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BIOPHYSICAL PERSPECTIVE OF CHITOSAN FILMS

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Abstract: Different compositions of chitosan films using 1%, 2%, 3% and 5% Acetic acid, .01% butanol+1% acetic acid and lauric acid+1% acetic acid+0.1%butanol were prepared by solution casting method. These six compositions of chitosan films were studied, their properties were tested and applications explored. The prepared films were characterized by testing their hydrophobicity, antimicrobial activity and effect on food. Hydrophobicity of films was checked by contact angle measurement. Films containing lauric acid proved to be the most hydrophobic. Antimicrobial and antibiofilm activities were verified with the help of adhesion assay which was performed by using some common pathogenic microorganism such as S.typhi, S.aureus, A.niger, R.rubra, A.flavis and S.cerivisae. Very good antimicrobial activity of films against A.flavis, A.niger, S.cerivisae and S.aureus was observed. The potential application of chitosan films as food wrappings to keep the food fresh was studied. Different foods were wrapped up in the films and kept under observation for 24 hours after which changes in the taste, texture and color of the foods was noted to determine the degree of freshness. The efficacy of the different chitosan films for this application was compared. Observations showed that food samples wrapped in the 1%, 2%, 5% acetic acid and 0.1% butanol films were found to be in good condition after 24 hours. However, those wrapped in 3% acetic acid and lauric acid films had completely deteriorated and were not fit for consumption. Thus pure acetic acid and acetic acid+butanol films were considered to be effective as food wrappings.

Keywords: chitosan, films; A.A.-acetic acid; L.A.-lauric acid; 0.1%butanol-1% acetic acid and 0.1%butanol; L.A.+A.A.-1% acetic acid and 1.5% lauric acid.

I. INTRODUCTION

Chitosan is a natural carbohydrate biopolymer derived by deacetylation (DA) of chitin which is a major component of the shells of crustacean such as crab, shrimp, and crawfish. After cellulose, chitin is the second most abundant natural biopolymer found in nature. Chitosan possesses some unique characteristics like ability to form films and nanoparticles, optical structural characteristics, antimicrobial activity, biodegradability etc. Chitosan also has a positive ionic charge, due to which it is able to bind with negatively charged species such as fats and lipids.

Chitosan is a non-toxic, biodegradable and biocompatible polymer. Over the last several years, chitin polymers, especially chitosan, have received increased attention as one of the promising renewable polymeric materials for their extensive applications in the pharmaceutical and biomedical industries for enzyme immobilization and purification, in chemical plants for wastewater treatment, and in food industries for food formulations as binding, gelling, thickening and stabilizing agent (Knorr, 1984). Chitosan is prepared by chemical N-deacetylation of chitin. Both of them are observed to have biological functions. Chitin and its derivatives have many properties that make them attractive for a wide variety of applications from food, nutrition and cosmetics to biomedicine, agriculture and environment. Chitosan is biodegradable, biocompatible and exhibits bioadhesive characteristics. It is a copolymer of glucosamine and N-acetyl glucosamine linked by β 1→4 glucosidic bonds obtained by N-deacetylation of chitin. Chitosan forms good films and membranes. Chitosan film has a potential to be employed for packaging, particularly as an edible packaging. This is due to its excellent properties like oxygen, carbon dioxide barrier and antimicrobial activity. For example, biodegradable and edible chitosan films were produced to protect foods from fungal decay and modify the atmospheres of fresh fruits. Chitosan is a semi crystalline polysaccharide that, unlike many biodegradable polymers it is insoluble in water at neutral pH (6-7). Chitosan films are described as being tough, long lasting and flexible. This presents an opportunity for developing a variety of films using chitosan and organic acid solvents. A detailed study of sorption behaviors of such films would allow an efficient selection of composition of chitosan films that are less sensitive to humidity. Hence, studies were conducted to determine the effects of different concentrations and types of solvents on sorption behavior of chitosan films and to establish models to predict sorption behaviors of such films. The development of chitosan films as new biodegradable packaging films is an innovative alternative to petroleum-based plastics for pollutionless environment. Unfortunately, there are some limitations to the application of chitosan film for packaging, because of its sensitivity to moisture as it has a large number of hydrogen bonds. One way to overcome this drawback is to associate chitosan with a moisture resistant polymer, while maintaining the overall biodegradability of the product. Lauric acid is associated with chitosan to increase the moisture resistance of the films. For many food applications, the most important functional characteristic of an edible film is its resistance to the migration of moisture. This is because critical levels of water activity must be maintained in many foods to exhibit optimum quality and acceptable safety. Among the various properties of chitosan, the physicochemical properties of its films are interesting for pharmaceutical applications, the food industry, and membrane separation. Chitosan is known as an antimicrobial compound, due to the interaction between the cationic amine group of chitosan and anionic functional groups that typically reside on microbes. It is postulated that during this

interaction between chitosan and microbes, the cell permeability increases, such that the microbe will lose vital nutrients, proteins, and electrolytes. Another possible reason for chitosan antimicrobial effects is that chitosan could inhibit both mRNA and protein synthesis by interacting with the microbial DNA. Regardless of the exact mechanism with which chitosan attains its antimicrobial properties, several parameters will determine chitosan antimicrobial activity, including DD, molecular weight (MW), temperature, and pH of solution. Chitosan, therefore, has been studied extensively for the food packaging industry. Also, because of its good fungicidal properties, chitosan, as well as chitin, has been investigated for cosmetics, such as creams and lotions. Single celled organisms are present in two forms as plank tonic (free floating cells) and sessile cells (surface adherent cells). Surface adherent cells form structured complexes called as biofilms (Costerton, 1995). Microbial biofilms play an important role in environment, industries and medical systems (Melo et al; 1997, Ebihara et al; 1999, Moss et al; 2006, Singh et al., 2006). They are mostly associated with water distribution lines, cooling towers, heat exchanger surfaces and oil recovery processes causing biofouling and biodeterioration (LeChevallier et al., 1987; Elvers et al., 2002). Biofilms often resist different clearance mechanisms, antibiotics and disinfectants. They are thus relatively more difficult to eradicate than their planktonic forms (Meluleni, et al, 1995; Evans, et al; 1991, d'Enfert, 2006). One possible mechanism suggested for detachment of biofilms is starvation. This is still an important area for further research (O'Toole, 2000). It is evident that biofilms are often detrimental and there is a need to disrupt them. There are reports on the use of mechanical devices and antimicrobial agents (disinfectants, heavy metals and natural biomolecules) for biofilm disruption. There are limitations for the use of some of these disruptors. For example, heavy metals as antifouling agents pose a threat on account of their toxic nature and the use of such metals has been banned (Lewis, 1998; Faria, et al; 2004). As the chitosan films show antimicrobial activity; they might act against biofilm formation property of some bacteria and fungi. This activity is also been checked for chitosan films in present project work. In this study lauric acid dissolved in butanol, different concentrations of acetic acid and butanol are used as solvents for different compositions of chitosan films.

II. MATERIALS AND METHODS

2.1 Materials:

Chitosan used was of medium molecular weight and purchased from Sigma. Acetic acid used was purchased from Merck. Acetic acid was diluted to different dilutions as 1%, 2%, 3% and 5% using distilled water. Lauric acid and butanol used were purchased from Fisher. Glucose, peptone, yeast extract from HIMEDIA, sodium chloride from SRL were used to make different types of media to grow different microorganisms.

Glass and plastic petri plates, paraffin wax from Fisher, sterile 96 well tissue culture plates with lid purchased from HIMEDIA.

2.2 Methods:

2.2.1 Preparation of chitosan solution:

2 gm of chitosan powder was added in 100 ml of 1%, 2%, 3% and 5% acetic acid solution in Erlenmeyer flask. The chitosan powder dissolved in acetic acid by continuous stirring for 30 minutes on magnetic stirrer. 30 gm of lauric acid powder was dissolved in 100ml of butanol. Lauric acid solution was mixed with chitosan/acetic acid solution and it was kept for stirring for 48hrs. Two layers were formed, from which the bottom viscous layer was used to cast the chitosan /lauric acid films.

2.2.2 Preparation of chitosan films:

The homogeneous solution was poured in glass/plastic petri plates. The plates were maintained at 40oC for 48 hrs in oven for drying. The dry, thin and transparent chitosan films were subjected to different characterization tests. Plates were wrapped to prevent the entry of dust particles. The films were immersed in 1% sodium acetate for 10-15min in order to remove from glass plates and the removed films were kept for drying in oven for 24hrs. It was quite difficult to remove films from the glass surface using the 1%sodium acetate method. The chitosan film surface is hydrophobic so these films also casted on plates with paraffin wax and plastic petri plates; and it proved to be easy way for removal of the films from the casting surface and there was no need for further drying also.

2.2.3 Preparation of chitosan films with acetic acid:

Weigh 2 gm of chitosan powder. Dilute acetic acid to 1%, 2%, 3% and 5% (v/v).Add weighed chitosan powder to each 100ml of diluted acetic acid. Mix the solution with the help of magnetic stirrer. Stir the solution continuously for 30 minutes on magnetic stirrer. Pour the 10ml of homogeneous solution in clean glass/plastic Petri plates. Keep the plates at 40oC for 48 hrs in oven.

2.2.4. Preparation of chitosan films with lauric acid and acetic acid:

Weigh 2 gm of chitosan powder. Add weighed chitosan powder to 100ml of 1% acetic acid acid solution. Make 1.5% solution of lauric acid in butanol. Add lauric acid solution in to chitosan/acetic acid solution. Mix the solution with the help of magnetic stirrer. Stir the solution continuously for 48 hours on magnetic stirrer. Separate two layers and pour viscous layer in the petri plate. Keep the plates at 40oC for 48 hrs in oven.

2.2.5 Preparation of chitosan films with acetic acid and butanol:

Weigh 2 gm of chitosan powder. Dilute acetic acid to 1% (v/v). Add weighed chitosan powder to 100ml of 1% acetic acid. Add 1ml of butanol in the above solution. Mix the solution with the help of magnetic stirrer. Stir the solution continuously for 30 minutes on magnetic stirrer. Pour the 10ml of homogeneous solution in clean glass Petri plates. Keep the plates at 40oC for 48 hrs in oven.

2.3 Characterization of chitosan films

2.3.1 Hydrophobicity test:

A drop of water was put on the dry chitosan films to test hydrophobicity by measuring the contact angle. The contact angle between chitosan film surface and water drop was greater compared to contact angle between clean glass slide surface and water drop. The contact angles on different films (1%A.A, 2%A.A,3%A.A, 5% A.A,L.A. + 1%A.A and 0.1% butanol) were measured using Goniometer. A drop of distilled water was placed on the film surface. The evolution of the droplet shape was recorded. Experiment was carried out by taking seven measurements of contact angle at different positions on the film. The contact angle was measured on both side of the drop and was averaged. Any reading above 90° is hydrophobic and below 90 is hydrophilic.

2.3.2 Antimicrobial activity and antibiofilm test:

In this test, we have used six microorganismswhich produce biofilms and can harm human being in different ways,Staphylococcus aureus, Salmonella typhi, Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae and Rhodotorula rubrato check the antibacterial and antifungal activity of the films. The preliminary check for the antimicrobial activitywas performed by using tissue culture plate having 24 wells. The same test was performed using 96 well tissue culture plates, different microorganisms and different compositions of the films. In each well of plates, suitable amount of chitosan/acetic acid solution was poured in each well except controls. The plate was kept for drying for 48 hours at 400 C in the oven. After drying, cultures of microorganisms were inoculated on the films. Six different microorganisms were allowed to grow in wells of the tissue culture plates. The 96 well tissue culture plates were read using ELISA reader at time period of 0, 24, 48 and 72 hours.

Staphylococcus aureus was grown in McConkey broth, A.niger and A.flavus were grown in Sabourd's broth, S. cerevisiae and S.typhi were grown in Nutrient broth and R.rubra was grown in MGYP broth.

Antimicrobial testing of chitosan films of different composition using sterile 96 well tissue culture plates:

Prepare chitosan solutions with different compositions. Add suitable amount of chitosan solution in each well of the plate (except control well for microorganism). Keep the plates at 40oC for 48 hours in oven for drying. Inoculate the wells with suitable amt of microorganism culture and medium. Incubate for different time periods (such as 0, 24, 48 and 72 hours). Read the plates at different time period using ELISA reader.

2.3.3 Effect of chitosan films packaging on food:

Chitosan coatings can be used for packaging of food as they show antimicrobial property as well as resistance to water; increasing shelf life of fruits, vegetables and processed foods. Packaging material should not affect the sensory as well as nutritional characteristics of food. Spoilage of food occurs mainly due to water activity and chitosan films being hydrophobic, act as barrier to water.

Chitosan films were tested for this with apple and potato by wrapping small pieces in all compositions of chitosan films for 24 hours. After 24 hours, sensory characteristics were checked by group of students. And the results were compared with the pieces of food kept without packaging. The chitosan film may be used to wrap foods that are highly susceptible to microbial growth or directly used as a surface coating on perishable fruits and vegetables to enhance microbial safety and extend food shelf life.

III. RESULTS AND DISCUSSION

3.1 Hydrophobicity Test:

The hydrophobicity of the chitosan films is checked by the contact angle between water drop and surface of the films.

On comparison with water drop on clean glass slide, it is found that contact angle between water drop and Chitosan film surface was larger. It shows that Chitosan films are hydrophobic. During drying of the films, the plates were not covered. So some part of the film got dust on it. When the water drop was put on this dusty surface, water is taken in and the volume of the film at that part is increased. And after some time the surface of that part became wrinkled. The reason for this, the hydrophobic groups are oriented on the outer surface of the film and the glass slide has all the hydrophilic residues lined up. So water is taken in through the leakage due to dust particles and surface of the film got wrinkled. Among the all compositions of chitosan films, films with composition of 1%A.A. + L.A. and 1%A.A. + 0.1% butanol have more water resistance as compared to 1%, 2%, 3% and 5% A.A. films. By these results, we can say that, butanol can be used to increase the hydrophobicity of the chitosan films. And chitosan films composited with Lauric acid are also highly resistant to water but these films were having lauric acid crystals deposited on the surface.

Basically by this test, hydrophobicity of films that is resistance to water is checked. The chitosan films with butanol and films with lauric acid are more hydrophobic which overcome the issues due to water uptake in the applications like packaging. The contact angle was found to be 115 ° for 1% A.A and was reduced to 101° for 2-5% A.A chitosan films. Thus the contact angle got reduced with the increasing H+ ion concentration in the films i.e. 2% A.A, 3% A.A, and 5% A.A. The contact angle of lauric acid films was found to be 125°.So lauric acid films were the most hydrophobic films followed by 1% A.A films.

3.2 Antimicrobial test:

Chitosan itself has antimicrobial activity, so the different compositions of chitosan filmsalso show antimicrobial activity. To perform this test, 96 well sterile tissue culture plates were used.The antimicrobial activity of different chitosan films with different concentrations of acetic acid, and also chitosan/acetic acid solution composited with lauric acid as well as butanol films were studied using the adhesion assay and absorbance of those were plotted against different time periods and concentrations. From this test of antimicrobial and antibiofilm activity, the chitosan films are showing good results against A.niger, S.cerivisae and

A.flavus as compared to S.typhi, R.rubra and S.auereus. So by these results we thought of moving on to packaging of food products using those films. We found that the chitosan films were more active against fungi as compared to the bacterial species. The chitosan films are inhibiting the biofilm formation of these micro-organisms. In some cases above, we can see that there was no growth of microorganism even at 0 hour also; this is because the microbial cells couldn't adhere on chitosan films. On the other hand, in some graphs, we can see growth because those cells were adhered to chitosan films. All the films are inhibiting the growth of each strain that was used in our experimental analysis. From the graphs, we can say that all compositions of chitosan films are antimicrobial specifically inhibiting the fungal growth to a great extent. Also showing effect on growth of bacteria but antibacterial effect is not much promising that of antifungal effect. As the acidity of films increased that means pH of the films is decreased the antimicrobial effect is increased.

3.3 Effect of packaging with chitosan films on food:

Small pieces of apple and potato are used as food samples to check effect of packaging with different compositions of chitosan films. The food samples wrapped in the chitosan films were kept for 24 hours to observe the effect of chitosan films on sensory characteristics of the food. On 24 hours inspection, food samples wrapped in 1%A.A., 2%A.A.,5%A.A. and 1%A.A. + 0.1% butanol films of chitosan were found to be in good condition and almost fresh. Very little effect on the sensory characteristics such as color, texture and taste were seen. These compositions of films are showing good results may be because of the acidity of the films. As pH decreases or acidity increases antimicrobial effect also increases. Many bacteria as well as fungi cannot survive in low pH environment. On the other hand (1%A.A. + L.A.) films are not showing good results. Food samples wrapped in this composition of films were found to somewhat spoiled due to microbial growth on them. The sensory characteristics of apple and potato pieces were also affected. They were became brownish in color, softened and tasted like vinegar and somewhat alcoholic. This is happened because lauric acid itself acts as nutrient for the growth of microorganisms, although it shows great resistance to water.

IV. CONCLUSIONS

Chitosan, a polysaccharide most abundant in nature and is biodegradable, biocompatible and easily available. It is very good biomaterial and can be used in lots of applications. Considering its film forming ability and partial hydrophobicity, chitosan has been used for making different compositions of films in this project work. So as the hydrophobic films made with biodegradable chitosan can be used as bioplastic. Chitosan films complex with lauric acid are most hydrophobic one followed by chitosan films with 0.1% butanol. Films with different dilutions of acetic acid (as 1%, 2%, 3%& 5%) were showing less hydrophobicity. And this has been proved by contact angle measurement. Chitosan films casted on glass plates were difficult to remove; so we have used wax plates for easy removal of the films from the casting surface. Wax surface is proved to be the best casting surface for preparation of chitosan films. Chitosan also shows antimicrobial property and hence the films formed by chitosan should also be antimicrobial. We have tested the films for antimicrobial activity as well as for antibiofilm activity. From the antimicrobial test we found best results against A.niger, A.flavus and S.cerevisiae and all the films showed high amount of inhibition against those microbes. The lauric acids films(L.A.+ 1% A.A.) were amongst the films showing least inhibition against the different microbes because the lauric acid films while casting had lauric acid crystal deposited on it, so the crystals themselves act as a substrate for the growth of microorganisms thereby giving the least antimicrobial activity. On the contrary the lauric acid films had high hydrophobic characteristic as compared to the other acetic acid and films prepared with 0.1% butanol. So deposition of lauric acid crystals made the films susceptible for the action of microorganisms. The antimicrobial activity of chitosan films is also based on the adherence of microbial cells on the film surface. Some films can adhere and some cannot. Also those adhere on the film surface killed due to electrostatic interaction between cationic film surface and anionic cell surface. Cells undergo expansion throwing out all the matter and get killed. So because of this antimicrobial activity of chitosan films is limited for some microorganisms; and which shows good results for microorganisms which are harmful for food and human body skin. With the results of hydrophobicity and antimicrobial test, packaging of food material using those films was suggested. Small pieces of apple and potato were used as food samples. The food samples wrapped in the 1%, 2%, 5% acetic acid and 0.1%butanol films were found in good condition even after 24hours. The texture, color and taste of the food samples were found to be same in those films. The food samples wrapped in the films of 3% acetic acid and lauric acid had complete deterioration and were not fit for consumption. Thus the films (1%, 2%, 5%A.A and 0.1% butanol) acted as a good barrier against the water and thus reducing the water activity which is the major cause of food spoilage. The lauric acid films were highly hydrophobic but due to least inhibitory action of microorganisms it is not worth enough for food packaging. So 1%, 2%, 5% and 0.1%butanol films can be used for packaging of food materials. And if in any way the lauric acid crystals can be dissolved properly then the chitosan film with lauric acid will be great composition for food packaging as well as bioplastic. The films had wide variety of applications in food, paint and various industries. The films can be used in paints and varnishes because of their antimicrobial activity present, they can inhibit the biofilm formation as well as the moisture resistance. The films of lauric and acetic acids can be coated on window panes.

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