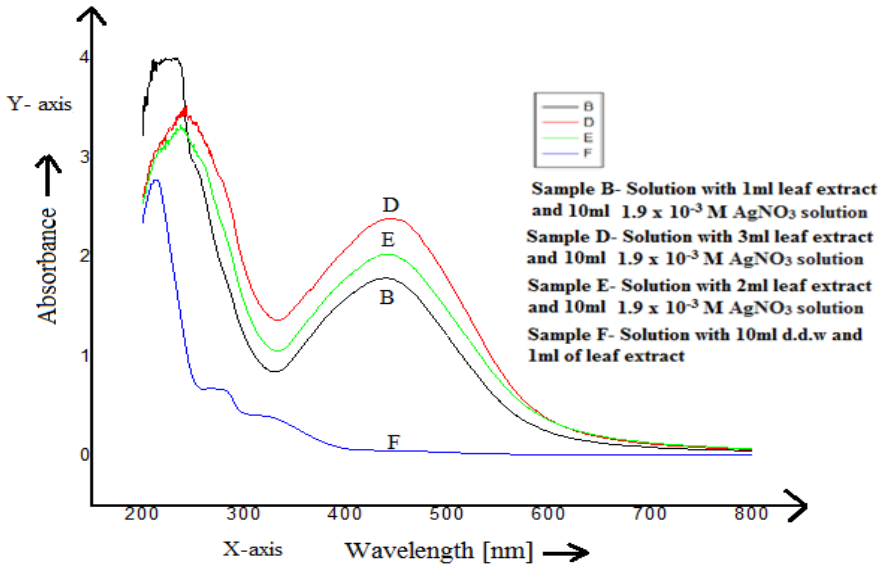


Figure 1- The above image shows the graphical representation of the UV-visible spectroscopic analysis of the samples, B-Sample 1, E- Sample 2, D- Sample 3 and F- Sample 4

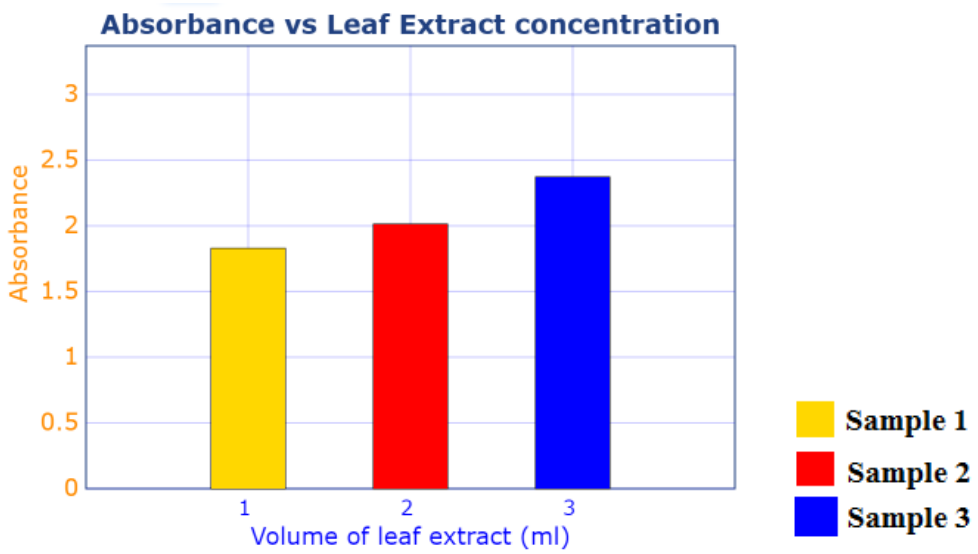


Figure 2- Graphical representation showing the increase in the absorbance of the samples with increase in the volume of lamboo tree leaf extract added to them.

Table 02: -

Serial No.	Maximum Wavelength (nm)	Absorbance	Amount of leaf extract present (ml)
1. Sample 1	437	1.827	1
2. Sample 2	437	2.011	2
3. Sample 3	437	2.370	3

Discussions: -

The graphical data shows formation of silver nanoparticles and with increase in amount of lamboo tree leaf extract to the silver nitrate, more absorbance is recorded which indicates the more generation of silver nanoparticles. The localized surface plasmon resonance was observed at 437 nm which is in accordance with the results obtained by the previous works stating that localized surface plasmon resonances observed where for silver nanoparticles generally tend to occur within a wavelength range of 410-450nm. For the control setup no absorbance was observed, but from the graphical analysis it is seen that small peeks are noticed near the ultraviolet region which indicates that this sample may show fluroscence when exposed to proper ultraviolet light.

Incorporation of Ag-nanoparticles to starch films and analysis of mechanical properties: -

Reagents-

Double distilled water (d.d.w), Starch powder (Merck Life Science Private Limited), Polyethylene glycol (LOBA CHEMIE PVT. LTD)

Equipments and Instruments-

REMI 2MLH Magnetic stirrer, heating mantle, conical flasks 250 ml and 100ml (borosil), borosil petri plates of diameter 10 cm, laboratory thermometer (Manufactured by LABWORLD, Range 0-300°C), TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems), Mettler Toledo ML 203E Digital Weighing balance .

Preparation of Starch Solution: -

2 gm of starch was dissolved in 50ml of d.d.w water and was mixed uniformly in a 100ml borosil conical flask. In this solution 2ml of 4% acetic acid was added and gradually it was heated from room temperature (37°C) to 90°C by keeping it on a magnetic stirrer followed with continuous stirring at 500 rpm. Temperature was monitored using a laboratory thermometer. 4ml of polyethylene glycol was added in this solution. This heating process was done for one hour.

Addition of the previously made silver nanoparticles of the starch solution:-

After 1 hour, from the above solution, 10 ml of solution was taken five times in five 100 ml conical flasks and to each of them silver nanoparticles made previously was added in different concentrations and were stirred at 350 r.p.m without any heat treatment until they got uniformly mixed which took approximately half an hour.

Casting of Film Solution: -

Each of the five mixtures were now spread on five borosil petriplates and kept at room temperature (37°C) for 24 hours for drying.

Table 03: -

Serial No.	**Vol. of Solution 1 (ml)**	**Vol. of AgNP added (ml)**
1. Sample A	10	1
2. Sample B	10	2
3. Sample C	10	4
4. Sample D	20	2
5. Sample E	20	4

Observation: - Films obtained after drying: - Translucent, light white colored films were obtained having smooth surfaces.

				
Sample A	**Sample B**	**Sample C**	**Sample D**	**Sample E**

Image 02- Sample A – 10 ml starch solution and 1 ml AgNP, Sample B- 10 ml starch solution and 2ml AgNP, Sample C- 10ml starch solution and 4ml AgNP, Sample D- 20ml starch solution and 2ml AgNP and Sample E- 20 ml starch solution and 4ml AgNP.

Analysis of mechanical property of the films obtained: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the following parameters: -
T.A Variable No: 1: Compression
Pre-test speed- 1.00 mm/second
Test speed- 5.00 mm/second
Post-test speed- 5.00 mm/second
T.A Variable No: 5: 0.0gm
Target mode- Distance
Distance-10.0mm
Strain- 75.0%
Trigger type- Auto (Force)
Trigger force- 5.00gm
Probe- P/5 ; 5mm DIA CYLINDER
STAINLESS
Points per second- 200
Test run by – user

Table 04: -

Serial No.	Sample Analyzed	Hardness (MPa) recorded
1	Sample A	4.0386
2	Sample B	4.3264
3	Sample C	5.2432
4	Sample D	5.8381
5	Sample E	6.4775

Reference: Films: -	Hardness (MPa)	Source: -
1.Films made with coatings of Niobium Pentoxide	6.1	Reference 93
2.Graphene-Fluoroethylene Vinyl Ether composite films	4.24	Reference 94
3.Low density polyethylene films	4.1	Reference 95
4.HDPE-LDPE Composite film (20% HDPE and 80% LDPE)	2.47	Reference 96

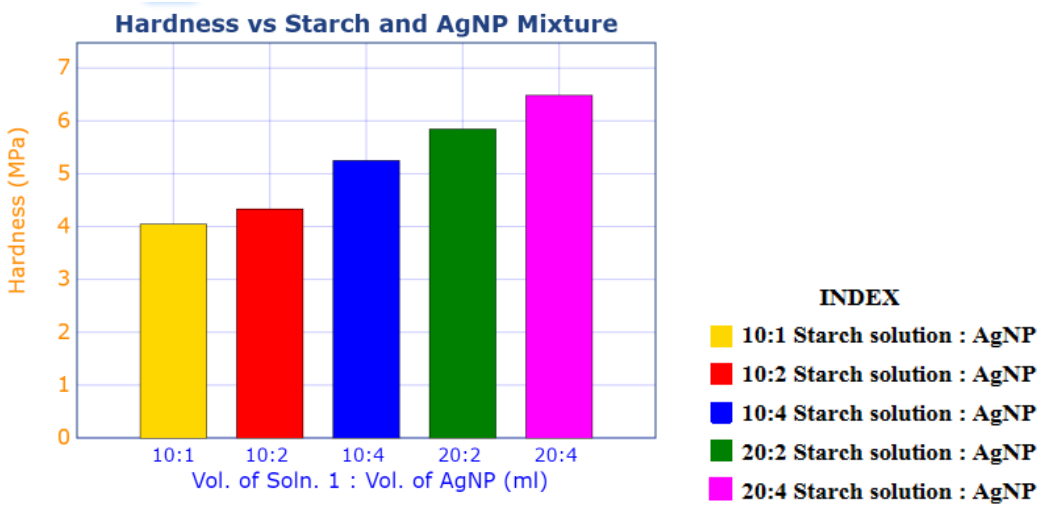


Figure 03- Graphical representation showing the increase in the hardness of the starch-silver nanocomposite films with the increase in the concentration of the silver nanoparticle added.

Results and discussion-

The above obtained data shows that hardness of the starch films increases with the concentration of silver nanoparticles added to it which is a positive indication as packaging films with better hardness are recommended to be used as packaging materials as they have more withstanding capacity towards external forces. Above graph shows, for three consecutive data the hardness increases when the amount of starch solution added remains same i.e 10ml but the amount of silver nanoparticle added is increased twice which shows that rise in hardness is due to the increase in the amount of AgNP added which were in accordance with the works of [97] and [98].

Preparation of sodium alginate and starch composite films: -

Reagents: -

Sodium alginate (Sisco Research Laboratories Pvt. Ltd.), Starch powder (Merck Life Science Private Limited), double distilled water, Polyethylene glycol (LOBA CHEMIE PVT. LTD)

Equipments and Instruments: -

Mettler Toledo ML 203E Digital Weighing balance, borosil conical flasks (100ml and 250ml), borosil petri plates of diameter 10cm, REMI 2MLH Magnetic stirrer, heating mantle, laboratory thermometer (Range 0-300°C), Screw Gauge (Manufactured by Mitutoyo, L.C-0.01mm).

Preparation of Sodium alginate solution: -

3 gm of sodium alginate was weighed and added with 25ml of double distilled water in a 100ml conical flask (Solution A).

Preparation of Starch solution: -

2gm of starch was weighed and added with 25ml of double distilled water in a 100 ml conical flask (Solution B). Both the solution A and B were magnetically stirred at 40°C (monitored by using laboratory thermometer) at 400 r.p.m for one hour. After one hour 4.5 ml of polyethylene glycol was added to both the solutions A and B, which will be acting as a plasticizer.

Preparation of the Final Solution, Solution C: -

Now, both Solution A and Solution B were mixed together in a 250ml conical flask and they were magnetically stirred for half an hour at 300 r.p.m till both of them got uniformly mixed together.

Casting of the Film Solution: -

From Solution C, nearly 12.5 ml of solution was taken four times and spread on four borosil petri plates and were kept at room temperature (36°C) for 24 hours and left to dry.

Observations: - Films obtained after drying: -

Sample 01 **Sample 02** **Sample 03** **Sample 04**

Image 03: - The above image shows the starch and Na-alginate composite films obtained after drying, white colored films with smooth surface was obtained.

Analysis of thickness of the composite films obtained: -

Here, the amount of film solution being same, thickness was analyzed using a screw gauge and the data was recorded.

Analysis of mechanical property of the films obtained: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the parameters as described earlier: -

Results and Discussions: -

Table 05: - The following table shows the data obtained for thickness measured using Screw Gauge: -

Serial No.	Thickness (mm)
1. Sample 1	0.080
2. Sample 2	0.084
3. Sample 3	0.083
4. Sample 4	0.053

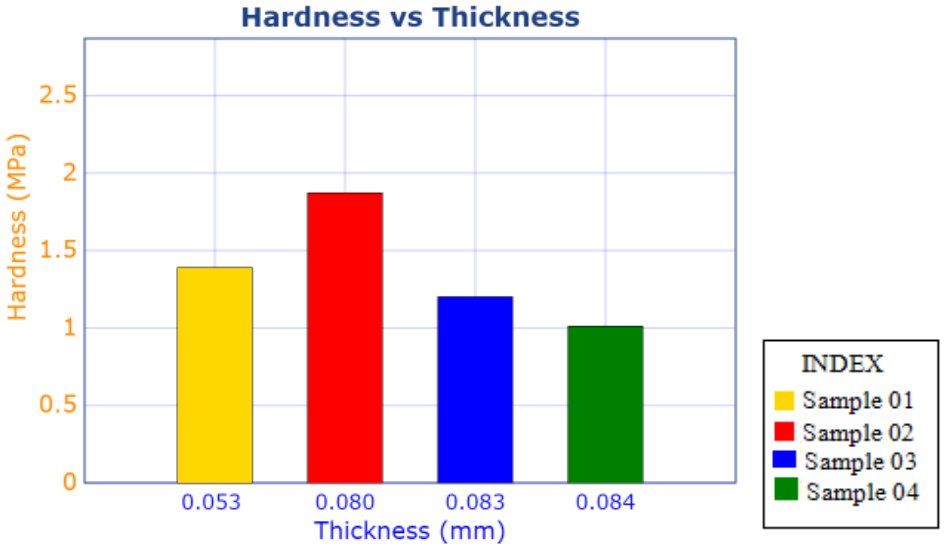
Table 06: - The following table shows the data obtained for hardness measurement using TA.XT Texture Analyzer: -

Serial No.	Hardness recorded (MPa)
1. Sample 1	1.8693
2. Sample 2	1.009
3. Sample 3	1.199
4. Sample 4	1.388

Reference table (TR) showing the hardness (MPa) of different composite films: -

Reference Films	Hardness (MPa)	Source: -
Gelatin-Corn starch-Glycerin-Papaya-Soy protein Composite film	1.74	Reference 99
Starch-activated carbon powder composite film	1.64	Reference 100
Lambda-2-Stannane—nickel (Ni_3Sn_4) intermetallic thin films	2.11	Reference 101
Films prepared with polyurethaneurea	0.35	Reference 102
Bismaleimide-triazine resin films	1.59	Reference 103
Glass-fiber-reinforced Bismaleimide-triazine resin composites	2.70	Reference 103

Figure 04: - Graphical representation is showing the relationship of the hardness (MPa of the Na-alginate-starch composite films with thickness (mm) : -



Discussions: -

The above results show us that as the film thickness increases gradually the hardness increases as observed for film 4 and film 1 but with film 3 and film 2 it falls with the increase in thickness from where it can be noted that too much thick film has comparatively lower hardness than thinner films with the same composition as obtained through the works of [104] and [105].

Preparation of Na-alginate and starch composite films with different compositions: -

Reagents: -

Sodium alginate powder, Starch powder (Merck Life Science Private Limited), double distilled water, Polyethylene glycol (LOBA CHEMIE PVT. LTD)

Materials and Instruments: -

Mettler Toledo ML 203E Digital Weighing balance, borosil conical flasks (100ml and 250ml), borosil petri plates of diameter 10cm, REMI 2MLH Magnetic stirrer, heating mantle, laboratory thermometer (Range 0-300°C), Screw Gauge (Manufactured by Mitutoyo, L.C-0.01mm).

Preparation of Na-alginate solution: -

3.5gm Na alginate was added with 50ml d.d.w and heated on a magnetic stirrer at 45°C with moderate stirring (250 rpm) for 1 hour. After properly mixing, 3ml polyethylene glycol was added to it and stirred for 30 minutes without heating. – Solution X

Preparation of Starch Solution: -

2.5gm starch was added with 50ml d.d.w and heated on a magnetic stirrer at 45°C with moderate stirring (250 rpm) for 1 hour. After properly mixing, 3ml polyethylene glycol was added to it and stirred for 30 minutes without heating. – Solution Y

Preparation of composite films: -

Different proportions of sodium alginate and starch solution were added together as given in the table below and were magnetically stirred at 400 r.p.m for thirty minutes until uniform mixing was completed.

Table 07: -

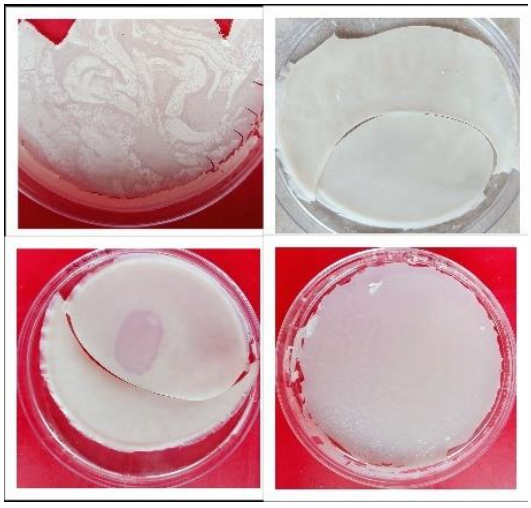
Serial No.	Vol. of Soln. X (ml)	Vol. of Soln. Y (ml)
1. Film 1	10	10
2. Film 2	20	10
3. Film 3	30	10
4. Film 4	10	20

Casting of the film solutions: -

After preparing the film compositions as per the parameters mentioned in Table 07, the solutions were spread uniformly on borosil plates of diameter 10cm and kept at room temperature (38°C) and left for 48 hours to dry.

Image 04- Films obtained after drying, opaque, white soft and smooth textured films were obtained as shown in the image below: -

Film 1 **Film 2**



Film 3 **Film 4**

Image 04: - The image shows the films obtained after drying, Film 1 (X: Y is 1:1) Film 2 (X: Y is 2:1), Film 3 (X: Y is 3:1) and Film 4 (X: Y is 1:2).

Thickness of the above films: -

Thickness of the above films was measured using a screw gauge (Manufactured by Mitutoyo, L.C-0.01mm) and the thickness was recorded.

Analysis of mechanical property of the composite films obtained: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the parameters as described earlier: -

Results and Discussions: -

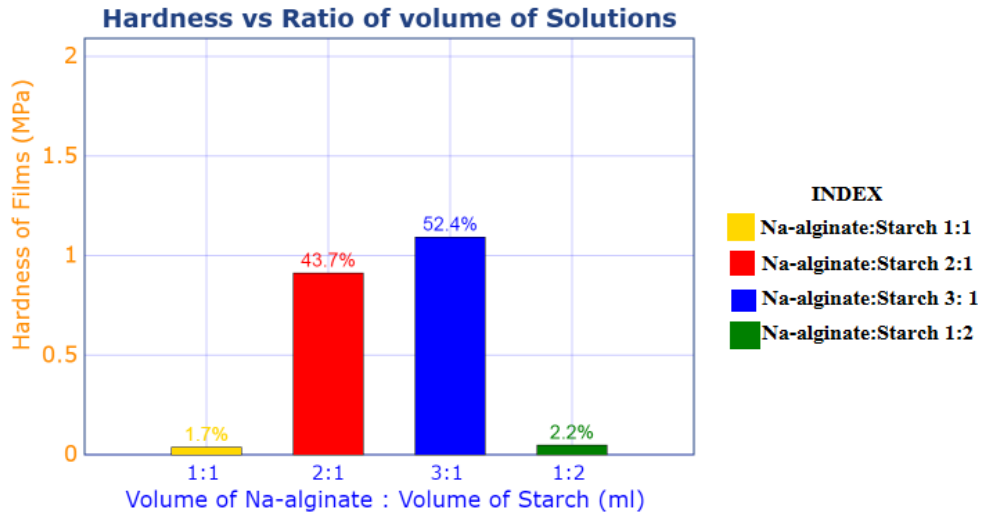
Table 08- The following table shows the data obtained for the thickness and the hardness measurement of the above films: -

Serial No.	Thickness (mm) Avg.	Hardness (MPa)
Film 1	0.06	0.0358
Film 2	0.256	0.9103
Film 3	0.376	1.0907
Film 4	0.074	0.0463

Table TR: -

Reference Films	Hardness (MPa)	Source: -
Gelatin-Corn starch-Glycerin-Papaya-Soy protein Composite film	1.74	Reference 99
Starch-activated carbon powder composite film	1.64	Reference 100
Lambda-2-Stannane—nickel (Ni_3Sn_4) intermetallic thin films	2.11	Reference 101
Films prepared with polyurethaneurea	0.35	Reference 102
Bismaleimide-triazine resin films	1.59	Reference 103
Glass-fiber-reinforced Bismaleimide-triazine resin composites	2.70	Reference 103

Figure 05: - Graphical representation shows the relationship of hardness (MPa) of the films with the change in Na-alginate and starch concentration: -



Discussions: -

Observations from Figure 05 shows that with increase in concentration of sodium alginate, the hardness and thickness of the composite films also increases but when the proportion of starch becomes twice than the Na-alginate composition then a sudden drop in the hardness is observed, which is not desirable for composite films, especially when the purpose is of food packaging. It was observed from the works of [106] and [107] where an excess of blends of other biopolymers enhanced the hardness of the starch films.

Preparation of sodium alginate and gelatin based composite films: -

To observe the properties of Gelatin-Na alginate composite films made with different concentrations,

Reagents: -

Sodium alginate powder (Sisco Research Laboratories Pvt. Ltd.), gelatin powder (Manufactured by MERCK), polyethylene glycol (LOBA CHEMIE PVT. LTD), double distilled water.

Equipments and Instruments: -

Same equipments and instruments were used as per the previous experiment.

Preparation of Na-alginate Solution: -

3.5gm of Na alginate was weighed and added with 50ml double distilled water. It was placed on a magnetic stirrer and magnetically stirred at 300r.p.m and the temperature was monitored by thermometer and kept at 40°C. After proper mixing, 3ml of polyethylene glycol was added and again stirred for half an hour. (Solution $_{alginate}$)

Preparation of Gelatin Solution: -

2.5gm gelatin was weighed and added with 50ml d.d.w and was stirred on a magnetic stirrer with moderate stirring (250 rpm) for one hour without any heating, at room temperature. After properly mixing, 3ml polyethylene glycol was added to it and stirred for half an hour with mild heating at 30°C. (Solution $_{Gelatin}$)

Preparation of the Composite Film: -

The composite films were made by mixing Solution $_{alginate}$ and Solution $_{Gelatin}$ as per the proportions given in Table 09 and they were magnetically stirred at 500 r.p.m for half an hour without heating. After proper and uniform mixing occurred, the composite film mixtures were spread on borosil petri plates of diameter 10cm and kept for 24 hours at room temperature (37.3°C) so that the films get dry.

Table 09: - The following table represents the ratios of gelatin: Na-alginate which is being used to prepare the composite films: -

Serial No.	Ratio of Gelatin : Na-alginate (ml)
1	5:5
2	5:10
3	10:10
4	10:15
5	15:10

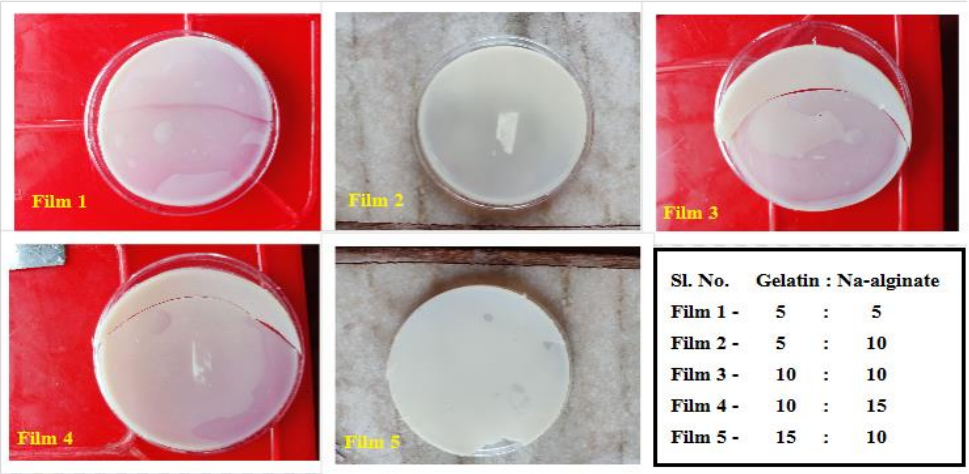


Image 05: - The above image shows the films obtained after 24 hours of drying in room temperature (37.3°C).

Analysis of hardness property of the Gelatin-Na alginate films: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the parameters as described earlier: -

Observations: -

Table 10 – The following table shows the results of the hardness observed for the films made with different concentration proportions of gelatin and sodium alginate: -

Serial No.	Gelatin : Na-alginate Ratio of films -	Hardness recorded (MPa)
Film 1	5 : 5	1.0124
Film 2	5: 10	0.8301
Film 3	10 : 10	1.1256
Film 4	10 : 15	1.4334
Film 5	15 : 10	0.9502

Table TR: -

Reference Films	Hardness (MPa)	Source: -
Gelatin-Corn starch-Glycerin-Papaya-Soy protein Composite film	1.74	Reference 99
Starch-activated carbon powder film	1.64	Reference 100
Lambda-2-Stannane—nickel (Ni_3Sn_4) intermetallic thin films	2.11	Reference 101
Films prepared with polyurethaneurea	0.35	Reference 102
Bismaleimide-triazine resin films	1.59	Reference 103

Glass fiber-reinforced Bismaleimide-triazine resin composites	2.70	Reference 103

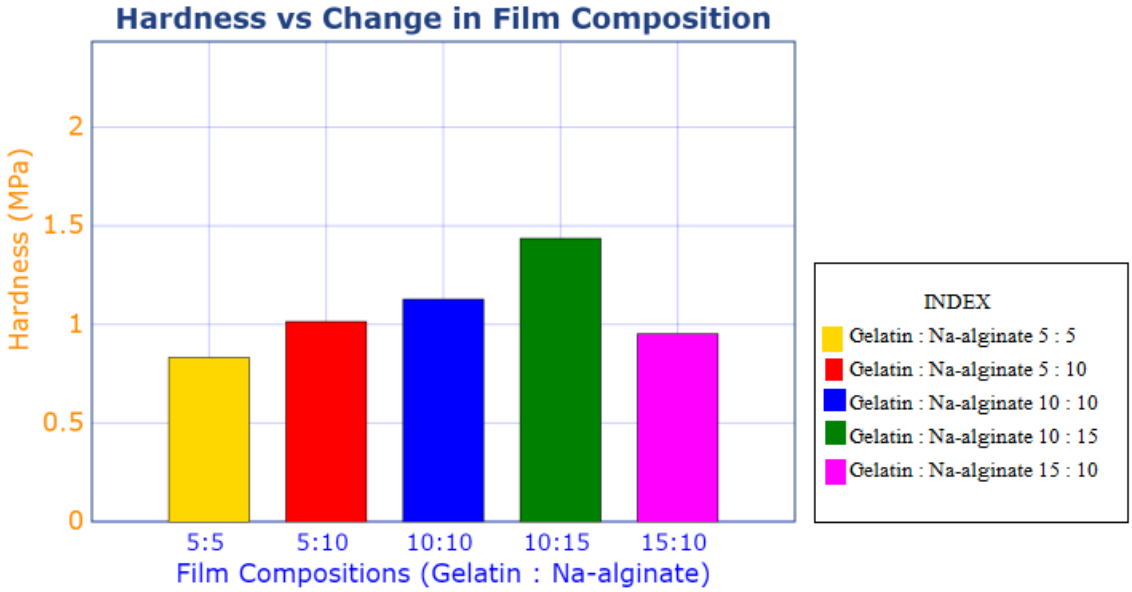


Figure 06: - The above graphical representation shows the relationship between the hardness (MPa) of the films analyzed with the change in composition of sodium alginate and gelatin in the film forming solutions: -

Discussions: -

Above analysis shows that a five times increase in the Na-alginate amount from film 1 to film 2 increases the hardness to 0.1182 times and for film 2 to film 3 a 0.1132 times increase in the hardness is observed which were in accordance with works of [108] and [109] where tensile properties and thickness were enhanced by using blends of Na-alginate with gelatin. Maximum hardness was observed for ratio of 2: 3 of gelatin: Na- alginate, after which it is seen that a 5 times increase in the gelatin concentration decreases the hardness to 0.48432 times, as shown in works of [110] where Na-alginate : gelatin ratio with highest gelatin content had lowest mechanical strength.

Comparing the hardness results obtained in Table 8, better hardness results are obtained for composite films with gelatin and sodium alginate.

Preparation of Sodium alginate-gelatin-silver nanocomposite films: -

Reagents: -

Same reagents used as previous experiment only silver nanoparticles which were previously synthesized were used.

Equipments and Instruments: -

Same equipments and instruments used as previous experiment.

Preparation of Sodium Alginate Solution: -

4 gm of sodium alginate was weighed and mixed with 80 ml of d.d.w and magnetically stirred at 400 r.p.m where heating was done at 40°C, monitored by a laboratory thermometer. After proper mixing 3 ml of polyethylene glycol was added to it and allowed to mix properly for half an hour without any heat treatment at 400 r.p.m. (Solution P_1)

Preparation of Gelatin Solution: -

4 gm of gelatin was weighed taken and mixed with 80 ml of d.d.w and magnetically stirred at 400 r.p.m and allowed to mix in room temperature only. After proper mixing 3 ml of polyethylene glycol was added to it and allowed to mix properly for half an hour without any heat treatment at 400 r.p.m. (Solution P_2)

Preparation of the mixture of Sodium alginate and Gelatin Solution: -

Four 100 ml borosil beakers were taken. In each beaker, 20 ml of solution from solution P_1 and Solution P_2 was added four times, i.e each beaker now contained 40 ml of solution (20 ml Solution P_1 and 20ml Solution P_2).

Addition of silver nanoparticles to the solution of gelatin and alginate: -

In each of the beakers respectively, 1ml, 2ml, 3ml and 4ml of previously prepared silver nanoparticles were added and they were magnetically stirred at 290 r.p.m for half an hour without any heat treatment.

Table 11: - The following table shows the compositions of the four film solutions that are prepared: -

Serial No.	**Vol. of Solution P_1 (ml)**	**Volume of Solution P_2 (ml)**	**Volume of AgNP added (ml)**	**Final Volume (ml)**
Film 1	20	20	1	41
Film 2	20	20	2	42
Film 3	20	20	3	43
Film 4	20	20	4	44

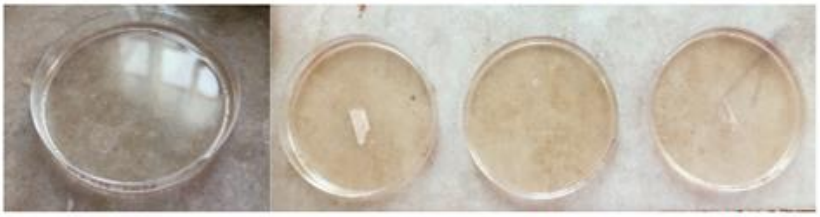
Casting of the film solutions: -

Four borosil petriplates of 10cm diameter were taken. Each of the four beakers was emptied by pouring the silver nanoparticle incorporated solution on the plates and were uniformly spread. After uniform spreading, the petriplates were kept at room temperature (37.5°C) for 48 hours so that the films get dry.

Observations: -

Image 06: - The image shows the film solutions being casted on the petriplates and left to dry.

After proper mixing poured on 4 borosil petriplates and left to dry for 48 hours at room temperature (37°C)

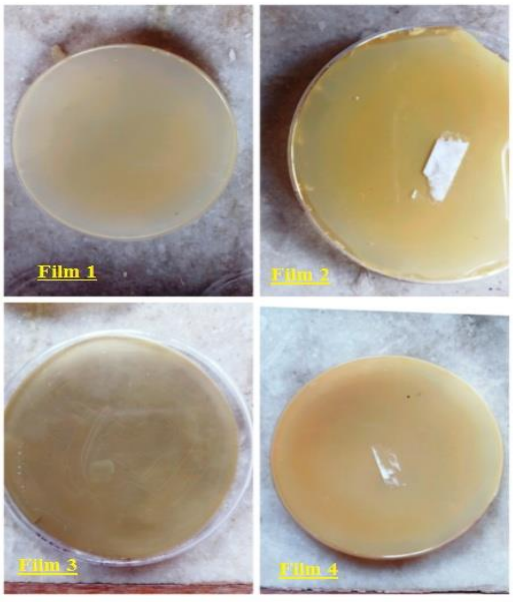


Film 1 Film 2 Film 3 Film 4

Films left to dry at room temperature for 48 hours.

Image 07: - The image shows the films obtained after drying for 42 hours at room temperature: -

Films obtained after drying : -



Analysis of hardness property of the Gelatin-Na alginate films: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the parameters as described earlier: -

Results and Discussions: -

Table 12: - The following table shows the data obtained for the hardness test performed for the following films: -

Serial No.	Ratio of Gelatin : Na-alginate (ml)	Vol. of AgNP added (ml)	Hardness recorded (MPa)
Film 1	1:1	1	1.1682
Film 2	1:1	2	1.4749
Film 3	1:1	3	1.9524
Film 4	1:1	4	2.6341

Table TR-

Reference Films	Hardness (MPa)	Source: -
Gelatin-Corn starch-Glycerin-Papaya-Soy protein Composite film	1.74	Reference 99
Starch-activated carbon powder composite film	1.64	Reference 100
Lambda-2-Stannane—nickel (Ni_3Sn_4) intermetallic thin films	2.11	Reference 101
Films prepared with polyurethaneurea	0.35	Reference 102
Bismaleimide-triazine resin	1.59	Reference 103
Glass fiber-reinforced bismaleimide-triazine resin composites	2.70	Reference 103

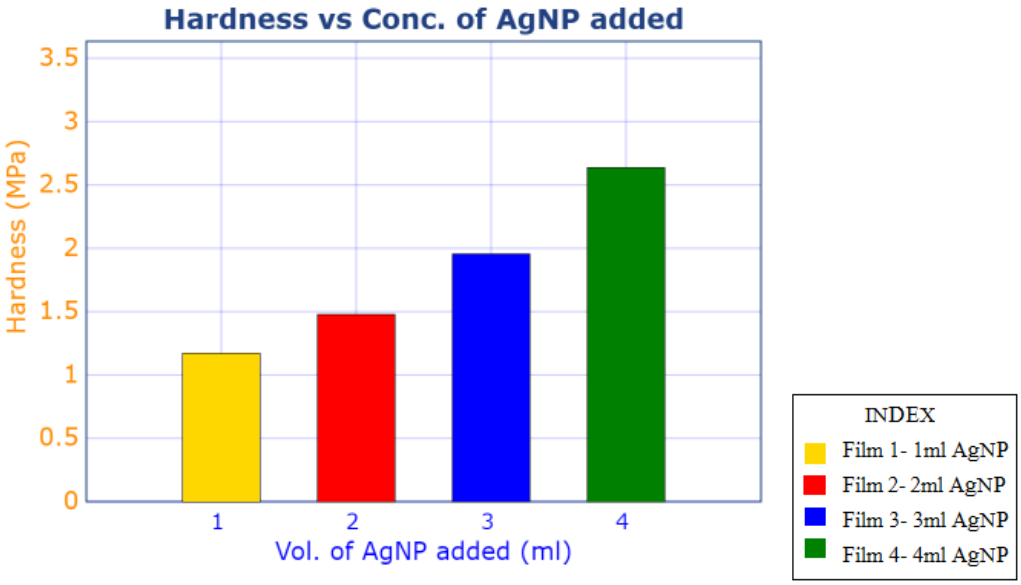


Figure 07: - The above graphical representation shows the relationship between the hardness (MPa) of the films analyzed with the addition of amount of silver nanoparticles to the film making solutions: -

Discussions: -

On comparing the results of Table 12 with previous results obtained from Table 10 it is found that for composite films which are devoid of nanoparticle, a five times increase in the Na-alginate concentration leads to a 0.177 times increase in the hardness whereas keeping the concentration ratio of the gelatin and Na-alginate mixture same and increasing the AgNP concentration in the film composition from 1ml to 2ml a 0.306 times increase is observed in the hardness of the film and increasing the AgNP from 2ml to 3ml, the hardness increased by 0.477 times without any deviation in the Na-alginate or gelatin concentration as obtained from works of [111] and [112] where increasing AgNP concentration leads to a increase in hardness and tensile strength.

1ml increase in the AgNP concentration can increase hardness of the films more than it had increased by changing the Na-alginate concentration 5 times which was devoid of any nanoparticle addition.

Solubility test of the nanocomposite films obtained as shown in the Image 07: -

The solubility test was performed according to the methods showed in the research works of [113] and [114].

Equipments required: -

100 ml borosil beakers, 100ml conical flasks, borosil funnels, Whatman Filter Paper (Size 110mm), Hot air oven (Bhattacharya and CO., Kolkata), and REMI 2MLH Magnetic stirrer.

All the four films as shown in Image 07 were placed in 100ml borosil beakers and 50 ml of d.d.w water was added to them. All of the four beakers were kept on magnetic stirrers and moderate stirring was done at 350 r.p.m until all of the films got completely dissolved in the water.

Passing of the dissolved films through filter paper: -

Now, four conical flasks were arranged with four borosil funnels on them and four filter paper cones were placed on the funnels. The film solutions from each four beaker was poured on them and was allowed to completely pass through the filter paper attached funnel.

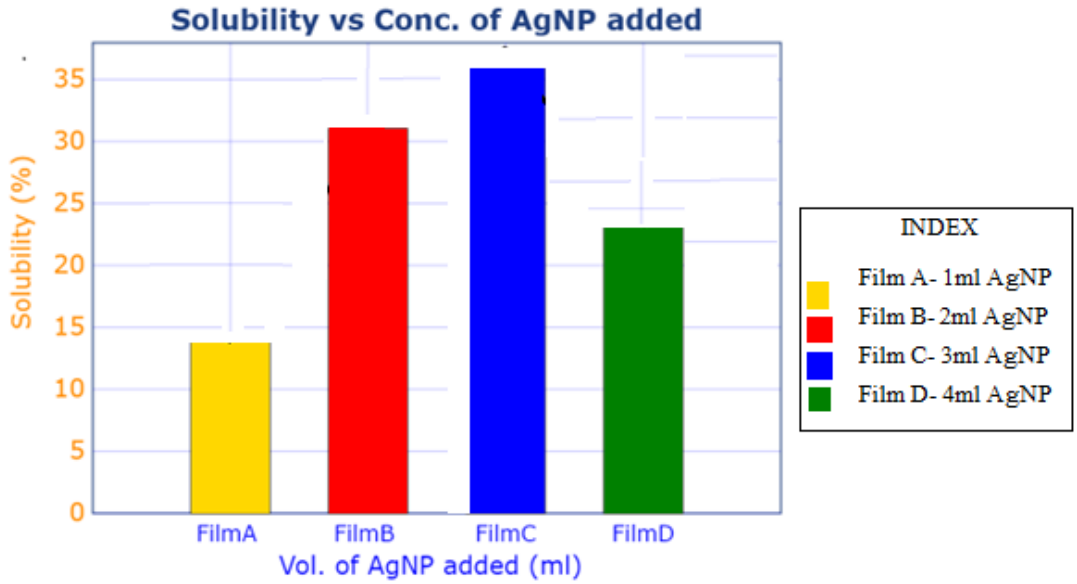
After this, the four filter papers were dried inside a hot air oven at 110°C and their weight was recorded. An increase in each of the weight of the filter papers indicated that a certain amount of film component has dissolved in the water which is being caught up by the filter paper.

Results and Discussions: -

Table 13- The following table shows the data obtained for the solubility tests performed for the four films obtained as shown in Image 07: -

Serial No.	Wt. of Films (g)	Wt. of Filter paper (g)	Wt. of filter paper after film solution passed and drying (g)	Increased Wt. of the filter paper (g)	% solubility of Films (100 ml d.d.w)
1.Film1	1.5166	1.1428	1.3585	0.2157	28.444
2.Film2	1.7774	1.1429	1.6944	0.5515	62.056
3.Film3	1.9704	1.1431	1.8714	0.7283	73.924
4.Film4	2.8624	1.1438	1.8150	0.6712	46.918

Figure 08: - The below figure shows the graphical representation of the relationship between the percentage of solubility with the volume of silver nanoparticles added: -



Discussions: -

The above observations show that with the gradual increase in the amount of silver nanoparticle being added the solubility gradually increases as obtained in the works of [115] where increase in solubility was observed with increase in NP proportion. When AgNP concentration was 4ml, a drop in the solubility was observed, works of [116] showed 31.1% fall in solubility when NP concentration was increased from 2 to 6%, and in above analysis 14% fall in solubility was seen for 2.12 times increase in NP concentration. Works of [117] explain that when AgNPs are added, very strong bonding is created between the polymeric matrices of the composite film mixtures and the AgNPs which makes the internal framework at the molecular level stronger leading to a lower solubility as the bonds are not easily broken down by water.

Hardness analysis comparisons of two set of films: with and without incorporation of silver nanoparticles: -

Sodium alginate-gelatin-silver nanocomposite films were made in two sets; each set with same composition, each film mixture in the second set was incorporated with silver nanoparticle extract.

Reagents: -

Sodium alginate powder (Sisco Research Laboratories Pvt. Ltd.), gelatin powder (Manufactured by MERCK), polyethylene glycol (LOBA CHEMIE PVT. LTD), previously prepared silver nanoparticles, double distilled water.

Equipments and Instruments: -

Mettler Toledo ML 203E Digital Weighing balance, borosil conical flasks (100ml and 250ml), borosil petri plates of diameter 10cm, REMI 2MLH Magnetic stirrer, heating mantle, laboratory thermometer (Range 0-300°C).

Preparation of Na-alginate solution: -

5gm sodium alginate was weighed and added in 85ml of d.d.w. It was placed on a magnetic stirrer to mix properly where r.p.m of 500 was set with a temperature of 45°C which was monitored by use of laboratory thermometer. After proper mixing, 4 ml of polyethylene glycol was added as plasticizer and allowed to mix for half an hour without heat treatment.

Preparation of Gelatin solution: -

5gm of gelatin was weighed and added in 85 ml of d.d.w. It was placed on a magnetic stirrer to mix properly where r.p.m of 500 was set with a temperature of 45°C which was monitored by use of laboratory thermometer. After proper mixing, 4 ml of polyethylene glycol was added as plasticizer and allowed to mix for half an hour without heat treatment.

Preparation of film casting Solutions: -

After the sodium alginate and gelatin solutions were prepared, two sets of film making solutions were prepared with different compositions of solutions of Na-alginate and gelatin as given in the tables below: -

Table 14-

Serial No.	**Vol. of Na- alginate solution (ml)**	**Vol. of Gelatin solution (ml)**	**Total Volume (ml)**
1.Film C1	2.5	2.5	5
2.Film C2	5	5	10

3.Film C3	7.5	7.5	15
4.Film C4	10	10	20
5.Film C5	12.5	12.5	25

Table 15: -

Serial No.	Vol. of Na- alginate solution (ml)	Vol. of Gelatin solution (ml)	Vol. of AgNP (ml)	Total Volume (ml)
1.Film D1	2.5	2.5	2.5	7.5
2.Film D2	5	5	2.5	12.5
3.Film D3	7.5	7.5	2.5	17.5
4.Film D4	10	10	2.5	22.5
5.Film D5	12.5	12.5	2.5	27.5

Casting of the film solutions: -

Both set of the film making solutions were casted on borosil petri plates of 10cm diameter and were left to dry at room temperature (37°C) for 48 hours.

Observations: -

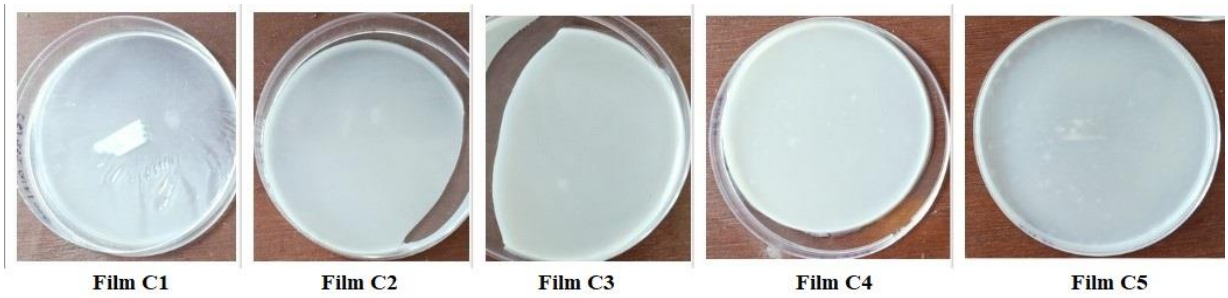
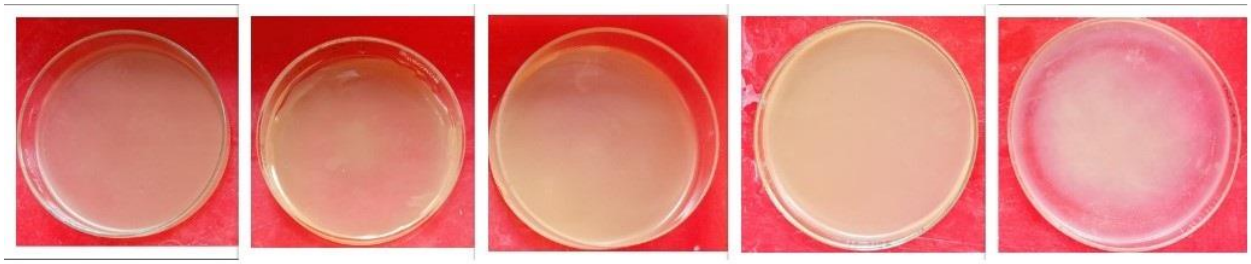


Image 08: -The above set of composite films obtained after drying which are prepared without use of any nanoparticles, films obtained white in color having smooth surface.

Image 09: - The below image shows set of composite films obtained after drying which are prepared by incorporation of silver nanoparticles, each set of films prepared contains 2.5 ml of silver nanoparticle in each of them which resulted in dark colored films.



Film D1 Film D2 Film D3 Film D4 Film D5

Hardness property analysis of the above set of films obtained: -

The films were placed on the analyzing surface of a TA.XT Express Enhanced texture analyzer manufactured by SMS (Stable Micro Systems) with the parameters as described earlier: -

Results and Discussions: -

Table 16-

Serial No.	Hardness Recorded (MPa)
Film C1	1.588
Film C2	1.565
Film C3	1.504
Film C4	1.923
Film C5	1.409
Film D1	1.968
Film D2	2.514
Film D3	2.701
Film D4	2.952
Film D5	3.997

Table TR-

Reference Films	Hardness (MPa)	Source: -
Gelatin-Corn starch-Glycerin-Papaya-Soy protein Composite film	1.74	Reference 99
Starch-activated carbon powder composite film	1.64	Reference 100
Lambda-2-Stannane—nickel (Ni_3Sn_4) intermetallic thin films	2.11	Reference 101
Films prepared with polyurethaneurea	0.35	Reference 102

Bismaleimide-triazine resin	1.59	Reference 103
Glass fiber-reinforced bismaleimide-triazine resin composites	2.70	Reference 1033

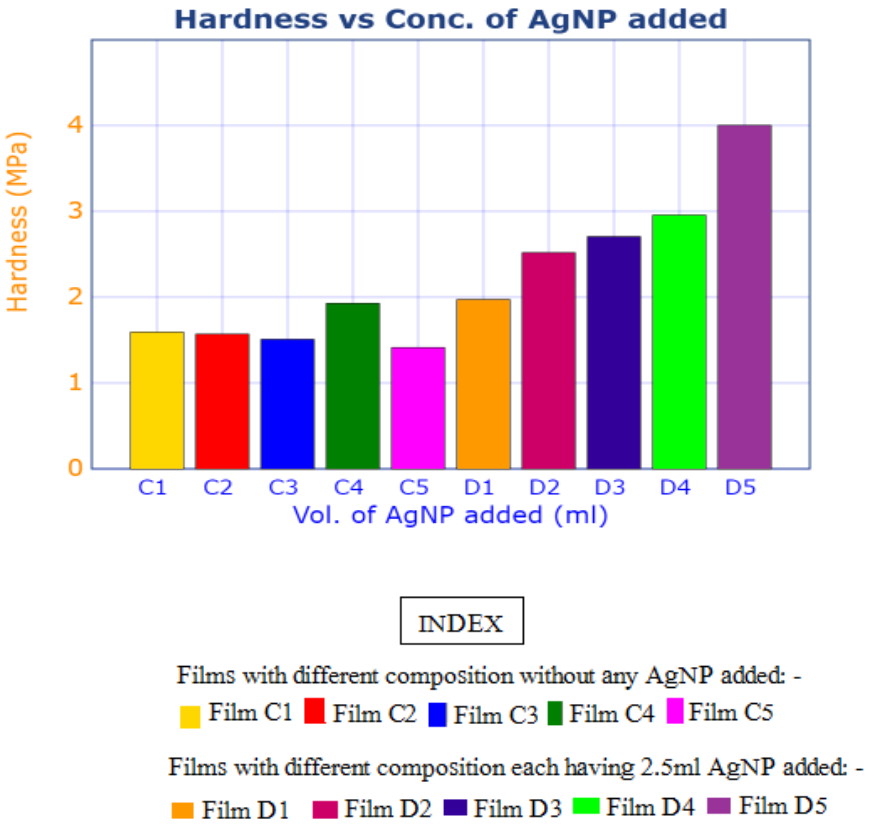


Figure 09: - The above graphical representation shows the relationship of hardness of the two set of films, with and without the incorporation of silver nanoparticles: -

Serial No.	**Films (No AgNP added)**	**Films (each film has 2.5ml AgNP added)**	**% increase in hardness (MPa)**
1	C1	D1	80.6
2	C2	D2	62.2
3	C3	D3	55.6
4	C4	D4	65.1
5	C5	D5	35.2

The above table shows a comparison between each set of Films C and Film D where film mixture composition volume is same for every individual set such as C3 and D3 , only for every set of D-Films, 2.5 ml of AgNP is added: -

Discussions: -

Above results show that films with same composition of gelatin and sodium alginate with silver nanoparticles had more hardness compared to the films made devoid of any AgNPs but with the same compositions of sodium alginate and gelatin solution.

This shows that by incorporating nanoparticles the hardness gets developed for the composite films as obtained in works of [118] where increasing AgNP amount by 0.0075% leaded to 94.5% increase in the hardness, enhancement in the mechanical properties, mainly tensile strength and hardness was observed with addition of AgNP for agar-gelatin-silver nanocomposite films [119]. Enhanement was observed for gelatin-curcumin-silver nanocomposite films with a 0.05% increase in amount of AgNP added [120], from where it can be concluded that AgNP addition enhances hardness of composite films.

Study of quality of food packed in composite films incorporated with silver nanoparticles-

Reagents: -

Reagents are same as used in the previous experiment.

Equipments and Instruments: -

All the equipments and instruments used were same as previous experiment; here TA.XT texture analyzer was not required.

Preparation of Sodium alginate solution: -

5gm of sodium alginate was weighed and added to 60 ml of d.d.w. It was kept on magnetic stirrer and allowed to mix properly at 550 r.p.m for 1 hour, temperature kept at 45°C which was monitored using laboratory thermometer. After proper mixing, 4ml of polyethylene glycol was added and again mixed for half an hour without any heat treatment on magnetic stirrer (at 500 r.p.m).

Preparation of Gelatin solution: -

5 gm of gelatin was weighed and added to 60 ml of d.d.w. It was kept on magnetic stirrer and allowed to mix properly at 550 r.p.m for 1 hour, without any application of heat. After proper mixing, 4ml of polyethylene glycol was added and again mixed for half an hour without any heat treatment on magnetic stirrer (at 500 r.p.m).

Preparation of the film forming solutions: -

Now 30 ml from each Na-alginate solution and gelatin solution were taken and mixed in two different conical flasks, with one container having 4.5 ml silver nanoparticle being added to it and these two containers were again stirred on magnetic stirrer for 1 hour without heat treatment.
Container 1- 30ml of gelatin solution + 30ml of Na-alginate solution
Container 2- 30ml of gelatin solution + 30ml of Na-alginate solution + 4.5 ml AgNP

Casting of the film forming solutions: -

The mixtures from the above containers were uniformly spread on two borosil petri plates of diameter 20cm and kept at room temperature (37.5℃) for 48 hours to dry.

Observations: - Films obtained after drying

Image 10: - Film A_N – Film incorporated with silver nanoparticle

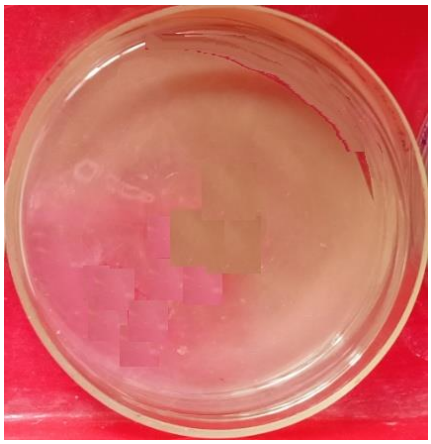
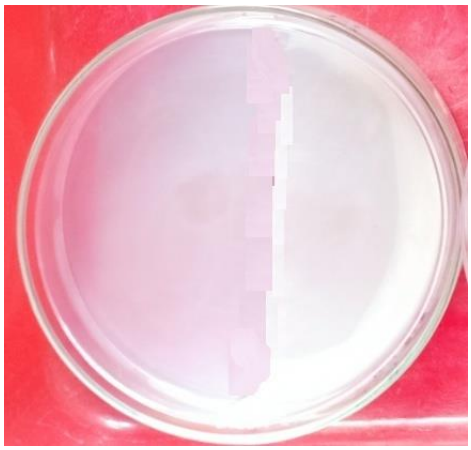


Image 11: - Film A_C – Film devoid of silver nanoparticle



Fresh tomatoes were used to study the packaging property of the above obtained composite films. Fresh tomatoes were obtained from the local market of Jadavpur, Kolkata-700032 and were placed on the above films. They were uniformly wrapped in the films and cello tape was use for making the films sealed and holding them in place and was kept at open room temperature (37°C) for five days.

Observations: -

Image 11- Tomato to be wrapped in gelatin-alginate composite film

Image 12: - Tomato to be wrapped in gelatin-alginate-Ag nanocomposite film

Image 13: - The above image shows fresh tomato being wrapped in Film A_C (composite film devoid of silver nanoparticle) and stored for five days at room temperature (Average temperature 37°C).

Image 14: - The above image shows fresh tomato being wrapped in Film A_N (composite film incorporated with silver nanoparticle) and stored for five days at room temperature (38°C)

Observation-

After 5 days of being stored in room temperature, on Day 5 the films were unwrapped to observe the tomatoes being wrapped in them. The observation is being shown in the Image 15 given below –

Image 15: -

Tomato unwrapped from gelatin-alginate-Ag nanocomposite film

Tomato unwrapped from gelatin-alginate composite film

Discussions: -

The above image shows that tomato wrapped in gelatin-Na-alginate composite film incorporated with AgNP was obtained in a much better condition compared to the tomato which was wrapped in gelatin-Na-alginate composite film devoid of AgNP.

Comparison of the organoleptic properties of the tomatoes unwrapped from the two Na-alginate-gelatin composite films, with and without incorporation of silver nanoparticles: -

Tomato wrapped in NP Composite Film	**Tomato wrapped in Composite Film**
Color retention is better.	Color gradually faded and became pale
Texture maintained well.	Texture became soft and wrinkled.
The internal components (seeds, pulp, juices) remained within the product without any visible loss.	The internal components (seeds, pulp, juices) oozed out from the food product showing loss of product quantity and damage to quality of product as well.

Research works showed that fruits packed in silver-nanocomposite films showed better retention of their organoleptic and nutritional properties as well [121]. Composite films incorporated with AgNP showed comparatively better retention of the overall visible properties of the food product being packed rather than the food product packed in composite films devoid of AgNP.

Study of the antimicrobial properties of the Na-alginate-gelatin silver nanocomposite films by using streak plate method of microbial inoculation: -

Microorganism used was *Escherichia coli.*

A very important aspect and property is to analyse the antimicrobial property of any packaging material to be used in the sector of food packaging.

Preparation of Nutrient agar media: -

Reagents: -

Nutrient agar, Beef Extract, Peptone, Double distilled water.

Equipments and Instruments: -

REMI 2MLH Magnetic stirrer, heating mantle, conical flasks 250 ml and 100ml (borosil), borosil petri plates of diameter 10 cm, laboratory thermometer (Manufactured by LABWORLD, Range 0-300°C), Autoclave (Bhattacharya and Co.), B.O.D Shaker Incubator, Refrigerator.

Nutrient agar media was prepared as per the composition given in the table below-

Table 18: -

Materials: -	Material Composition: -
Agar	3.75 g
Beef Extract	0.75 g
Peptone	1.25 g
D.D.W (double distilled water)	250 ml

Preparing the mixture of agar medium: -

All above components were put together inside a conical flask and were made to mix uniformly by using magnetic stirrer (450 r.p.m) and were boiled at 50°C (temperature was being monitored using laboratory thermometer) to form a uniform homogenous mixture.

This dissolved medium was then autoclaved at 15 psi pressure at 121°C temperature for 15 minutes. After this, the conical flask was taken out of the autoclave and allowed to cool moderately at room temperature (cooling was done moderately which would otherwise lead to the solidifying of the agar media).

Pouring of the molten agar media on borosil petriplates: -

The agar media now from the conical flask was poured on borosil petri plates and the petri plates were kept inside an incubator at 37.5°C for 24 hour so that the media gets dried and solidified.

Storage of the solidified nutrient agar medium: -

After 24 hours, the petriplates containing the solidified agar media was taken out of the incubator and was kept inside a refrigerator and stored there to be used later.

Image 16: - Image showing nutrient agar media obtained: -

Inoculation of Bacterial culture: -

Streak plate method was used to inoculate bacterial culture of *Escherichia coli* to study their growth on the composite films. During this experiment composite films made by using Na-alginate and gelatin and composite films made by using Na-alginate and gelatin along with incorporation of silver nanoparticles were used to see the respective antimicrobial properety of both the films. Inoculation was also done on nutrient agar media which was used as a reference to compare the microbial growth with those of the composite films prepared.

Equipments and Instruments required: -

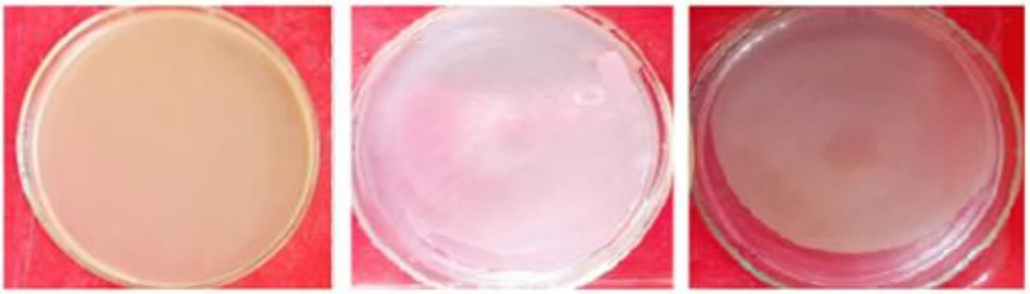
Metal wire loop, spirit lamp burner, B.O.D shaker incubator, laminar flow chamber.

Inoculation of bacterial culture of *Escherichia coli* into the nutrient agar media, Na-alginate-gelatin composite film and Na-alginate-gelatin-AgNP composite film: -

1. Metal wire loop was sterilized using flame from burner.
2. *Escherichia coli* were obtained from previously made test tube suspension cultures present in laboratory of Department of Food Technology and Bio-Chemical Enginnering, Jadavpur University.
3. The 3 petri plates (Nutrient agar media, Na-alginate/Gelatin film, Na-alginate/Gelatin film/AgNP) were placed with their covers opened.
4. The sterilized loop was dipped deep inside the test tube having the microbial culture and quickly taken out and with it different zigzag patterns were drawn on each three petri plates by scratching them with the metal loop.
5. This process was done three times for each petri plates.
6. All the petriplates were again covered with their lids and kept on an incubator at 38°C temperature for 24 hours.
7. After 24 hours, all three petri plates were taken out of the incubator to observe the microbial growth which occurred in them.
8. All the equipments and the entire process of inoculation were carried out inside a laminar flow chamber so as to keep out any external contamination which would interfere with the desired results.

Observation –

Image 17- Image showing the three petri plates before inoculation of microbial culture.

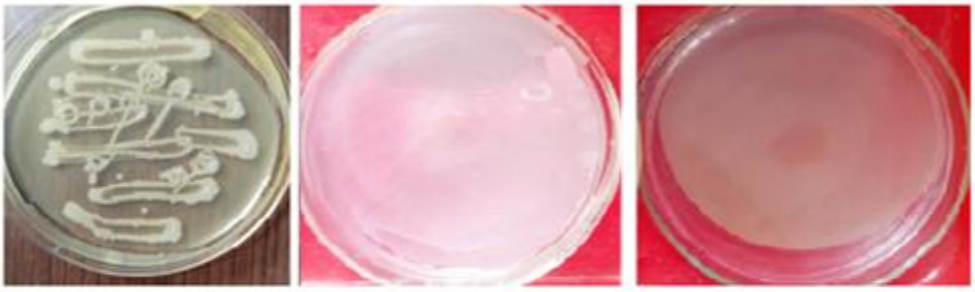


Nutrient agar Media (Pre-inoculation)

Na-alginate-gelatin film (Pre-inoculation)

Na-alginate-gelatin-Ag nanocomposite (Pre-inoculation)

Image 18- Image showing the three petri plates after inoculation of microbial culture and being taken out from incubation chamber after incubating at 38°C for 24 hours.



Nutrient agar Media (Post-inoculation) | Na-alginate-gelatin film (Post-inoculation) | Na-alginate-gelatin-Ag nanocomposite (Post-inoculation)

Discussions: -

The above image show the growth of colonies of *Escherichia coli* on the surface of nutrient agar media whereas no observable growth on the composite films. Films with AgNP showed no growth of *Escherichia coli* colonies indicating the antimicrobial property of silver nanoparticles included composite films which are an essential property of packaging films to be used for food packaging as showed in works of [122] where 91% inhibition in growth of *Escherichia coli* was observed with AgNP addition, researches also showed better and enhanced antibacterial effect towards *Escherichia coli* with addition of AgNP as shown in works of [123] and [124]. Therefore it can be concluded that silver nanoparticle incorporated composite films show very prominent antimicrobial property towards the growth of *Escherichia coli* bacteria.

Study of the antimicrobial properties of the Na-alginate-gelatin silver nanocomposite films by using streak plate method of microbial inoculation: -

Microorganism used: - *Staphylococcus aureus*

Preparation of Nutrient agar media: -

Reagents: -

Nutrient agar, Beef Extract, Peptone, Double distilled water.

Equipments and Instruments: -

REMI 2MLH Magnetic stirrer, heating mantle, conical flasks 250 ml and 100ml (borosil), borosil petri plates of diameter 10 cm, laboratory thermometer (Manufactured by LABWORLD, Range 0-300°C), Autoclave, B.O.D Shaker Incubator, Refrigerator.

Table 19: -

Serial No.	Material Composition
Agar	3.75 g
Beef Extract	0.75 g
Peptone	1.25 g
D.D.W (double distilled water)	250 ml

Nutrient agar media was prepared as per the composition given in the above table.

Preparing the mixture of agar medium: -

All above components were put together inside a conical flask and were made to mix uniformly by using magnetic stirrer (450 r.p.m) and were boiled at 50°C (temperature was being monitored using laboratory thermometer) to form a uniform homogenous mixture.
This dissolved medium was then autoclaved at 15 psi pressure at 121°C temperature for 15 minutes. After this, the conical flask was taken out of the autoclave and allowed to cool moderately at room temperature (cooling was done moderately which would otherwise lead to the solidifying of the agar media).

Pouring of the molten agar media on borosil petriplates: -

The agar media now from the conical flask was poured on borosil petri plates and the petri plates were kept inside an incubator at 37.5°C for 24 hour so that the media gets dried and solidified.

Storage of the solidified nutrient agar medium: -

After 24 hours, the petriplates containing the solidified agar media was taken out of the incubator and was kept inside a refrigerator and stored there to be used later.

Image 19: - Image showing nutrient agar media obtained: -

Streak plate method was used to inoculate culture of *Staphylococcus aureus* to study their growth on composite films. Composite films made by using Na-alginate and gelatin and composite films made by using Na-alginate and gelatin along with incorporation of silver nanoparticles were used

to see the respective antimicrobial properety of both the films. Inoculation was done on nutrient agar media which was used as a reference to compare the microbial growth with those of the composite films prepared.

Equipments and Instruments required: -

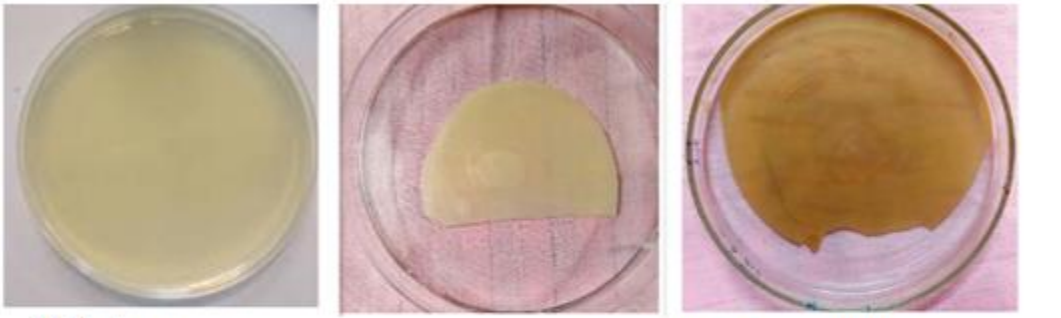
Metal wire loop for inoculation, Spirit lamp burner, B.O.D Shaker Incubator, Laminar flow chamber.

Inoculation of bacterial culture of *Staphylococcus aureus* into the nutrient agar media, Na-alginate-gelatin composite film and Na-alginate-gelatin-AgNP composite film: -

1. Metal loop was sterilized using flame from burner.
2. *Staphylococcus aureus* were obtained from previously made test tube suspension cultures present in laboratory of Department of Food Technology and Bio-Chemical Enginnering, Jadavpur University.
3. The 3 petri plates (Nutrient agar media, Na-alginate/Gelatin film, Na-alginate/Gelatin film/AgNP) were placed with their covers opened.
4. The sterilized loop was dipped deep inside the test tube having the microbial culture and quickly taken out and with it different zigzag patterns were drawn on each three petri plates by scratching them with the metal loop.
5. This process was done three times for each petri plates.
6. All petriplates were again covered with their lids and kept on an incubator at 37°C temperature for 24 hours.
7. After 24 hours, all three petri plates were taken out of the incubator to observe the microbial growth which occurred in them.
8. All the equipments and the entire process of inoculation were carried out inside a laminar flow chamber so as to keep out any external contamination which would interfere with the desired results.

Observation –

Image 19- Image showing the three petri plates before inoculation of microbial culture.

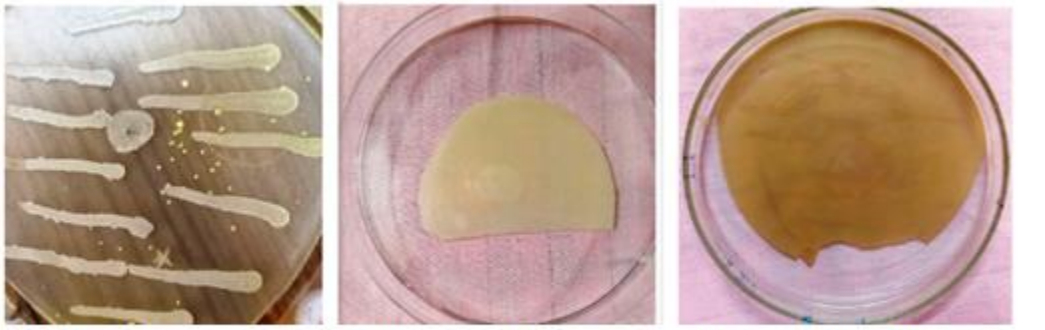


Nutrient agar Media (Pre-inoculation)

Na-alginate-gelatin film (Pre-inoculation)

Na-alginate-gelatin-Ag nanocomposite (Pre-inoculation)

Image 20: - Image showing the three petri plates after inoculation of microbial culture and being taken out from incubation chamber after incubating at 37°C for 24 hours.



Nutrient agar Media (Post-inoculation) | Na-alginate-gelatin film (Post-inoculation) | Na-alginate-gelatin-Ag nanocomposite (Post-inoculation)

Discussions: -

The above images show the growth of *Staphylococcus aureus* on the surface of nutrient agar media whereas no observable growth on the composite films. Film made with incorporation of AgNPs showed no growth of *Staphylococcus aureus* colonies indicating the antimicrobial property of silver nanoparticle included composite films as showed by works of [125] where 0.3% increase in the AgNP amount leaded to a 4 times inflation in the antimicrobial property against *Staphylococcus aureus* species compared to films without AgNPs and [126] which showed that suspensions having *Staphylococcus aureus* when AgNP was added, no further growth of the organism was found in that suspension for 1 week, [127].

Therefore it can be concluded that AgNP incorporated composite films show very prominent antimicrobial property towards the growth of *Staphylococcus aureus* bacteria and can be used as composite films for effective antimicrobial packaging.

Conclusion: -

In the present work, it has been shown that leaf extract of lamboo tree can effectively produce AgNPs when it reacts with aqueous $AgNO_3$. The as-synthesized silver colloids show distinct SPR peak at 437nm which is the signature peak of silver nanoparticles. Films with enhanced hardness were observed when films were incorporated with starch. 2.5 times increase in the hardness (MPa) of the composite films was observed when AgNP content was increased from 1ml to 4ml. For composite films, Na-alginate-gelatin film showed 0.6 times more hardness compared to Na-alginate-starch composite films when Na-alginate and biopolymer ratio is same

Na-alginate-gelatin-silver nanocomposite films showed an average 0.5 times increase in hardness with every 1ml addition of AgNP showing that AgNPs can enhance the hardness property which as described by previous works is due to extensive bonding between the AgNPs with the polymeric matrices. Ag-nanocomposite films showed maximum solubility of 73.8% in 100ml d.d.w., although with more AgNP addition the solubility decreased to 46.9% which is due to the excess silver NPs that makes the polymer matrix bonds stronger and difficult to be broken down by water. Tomatoes packed in Na-alginate-gelatin-AgNP composite films kept for 5 days at room temperature (avg.37°C) showed better retention in textural and visual properties compared to composite films devoid of AgNPs. Remarkable antibacterial activity was observed for the Na-alginate-gelatin-AgNP composite films against *Escherichia coli* and *Staphylococcus aureus*, inoculated using streak plate method on films, and no observable growth was seen after incubation period.

Therefore Na-alginate-gelatin-AgNP composite films prepared as described in the above research work can be used as effective hard and biodegradable packaging films with good antimicrobial properties.

References: -

1. A. Mitra, G. De, 2016, Sol-Gel Synthesis of Metal Nanoparticle Incorporated Oxide Films on Glass, William Andrew Publishing, and Chapter 06, Page no. 145-163, DOI- https://doi.org/10.1016/B978-0-323-39309-6.00006-7.

2. Habibullah, G., Viktorova, J. & Ruml, T., 2021, Current Strategies for Noble Metal Nanoparticle Synthesis, Nanoscale Res. Letter, DOI- https://doi.org/10.1186/s11671-021-03480-8.

3. Reverberi AP, Vocciante M, Salerno M, Ferretti M, Fabiano B., 2020, Green Synthesis of Silver Nanoparticles by Low-Energy Wet Bead Milling of Metal Spheres, Multidisciplinary Digital Publishing Institute, Volume 13, Issue 1, DOI- *https*://doi.org/10.3390/ma13010063.

4. Saratale, Rijuta & Saratale, Ganesh & Shin, Hanseung & Jacob, Jaya & Pugazhendhi, Arivalagan & Bhaisare, Mukesh & Kumar, Gopalakrishnan, 2018, "New insights on the green synthesis of metallic nanoparticles using plant and waste biomaterials: current knowledge, their agricultural and environmental applications", Environmental Science and Pollution Research, Volume 25, 10.1007/s11356-017-9912-6.

5. Goutam, Surya & Saxena, Gaurav & Roy, Diptarka & Yadav, Anil & Bharagava, Ram, 2019, "Green Synthesis of Nanoparticles and Their Applications in Water and Wastewater Treatment, Publisher- Spriner Nature, Singapore, Chapter 16, Edition 01, DOI-10.1007/978-981-13-1891-7_16.

6. Abdelnour SA, Alagawany M, Hashem NM, Farag MR, Alghamdi ES, Hassan FU, Bilal RM, Elnesr SS, Dawood MAO, Nagadi SA, Elwan HAM, ALmasoudi AG, Attia YA, 2021, "Nanomaterials: Fabrication Methods, Benefits and Hazards, and Their Applications in Ruminants with Special Reference to Selenium and Zinc Nanoparticles, Animals, MDPI, Volume 11, Issue 07, DOI- https://doi.org/10.3390/ani11071916.

7. Khadeeja Parveen, Viktoria Banse, and Lalita Ledwani, 2016, "Green synthesis of nanoparticles: Their advantages and disadvantages", AIP Conference Proceedings 1724, Volume 1724, Issue 01, DOI-https://doi.org/10.1063/1.4945168.

8. Jagpreet Singh, Tanushree Dutta, Ki-Hyun Kim, Mohit Rawat, Pallabi Samddar & Pawan Kumar, 2018, "Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation", Journal of Nanobiotechnology, Volume 16, Issue 84, DOI-https://doi.org/10.1186/s12951-018-0408-4.

9. Sudip Mukherjee and Chitta Ranjan Patra, 2017, "Biologically synthesized metal nanoparticles: recent advancement and future perspectives in cancer theranostics", Future Science Journal Volume 03, Issue 03, DOI-https://doi.org/10.4155/fsoa-2017-0035

10. Vijay Devra, 2022, “Synthesis of metal nanoparticles by microbes and biocompatible green reagents”, Chapter 02, Page no. 17-45, ISBN 9780128235751, DOI-https://doi.org/10.1016/B978-0-12-823575-1.00013-5.

11. Gaurav Pal, Priya Rai, Anjana Pandey, 2019, “Green synthesis of nanoparticles: A greener approach for a cleaner future, Publisher-Elsevier, Chapter 01, Pages 1-26, ISBN 9780081025796, DOI-https://doi.org/10.1016/B978-0-08-102579-6.00001-0.

12. Kurcheti PP, Dhayanath, Mary AJ, Jeena, Alim H, 2020, “Biosynthesis of Nanoparticles - A New Horizon in Fish Biomedicine”, HSOA Journal of Aquaculture and Fisheries 4: 035.

13. Sanket, Swaroop & Das, Swagat Kumar, 2021, “Role of Enzymes in Synthesis of Nanoparticles, Book of Biomedical and Life Sciences, Published by Springer, Singapore, DOI-10.1007/978-981-33-4195-1_7.

14. Jing Huang, Yue Zheng, Joseph D. Groom, Zheng Yu, Ludmila Chistoserdova, 2021, “Expression, purification and properties of the enzymes involved in lanthanide-dependent alcohol oxidation: XoxF4, XoxF5, ExaF/PedH, and XoxG4”, Methods in Enzymology, Academic Press, Volume 650, Pages 81-96,ISSN 0076-6879, ISBN 9780128238561, DOI-https://doi.org/10.1016/bs.mie.2021.01.041.

15. Amit Kumar Mittal, Yusuf Chisti, Uttam Chand Banerjee, 2013, “Synthesis of metallic nanoparticles using plant extracts, Biotechnology Advances”, Volume 31, Issue 2, Pages 346-356, ISSN 0734-9750, DOI-https://doi.org/10.1016/j.biotechadv.2013.01.003.

16. Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., & Kalinina, N. O., 2014, "Green" nanotechnologies: synthesis of metal nanoparticles using plants, Acta naturae, Volume 06, Issue 01, Page no. 35–44.

17. Fierascu, R.C. & Ion, Rodica-Mariana & Dumitriu, I., 2010, “Noble metals nanoparticles synthesis in plant extracts”, Optoelectronics and Advanced Materials, Rapid Communications, Volume 04, Page no. 1297-1300.

18. Rai, Mahendra & Yadav, Alka, 2013, “Plants as potential synthesizer of precious metal nanoparticles: Progress and prospects”, IET nanobiotechnology / IET. 7. 117-24. 10.1049/iet-nbt.2012.0031.

19. Rawat, R.S., 2015, “Dense Plasma Focus - From Alternative Fusion Source to Versatile High Energy Density Plasma Source for Plasma Nanotechnology”, Journal of Physics: Conference Series. 591. 012021, DOI-10.1088/1742-6596/591/1/012021.

20. Namra Abid, Aqib Muhammad Khan, Sara Shujait, Kainat Chaudhary, Muhammad Ikram, Muhammad Imran, Junaid Haider, Maaz Khan, Qasim Khan, Muhammad Maqbool, 2022, “Synthesis of nanomaterials using various top-down and bottom-up approaches, influencing factors, advantages, and disadvantages: A review”, Advances in Colloid and Interface Science, Volume 300, 102597, ISSN 0001-8686, DOI-https://doi.org/10.1016/j.cis.2021.102597.

21. Kumar, A., Gupta, K., Dixit, S. et al., 2019, "A review on positive and negative impacts of nanotechnology in agriculture", International Journal of Environmental Science and Technology Volume 16, Page no. 2175–2184, DOI-https://doi.org/10.1007/s13762-018-2119-7.

22. Jadoun, Sapana & Arif, Rizwan & Jangid, Nirmala & Meena, Rajesh, 2021, "Green synthesis of nanoparticles using plant extracts: a review", Environmental Chemistry Letters, Volume 19, Page no. 355–374, DOI-10.1007/s10311-020-01074-x.

23. Banhegyi, Gyorgy, 2015, "The difference between top down and bottom up methods for creating nano-structures", retrieved from: https://www.researchgate.net/post/What-is-the-difference-between-top-down-and-bottom-up-methods-for-creating-nano-structures/54af9baad685cccd3e8b462a/citation/download.

24. Singh, J. P., Kumar, M., Sharma, A., Pandey, G., Chae, K., & Lee, S., 2020, "Bottom-Up and Top-Down Approaches for MgO, Sonochemical Reactions", IntechOpen, DOI-https://doi.org/10.5772/intechopen.91182.

25. Habiba, Khaled & Makarov, Vladimir & Weiner, Brad & Morell, Gerardo, 2014, "Fabrication of Nanomaterials by Pulsed Laser Synthesis", Book of Manufacturing Nanostructures, Chapter 10, DOI-10.13140/RG.2.2.16446.28483.

26. Ovais, Muhammad & Raza, Abida & Naz, Shagufta & Islam, Nazar Ul & Khalil, Ali & Ali, Shaukat & Khan, Muhammad & Shinwari, Zabta, 2017, "Current state and prospects of the phytosynthesized colloidal gold nanoparticles and their applications in cancer theranostics. Applied Microbiology and Biotechnology", Volume 101, DOI-10.1007/s00253-017-8250-4.

27. Olivia Dawson, 2011, "Difference between top down and bottom up approach in nanotechnology", online knowledge hub, https://www.differencebetween.com/difference between top down and bottom up approach in nanotechnology.

28. Vadahanambi, Sridhar, 2017, "Re: Discuss the advantages and disadvantages of Top-down and Bottom-up approaches in nano fabrication", Retrieved from: https://www.researchgate.net/post/Discuss_the_advantages_and_disadvantages_of_Top-down_and_Bottomup_approaches_in_nano_fabrication/58b4fce3615e271f052c9b98/citation/download.

29. Kumar, S., Bhushan, P., Bhattacharya, S., 2018, "Fabrication of Nanostructures with Bottom-up Approach and Their Utility in Diagnostics, Therapeutics, and Others", Environmental, Chemical and Medical Sensors, Energy, Environment and Sustainability, Springer, Singapore, DOI-https://doi.org/10.1007/978-981-10-7751-7_8.

30. Peiqin Li, Chao Luo, Weibo Sun, Shiqiong Lu, Yan Mou, Youliang Peng and Ligang Zhou, 2011, "In vitro antioxidant activities of polysaccharides from endophytic fungus Fusarium oxysporum Dzf17", Journal of Microbiology Research, Vol. 5 Issue 32, DOI: 10.5897/AJMR11.1342.

31. Oxana V. Kharissova, H.V. Rasika Dias, Boris I. Kharisov, Betsabee Olvera Pérez, Victor M. Jiménez Pérez, 2013," The greener synthesis of nanoparticles", Trends in Biotechnology, Volume 31, Issue 4, Pages 240-248, ISSN 0167-7799, DOI- https://doi.org/10.1016/j.tibtech.2013.01.003.

32. Tang, S., Liu, Y., Zheng, N. et al., 2020, "Temporal variation in nutrient requirements of tea (*Camellia sinensis*) in China based on QUEFTS analysis", Journal of Scientific Reports, Article No. 1745, DOI- https://doi.org/10.1038/s41598-020-57809-x.

33. Han, Wenyan & Li, Xin & Yan, Peng & Zhang, Liping & Ahammed, Golam Jalal, 2018, "Tea cultivation under changing climatic conditions", Pages 455-472, DOI- 10.19103/AS.2017.0036.19.

34. Laskar, Nurujjaman, 2018, "Productivity analysis of tea production in tea Industry of Assam".

35. M V, Durai, 2019, "Mahogany-Vansangyan", Institute of Wood Science and Technology, Bengaluru, https://www.researchgate.net/publication/337032462_Mahogany-Vansangyan.

36. Kallio, Maarit & Kanninen, Markku & Krisnawati, Haruni, 2011, "Swietenia macrophylla King Ecology, silviculture and productivity", Center for International Forestry Research, Bogor, Indonesia.

37. Udayana, Cicik, karpe, Christina, Solberg, Svein Ø., Mathisen, Karen M., Andreassen, Harry P., 2019, "Soil properties after forest rehabilitation by planting teak and mahogany in Java, Indonesia", Forest Science and Technology, Volume 15, Issue 4, Page number 230-237, doi: 10.1080/21580103.2019.1673220

38. A. Bikoro Bi Athomo, S.P. Engozogho Anris, R. Safou-Tchiama, F.J. Santiago-Medina, T. Cabaret, A. Pizzi, B. Charrier, 2018, "Chemical composition of African mahogany (K. *ivorensis* A. *Chev*) extractive and tannin structures of the bark by MALDI-TOF, Industrial Crops and Products, Volume 113, Pages 167-178, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2018.01.013

39. Vikram Paritala, Kishore K. Chiruvella, Chakradhar Thammineni, Rama Gopal Ghanta, Arifullah Mohammed, 2015, " Phytocehmical and antimicrobial potentials of mahogany family", Revista Brasileira de Farmacognosia, Volume 25, Issue 1, Pages 61-83, https://doi.org/10.1016/j.bjp.2014.11.009.

40. M. Ajanal, M. Gundkalle, and S. Nayak, 2012, "Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer," Ancient Science of Life, Volume 31, Issue no. 4, Page no. 198-201.

41. B. Mahdi-Pour, S. L. Jothy, L. Y. Latha, Y. Chen, and S. Sasidharan, 2012, "Antioxidant activity of methanol extracts of different parts of *Lantana camara*," Asian Pacific Journal of Tropical Biomedicine, Volume 02, Issue No. 12, Page no. 960-965.

42. Sun, Chunli, Wu, Zhengshuang, Wang, Ziyan, Zhang, Hongcheng, 2015, "Effect of Ethanol/Water Solvents on Phenolic Profiles and Antioxidant Properties of Beijing Propolis Extracts", Hindawi Publishing Corporation, Volume 2015, DOI - 10.1155/2015/595393

43. Brglez Mojzer, E.; Knez Hrnčič, M.; Škerget, M.; Knez, Ž. Bren, U., 2016, "Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects", Molecules, MDPI Journal, Volume *21*, Issue 7, Page no.901, https://doi.org/10.3390/molecules21070901

44. Rastogi, R.P. and Mehrotra, B.N., 1998-2002, "Compendium of Indian medicinal plants", Journal of Scientific Research, Volume. 1-6, Central Drug Research Institute/National Institute of Science Communication, Lucknow/New Delhi.

45. P. S. C. Rao,R. E. Jessup,D. E. Rolston,J. M. Davidson,D. P. Kilcrease, 1980, "Experimental and Mathematical Description of Nonadsorbed Solute Transfer by Diffusion in Spherical Aggregates", Soil Science Society of America Journal, Volume 44, Issue 4, Page no. 684-688, https://doi.org/10.2136/sssaj1980.03615995004400040004x.

46. Van Hoyweghen, Laura & De Beer, Thomas & Deforce, Dieter & Heyerick, Arne, 2012, "Phenolic Compounds and Anti-oxidant Capacity of Twelve Morphologically Heterogeneous Bamboo species, Phytochemical analysis", PCA, Volume 23, Issue 5, Page no.433-43, DOI 10.1002/pca.1377.

47. María Castro-Puyana, María Luisa Marina, Merichel Plaza, 2017,"Water as green extraction solvent: Principles and reasons for its use, Current Opinion in Green and Sustainable Chemistry, Volume 5, Pages 31-36, ISSN 2452-2236,https://doi.org/10.1016/j.cogsc.2017.03.009.

48. Anandalakshmi, K., Venugobal, J. & Ramasamy, V., 2016, "Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity", Journal of Applied Nanoscience, Volume 6, Page no. 399–408 https://doi.org/10.1007/s13204-015-0449-z.

49. François Eya'ane Meva, Marcelle Loretta Segnou, Cecile Okalla Ebongue, Agnes Antoinette Ntoumba, Philippe Belle Ebanda Kedi, Vandi Deli, Marie-Annie Etoh, Emmanuel Mpondo Mpondo, 2016, "Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from Megaphrynium macrostachyum leaf extract", Revista Brasileira de Farmacognosia, Volume 26, Issue 5, Pages 640-646, ISSN 0102-695X, https://doi.org/10.1016/j.bjp.2016.06.002.

50. A.M. Awwad, N.M. Salem, A.O. Abdeen, 2013, "Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity", International Journal of Industrial Chemistry, Volume 4, Issue 29, https://doi.org/10.1186/2228-5547-4-29.

51. Prachi Kumari, Chirom Aarti, Arun Renganathan, 2013, "Synthesis and characterization of silver nanoparticles using cannonball leaves and their cytotoxic activity against MCF-7 cell line", Journal of Nanotechnology, Volume 2013, Article ID 598328,

https://doi.org/10.1155/2013/598328.

52. P. Mulvaney, 1996, “Surface plasmon spectroscopy of nanosized metal particles”, Langmuir, Volume 12, Issue 03, Page no. 788-800.

53. G. Thirumurgan and M.D Dhanaraju, 2011, “Novel biogenic metal nanoparticles for pharmaceutical applications”, Advanced Science Letters, Volume 04, Issue 02, Page no. 339-348.

54. K. Govindaraju, S. Tamilselvan, V. Kiruthiga and G. Singaravelu, 2010, “Biogenic silver nanoparticles by *Solanumtorvum* and their promising antimicrobial activity”, journal of Biopesticides, Volume 03, Issue 01, Page no. 394-399.

55. Shankar, Shiv &Rhim, Jong-Whan, 2018, “Bionanocomposite Films for Food Packaging Applications”, Online reference module in Food Science, Elsevier, Page no. 1-10, 10.1016/B978-0-08-100596-5.21875-1.

56. Dalberg: Global Consulting Firm, 2019, “Solving Plastic Pollution through Accountability”.

57. Anna (Anya) Phelan, Katie Meissner, Jacquelyn Humphrey, Helen Ross, 2022, "Plastic pollution and packaging: Corporate commitments and actions from the food and beverage sector, Journal of Cleaner Production, Volume 331, ISSN 0959-6526,https://doi.org/10.1016/j.jclepro.2021.129827.

58. Rhim, J.W, Wang, L.F, Hong, S.I, 2013, “Bionanoparticles for food packaging applications”, Progress in Polymer Science, Page no. 629-1652.

59. M.J. Fabra, A. López-Rubio, J.M. Lagaron, 2014, "Biopolymers for food packaging applications", Smart Polymers and their Applications, Woodhead Publishing, Pages 476-509, ISBN 9780857096951, https://doi.org/10.1533/9780857097026.2.476.

60. Senka Z. Popović, Vera L. Lazić, Nevena M. Hromiš, Danijela Z. Šuput, Sandra N. Bulut, 2018, “Biopolymer Packaging Materials for Food Shelf-Life Prolongation”, Handbook of Food Bioengineering, Biopolymers for Food Design, Academic Press, Chapter 08, Pages 223-277, ISBN 9780128114490, https://doi.org/10.1016/B978-0-12-811449-0.00008-6.

61. Porta R, Sabbah M, Di Pierro P., 2020, “Biopolymers as Food Packaging Materials”, International Journal of Molecular Sciences, Volume 21, Issue 14, https://doi.org/10.3390/ijms21144942.

62. Krishnan, Gowthaman & Lim, Hn & Sreeraj, T.R. & Amalraj, Augustine & Gopi, Sreeraj, 2021, “Advantages of biopolymers over synthetic polymers”, Book of Biopolymers and their industrial applications, Page no. 351-372, 10.1016/B978-0-12-819240-5.00015-8.

63. Abe MM, Martins JR, Sanvezzo PB, Macedo JV, Branciforti MC, Halley P, Botaro VR, Brienzo M., 2021, “Advantages and Disadvantages of Bioplastics Production from Starch and

Lignocellulosic Components", *Polymers, MDPI*, Volume 13, Issue 15 2021, https://doi.org/10.3390/polym13152484

64. Maximilian Lackner, 2015, "Bioplastics", Kirk-Othmer Encyclopedia of Chemical Technology, Chapter 06, Page no. 01-41, https://doi.org/10.1002/0471238961.koe00006

65. Majid Jamshidian, Elmira Arab Tehrany, Muhammad Imran, Muriel Jacquot, Stéphane Desobr, 2010, "Poly-Lactic Acid: Production, Applications, Nanocomposites, and Release Studies", Comprehensive Reviews in Food Science and Food Safety, Volume 09, Issue 05, Page no. 552-571, https://doi.org/10.1111/j.1541-4337.2010.00126.x

66. Ulf Neuling and Martin Kaltschmitt, 2017, "Review of Biofuel production, feedstock, processes and markets", Journal of Oil Palm Research, Volume 29, Issue 02, Page no. 137-167, https://doi.org/10.21894/jopr.2017.2902.01

67. Carmen M.O. Müller, João Borges Laurindo, Fabio Yamashita, 2011, "Effect of nanoclay incorporation method on mechanical and water vapor barrier properties of starch-based films, Industrial Crops and Products, Volume 33, Issue 3, Page no. 605-610, ISSN 0926-6690, https://doi.org/10.1016/j.indcrop.2010.12.021.

68. Nanou Peelman, Peter Ragaert, Bruno De Meulenaer, Dimitri Adons, Roos Peeters, Ludwig Cardon, Filip Van Impe, Frank Devlieghere, 2013, "Application of bioplastics for food packaging, Trends in Food Science & Technology, Volume 32, Issue 2, Pages 128-141, ISSN 0924-2244, https://doi.org/10.1016/j.tifs.2013.06.003.

69. Ana M. Díez-Pascual, 2022, "Biopolymer Composites: Synthesis, Properties, and Applications", International Journal of Molecular Sciences, Volume 23, Issue 04, 10.3390/ijms23042257.

70. Yang, Bingyan, Yixin Dong, Fei Wang, and Yu Zhang, 2020, "Nanoformulations to enhance the bioavailability and physiological functions of polyphenols, Molecules, Volume 25, Issue 20, https://doi.org/10.3390/molecules25204613

71. El-Kaliuoby MI, Amer M, Shehata N, 2021, "Enhancement of Nano-Biopolymer Antibacterial Activity by Pulsed Electric Fields", Polymers (Basel), Volume 13, Issue 11, Doi: 10.3390/polym13111869.

72. Mohit Batra, Gulshan K. Malik, Jayeeta Mitra, 2020, "Enhancing the properties of gelatin–chitosan bionanocomposite films by incorporation of silica nanoparticles", Journal of Food Process Engineering, Volume 43, Issue 02, https://doi.org/10.1111/jfpe.13329.

73. Swarup Roy and Jong-Whan Rhim, 2021, "Gelatin-Based Film Integrated with Copper Sulfide Nanoparticles for Active Packaging Applications", MDPI Journal of Applied Sciences, Volume 11, Issue 14, https://doi.org/10.3390/app11146307.

74. Marangoni Júnior L, Rodrigues PR, Silva RGD, Vieira RP, Alves RMV, 2021, "Improving the mechanical properties and thermal stability of sodium alginate/hydrolyzed collagen films through the incorporation of SiO_2", Current Research in Food Science, Volume 05, Page No. 96-101, Doi- 10.1016/j.crfs.2021.12.012.

75. Han Ching Voon, Rajeev Bhat, Azhar Mat Easa & A. A. Karim, M. T. Liong, 2012, "Effect of Addition of Halloysite Nanoclay and SiO_2 Nanoparticles on Barrier and Mechanical Properties of Bovine Gelatin Films", Journal of Food and Bioprocess Technology, Volume 05, Page no. 1766-1774, https://doi.org/10.1007/s11947-010-0461-y.

76. Thiagamani, Senthil Muthu Kumar & Rajini, N. & Siengchin, Suchart & Rajulu, A.V. & Hariram, Natarajan, 2019, "Influence of silver nanoparticles on the mechanical, thermal and antimicrobial properties of cellulose-based hybrid nanocomposites, Journal of Composites Part B: Engineering, Volume 165, Page no. 516-525, https://doi.org/10.1016/j.compositesb.2019.02.006.

77. Moses M. Solomon, Husnu Gerengi, and Saviour A. Umoren, 2019, "Carboxymethyl Cellulose/Silver Nanoparticles Composite: Synthesis, Characterization and Application as a Benign Corrosion Inhibitor for St37 Steel in 15% H_2SO_4 Medium", ACS Applied Materials & Interfaces, Volume 09, Issue 07, Page no. 6376-6389, DOI: 10.1021/acsami.6b14153.

78. Shankar S., Wang L.F., Rhim J.W, 2016, "Preparations and characterization of alginate/silver composite films: Effect of types of silver particles, Journal of Carbohydrate Polymers, Volume 146, Page no. 208–216, doi: 10.1016/j.carbpol.2016.03.026.

79. Shiji Mathew, S. Snigdha, Jyothis Mathew, E.K. Radhakrishnan,2018,"Poly(vinyl alcohol): Montmorillonite: Boiled rice water (starch) blend film reinforced with silver nanoparticles; characterization and antibacterial properties", Journal of Applied Clay Science, Volume 161, Pages 464-473, ISSN 0169-1317, https://doi.org/10.1016/j.clay.2018.05.009.

80. Yadav M, Liu YK, Chiu FC, 2019, "Fabrication of Cellulose Nanocrystal/Silver/Alginate Bionanocomposite Films with Enhanced Mechanical and Barrier Properties for Food Packaging Application", Nanomaterials (Basel), National Library of Medicine, Volume 09, Issue 11, doi: 10.3390/nano9111523.

81. Tabari, M. 2018, "Characterization of a new biodegradable edible film based on Sago Starch loaded with Carboxymethyl Cellulose nanoparticles", *Nanomedicine Research Journal*, Volume 03, Issue 01, Page no. 25-30, doi: 10.22034/nmrj.2018.01.004.

82. Sharmin, N., Pang, C., Sone, I., Walsh, J.L., Fernández, C.G., Sivertsvik, M., Fernández, E.N, 2021, "Synthesis of Sodium Alginate–Silver Nanocomposites Using Plasma Activated Water and Cold Atmospheric Plasma Treatment", Journal of Nanomaterials, Volume 11, https:// doi.org/10.3390/nano11092306.

83. Shankar, S.; Teng, X.; Li, G.; Rhim, J.-W., 2015, “Preparation, characterization, and antimicrobial activity of gelatin/ZnO nanocomposite films, Food Hydrocolloids, Volume 45, Page no. 264–271.

84. Kshirod Kumar Dash, K. Kumar Dash, Ashwani Kumar, A. Kumar, Sonam Kumari, S. Kumari, & Mudasir Ahmad Malik, M. Ahmad Malik, 2021, “Silver Nanoparticle Incorporated Flaxseed Protein-Alginate Composite Films: Effect on Physicochemical, Mechanical, and Thermal Properties, Journal of polymers and the environment, Volume 29, Issue 11, Page no. 3649-3659, doi: 10.1007/s10924-021-02137-y.

85. Motelica L, Ficai D, Oprea OC, Ficai A, Ene VL, Vasile BS, Andronescu E, Holban AM., 2021, “Antibacterial Biodegradable Films Based on Alginate with Silver Nanoparticles and Lemongrass Essential Oil-Innovative Packaging for Cheese”, Nanomaterials (Basel, Switzerland), Volume 11, Issue 09, DOI: 10.3390/nano11092377.

86. Kumar R, Ghoshal G, Goyal M., 2019, “Synthesis and functional properties of gelatin/CA-starch composite film: excellent food packaging material. Journal of Food Science and Technology, Volume 56, Issue 4, Page no.1954-1965, doi: 10.1007/s13197-019-03662-4.

87. Thakur, Rahul & Pristijono, Penta & Scarlett, Christopher & Bowyer, Michael & Singh, Sukhvinder & Vuong, Quan., 2019, “Starch-based films: Major factors affecting their properties, International Journal of Biological Macromolecules, Volume 132, Page no. 1079-1089, 10.1016/j.ijbiomac.2019.03.190.

88. I. Arvanitoyannis, E. Psomiadou, A. Nakayama, S. Aiba, N. Yamamoto, 1997, "Edible films made from gelatin, soluble starch and polyols", Part 3, Food Chemistry, Volume 60, Issue 4, Page no. 593-604, ISSN 0308-8146, https://doi.org/10.1016/S0308-8146(97)00038-1.

89. Syazwani Aqilah Alias and Norizah Mhd Sarbon, 2019, “Rheological, physical and mechanical properties of chicken skin gelatin films incorporated with potato starch”, NPJ Science of Food, Volume 03, Issue 26, https://doi.org/10.1038/s41538-019-0059-3.

90. Zhanfeng Dong, Qun Wang, Yumin Du, 2006, "Alginate/gelatin blend films and their properties for drug controlled release”, Journal of Membrane Science, Volume 280, Issues 1–2, Pages 37-44, ISSN 0376-7388, https://doi.org/10.1016/j.memsci.2006.01.002.

91. Mithilesh Yadav, Fang-Chyou Chiu, 2019, “Cellulose nanocrystals reinforced κ-carrageenan based UV resistant transparent bionanocomposite films for sustainable packaging applications”, Carbohydrate Polymers, Volume 211, Pages 181-194, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2019.01.114.

92. Zhang, P.; Zhao, Y.; Shi, Q., 2016, “Characterization of a novel edible film based on gum ghatti: Effect of plasticizer type and concentration”, Carbohydrate Polymers, Volume 153, Page no.345–355.

93. Tang, Chien-Jen & Jaing, Cheng-Chung & Tien, Chuen-Lin & Sun, Wei-Chiang & Lin, Shih-Chin, 2017, “Optical, structural, and mechanical properties of silicon oxynitride films

prepared by pulsed magnetron sputtering", Journal of Applied Optics, Volume 56, Issue 04, C168-C174. 10.1364/AO.56.00C168.

94. Fu, Peng & Teri, Ge-Le & Chao, Xiao-Lian & Li, Jing & Li, Yu-Hu & Yang, Hong., 2020, "Modified Graphene-FEVE Composite Coatings: Application in the Repair of Ancient Architectural Color Paintings, MDPI, Volume 10, Issue 1162, 10. 1162. 10.3390/coatings10121162.

95. Dilara, P.A., & Briassoulis, D., 2000, "Degradation and stabilization of low-density polyethylene films used as greenhouse covering materials*"*, Journal of Agricultural Engineering Research, Volume 76, 309-321.

96. Shebani, Anour & Klash, Abdalah & Elhabishi, Rabea & Abdsalam, Shalh & Elbreki, Hassan & Elhrari, Wael., 2018, "The Influence of LDPE Content on the Mechanical Properties of HDPE/LDPE Blends, Research & Development in Material Science, Volume 07, 10.31031/RDMS.2018.07.000672.

97. Florencia Ortega, Valeria B. Arce, M. Alejandra Garcia, 2021, "Nanocomposite starch-based films containing silver nanoparticles synthesized with lemon juice as reducing and stabilizing agent, Journal of Carbohydrate Polymers, Volume 252, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2020.117208.

98. Srikhao, N., Kasemsiri, P., Ounkaew, A., Narubeth Lorwanishpaisarn, Manunya Okhawilai, Uraiwan Pongsa, Salim Hiziroglu and Prinya Chindaprasirt, 2021, "Bioactive Nanocomposite Film Based on Cassava Starch/Polyvinyl Alcohol Containing Green Synthesized Silver Nanoparticles", Journals of Polymers and the Environment, Volume 29, Page no.672–684, DOI-https://doi.org/10.1007/s10924-020-01909-2.

99. Ashfaq J, Channa IA, Shaikh AA, Chandio AD, Shah AA, Bughio B, Birmahani A, Alshehri S, Ghoneim MM, 2022, "Gelatin- and Papaya-Based Biodegradable and Edible Packaging Films to Counter Plastic Waste Generation, Journal of Materials Basel, Volume 15, Issue 3, doi: 10.3390/ma15031046.

100. Kwaśniewska A, Świetlicki M, Prószyński A, Gładyszewski G., 2021, "Physical Properties of Starch/Powdered Activated Carbon Composite Films", Journal of Polymers Basel, Volume 13, Issue 24, doi: 10.3390/polym13244406.

101. Chen, Zhong, He, Min, Balakrisnan, Bavani, Chum, Chan Choy, 2006, "Elasticity modulus, hardness and fracture toughness of Ni3Sn4 intermetallic thin films", MSE Journal Articles, Volume 423, Issue 1-2, Page no. 107-110, 10.1016/j.msea.2005.12.038.

102. Lin, Jiang & Qiang, Xu & Hu, Chun, 2006, "Preparation and Characterization of Waterborne Polyurethaneurea Composed of Dimer Fatty Acid Polyester Polyol", Journal of Nanomaterials, Volume 2006, DOI- 10.1155/JNM/2006/14906.

103. Zeng, Xiaoliang & Yu, Shuhui & Lai, Maobai & Sun, Rong & Wong, C.P., 2013, “Tuning the mechanical properties of glass fiber-reinforced bismaleimide-triazine resin composites by constructing a flexible bridge at the interface”, Science and Technology of Advanced Materials, Volume 14, Issue 06, DOI- 10.1088/1468-6996/14/6/065001.

104. Zhang, Weiguang, Jijun Li, Yongming Xing, Xiaomeng Nie, Fengchao Lang, Shiting Yang, Xiaohu Hou, and Chunwang Zhao, 2021, "Experimental Study on the Thickness-Dependent Hardness of SiO_2 Thin Films Using Nanoindentation" Journal of *Coatings*, Volume 11, Issue 01, DOI- https://doi.org/10.3390/coatings11010023.

105. Kariper, İ. A., 2016, “Hardness of Thin Films and the Influential Factors”, Book of Diamond and Carbon Composites and Nanocomposites, IntechOpen, ISBN 978-953-51-2454-2, DOI- https://doi.org/10.5772/63302.

106. Chayapa Weerapoprasit,Jutarat Prachayawarakorn, 2016, Properties of biodegradable thermoplastic cassava starch/sodium alginate composites prepared from injection molding”, Journal of Polymer Composites, Volume 37, Issue 12, Page no. 3365-3372, DOI-https://doi.org/10.1002/pc.23534.

107. Jha, P., 2020, “Effect of plasticizer and antimicrobial agents on functional properties of bionanocomposite films based on corn starch-chitosan for food packaging applications”, International Journal of Biological Macromolecules, Volume 160, Page no. 571–582, DOI-https://doi.org/10.1016/j.ijbiomac.2020.05.242.

108. Xiao, Chaobo & Liu, Hongjuan & Lu, Yongshang & Na, Na, 2001, “Blend films from sodium alginate and gelatin solutions”, Journal of Macromolecular Science, Part A, Volume 38, Page no. 317-328, DOI-10.1081/MA-100103352.

109. Abbasiliasi, Sahar, Shun, Tan Joo, Tengku Ibrahim, Tengku Azmi, Ismail, Nurdiana Ariff, Arbakariya B., Mokhtar, Nurfadhilah Khairil, Mustafa, Shuhaimi, 2019, "Use of sodium alginate in the preparation of gelatin-based hard capsule shells and their evaluation in vitro", The Royal Society of Chemistry, Volume 09, Issue 28, DOI- 10.1039/C9RA01791G.

110. Yue Shan, Chaoyue Li, Yongzhi Wu, Qiwen Li and Jinfeng Liao, 2019, “Hybrid cellulose nanocrystal/alginate/gelatin scaffold with improved mechanical properties and guided wound healing”, The Royal Society of Chemistry, Volume 09, Page no. 22966-22979, DOI: 10.1039/C9RA04026A.

111. Wang, Y., Luo, W., Tu, Y., & Zhao, Y., 2021, “Gelatin-Based Nanocomposite Film with Bacterial Cellulose–MgO Nanoparticles and Its Application in Packaging of Preserved Eggs”, Journal of Coatings, Volume 11, Issue 01, Page no. 39, DOI-https://doi.org/10.3390/coatings11010039.

112. Yang, Mao, Zhang, Min, Wang, Yu, Li, Yanchun, Han, Wenjia, Dang, Xugang, 2022, Silver Nanoparticle-Loaded Gelatin-Based Nanocomposite Films toward Enhanced Mechanical

Properties and Antibacterial Activity", Volume 05, Issue 05, American Chemical Society, DOI-10.1021/acsabm.2c00039.

113. Mithilesh Yadav, Fang-Chyou Chiu, 2019, "Cellulose nanocrystals reinforced κ-carrageenan based UV resistant transparent bionanocomposite films for sustainable packaging applications", Carbohydrate Polymers, Volume 211, Pages 181-194, ISSN 0144-8617, https://doi.org/10.1016/j.carbpol.2019.01.114.

114. Zhang, P.; Zhao, Y.; Shi, Q., 2016, "Characterization of a novel edible film based on gum ghatti: Effect of plasticizer type and concentration", Carbohydrate Polymers, Volume 153, Page no.345–355.

115. Sifuentes-Nieves, Israel & Rendon, Rodolfo & Jiménez-Aparicio, Antonio & Camacho-Díaz, Brenda & López, Gustavo & Solorza-Feria, Javier, 2015, "Physical, Physicochemical, Mechanical, and Structural Characterization of Films Based on Gelatin/Glycerol and Carbon Nanotubes", International Journal of Polymer Science, Volume 15, Page no.1-8, DOI 10.1155/2015/763931.

116. Gui Chan Lee and Norizah Mhd. Sarbon, 2020, "Mechanical and physical properties of Bio-Nanocomposite films based on chicken skin gelatin with different concentration of chitosan nanoparticles", Universiti Malaysia Terengganu Journal of Undergraduate Research, Volume 02, Issue 03, Page 1-14.

117. Mehdi Abdollahi, Mehdi Alboofetileh, Rabi Behrooz, Masoud Rezaei, Reza Miraki, 2013, "Reducing water sensitivity of alginate bio-nanocomposite film using cellulose nanoparticles, International Journal of Biological Macromolecules, Volume 54, Pages 166-173, ISSN 0141-8130, DOI- https://doi.org/10.1016/j.ijbiomac.2012.12.016.

118. Ediyilyam S, George B, Shankar SS, Dennis TT, Wacławek S, Černík M, Padil VVT, 2021, "Chitosan/Gelatin/Silver Nanoparticles Composites Films for Biodegradable Food Packaging Applications", Journal of *Polymers*, Volume 13, Issue 11, DOI https://doi.org/10.3390/polym13111680.

119. J.W. Rhim, L.F. Wang, S.I. Hong, 2013 "Preparation and characterization of agar/silver nanoparticles composite films with antimicrobial activity", Journal of Food Hydrocolloids, Volume 33, Issue 2, Pages 327-335, ISSN 0268-005X, DOI https://doi.org/10.1016/j.foodhyd.2013.04.002.

120. Ediyilyam S, George B, Shankar SS, Dennis TT, Wacławek S, Černík M, Padil VVT, 2021, "Chitosan/Gelatin/Silver Nanoparticles Composites Films for Biodegradable Food Packaging Applications", Journal of Polymers, Volume 13, Issue 11, DOI https://doi.org/10.3390/polym13111680.

121. Sharmin N, Pang C, Sone I, Walsh JL, Fernández CG, Sivertsvik M, Fernández EN, 2021, "Synthesis of Sodium Alginate–Silver Nanocomposites Using Plasma Activated Water and Cold Atmospheric Plasma Treatment", Journal of *Nanomaterials*, Volume 11, Issue 09, DOI-https://doi.org/10.3390/nano11092306.

122. Pawika Mahasawat, Ketsarin Hlongkeaw and Sutthida Charoenrit, 2017, "Effect of Chitosan and Alginate Concentration on Size and Bactericidal Activity against Escherichia coli of Chitosan/Alginate/Silver Nanoparticle Beads", Journal of Applied Mechanics and Materials, Volume 855, Page no. 54-59, DOI-10.4028/www.scientific.net/AMM.855.54

123. Acharya, Chinmayee & Panda, Chitta & Bhaskara, Prakash & Sasmal, Abhisek & Shekhar, Suman & Sen, Akhil, 2017, "Physicochemical and anti microbial properties of sodium alginate/gelatin based silver Nanoformulations", Journal of Polymer Bulletin, DOI-10.1007/s00289-0 16- 1738-8.

124. E Susilowati, M Masykuri, M Ulfa and R D Hardini, 2020, "Synthesis of Silver-Alginate Nanocomposites Colloidal Assisted by Microwave Irradiation as Antibacterial Material", IOP Publishing Limited, Volume 1912, DOI- 10.1088/1742-6596/1912/1/012016.

125. Galdoporpora, Juan & Morcillo, Marina & Ibar, Angelina & Pérez, C.J. & Tuttolomondo, María & Desimone, Martin, 2019, "Development of Silver Nanoparticles/Gelatin Thermoresponsive Nanocomposites: Characterization and Antimicrobial Activity, Journal of Current Pharmaceutical Design, Volume 25, DOI- 10.2174/1381612825666191007163152.

126. Patricia Hubner, Nicoly Donati, Luci Kelin de Menezes Quines, Isabel Cristina Tessaro, Nilson Romeu Marcilio, 2020, "Gelatin-based films containing clinoptilolite-Ag for application as wound dressing", Materials Science and Engineering: C, Volume 107, ISSN 0928-4931, DOI-https://doi.org/10.1016/j.msec.2019.110215.

127. Diniz FR, Maia RCAP, Rannier L, Andrade LN, V Chaud M, da Silva CF, Corrêa CB, de Albuquerque Junior RLC, P da Costa L, Shin SR, Hassan S, Sanchez-Lopez E, Souto EB, Severino P, 2020, "Silver Nanoparticles-Composing Alginate/Gelatine Hydrogel Improves Wound Healing In Vivo", Journal of Nanomaterials (Basel), Volume 10, Issue 02, DOI-10.3390/nano10020390.