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# Release of nifedipine through crosslinked chitosan membranes

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## Summary

The permeability characteristics of a chitosan membrane using the anti-hypertensive drug nifedipine have been investigated. Chitosan membranes were crosslinked with different concentrations of glutaraldehyde and the effect of crosslinking on the permeability characteristics was studied. Permeation studies were carried out in static glass diffusion cells. With increasing degree of crosslinking a definite decrease in the diffusion coefficient, equilibrium swelling, partition coefficient and permeability coefficient was observed. These data supported the involvement of both pore and partition mechanisms in the transport of a hydrophobic drug such as nifedipine through chitosan/crosslinked chitosan membranes. It has been shown that by altering the permeability of chitosan membranes by different degrees of crosslinking the release of bioactive agents could be programmed.

## Introduction

Chitosan ([1–4] 2-amino-2-deoxy-β-D-glucan) is a natural polycationic polymer which possesses valuable properties as a biomaterial (Hirano et al., 1987; Takayama et al., 1990; Lehr et al., 1992). It is the *N*-deacetylated product of chitin(1–4-linked-2-acetamide-2-deoxy-β-D-glucan) which is abundant in nature. Chitosan's unique solubility, solution properties, polycationic character, physical attributes and chemical and biological activity make it an attractive biopolymer for many biomedical applications. Moreover, the biocompatibility and biodegradability of this novel polysaccharide have been well established (Amano and Ito, 1978; Muzzarelli et al., 1988; Hirano et al., 1990; Ouchi et al., 1991). Increasingly over the last few years, chitosan has been used in the pharmaceutical industry for its potential use in controlled drug delivery systems (Miyazaki et al., 1988, 1990; Nigalaye et al., 1990).

The film-forming property of chitosan finds many applications in various fields (Krajewska et al., 1990; Kubota et al., 1991). Only a few studies have so far been performed on the usefulness of chitosan films as drug delivery systems (Miyazaki et al., 1990; Nakatsuka and Andrady, 1992). The present study was focussed towards the development of chitosan membranes with different permeability characteristics by crosslinking with glutaraldehyde and its utilization in controlled drug

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delivery systems. Permeability studies of these membranes were carried out using the antihypertensive drug nifedipine.

## Materials and Methods

*Materials*

Chitosan was a gift sample from Central Food Technological Research Institute (CFTRI), Mysore, India. Glutaraldehyde (Riedel) and nifedipine (Medimix Laboratories, India) were used as obtained. All other chemicals used were of analytical grade.

*Preparation of chitosan membranes*

Chitosan (1 g) was dissolved in 10% acetic acid with constant stirring for 48 h. The resultant viscous solution was filtered. The filtrate was left to stand until all air bubbles had disappeared. The bubble-free liquid was spread on a clean dry glass plate in a dust-free atmosphere and left to dry at room temperature. The membrane thus obtained was neutralized by immersion in aqueous NaOH (4%) for 1 h, washed several times with distilled water and dried. The dry membranes thus obtained having a thickness of 20 $\mu$m were used in further studies.

*Crosslinking of chitosan membranes*

The chitosan membranes were crosslinked with different glutaraldehyde concentrations ranging from 0.01 to 0.3% at room temperature. After crosslinking, the membranes were washed extensively with distilled water to remove excess aldehyde, dried and stored at room temperature. From the permeability studies of chitosan membranes of different degrees of crosslinking it was found that the permeability to nifedipine was reduced markedly when the membrane were treated with 0.01% glutaraldehyde. Hence, no efforts were made to use glutaraldehyde beyond 0.3% in the present study.

*Equilibrium swelling studies*

The water sorption capacity of chitosan membranes was determined by swelling the membranes in water and in aqueous acetone at room temperature. A known weight of chitosan membrane was placed in either water or aqueous acetone for the required period of time. The swollen weight of the membrane was determined by first blotting the membrane with filter paper to remove adsorbed water or aqueous acetone and weighed immediately on an electronic balance. The weight of the swollen membrane was recorded at various time periods of 3, 6, 24 and 48 h. The percent swelling of chitosan membranes in both the media was then calculated from the formula:

$$\text{Esw} = \frac{W_e - W_0}{W_0} \times 100$$

where Esw is the percent swelling of membrane at equilibrium, $W_e$ denotes the weight of the membrane at equilibrium swelling and $W_0$ is the initial weight of the membrane. The swelling experiments were also repeated with crosslinked chitosan membranes. Each experiment was repeated three times and the average value was taken as the percent swelling value.

*Scanning electron microscopic (SEM) studies*

Excess moisture in the membranes was removed by vacuum drying at 60°C for 1 week. The dry membranes were then mounted on metal stubs with double pressure sensitive adhesive tape and coated with a thin layer of gold to improve the conductivity. The samples were observed with a Stereo Scann S-150 scanning electron microscope.

*Infrared spectroscopic (IR) studies*

Infrared analyses of chitosan membranes (crosslinked and uncrosslinked) were carried out using a Nicolet DX-20 Infrared spectrometer. The excess moisture in the membranes was removed by vacuum drying at 60°C for 1 week and the membranes were directly used for recording the infrared spectra.

*Permeation studies of chitosan membranes*

All permeation studies were carried out using static glass diffusion cells (6 ml half cell volume

and 1.13 $cm^2$ area of diffusion). Since nifedipine is insoluble in water 50% aqueous acetone was used as the solvent for nifedipine throughout these experiments. The chitosan membrane previously treated with the above solvent for 12 h was mounted carefully between the two half cells of the diffusion cell and fastened with a rigid clamp. The donor compartment was charged with a 1% solution of nifedipine in 50% aqueous acetone and the receiver compartment contained the pure solvent, 50% aqueous acetone. The stirring rate was set at 100 rpm throughout the experiment. At predetermined times the entire contents of the receiver were withdrawn and replaced with fresh solvent. The samples were assayed for nifedipine spectrophotometrically at 336 nm with a Shimadzu model UV-2100-S instrument. Experiments were repeated with chitosan membranes of different degrees of crosslinking. Each permeation experiment was repeated six times to obtain concordant readings. Since nifedipine is light sensitive the diffusion cells and all glassware used in this experiment were wrapped in black paper in order to avoid photodecomposition.

*Data analysis of permeation studies*

The data were plotted as the cumulative amount of drug collected in the receptor as a function of time (Fig. 1). The permeability coefficient for a given run was calculated from Fick's first law:

$$\frac{dM}{dt} = J_t = APC$$

where $J_t$ is the total flux of drug at steady state (mg/h), $A$ represents the area of diffusion ($cm^2$), $P$ is the permeability coefficient ($cm^2$/h) and $C$ denotes the initial concentration difference expressed between the diffusion cell's chambers which was taken as the initial donor side concentration.

Diffusion coefficients were calculated from the Daynes and Barrer lag time equation (Roy and Flynn, 1989):

$$D = \frac{h^2}{6t_L}$$

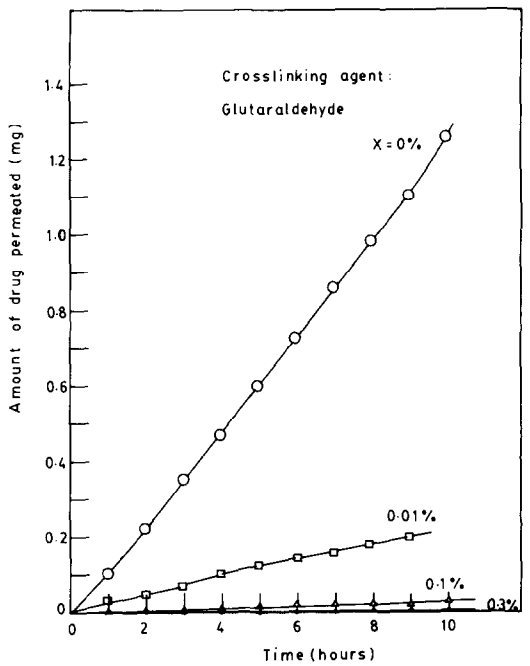


Fig. 1. Variation of cumulative amount of nifedipine in permeate with time for chitosan membranes of different degrees of crosslinking. Each point represents the average of six determinations.

where $t_L$ is the lag time and $h$ represents the thickness of the membrane.

The permeability coefficient is a function of the diffusion coefficient and partition coefficient ($K$)

$$P = DK$$

Since $P$ and $D$ are known the partition coefficient was directly deduced from the above equation.

## Results and Discussion

*Equilibrium swelling studies*

Under equilibrium conditions, the degree of swelling in an ideal membrane is governed by crosslinking density, temperature, molar volume of the solvent and the polymer solvent interactions. Swelling can be altered over a wide range by crosslinking the membrane. The swelling be-

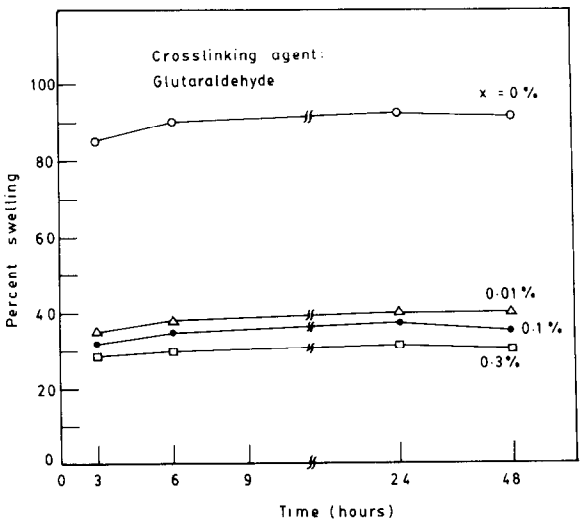


Fig. 2. Percent swelling of chitosan membranes of different degrees of crosslinking in water.

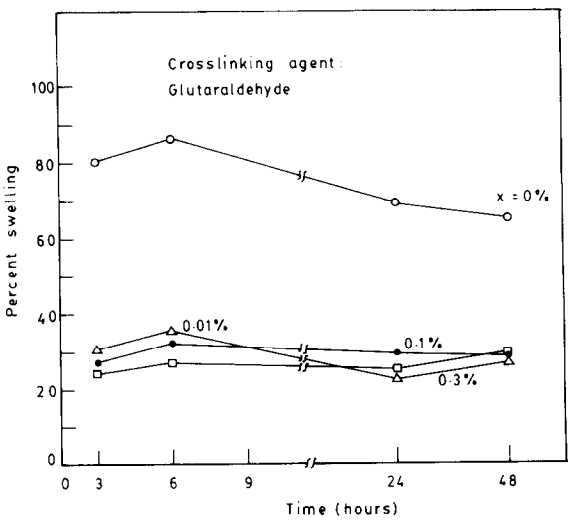


Fig. 3. Percent swelling of chitosan membranes of different degrees of crosslinking in 50% aqueous acetone.

haviour of chitosan membranes (uncrosslinked and crosslinked with different concentrations of glutaraldehyde) in both water and aqueous acetone is shown in Figs 2 and 3, respectively. The equilibrium swelling in water was about 90% with the uncrosslinked chitosan membrane. It was reduced to 38% when the membrane was treated with 0.01% glutaraldehyde, which is significant. When the concentration of glutaraldehyde was increased to 0.3% swelling was further reduced to 30%. In aqueous acetone medium, the swelling data followed the same pattern. A slight decrease

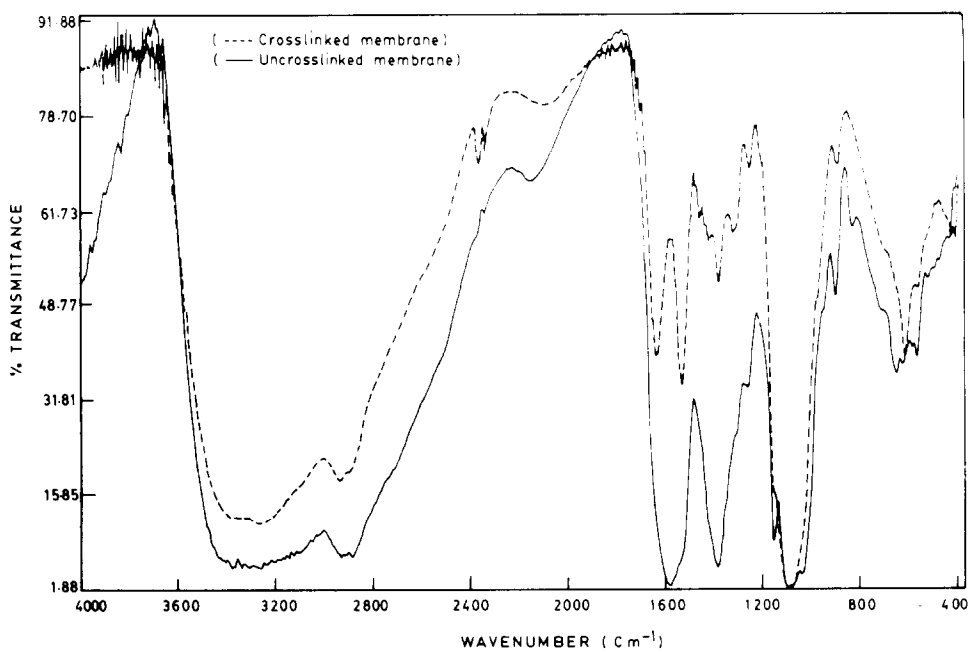


Fig. 4. IR spectra of chitosan membranes. (———) Uncrosslinked membrane, (- - - - -) crosslinked membrane.

in swelling was noted in aqueous acetone after reaching maximum swelling at 6 h. This may be due to the replacement of water from the membrane by acetone after initial swelling.

*Morphological characteristics of chitosan membranes*

Morphological characteristics of crosslinked and uncrosslinked chitosan membranes were observed using SEM. The surface of the crosslinked chitosan membrane appeared very smooth and compact whereas the uncrosslinked membrane showed a porous and granular structure. The increased compactness of a membrane due to crosslinking generally reduces its permeability to solutes. In the present study, it was observed that the release of nifedipine from the chitosan membranes was altered significantly due to crosslinking, as discussed in the later part of the study. The results obtained in the present investigation are in good agreement with earlier reports (Nakatsuka et al., 1992; Thacharodi and Panduranga Rao, 1992).

*Infrared spectroscopic studies*

The IR spectra of chitosan membranes, both crosslinked and uncrosslinked, are shown in Fig. 4. The IR spectrum of the uncrosslinked membrane showed a strong peak of the primary amino group at 1574 $cm^{-1}$ whereas, in the case of the crosslinked chitosan membrane, a doublet between 1660 and 1590 $cm^{-1}$ appears which is characteristic of the C ≈ N group (Simons, 1978). The IR spectra clearly indicated the presence of a Schiff base type of crosslink in the glutaraldehyde-treated membranes.

*Permeation studies of chitosan membranes*

Permeation of solutes in a polymeric membrane is described in terms of two mechanisms: the partition and pore mechanisms. The two mechanisms may not operate exclusively but one may be expected to dominate over the other for a given drug/membrane pair. In those membranes where the partition mechanism predominates, the solute dissolves in the polymer itself and progresses across the membrane via diffusion in the polymer fractions. Permeability is then mainly determined by the solubility of the solute in the polymer. In the pore mechanism the solute is presumed to diffuse through microchannels within the membrane structure. The permeability would then be determined by the average pore size in relation to the molecular volume of the solute and the solubility of the solute in the solvent used for diffusion studies.

Crosslinking a polymer membrane generally leads to a reduction in permeability to solutes and the decrease may be due to a number of causes. The crosslinked regions may act as excluded volumes for the sorption process and impermeable barriers for the diffusion process. In the diffusion process, the impermeable regions require penetrant migration around them which increases the average path length relative to the nominal dimensions of the membrane. These tortuosity and barrier effects reduce the permeability of the membrane (Rogers, 1985). Chemical modification of the original polymer matrix by the altered chemistry at crosslinking points (functional groups) is another cause of this behaviour. Reduced solubility of the solutes in the polymer due to crosslinking may also lead to reduced

TABLE 1

*Variation in permeability characteristics of chitosan membranes due to crosslinking with glutaraldehyde*

| Nature of membrane | $P$ ($\times 10^{-3}$) ($cm^2/h$) | $D$ ($\times 10^{-7}$) ($cm^2/h$) | $J$ ($mg/cm^2$ per h) | $K$ ($\times 10^5$) |
|---|---|---|---|---|
| Uncrosslinked | 11.2 ± 1 | 31.17 ± 4 | 0.112 ± 0.01 | 4.055 ± 1 |
| Crosslinked with 0.01% glutaraldehyde | 2.047 ± 0.5 | 29.33 ± 5 | 0.021 ± 0.005 | 0.672 ± 0.3 |
| Crosslinked with 0.1% glutaraldehyde | 0.179 ± 0.007 | 20.00 ± 3 | 0.002 ± 0.0002 | 0.14 ± 0.1 |
| Crosslinked with 0.3% glutaraldehyde | 0.075 ± 0.01 | 5.83 ± 0.07 | 0.0008 ± 0.0001 | 0.125 ± 0.03 |

$P$, permeability coefficient; $D$, diffusion coefficient; $J$, drug flux; $K$, partition coefficient. Values represent the mean + SD for six determinations.

permeability of the polymer membranes to solutes. In the present study, the permeability coefficient of chitosan membranes showed a definite decrease with increase in the degree of crosslinking, the partition coefficient also being significantly affected by crosslinking (Table 1). The same trend was observed both in diffusion coefficient (Fig. 5) and in the percent swelling values. These significant changes in the physical properties of membranes demonstrated a definite effect on drug flux; it decreased with increase in the degree of crosslinking (Fig. 6).

Since there is a large decrease in the partition coefficient it is possible that both partition and pore mechanisms operate concurrently in the transport of nifedipine through chitosan/crosslinked chitosan membranes. In our earlier study (Thacharodi and Panduranga Rao, 1992), it was shown that highly water soluble drugs such as propranolol hydrochloride could be transported through chitosan membranes predominantly by the pore mechanism. The partition coefficient was not affected in this case. From these data, it

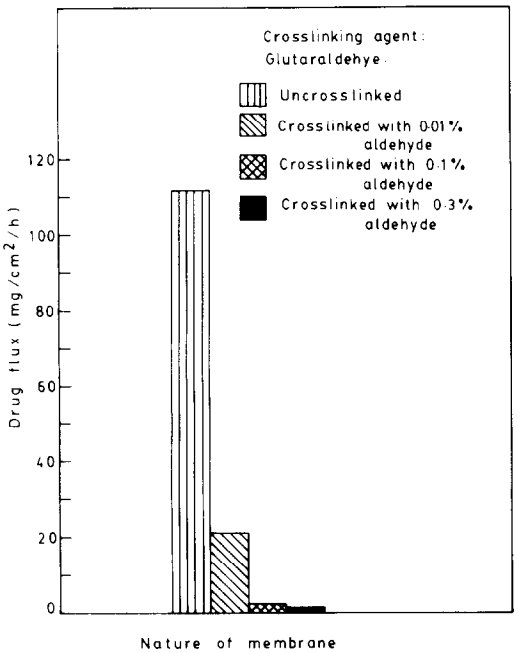


Fig. 6. Flux of nifedipine through chitosan membranes of different degrees of crosslinking.

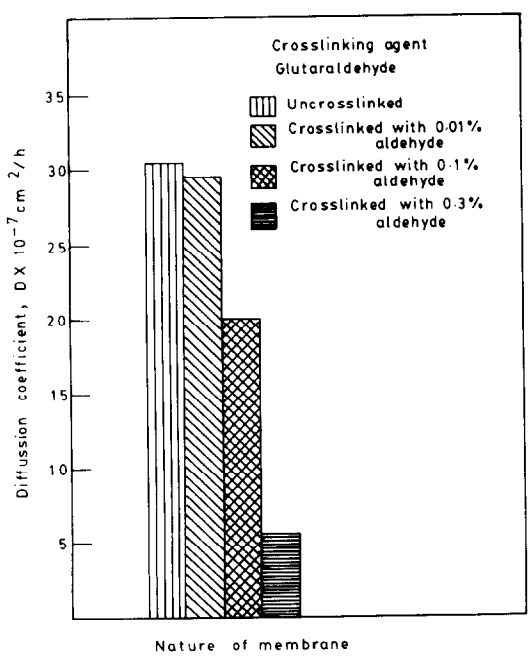


Fig. 5. Diffusion coefficient of nifedipine through chitosan membranes of different degrees of crosslinking.

may be concluded that a highly water soluble drug or solute could be transported through chitosan membranes mainly via microchannels (pore mechanism) whereas the transport of a hydrophobic solute such as nifedipine through chitosan membranes will be influenced by both partition and pore mechanisms.

## Conclusion

The permeability characteristics of chitosan membranes can be altered by crosslinking with glutaraldehyde. The morphological structure of the membranes is changed by crosslinking and these changes in turn affect the permeation of drugs such as nifedipine. Chitosan membranes showed promising potential for use in the delivery of the antihypertensive drug nifedipine and work is in progress in our laboratory to use this system in the controlled delivery of bioactive substances.

## References


Amano, K. and Ito, E., The action of lysozyme on partially deacetylated chitin. *Eur. J. Biochem.*, 85 (1978) 97–104.

Hirano, S., Noishiki, Y., Kinugawa, J. Higashijima, H. and Hayashi, T., Chitin and chitosan for use as a novel biomedical material. In Gebelein, C.G. (Ed.), *Advances in Biomedical Polymers*, Plenum, New York, 1987, pp. 285–297.

Hirano, S., Seino, H., Akiyama, Y. and Nonaka, I., Chitosan: A biocompatible material for oral and intravenous administration. In Gebelein, C.G. and Dunn, R.L. (Eds.), *Progress in Biomedical Polymers*, Plenum, New York, 1990, pp. 283–290.

Krajewska, B., Leszko, M. and Zaborska, W., Urease immobilized on chitosan membrane: Preparation and properties. *J. Chem. Tech. Biotechnol.*, 48 (1990) 337–350.

Kubota, N., Ohga, K. and Moriguchi, M., Permeability properties of glycol chitosan modified with thiol groups. *J. Appl. Polym. Sci.*, 42 (1991) 495–501.

Lehr, C.M., Bouwstra, A.J., Schacht, H.E. and Junginger, E.H., In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.*, 78 (1992) 43–48.

Miyazaki, S., Ymaguchi, H., Yokouchi, C., Takada, M. and Hou, W., Sustained release and intragastric floating granules of indomethacin using chitosan in rabbits. *Chem. Pharm. Bull.*, 36 (1988) 4033–4038.

Miyazaki, S., Ymaguchi, H., Tkada, M., Hou, W., Takeichi, Y. and Yasubuchi, H., Pharmaceutical application of biomedical polymers: XX1X. Preliminary study on film dosage form prepared from chitosan for oral drug delivery. *Acta Pharm. Nord.*, 2 (1990) 401–406.

Muzzarelli, R., Baldassarre, V., Conti, F., Ferrara, P., Biagini, G., Gazzanelli, G. and Vasi, V., Biological activity of chitosan: Ultra structural study. *Biomaterials*, 9 (1988) 247–252.

Nakatsuka, S. and Andrady, L.A., Permeability of vitamin-B-12 in chitosan membranes. Effect of crosslinking and blending with poly(vinyl alcohol) on permeability. *J. Appl. Polym. Sci.*, 44 (1992) 17–28.

Nigalaye, A.G., Adusumilli, P. and Bolton, S., Investigation of prolonged drug release from matrix formulations of chitosan. *Drug Dev. Ind. Pharm.*, 16 (1990) 449–467.

Ouchi, T., Banba, T., Masuda, H., Matsumoto, T., Suzuki, S. and Suzuki, M., Design of chitosan-5 FU conjugate exhibiting antitumor activity. *J. Macromol. Sci-Chem. A*, 28 (1991) 959–975.

Rogers, C.E., Permeation of gases and vapours in polymers. In Comyn, J. (Ed.), *Polymer Permeability*, Elsevier, Amsterdam, 1985, pp. 11–75.

Roy, D.S. and Flynn, L.G., Transdermal delivery of narcotic analgesics: Comparative permeabilities of narcotic analgesics through human cadaver skin. *Pharm. Res.*, 6 (1989) 825–832.

Simons, W.W., *The Sadtler Handbook of Infrared Spectra*, Sadtler Research Laboratories, 1978, pp. 207–226.

Takayama, K., Hirata, M., Machida, Y., Masada, T., Sannan, T. and Nagai, T., Effect of interpolymer complex formation on bioadhesive property and drug release phenomenon of compressed tablet consisting of chitosan and sodium hyaluronate. *Chem. Pharm. Bull.*, 38 (1990) 1993–1997.

Thacharodi, D. and Panduranga Rao, K., Diffusional release of antihypertensive drug propranolol hydrochloride from natural polymeric films. *Trends Biomater. Artificial Organs*, 6 (1992) 52–56.