Study the Effect of some Physical Parameters on the Diffusion Properties of Prepared Alginate-Chitosan Capsule

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ABSTRACT

Colloid hydrogel spherical core of alginate-chitosan capsules which is used to surround a bovine serum albumin were prepared using coacervation method. The effective physical parameters on porosity or permeability of chitosan-alginate polyions membrane were determined by the manipulation of solution conditions such as pH, ionic strength, and polymer concentration of chitosan. Different pH (3.9, 4.9, 5.9) and different concentrations of chitosan (0.1 g, 0.2 g, 0.3 g) as well as with and without NaCl (0.0, 0.1, 0.2, 0.3 %) were used to study the release of bovine serum albumin (BSA, molecular weight= 69,000) in vitro from the capsule prepared from alginate-chitosan blended polymer. The overall diffusion of BSA through the chitosan capsule membrane increased as the pH increased from 3.9 to 5.9. Also, an increase in the concentration of NaCl in the chitosan solution used in the preparation of capsule caused an increase in the diffusion of the protein. In contrast, an increase in the concentration of chitosan solution from 0.1g to 0.3g caused a decrease in diffusion of the proteins.

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.   5.9   4.9   3.9

.   (%0.3 0.2 0.1 0.0)

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(0.3   0.1   0.2)

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INTRODUCTION

Chitosan is a polysaccharide-based bipolymer, poly-(1→4)-2-amino-2-deoxy-β-D-glucose, obtained by deacetylating of chitin which is the major constituent of exoskeleton of crustaceous such as crab and shrimp shells.

The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin leaving behind a compound of chitosan with a high degree of chemical reactive amino group. The degree of deacetylation DD is an important property in chitosan production as it affects the physicochemical properties, hence determines its appropriate applications, biodegradibility and immunological activity (Tolaimate, 2000). Chitosan can be applied in drug delivery system with different routes of administration as well in food industry, agriculture, biomedical, waste water treatment, membrane, and wall material of the capsule (Mattheus, 1997).

Methods to prepare capsule include; first complex coacervation, which is a process in which chitosan is used as a cationic polyelectrolyte to interact with an anionic polyelectrolyte such as alginate, carrageenan, or arabic gum to form the wall material (Murali *et al.*, 2001). Second, adjustment of the solution pH to beyond the isoelectric point of chitosan which causes precipitation and aggregation, then forming the wall material (Lim, 1991). Third, in *situ* polymerization: in which opposite charged compounds, such as polyphosphate and chitosan, mixed in the solution. Polymerization occurs in the interfacial membrane and forms the wall material of the capsule (Beaumont, 1987). Hwang *et al.*, (1986) proposed that the morphology of the chitosan plays a significant role in the structure of the capsule membrane. Thus, the morphology of the chitosan molecules can be changed to form a special network that results in the increase of the wall thickness (Hsieh *et al.*, 2007). Slioyn and Rha (1989) reported that the release rate of the chitosan-carboxymethylcelluulose capsule increased by increasing the concentration of sodium chloride or phosphate. The release rate increase due to addition of sodium chloride is greater than that due to addition of phosphate. However, the release rate of the capsule prepared from lower molecular weight chitosan is lower than that prepared from higher molecular weight of chitosan (George, 2006). The effect of chain flexibility of chitosan molecules on the preparation, physical properties, and release rate of the prepared capsule were investigated (Rong, 1997). The polyelectrolyte complex film of chitosan and alginate was used for sustained drug delivery of potent antimicrobial and antifungal drugs by transdermal drug delivery (Bhupendra *et al.*, 2009). Moreover, the main mechanism for forming the membrane is the electrostatic interaction between positively charged amino groups on chitosan chain and the negatively charged groups on counter polyanions backbone. Hence, theoretically, it should be possible to control the porosity and permeation characters of a membrane by the physical and processing conditions contributed to the

polymer chain conformation in solution (Hsieh *et al*., 2007). The objective of this study is to evaluate the diffusion properties of chitosan-alginate capsule prepared by complex coacervation method at different pH, ionic strength and concentration of chitosan solution.

MATERIALS AND METHODS

Materials and instruments

Chitosan was purchased from Orient Company Malaysia. Sodium alginate was supplied by Sigma. Sodium chloride and phosphate buffer were obtained from APS Chemicals. Calcium acetate was obtained from AJAX Chemicals, Australia. BSA was obtained from Sigma company.

Fourier Transform Infrared Spectrophotometer (FT-IR) spectra of chitosan was recorded on the FT-IR spectrophotometer (Perkin-Elmer 1725X at University Putra Malaysia), using potassium bromide pellet technique. BSA diffusion was determined by UV- Visible Spectrophotometer (Carrywinn U.V. Varian at college of Pharmacy, Mosul University).

Determination of the degree of deacetylation of chitosan

Chitosan and KBr at a ratio of 1 : 100 was mixed well, dried, and then made into a disc. The IR spectrum was measured by FT-IR spectrophotometer with a frequency range of 400-4000 cm.$^{-1}$ The following methods were proposed for determination the DD of chitosan depended on the base-line of the IR spectrum which are given via (1) Domszy and Roberts, 1985. (2) Sabinis and Block, 1997. (3) Baxter et. al,1992. (4) Rout, 2001 that considered in our calculation of DD value. Equation 1 is applied to determine the DD value.

$$DD \% = 118.883 - [40.1647 \times (A_{1655}/ A_{3450})] \qquad -------- (1)$$

where A_{1655} is the absorbance of the amide band at 1655 cm,$^{-1}$ and A_{3450} is the absorbance of the O-H band at 3450 cm^{-1} . The factor 40.1647 denotes the value of the ratio of A_{1655}/ A_{3450} for fully N-acetylated chitosan. The number 118.883 was proposed to be related to the baseline (Rout, 2001). It was reported that the DD is one of the more important chemical character (Li *et al*., 1997), which could influence the performance of chitosan in many of its application. In addition, the DD determines the content of free amino groups in the polysaccharides, which can be employed to differentiate between chitin and chitosan (Baxter, 1992). Tolaimate in 2000 has reported that material becomes an intereasting in pharmaceutical application with a DD value of 75% or above due to its biocompatibility and low toxicity.

Preparation of capsules using complex coacervation method

The preparation of chitosan capsules was based on the method of Hwang *et al*., (1986). Chitosan 0.1g was dissolved in 0.2 *M* acetic acid (5 ml), and 5 m *M* calcium acetate (5ml), then the pH adjusted at 3.9, 4.9, and/or 5.9 to obtain the optimum pH. The core material, 0.3 % bovine serum albumin, was mixed with 1.2 % of sodium alginate

containing NaCl salt. By using burette, the BSA alginate solution was dropped into the chitosan solution to form the capsule with no stirring (the volume of each dropwise about 0.1 ml). The droplet capsule was cured for 5 min. at room temperature and then collected.

To study the effect of ionic strength of chitosan solution on the transmembrane diffusion, NaCl in different concentrations (0.0, 0.1, 0.2, 0.3 % chitosan weight basis) were added to the chitosan solution and then adjusted at optimum pH that determines in previous step. Then the above experiments were repeated for the chitosan solutions of 0.2 and 0.3 g.

Diffusion of Protein

About 50 capsules containing albumin were transferred to 50 ml of potassium phosphate buffer solution (pH 7.4). The diffusion of protein from inside to outside the capsules was determined by measuring the absorbances of 5ml of release media at 280 nm (using UV- Visible Spectrophotometer) during a set time interval of each 20 min.. Protein concentrations at a particular time   were plotted against time. A plot of known concentrations of albumin against their absorbance were used to obtain a standard curve (Kim *et al*., 1999). Then, the standard curve was used to determine the unknown concentration of diffusive albumin outside the capsules.

RESULTS AND DICUSSION

Degree of Deacetylation

The process of deacetylation involves the removal of acetyl groups ($-COCH_3$) from the molecular chain of chitin, leaving behind a complete amino groups ($-NH_2$), and chitosan versatility depends mainly on the high degree chemical reactivity of amino group. Deacetylation may be achieved by chemical process (Sandford, 1987) or by fermentation using microorganism (Healy, 1994). The DD is simply calculated from equation 1 :

$$DD \% = 118.883 - [40.1647 \times (61.74 / 85.61)] = 89.917$$

The DD from FT-IR spectra of using chitosan, Fig.1, was found to be about 90 % which is commonly used in pharmaceutical applications and it is in agreement with the previous study (Tolaimate, 2000).
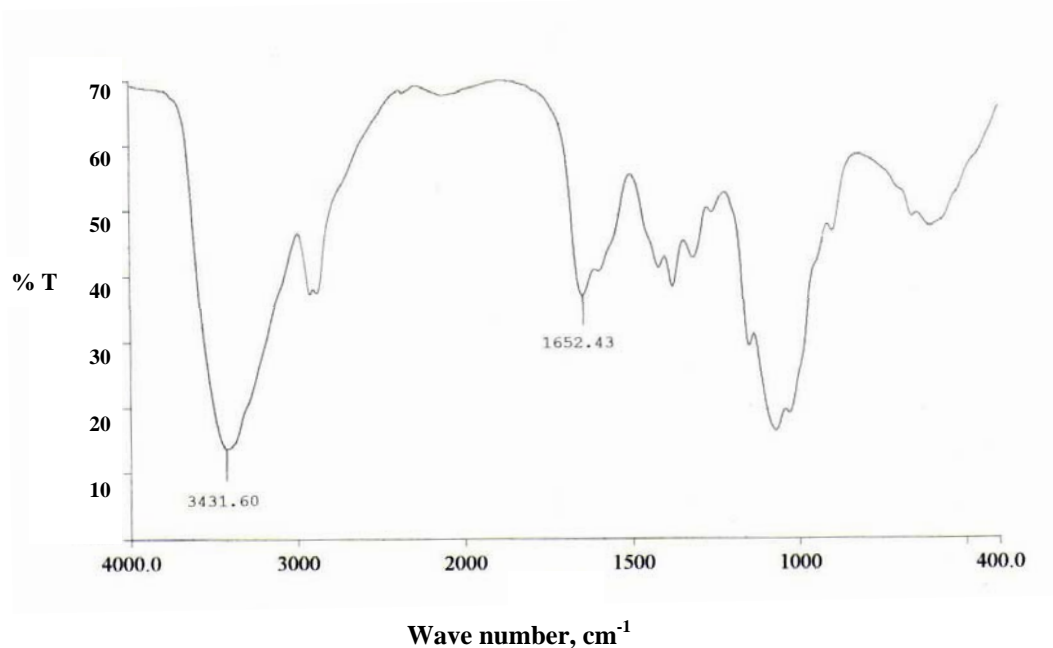
Wave number, cm^{-1}

Fig. 1: FT-IR Spectra of chitosan (Transmmitance vs. Wave Number in cm^{-1})

In-vitro diffusion of Protein

Different solution conditions including pH, ionic strength and chitosan concentrations were used. The variation in the constitution leads to change in the frequency of the electrostatic interactions between chitosan and alginate, and then the nature of the structure of membrane network (Erkesh, 2008).

The diffusion rate of BSA through membrane from the inside to outside the capsules at different pH is shown in Fig. 2. An increase in pH of the chitosan solutions from 3.9 , 4.9 and 5.9 will cause an increase in the diffusion rate from 48.76 , 52.85 and to 111.55 µg/ml. min, respectively. This may attributed to the intrinsic viscosities of chitosan in different pH solutions. A higher intrinsic viscosity indicates that molecule will be more extended in the solution (Li *et al*., 1992). The extended molecule will increase that chances to be involved in intermolecular entanglements. Thus, as pH increasing the counter ion interaction of chitosan-alginate decreased and that caused an increase in the diffusion rate of drug. Consequently, the relationship between pH and diffusion rate gives a good indication about the porosity of the membrane (Hsieh, 2007).

The effect of the ionic strength in term of different percent of NaCl on the transmembrane diffusion of protein in the capsule with time is shown in Fig. 3. The diffusion properties of capsules suggest inconsistent concentration at the beginning. After one hour, diffusion of BSA increased with time by increasing the NaCl concentration in chitosan solution. Capsules prepared from solutions containing 0.2% NaCl have larger diffusion rate than those containing 0.3% NaCl. This may be attributed to the counter-ion effect which depresses the third electroviscous effect and render the chitosan molecules to be more flexible with randomly coiled shape (Bhupendra *et al*., 2009).

The effect of the chitosan concentration on the diffusion of BSA is shown in Fig. 4. The diffusion rate decrease with an increase in chitosan concentrations but increased for

0.3 g of chitosan concentration. The diffusion of BSA in the capsules prepared with 0.1 g chitosan was higher than the capsule prepared with 0.2 g chitosan. However the diffusion rate is almost same after 3 hours. The diffusion of BSA in the capsules prepared with 0.3 g chitosan showed the inconsistent result. This result may due to the increase of algination created in the small concentarion (0.1 g) of chitosan compared to the higher concentration of chitosan (0.2 g and 0.3 g).
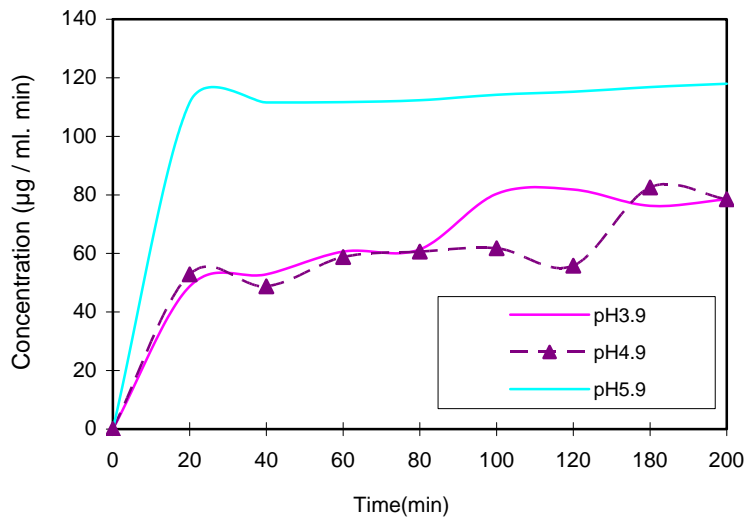

Fig. 2: Diffusion of BSA from the capsules prepared from chitosan solution at
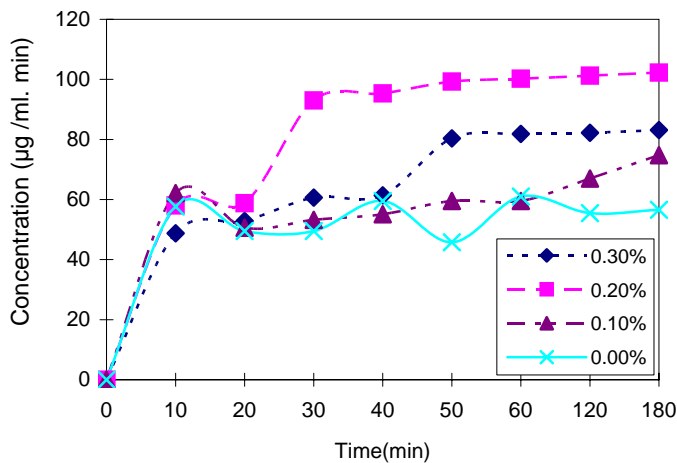pH 3.9, 4.9, 5.9 at room temperature using 0.1 g of chitosan.

Fig. 3: Effect of NaCl percent on BSA diffusion at room temperature from capsules
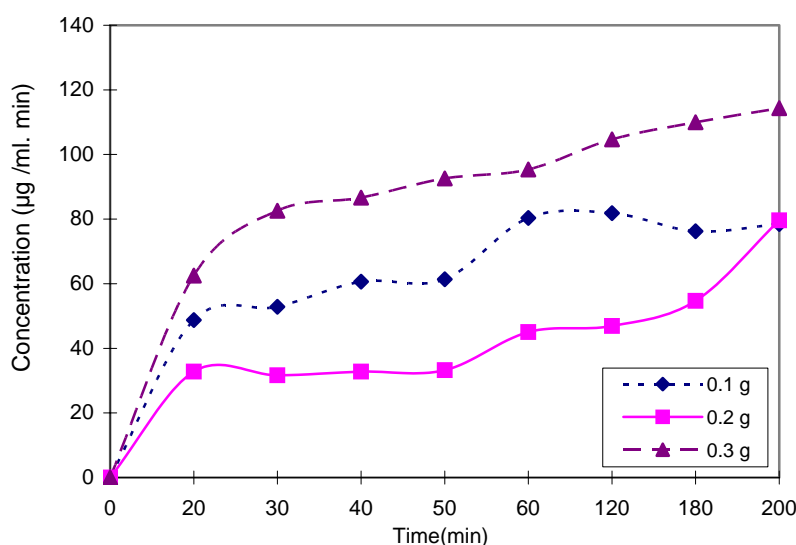prepared of 0.1g of chitosan solution at pH 5.9.

Fig. 4: Effect of chitosan concentrations on diffusion of BSA at room temperature and pH 5.9.

CONCLUSION

In this study the DD of chitosan was 90%. So that, chitosan is an interested material in pharmaceutical application due to its biocompatibility and low toxicity. The results showed that the physical parameters have an effects on the porosity of chitosan-counter polyion membrane which can be controlled by pH of chitosan solution (at 5.9) and the percent of NaCl concentration (fixed at the 0.2% NaCl). Chitosan of 0.3 g has a significance effect on drug diffusion. The use of such system makes it possible to develop systems with required rate of drug delivery to the body in accordance with the pre set time program.

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