CROSS-LINKED CHITOSAN FILMS: EFFECT OF CROSS-LINKING DENSITY ON SWELLING PARAMETERS

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ABSTRACT

Equilibrium water content (EWC), equilibrium swelling ratio (ESR) and mass gain ratio are the swelling parameters that are useful for correlating drug release characteristics from polymeric films, barriers etc. These parameters can be judiciously used in predicting and modifying drug release from dosage forms. Further, drug release is known to be influenced by cross-linking density. Therefore, the present investigation was conducted to study the influence of cross-linking density on swelling parameters. For this purpose, chitosan (CH) films were prepared by cross-linking with various concentrations of sodium tripolyphosphate (NaTPP) or sodium citrate (NaCit) solutions. The swelling parameters and in vitro permeation of model polar drug (5-FU) and nonpolar drug (INDO) cross-linked CH films were determined. The swelling characteristics revealed that cross-linking with 5% w/v NaTPP or 10% w/v NaCit solution of films containing 4% w/v CH as well as with 1% w/v NaTPP or 5% w/v NaCit solution of films containing 2.5% w/v CH exhibited minimum ESR and EWC. The *in vitro* permeation of both 5-FU and INDO across these films was lowest. However, these films showed maximum mass gain ratio. Further, lowest ΔH of the endothermic transition characteristics of water uptake in films suggested the role of cross-linking density in water uptake by these films. IR analysis showed two terminal $–PO_3^-$ moieties of NaTPP or $–COO^-$ moieties of NaCit to be linked with two $–NH_3^+$ ($OOCH_3^-$) moieties of CH monomers one on each side. Overall, the results indicate role of swelling parameters in predicting cross-linking density in polymeric films.

Keywords: Chitosan, Equilibrium water content, Equilibrium swelling ratio, 5-Fluorouracil, Indomethacin, *in vitro* permeation, Mass gain ratio, Swelling index.

INTRODUCTION

In the last few years, the use of natural hydrophilic polymers as drug carriers has received considerable attention, especially from the viewpoint of environmental pollution, biodegradability, safety and cost. Polysaccharides such as alginates and chitosans and their derivatives are being widely investigated for modifying drug-release from dosage form. Chitosan (CH) 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. CH is a polycationic polysaccharide in acidic medium (pKa 6.5) and is reported to form complex with negatively charged moieties such as sodium carboxymethylcellulose, citrates, pectin, acacia, agar, sodium caprylate, stearic acid, gluteraldehyde, sodium tri-polyphosphate, lactic acid, malic acid and alginic acid (Adusumilli and Bolton, 1991; Akbuga and Bergisadi 1996; Suheyla, 1997; Dureja *et al*., 2001and Wang *et al*., 2001). These CH complexes are insoluble in alkaline buffer. This property of complexed CH has been utilized in preparing beads (Bodmeier, 1989; Sezer and Akbuga, 1995), microspheres (Genta *et al*., 1997; Aiedeh *et al*., 1997) and artificial films (Berger *et al*., 2004; Wan *et al*., 2003 and Rana *et al*., 2004). In addition CH acts as a penetration enhancer by opening the tight epithelial junctions. This property of CH is intensively being explored for protein and vaccine delivery (Van der Lubben *et al*., 2001a, b). Complexed CH nanoparticles are being studied for delivery of polypeptides such as tetanus toxoid, diphtheria toxoid and insulin (Van der Lubben, 2003; Xu and Du, 2003; Calvo *et al*., 1997a, b; Janes and Alonso, 2003).

The modification of drug released from dosage forms utilizing CH is due to the networks formed from ionic/ covalent cross-linking of CH. Also, the drug release from pH-dependent drug delivery systems can be influenced by altering the experimental conditions during cross-linking CH. Cross-linked CH generally exhibit pH-sensitive swelling and release the drug by diffusion through their porous structure (Tapia *et al*., 2002; Remunan-Lopez and Bodmeier, 1997; Bhumkar and Pokharkar, 2006). Overall, in order to use these films for controlled-release applications, it is necessary to understand the swelling characteristics of CH films and their permeability to model drugs.

In the light of these reports, the present investigation aimed at preparing films using CH cross-linked with various concentrations of sodium tripolyphosphate

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(NaTPP) or sodium citrate (NaCit). A systematic study was conducted to gain insight into cross-linking density by investigating the swelling parameters.

MATERIALS AND METHODS

Materials

Chitosan, 85% deacetylation (Indian Sea Foods, Cochin, India), 5-fluorouracil (Dabur Research Foundation, Delhi, India), indomethacin (Crystal Pharmaceuticals, Ambala, India), were gift samples. Sodium tripolyphosphate (NaTPP), sodium citrate (NaCit) and glacial acetic acid were purchased from Loba Chemie, Bombay, India. Other chemical purchased were of analytical or HPLC grade.

Methods

Preparation of sodium citrate or sodium tripolyphosphate cross-linked chitosan films

CH (2.5% or 4% w/v) solutions were prepared in acetic acid (3% w/v) by homogenizing (Remi, Mumbai, India) at 2000 rpm. A portion (15 mL) was poured in a glass ring (cross sectional area 3.14 cm^2) fitted on polycarbonate petri plate and subjected to drying at 45^oC for 24 h. Dried films were stored in polyethylene bags till use. Cross-linking of CH films was done by dipping in a 10 mL solution of NaTPP (1-20% w/v) or NaCit (1-20% w/v) after adjustment to pH 5 for 45 min. These films were washed with water to remove excess NaTPP/ NaCit. Films that were insoluble in receptor solution (phosphate buffer pH 7.4) for more than 48 h were used for *in vitro* permeation experiments or for determination of swelling parameters.

In vitro permeation studies

Vertical Franz diffusion cell apparatus was designed and fabricated in our laboratory. It consisted of 8 glass diffusion cells (20 mL each) maintained at 37^oC ± 1^oC by water heating system. Stirring of receptor fluid in each cell was accomplished by magnetic stirrer (300 rpm). Each cross-linked film was clamped between donor and receptor compartments. The receptor compartment contained phosphate buffer (pH 7.4), sodium azide (0.5% w/v) and PEG 400 (5.0% v/v). Either drug was suspended in propylene glycol (4 mL) and loaded in the donor compartment. Aliquots (1 mL) withdrawn at various intervals were immediately analyzed for 5-FU or INDO by HPLC (Waters, 515 pump, USA) using Spherisorb C_{18} column (4.6 x 250 mm) and UV detector (2487 Dual wavelength). Sodium acetate (0.1% w/v) or methanol: citrate buffer 10 $mmol^{-1}$ (75:25) at flow rates of 0.6 mL min^{-1} or 1.0 mL min^{-1}, respectively, was used as mobile phase for 5-FU or INDO. The respective detection wavelength for 5-FU or INDO was 265 and 240 nm as reported by Sasaki *et al.*, 1991. Permeation experiments were replicated five times.

Infrared absorption spectroscopy (IR)

Samples of CH powder, CH-NaTPP cross-linked films or CH-NaCit cross-linked films were dried to constant weight and triturated with an equal quantity of KBr. Each sample was then compressed to obtain discs for IR analysis. The spectra of these discs were recorded on a Perkin Elmer RXI, IR spectrophotometer (USA) in the spectral region of 500 to 4000 cm^{-1}. The experiments were carried out in triplicate.

Swelling Parameters

Equilibrium water content (EWC)

EWC was calculated by using the formula:

$$EWC\ (\%) = [(Ws-Wd) / Wd] \times 100$$

Where, Wd is the weight of dried (in vacuo for 48h over P_2O_5) film, and Ws is the weight of swollen films (soaked for 24h in phosphate buffer, pH 7.4) after removing excess surface moisture with filter paper.

Equilibrium swelling ratio (ESR)

ESR is the ratio of swollen weight (Ws) to the weight of dried films (Wd).

Mass gain ratio

The mass gain ratio was calculated as the ratio of Wd to the initial mass of CH used for preparing the respective film.

Differential Scanning Calorimetric (DSC) Analysis

The swollen cross-linked CH films used for estimation of EWC were subjected to DSC analysis in a heating rate of 10°C/min from 20°C to 150°C.

RESULTS AND DISCUSSION

CH contains free $–NH_2$ groups. Interactions of different functional groups with these moieties are utilized in modifying drug release. Also, this $–NH_2$ group gains positive charge when dissolved in acidic medium. On the other hand, NaTPP and NaCit dissolved in water dissociate to give phosphoric ions and carboxylate ions, respectively. The cross-linking of CH is known to be dependent on the availability of the cationic sites and the negatively charged species. Therefore, two concentrations of CH and various concentrations of NaTPP or NaCit were used to study the relationship between swelling parameters and cross-linker concentration.

Swelling of CH films, mainly influenced by ionic interactions between CH 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*., 1997; Mi *et al*., 1999; Sezer and Akbuga, 1995).

EWC indicates percentage of water retained by the films as compared to dried films. Figs. 1 and 2 show the EWC of CH films cross-linked with various concentrations of NaTPP or NaCit (1-20% w/v) as a function of cross-linker concentration. CH films prepared by using 4% w/v CH and cross-linked with NaTPP (5 % w/v) or NaCit (10 % w/v) revealed the minimum EWC and hence maximum degree of cross-linking. However, different concentration of NaTPP (1% w/v) or NaCit (5% w/v) were required to produce similar results in CH films prepared using 2.5% w/v CH. Hence, it can be suggested that the cross-linking density of CH (4% w/v) films was increased to maximum in films cross-linked with 5% w/v NaTPP or 10% w/v NaCit as well as in films prepared with 2.5% w/v CH and cross-linked with 1% w/v NaTPP or 5 %w/v NaCit solutions.

ESR is the ratio of the weight of swollen film to the dried. ESR of the films prepared using 4% w/v CH and cross-linked with 5% w/v NaTPP or 10% w/v NaCit was found to be minimum. Similar films employing 2.5% w/v CH required 1% w/v NaTPP or 5% w/v NaCit to render them least swellable (Figs. 1 and 2). The decreased in ESR indicates reduced water retaining capacity of films. This in turn can be attributed to higher cross-linking density. Further these findings were correlated with mass gain ratio.

Mass gain ratio is the indicator of increase in the weight of cross-linked films as compared to uncross-linked films. The mass gain ratio increases only when the number of linkages between NaTPP or NaCit and CH molecules increases in CH films. Figure 1and 2 shows mass gain ratio to be maximum for CH films prepared from 4% w/v CH solution and cross-linked with 5% w/v NaTPP or 10% w/v NaCit solutions. The films prepared from 2.5% w/v CH solution required cross-linking with lower concentration of NaTPP (1% w/v) or

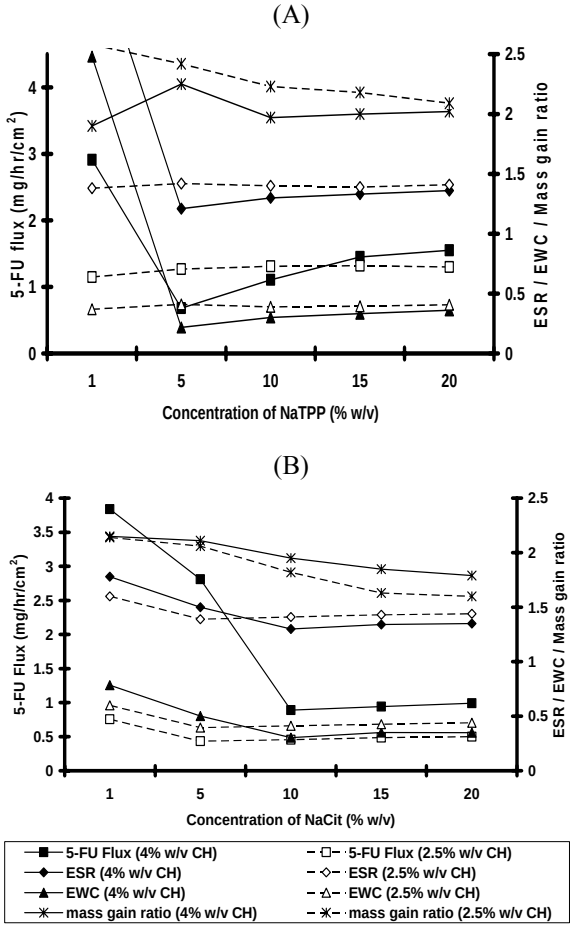

Fig. 1: Correlation of swelling parameters (EWC, ESR or mass gain ratio) with in vitro permeation of 5-FU across films cross linked with various concentrations of NaTPP (A) and NaCit (B).

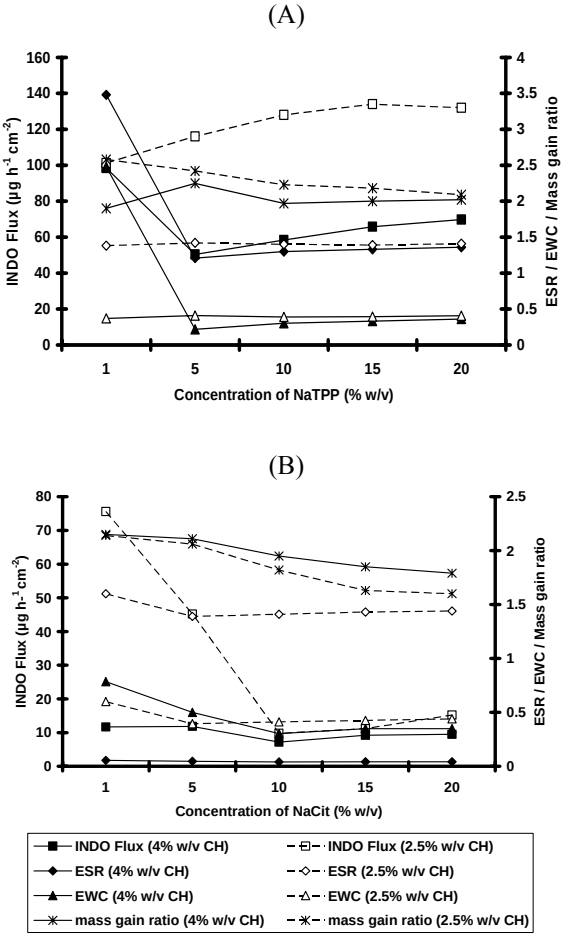

Fig. 2: Correlation of swelling parameters (EWC, ESR or mass gain ratio) with in vitro permeation of INDO across films cross linked with various concentrations of NaTPP (A) and NaCit (B).

NaCit (5% w/v) solution to exhibit maximum mass gain ratio. It is noteworthy that cross linking with 5% w/v NaTPP or 10% w/v NaCit solution of films prepared from 4% w/v solution of CH as well as with 1% w/v NaTPP or 5% w/v NaCit solution of films prepared from 2.5% w/v solution of CH yielded similar trend for all the three parameters namely EWC, ESR and mass gain ratio.

The *in vitro* permeation of model polar (5-FU) and non polar (INDO) drugs across these cross-linked films was studied. Flux of both 5-FU and INDO across CH films cross linked with 1% w/v, NaTPP was found to be highest (Figure 3). This could be attributed to improper cross linking at very low NaTPP concentration. Further increase in NaTPP concentration to 5% w/v is expected to decrease the positive charge on CH film. However, it can be envisaged to be still high enough to interact with and restrict the movement of negatively charged 5-FU and INDO species. Therefore, cross-linking CH films with 5% w/v, solution of NaTPP to decrease the permeation of both 5-FU and INDO a value lower than that across films cross linked with 1% w/v solution of NaTPP. Hence, cross linking with 5% w/v, NaTPP appears to indicate optimum cross linking of CH with NaTPP. However, further increase in NaTPP concentration (10 or 20% w/v) resulted in enhanced passage of 5-FU and INDO across these films. This might be due to increased negative charge on the film (due to excessive presence of $-PO_3^-$ moieties) that repelled the negatively charged 5-FU molecules across the film. It is important to note that Remunan-Lopez and Bodmeier (1997) observed the diffusion of chlorpheniramine maleate (a basic drug) that is positively charged in CH films to decrease with increase in concentration of NaTPP. Therefore, enhanced permeation of both 5-FU and INDO across CH films cross-linked with increasing concentration of NaTPP can be ascribed to their acidic nature due to which both drugs shall be negatively charged in the films and hence, repelled in to the receptor compartment. Therefore, these findings are in consonance with those of Remunan-Lopez and Bodmeier (1997). Figs. 1 and 2 indicate a positive correlation of *in vitro* permeation of 5-FU or INDO with ESR, EWC and mass gain ratio. Hence, the results suggest an overwhelming role of cross-linking density to be responsible for minimum EWC, ESR or *in vitro* permeation of both drugs and maximum mass gain ratio.

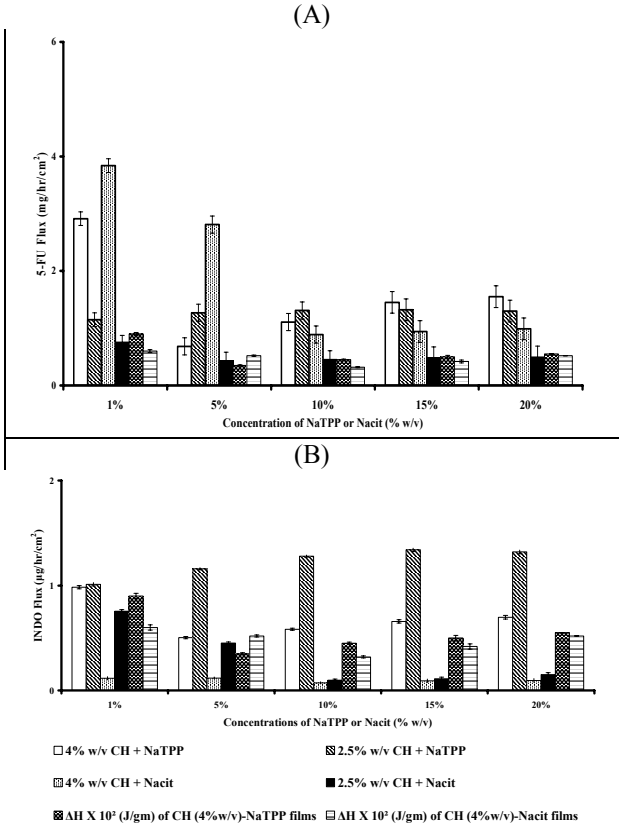

Fig. 3: Effect of concentration of NaTPP or NaCit on in vitro permeation of 5-FU (A) or on INDO (B) and on ΔH (J/g) of endothermic transition.

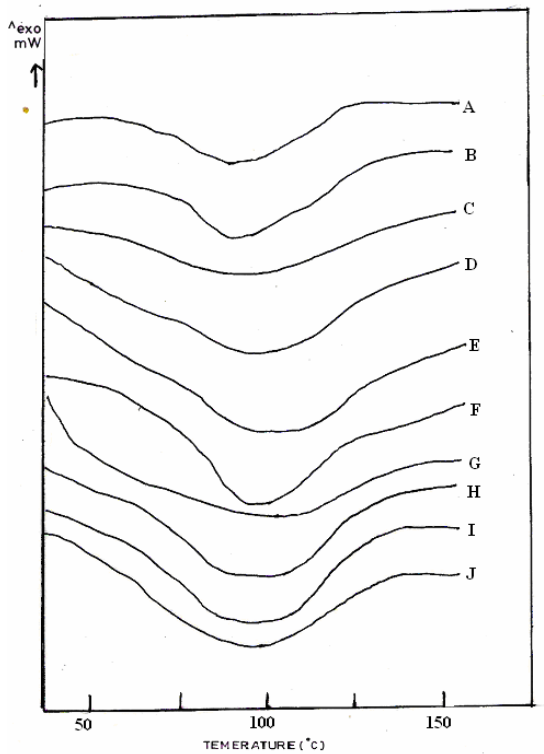

Fig. 4: DSC spectra of swallowed CH (4% w/v) films cross-linked with various concentrations of sodium citrate solutions after adjustment to pH 5: A- 1% w/v; B- 5% w/v; C- 10% w/v; D- 15% w/v and E- 20% w/v and NaTPP: F-1% w/v, G-5% w/v, H-10% w/v, I-15% w/v, J-20% w/v.

The DSC analysis was conducted to estimate the amount of moisture present in cross-linked CH films as a result of swelling. The observed endothermic transition at around 96°C could be attributed to moisture present in these films. Thermograms of swollen films prepared by cross-linking with 5% w/v NaTPP or 10% w/v NaCit showed lowest ΔH of the endothermic transition (Figs. 3 and 4) suggesting lowest moisture content. This seems to be due to low water uptake capacity and high cross-linking density of these films. In addition, lowest EWC and ESR of these films confirmed the overwhelming role of cross-linking density in reducing water uptake.

The IR spectra of all CH films (4% w/v) cross-linked with NaTPP showed peaks at 1060 – 1300 cm^{-1} suggesting presence of phosphonate linkages between $–NH_3^+$of chitosan and $–PO_3^-$ moieties of NaTPP during cross linking process. Only one peak at 1120 cm^{-1} was observed in the film cross linked with 1% w/v NaTPP (Fig. 5B) indicating no appreciable linkage. However, the films cross linked by 5% w/v, NaTPP showed two peaks, one at 1140 cm^{-1} and another at 1280 cm^{-1} (Fig. 5C), indicative of symmetric and antisymmetric stretching of phosphonate linkage, respectively. The latter peak (antisymmetric) is known to occur due to restricted rotation (Kemp, 1991). Therefore, the two terminal $–PO_3^-$ moieties of NaTPP molecule seem to be linked with two $–NH_3^+(OOCCH_3)^-$moieties of two CH monomers, one on each side. This cross linking perhaps restricted the permeation of drug molecules and resulted in lowest flux of both drugs across films crosslinked with 5% w/v, NaTPP. These two peaks were also observed in films cross linked with 10% w/v, NaTPP (Figure 5D). However, the intensity of antisymetric peak was significantly reduced. This antisymetric peak was found to merge in to a single broad band at 1060 cm^{-1} indicating absence of restricted rotation in films cross linked with 15% w/v or 20% w/v NaTPP (Fig. 5E-F). In consonance, greater permeation of both drugs was observed across CH films cross linked with 10% w/v, 15% w/v or 20% w/v of NaTPP. CH films (4% w/v) obtained after dipping in 1% w/v NaCit solution indicated antisymmetric and symmetric stretching at 1386 cm^{-1} and 1458 cm^{-1}, respectively along with peak at 1560 cm^{-1} of ammonium ion (Fig. 5G). Similar peaks were observed in films cross-linked by dipping in 5% w/v to 20% w/v NaCit solutions adjusted to pH 5 (Fig. 5H-K). The antisymmetric peaks are known to occur due to restricted rotation (Kemp, 1991). Therefore, the two terminal $–COO^-$ moieties of NaCit molecules seem to be linked with two $–NH_3^+$ $(OOCCH_3)^-$ moieties of two CH monomers, one on each side. This cross linking perhaps restricted the permeation of drug molecules and resulted in lower permeation of both drugs across films cross-linked with 1% w/v - 20% w/v NaCit solution. Unfortunately, the spectra of CH films cross-linked with increasing NaCit concentration (1% w/v to 20% w/v) did not differ significantly. Therefore, the extent of cross-linking with increasing concentration of NaCit could not be explained on basis of IR spectral data.

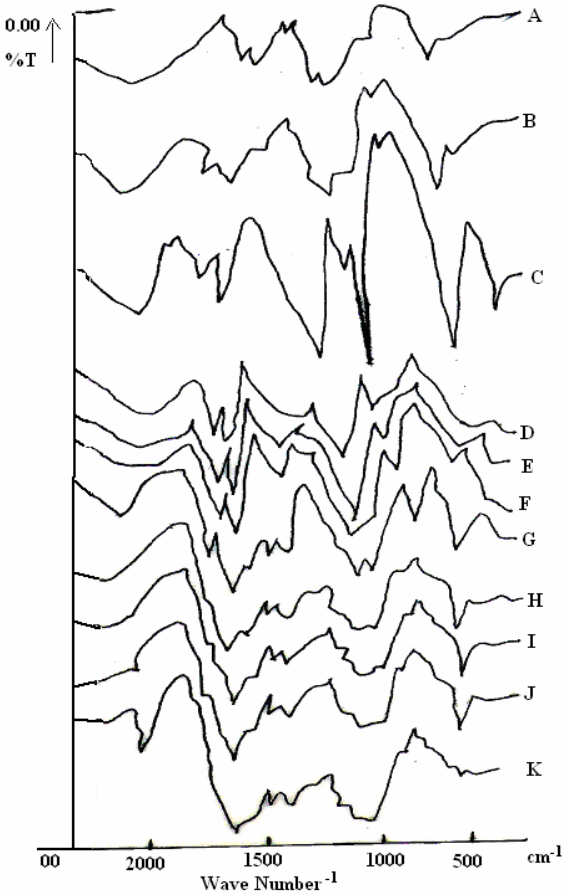

Fig. 5: IR spectra of CH powder (A) and CH (4% w/v) films cross-linked with various concentrations of NaTPP: B- 1% w/v; C- 5% w/v; D- 10% w/v; E- 15% w/v and F- 20% w/v and sodium citrate solutions after adjustment to pH 5 (G-1% w/v, H-5% w/v, I-10% w/v, J-15% w/v, K-20% w/v).

CONCLUSION

The swelling parameters estimated for the CH films prepared by cross linking with either NaTPP or NaCit revealed good consonance with cross linking density. DSC analysis of swollen cross linked films was also in agreement with swelling parameters. Further, IR spectral analysis supports maximum cross linking density. Hence, it could be envisaged that there is overwhelming role of swelling parameters in predicting cross linking density.

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