INTERACTIONS OF CROSS-LINKED AND UNCROSS-LINKED CHITOSAN HYDROGELS WITH SURFACTANTS FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

The swelling equilibrium of Chitosan and sodium tripolyphosphate (NaTPP) cross-linked chitosan hydrogels in aqueous solutions of surfactants differing in structure and hydrophobicity at 25°C is reported. Anionic surfactant sodium dodecylsulfate (SDS), the cationic surfactant hexadecyltrimethylammonium bromide (HTAB) and neutral surfactants Triton X-100 were employed. The surfactants induced abrupt change in the gel volume. The equilibrium swelling ratio first decreased sharply as the concentration of the surfactant increased and remained almost constant up to the critical micelles concentration (CMC) of the surfactants and then increased again as the concentration increased above the CMC of the surfactants used. The equilibrium volume change of hydrogel was significantly increased from HTAB > Triton X-100 > SDS> the mixed SDS/Triton X-100 system. A decrease in equilibrium swelling ratio of the gel in SDS/TX-100 mixtures was observed with an increase in the mole ratio of SDS. The results also revealed that cross-linking with 3% or 5% w/v NaTPP exhibited lower equilibrium swelling values. This swelling study showed that cross-linking density, surfactant type, and their respective concentrations were important parameters that could affect equilibrium swelling of chitosan gel. Structural elucidation of the nanocomposites was monitored using Infra-red Spectroscopy (IR), and X-Ray Diffractography (XRD).

Keywords: Chitosan Hydrogel, Surfactants, Swelling Equilibrium and Hydrophobicity

INTRODUCTION

Considerable attention has been drawn to the use of natural hydrophilic polymers as drug carriers, dialysis membranes and artificial organs from the view point of environmental pollution, biodegradability, safety and cost (Li *et al.* 1997). A wide range of hydrophilic polymers have been examined for preparing hydrogels and chitosan is one of them. Chitosan is biodegradable and biocompatible. It exhibits bio-adhesive characteristics. Chitosan is a copolymer of glucosamine and N-acetylglucosamine linked by β-1-4 glucosidic bonds obtained by N-deacetylation of chitin. It is also a polycationic polysaccharide that can form complexes in acidic medium with negatively charged moieties such as sodium tri-polyphosphate, glutaraldehyde, sodium carboxymethylcellulose, alginic acid, glutamate, and citrates but such complexes formed are insoluble in alkaline medium (Remunan-Lopez and Bodmeier, 1997; Mi *et al.,* 1999; Wang *et al.,* 2001; Lu *et al.,* 2007; Ahmet *et al.,* 2010 and Leonida *et al.,* 2011). In recent years, a number of studies have reported the use of chitosan complexes, for instance, in preparing microspheres (Jiang *et al.,* 2010 and Wang *et al.,* 2011), beads (Sezer and Akbuga, 1995; Chiou *et al.,* 2006 and Bamgbose *et al.,* 2013) and artificial films (Berger *et al.,* 2004; Wan *et al.,* 2004; Rana *et al.,* 2004 and Bamgbose *et al.,* 2012). Cross linking of chitosan are effective and convenient methods of improving the physical and mechanical properties of chitosan for practical applications (Jameela *et al.,* 1994) and considerable work has been done on the characterization and swelling behavior of hydrogel crosslinked with various crosslinking agents. (Karadag and Saraydin, 2002).

Surfactants are employed in most studies because they comprise of hydrophilic, ionic or non-ionic groups bound to a non-polar hydrophobic group (Tuncer and Melike, 2004). It is expected that both the peculiarities of charged groups on the surfactant and the network, and the hydrophobicity of the surfactants tails will influence the equilibrium extent of gel swelling. Interactions between surfactant molecule and a polymer gel network often result in a pronounced volume change of the gel with the surfactant effectively absorbed by the gel (Khokhlov *et al.,* 1992; Kokufuta *et al.,* 1993; Khokhov *et al.,* 1993 and Isogai *et al.,* 1996). Study response of the polymer network to external stimuli such as change in pH, ionic strength and temperature is of

interest not only for the design of novel sensing, switching, drug delivery and other devices, but also for better understanding of the interaction of polymer system (Ju *et al.,* 2001; Peppas *et al.,* 2000; Hirasa *et al.,* 1991; Martinez-Ruvalcaba *et al.,* 2009 and Ju *et al.,* 2002). It has been demonstrated that polymers swell if they interact with the solvents at all, and the degree of this interaction is significantly determined by the polymer structure, its environment and the degree of cross linking which is an index of porosity.

Although the swelling characteristics of chitosan hydrogel are known in literature (Tuncer and Melike, 2004; Chiou *et al.,* 2006; Ashok and Vikas, 2010 and Bamgbose *et al.,* 2012,), information regarding the interaction of chitosan gel with cationic, anionic and neutral surfactants is a subject of continuing interest. The anionic surfactant sodium dodecylsulfate (SDS), the cationic surfactant hexadecyltrimethylammonium bromide (HTAB) and neutral surfactants Triton X-100 were used. These surfactants were chosen because of their extreme differences in water solubility and in sensitivity to dissolved organic matters. The effects of these surfactants on the equilibrium swelling ratio of chitosan hydrogel were studied in aqueous system as a function of surfactant type and their respective concentrations at room temperature.

As a result of the need to develop a cheap non-toxic, readily available chitosan hydrogel which could be effectively used for controlled release applications in a wide range of concentrations under ambient temperature and pressure and at a moderate dose, the swelling characteristics of cross linked chitosan films were investigated.

MATERIALS AND METHODS

Materials

Sodium dodecylsulfate (SDS) with CMC = 8.22 x 10^{-3} moldm^{-3} was purchased from Surechem Product Ltd England and hexadecylmethylammonium bromide (HTAB) with CMC =11.0 x 10^{-3} moldm^{-3} , Sodium tripolyphosphate (NaTPP), sodium citrate (NaCit) and glacial acetic acid were obtained from Sigma-Aldrich Chemical Co (St Louis, USA). The Triton X-100 (TX-100) with CMC = 0.228 x 10^{-3} moldm^{-3} was a Rieldel-de-Hans product. All chemicals were of analytical grade and used without purification. In all the experiments, double distilled water was used. The CMC of each surfactant was determined by conductometric method using electric conductivity meter DDS-307 made by Jenway at controlled temperature $25^{O}C \pm 1$.

Gel Preparation

The protocols for the preparation of the various hydrogels were carried out using literature methods of Ahmet *et al.,* 2010. Chitosan produced from lobster shell wastes was offered as flakes from Aldrich Chemical Co. with 85% degree of deacetylation. 4% w/v chitosan was dissolved in 2% v/v acetic acid solution to prepare homogeneous chitosan solution at room temperature with stirring for 20 minutes. The solutions were cast into films in plastic petri dishes and air dried for 24 h. The dry films were immersed in 5% NaOH solution to neutralize acetic acid residues, and then washed with ethanol to remove excess NaOH. After rinsing with excess distilled water, the films were air dried for 24 hrs. Also, chitosan films were prepared by cross-linking with 3% and 5% w/v NaTPP solution of films containing 4% w/v chitosan. These films were washed with water to remove excess NaTPP.

Characterization

The XRD measurements were taken with Monochromatic CuKα radiation (wavelength = 1.5406) produced by MD Minidiffractometer model. The IR spectra were recorded on Perkin Elmer IR spectrophotometer in the spectral region of 350 to 4000 cm^{-1}.

Swelling Equilibrium Measurement

For the swelling experiments, 0.123*g* dry film with an average thickness of 16*m*μ was immersed in 10 ml of aqueous surfactants solution prepared using double distilled water and equilibrated for 24 hrs. Gravimetric method was employed to study the swelling process. The equilibrium swelling ratio of hydrogel, which signifies the expanding and retracting forces between the films at equilibrium, was determined. The weight of the swollen sample was measured after blotting excessive water gently with filter paper. Aqueous surfactants solution were kept at 25°C for 24 hours to allow

the film to reach the equilibrium state to examine surfactant composition effects on swelling properties of the film. The percentage swelling (S%) was calculated using the following equation (Uzum and Karadag, 2006);

$$S\% = \left(\frac{W_t - W_o}{W_o} \right) \times 100 \qquad (1)$$

Where W_t is the mass of the swollen film at time t, and W_o is the mass of the dry film. The surfactants were used in a wide concentration range from the pre-micellar to the post-micellar region.

RESULTS AND DISCUSSION

Characterization of Chitosan Hydrogel

The Chitosan hydrogel and NaTPP cross-linked hydrogel were characterized using IR spectroscopy. The results are presented in Figures (1, 2 & 3). In Figure 1, the spectral band for chitosan appears at 3760 cm^{-1} (axial OH group), 3271 cm^{-1} (N-H stretch of secondary amine) and 1153-1054 cm^{-1} (ether linkage, C-O-C band stretching). However, the peak is more pronounced at 3000 cm^{-1} which indicates that the (C-H) peak is more intense in this compound. There are also bands at 1649 cm^{-1} (C=O stretch of amide), 1531 cm^{-1} (NH angular deformation in CONH plane) and 2360 cm^{-1} (CN asymmetric band stretching). The bending vibrations between 1500 and 1000 cm^{-1} intensity indicate some interactions of the amino group.

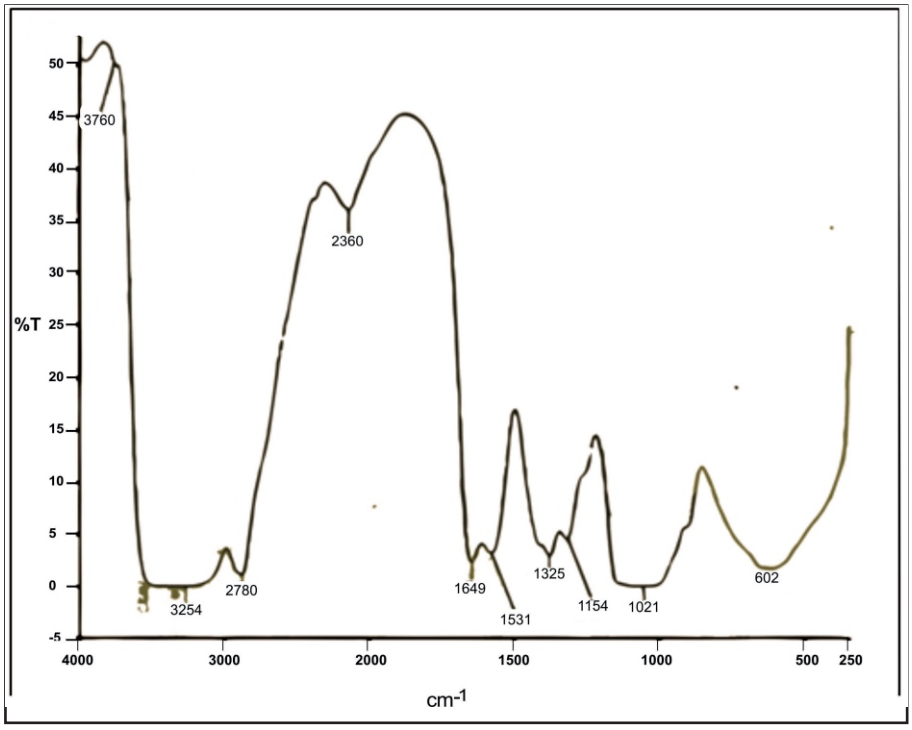

Figure 1: IR spectrum of Chitosan Hydrogel

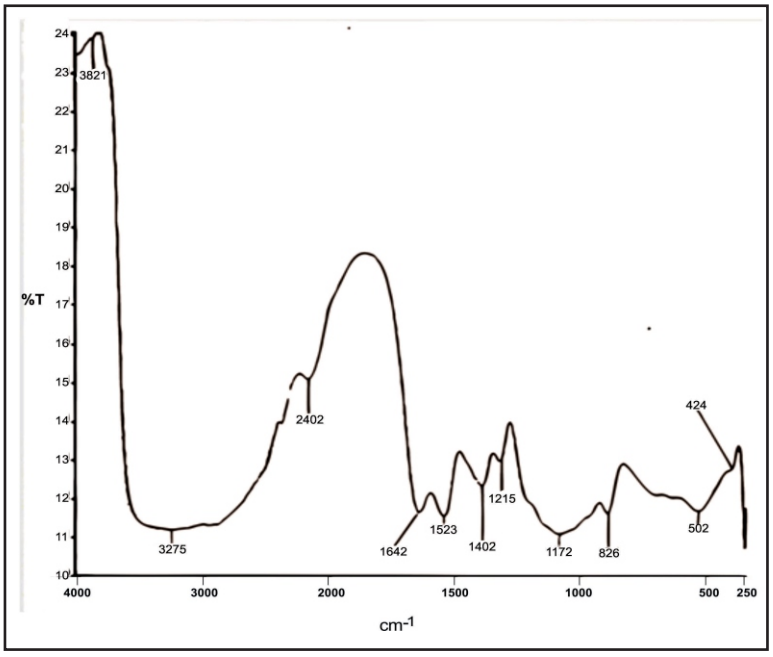

Figure 2: IR Spectrum of 3% NaTPP Cross-linked Chitosan Hydrogels.

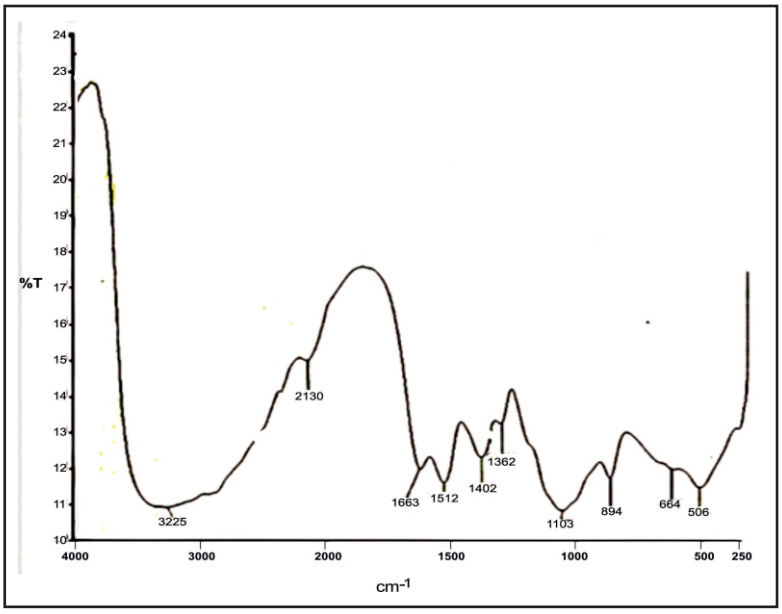

Figure 3: IR Spectrum of 5% NaTPP Cross-linked Chitosan Hydrogels.

The IR spectra of NaTPP cross-linked chitosan hydrogels showed peaks at 1054 – 1308 cm^{-1} suggesting the presence of phosphonate linkages between ammonium, $-NH_3^+$ of chitosan and $-PO_3^{2-}$ moieties of NaTPP during cross linking process. One peak was observed at 1195 cm^{-1} in the film cross linked with 3% w/v NaTPP (Fig. 2) indicating no appreciable linkage. Also, the films cross-linked by 5% w/v, NaTPP showed two peaks, one at 1140 cm^{-1} and another at 1279 cm^{-1} (Fig. 3), indicating symmetric and asymmetric stretching of phosphonate linkage, respectively (Ashok and Vikas, 2010). Kemp (1991) also observed that the asymmetric peak is known to occur due to restricted rotation.

The two terminals $-PO_3^{2-}$ groups of NaTPP molecule appears to be connected with two $-NH_3^+$,$-\left(CH_3COO^-\right)$ groups of two chitosan monomers.

Figure 4 (A, B, C, D) presents XRD spectra of the chitosan and chitosan hydrogels. The XRD spectrum of chitosan shows that the chitosan

exhibits diffraction peak at 2θ ~ 35° which is a typical fingerprint of semi-crystalline chitosan. However, the X-ray diffractogram of chitosan film shows that the film has a higher crystallinity than the original chitosan and also has a better amorphous domain which allows better accessibility of the solvent. When the film was exposed to X-ray diffraction analysis, it gives a diffractogram of five peaks characteristics of crystalline regions at 27.5°, 34°, 39°, 52°, and 68°, respectively. This suggested that the film is a porous membrane. The XRD patterns of a typical NaTPP cross-linked chitosan hydrogels synthesized in this study are almost identical regardless of experimental conditions Figure 4 (C & D), indicates a reflection of high degree of crystallinity.

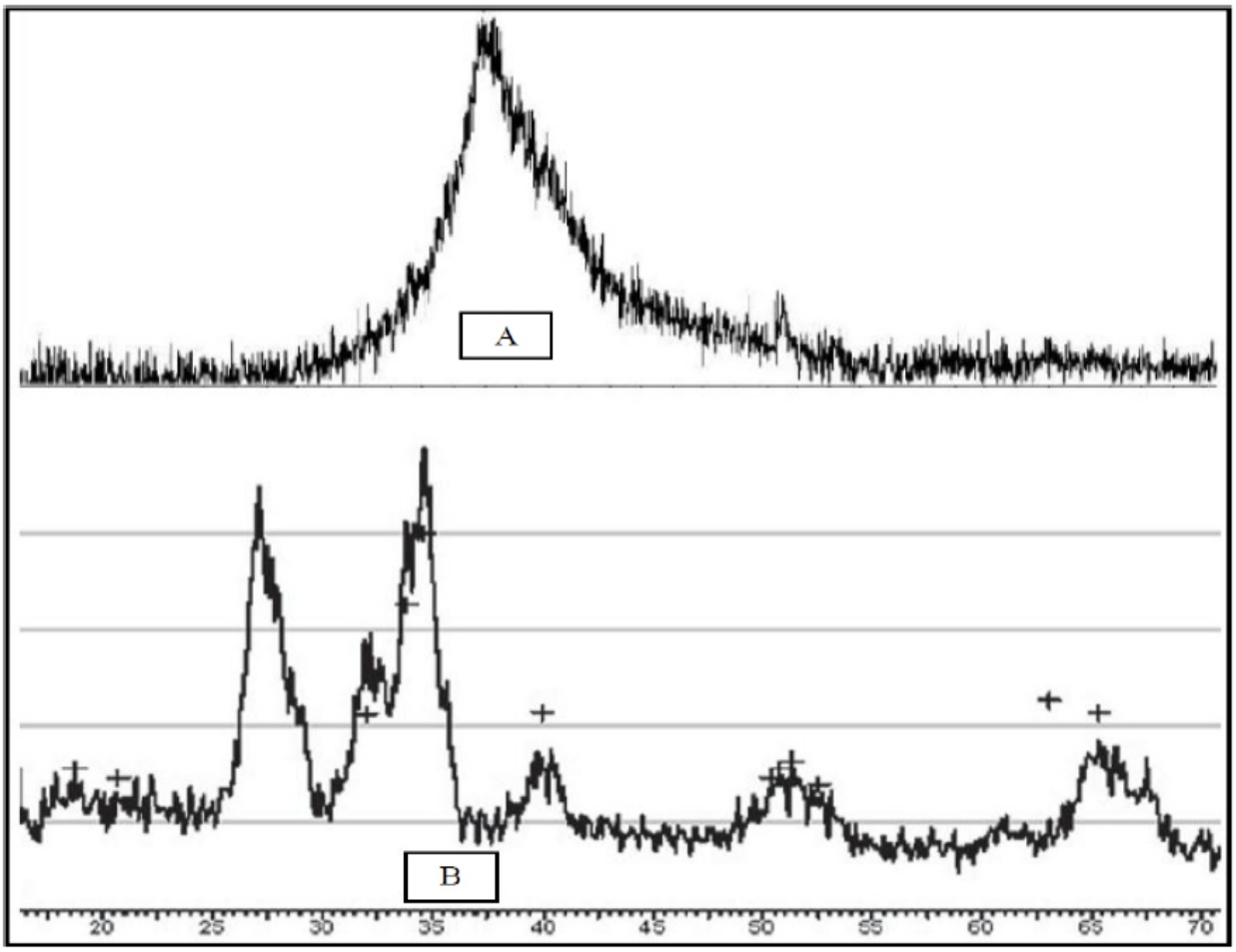

Figure 4: XRD Diffractogram of Chitosan (A) and Chitosan Hydrogel (B)

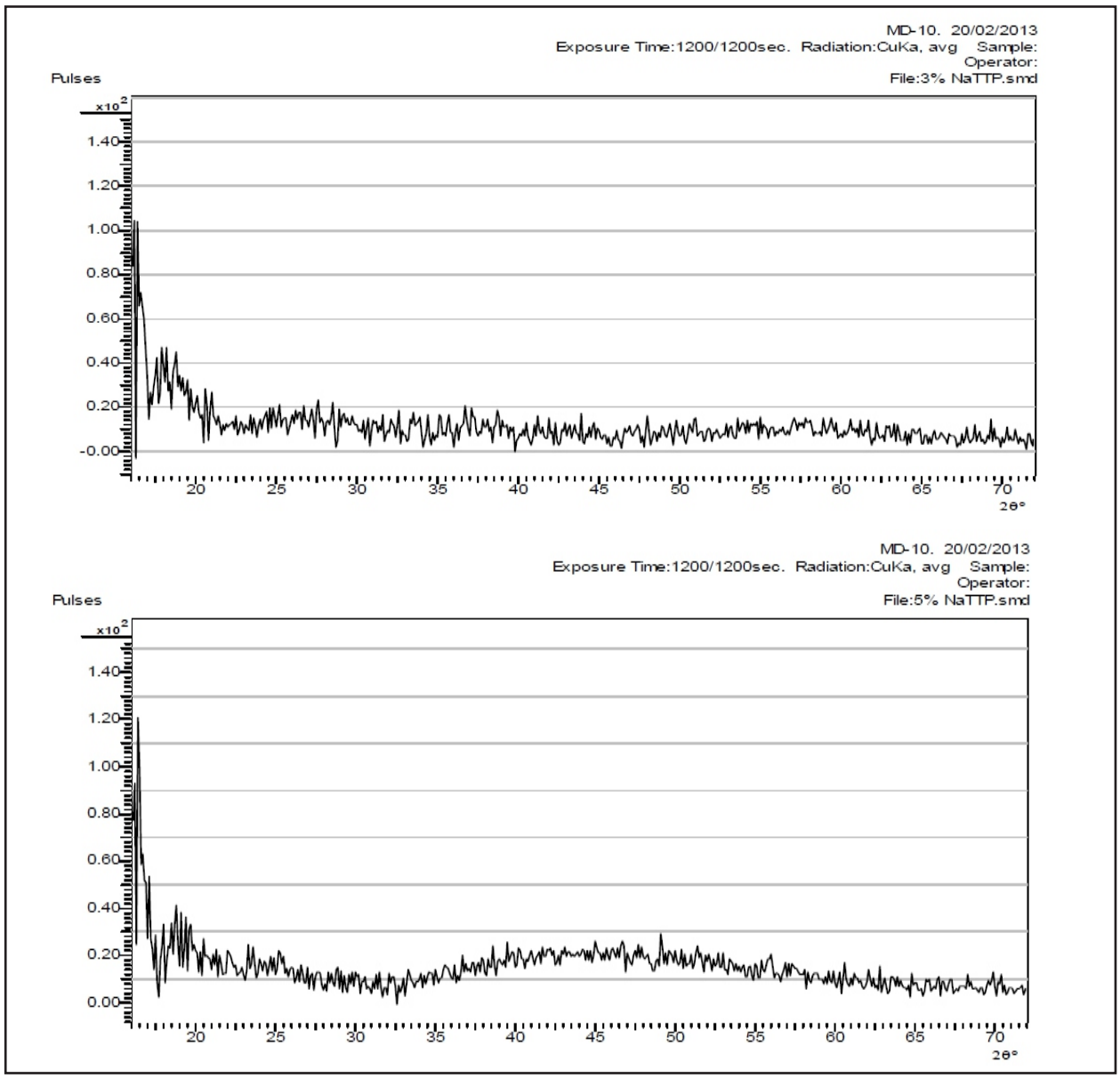

Figure 4: XRD Diffractogram of 3% NaTPP Cross-linked Chitosan (C) and 5% NaTPP Cross-linked Chitosan Hydrogel (D)

Effects of Surfactants on Swelling Equilibrium

Figure 5, shows the percentage equilibrium swelling volume of chitosan hydrogel in anionic surfactant (SDS), cationic surfactant (HTAB) and the neutral surfactant Triton X-100 systems, plotted as a function of the Surfactant solution concentrations below, at and above the CMC of the surfactants. All the surfactants induce abrupt change in the gel volume. The equilibrium swelling ratio first decreased sharply as the concentration of the surfactant increases and remained almost constant up to the CMC of the surfactants. It increased again as the concentration increases above the CMC of the surfactants (Figures 5 & 6). The increase is more at concentrations above the CMC of all the surfactants used in this study. The increase is more at concentrations above the critical micellar concentration (CMC = 8.22 x 10^{-3} for SDS, 11.0 x 10^{-3} for HTAB, and 0.228 x 10^{-3} moldm^{-3} for Triton X-100) of all the surfactants. This enhancement may be attributed to the successive micellization of the heterogeneous monomer species as a result of increase in the micellar size and geometry. Surfactants are assumed to exist in monomeric state below CMC. Above CMC, the concentration of monomers remains constant at CMC while the excess

surfactants leads to the formation of pseudo-.phase micelles in accordance to the conventional phase- separation model for surfactants in aqueous solution (Daniel and Cary, 1989). As shown in Figure 5, the chitosan hydrogel exhibited re-entrant conformational transitions depending on the concentrations below and above the CMC of the surfactants. The percentage equilibrium volume swelling of chitosan film first decreased with increase in the concentrations of SDS below the CMC of the surfactant from 0.00242 moldm^{-3} to 0.00822 moldm^{-3} (the CMC of SDS) and then increased again up to 0.0147 moldm^{-3}. At concentrations below 0.00822 moldm^{-3}, the interactions between the carboxylic acid groups of the chitosan hydrogel and surfactant molecule predominate over the interaction between polymer chains. This causes the hydrogel to swell. On the other hand the alkyl group in the chain backbone is hydrophobic in nature, these groups are exposed to form aggregates above 0.00713 moldm^{-3} concentration to the CMC giving rise to conformational changes. The polymer chains are forced into a globule conformation in which polymer-polymer interactions are dominant. Consequently, the continuous re-entrant phase transition at above the 0.00896 moldm^{-3} results from a change in the balance between various types of interactions especially hydrogen bonding and hydrophobic interactions. These trends are clearly observed for the chitosan hydrogel in all other surfactants and in the NaTTP cross-linked chitosan but with lower swelling percentage equilibrium volume values as shown in Figure 7.

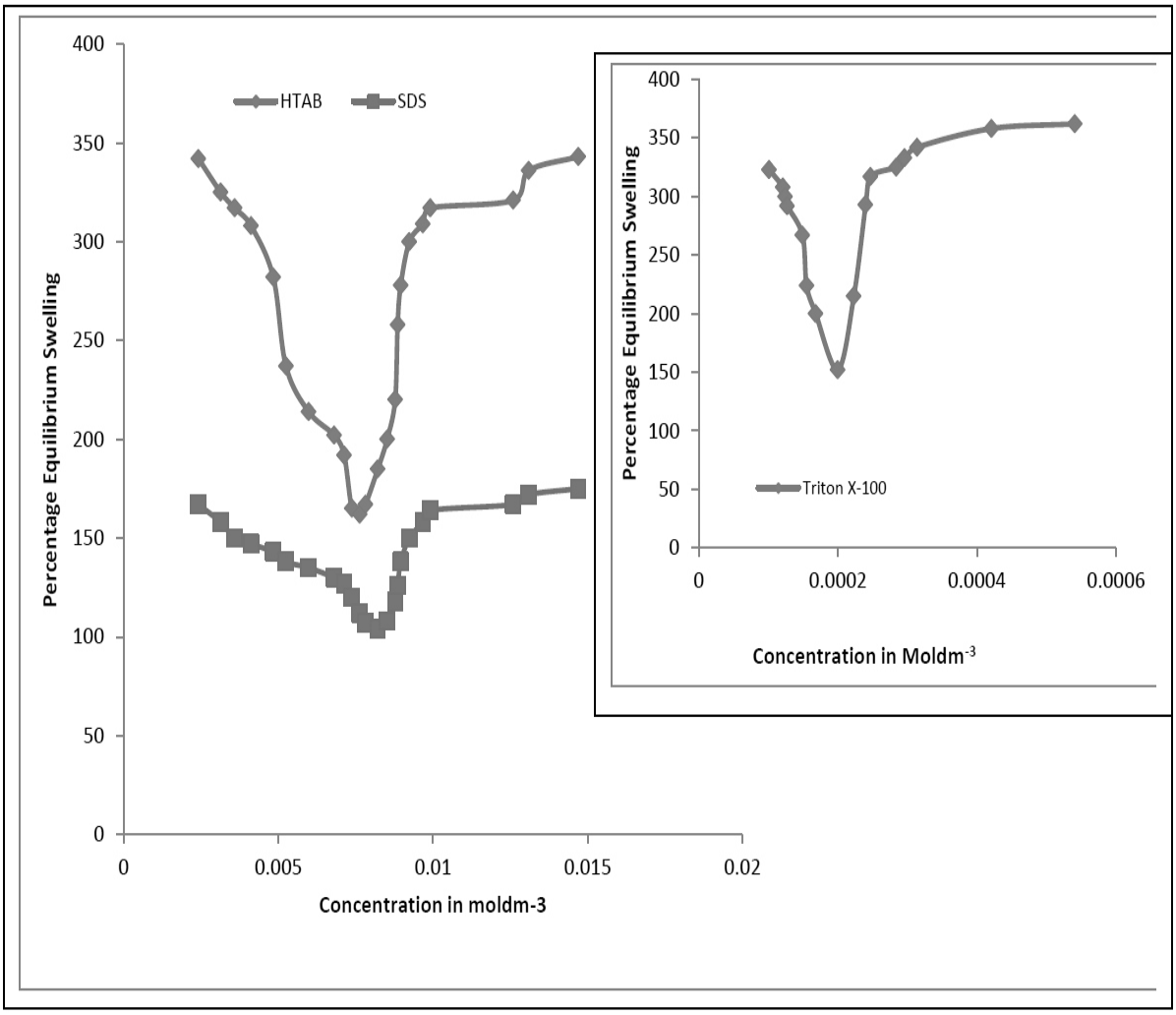

Figure 5: Percentage Equilibrium Swelling as a Function of Surfactant Concentrations. Inset (Triton X-100)

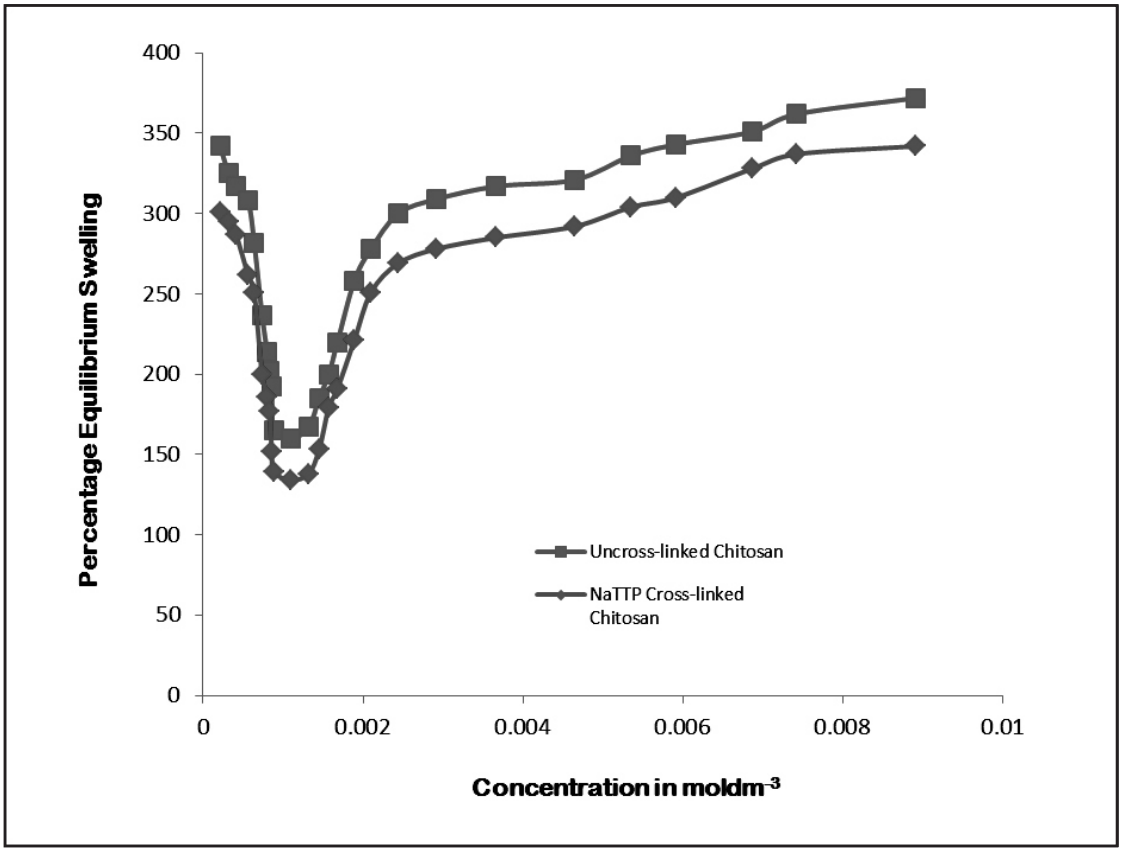

Figure 6: The Swelling Equilibrium of both Chitosan and NaTTP Cross-linked Chitosan Hydrogels in HTAB

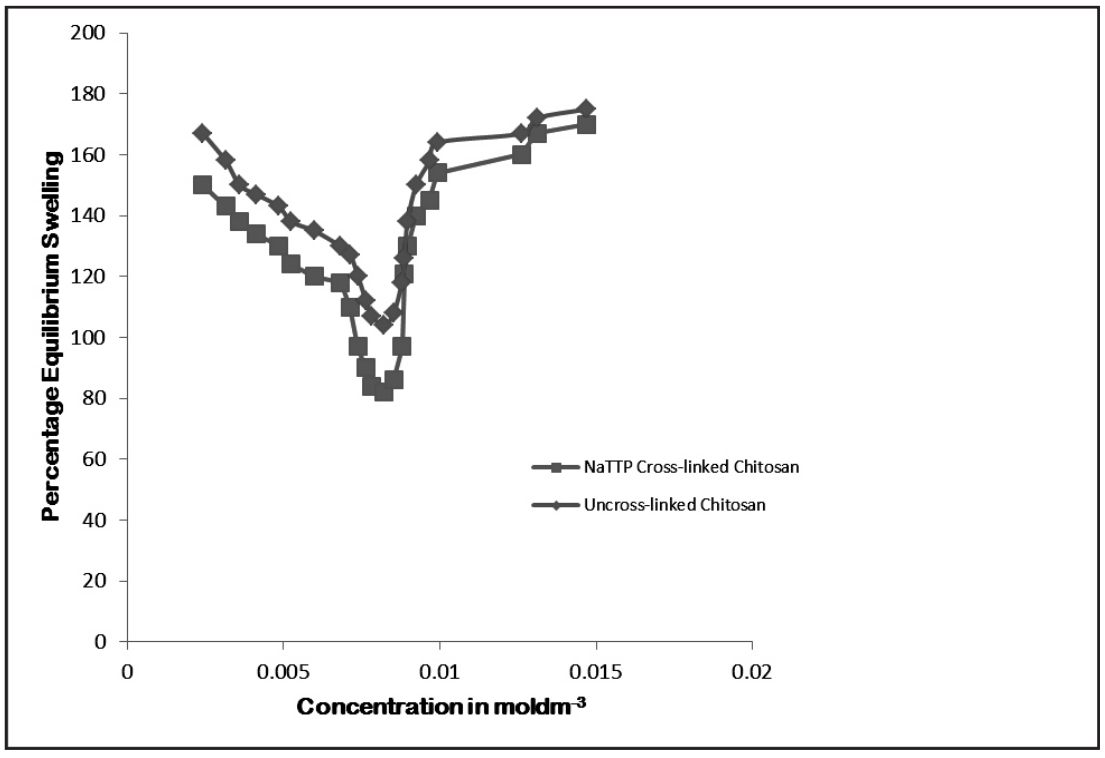

Figure 7: The Swelling Equilibrium of both Chitosan and NaTTP Cross-linked Chitosan Hydrogels in SDS

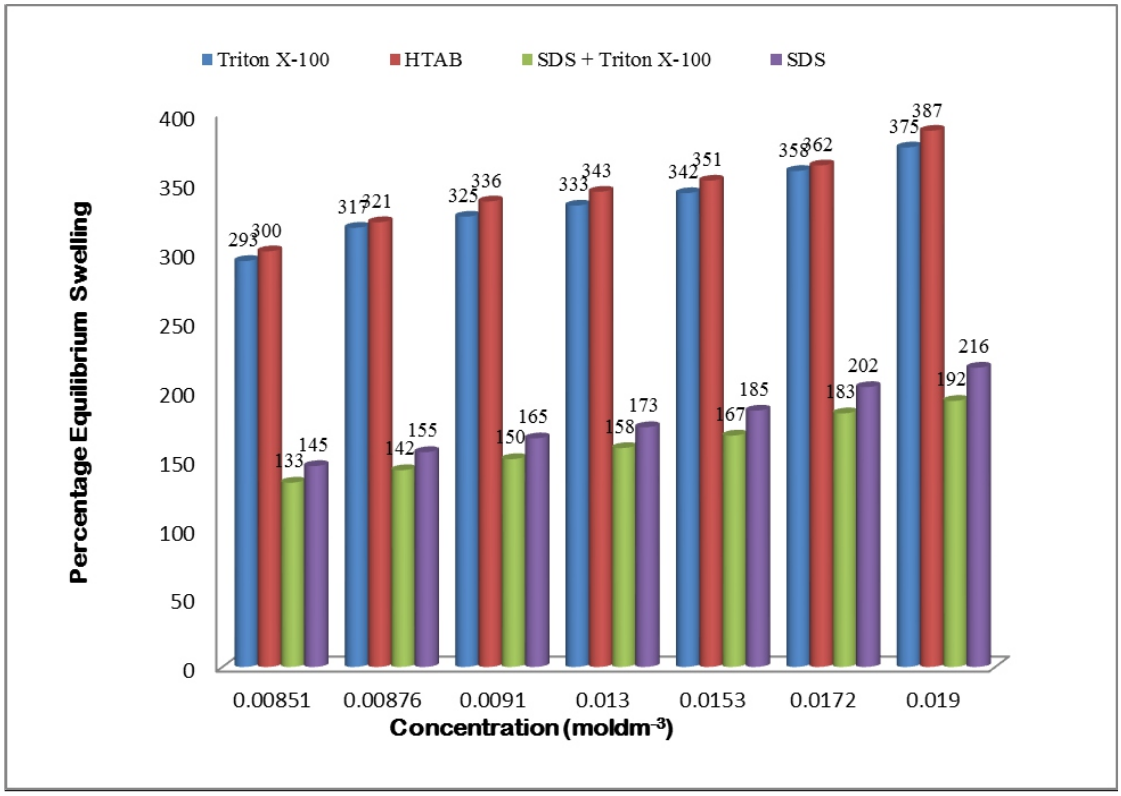

Figure 8: Percentage Equilibrium Swelling as a Function of Surfactant Concentrations

NaTPP dissolved in water and dissociate to give phosphoric ions. The cross-linking of chitosan is known to be dependent on the availability of the cationic sites and the negatively charged species. Swelling of chitosan films, mainly influenced by ionic interactions between chitosan chains, is reported to depend on the cross-linking density achieved during the formation of the network. An increase in cross-linking density is reported to induce a decrease in swelling (Mi *et al.,* 1999; Wang *et al.,* 2001 and Leonida *et al.,* 2011).

Figure 8, also clearly shows that, the percentage equilibrium volume change of chitosan hydrogel increased from HTAB > Triton X-100 > SDS > SDS/TX-100 mixed system. The results also show that the equilibrium swelling ratio of the gel decreases in SDS/TX-100 mixtures with an increase in the mole ratio of SDS from 0.00 to 0.85 and increased sharply as the concentration of the surfactant increases.

CONCLUSION

The swelling equilibrium of Chitosan and NaTPP cross-linked hydrogels in aqueous solutions of surfactants differing in structure and hydrophobicity has been studied. The chitosan hydrogels were synthesized from a non-toxic, low-cost biocompatible material. All the surfactants induce abrupt change in the gel volume. The percentage equilibrium swelling of the chitosan hydrogel increased sharply as the concentration of the surfactant increases and it is more at concentrations above the critical micellar concentration (CMC) of all the surfactants used in this study. These trends are clearly observed for the chitosan hydrogel in all other surfactants and in the NaTTP cross-linked chitosan but with lower swelling percentage equilibrium volume values. It was also observed that the surfactants induced profoundly different gel swelling profile, and both the structure and the hydrophobicity of the surfactant are the influential factors. This swelling study shows that the cross-linking density, the surfactant types, and their respective concentrations are important parameters affecting the equilibrium swelling ratio of the chitosan gel. As drug release is known to be influenced by cross-linking density, temperature, and pH, these factors can be judiciously used in predicting and modifying controlled drug release.

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