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Development and Characterization of Chitosan and Polymethylmethacrylate Interpenetrating Polymer Network Ophthalmic Inserts

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Abstract: The present research describes the development and in vitro testing of rod-shaped mucoadhesive ophthalmic inserts prepared from appropriate mixtures of Chitosan, Polymethylmethacrylate (PMMA) and sodium chloride as release modifier. Cylindrical devices (diameter 0.8 mm, length 8–10 mm, weight 3–8 mg) all containing 0.8 mg oxytetracycline HCl (OXT) were prepared using emulsion crosslinking technique. Mucoadhesion studies in vitro showed that the mucoadhesive properties increased significantly with increasing thickness of the IPN. The inserts were tested for drug release in vitro, and for retention in rabbit eyes. The presence of IPN, as well as of NaCl, in general increased the drug release rate. A nearly zero-order release rate for about 1 week was observed in vitro for some types of inserts while other showed a Fickian release profile. The in vitro minimum inhibitory concentration values (MIC 90%) of OXT against micro-organisms responsible of common ocular infections range from 0.8 to 2.0 μg/ml, while MIC 90% values in the range 14-50 μg/ml have been indicated for *Pseudomonas aeruginosa*. When tested in rabbits, the ocular retention of IPN samples was significantly higher with respect to the inserts prepared from the polymers alone. The presently described mucoadhesive inserts might prove efficient therapeutic systems for chemotherapy of ocular bacterial infections, such as trachoma.
Keywords — Chitosan/PMMA; IPN; mucoadhesion; ophthalmic inserts.

Introduction

Ophthalmic inserts with prolonged retention in the conjunctival sac can significantly increase the topical bioavailability of ophthalmic drugs[1]. Inserts can release drugs at sustained and even controlled rate, if properly engineered. These delivery systems provide improved therapeutic efficacy and a lower incidence of side-effects by significantly reducing systemic drug absorption. The cylindrical, rod-shape has been found to provide optimal retentive properties [2-4]. A rod-shaped silicone elastomer insert claimed to have long retention and sustained release properties [5]. It has been reported that while doxycycline given orally approaches effective concentrations in tears, tetracycline and oxytetracycline given by the same route do not achieve inhibition level in these fluids [6]. An evaluation on rabbits of topically applied tetracycline in different vehicles showed that ocular levels of drug were highest with a petrolatum mineral oil-ointment and lowest with isotonic saline vehicles [7]. However, standard ophthalmic ointments are characterized by a pulsed delivery and typically require twice daily applications for several weeks while ocular inserts might more efficiently produce the desired therapeutic results [8,9]. Gurtler et al. reported interesting results obtained with a bioadhesive ophthalmic drug insert containing gentamicin, which ensured an efficacious drug concentration in tears for 72 h [10]. The lack of adhesive interactions between hydrophobic polymers like silicon elastomers and the hydrophillic palpebral and scleral mucosae caused a relatively high rate of expulsion from rabbit eyes during long-term treatment as compared to hydrophilic polymers. The ocular retention of the inserts is hereby improved by developing interpenetrating polymer network (IPN) of mucoadhesive chitosan and polymethymethacrylate into rod shaped ophthalmic inserts. Hydrophilicity gradients

were introduced into the methylmethacylic acid network by allowing chitosan network to diffuse into it and simultaneously polymerize, thus forming a gradient interpenetrating polymer network layer. The preparation and in vitro/in vivo testing of IPN hydrogel inserts releasing oxytetracycline constitutes the object of the present report. The oxytetracycline is a broadspectrum antibacterial drug, active against many common gram-positive and gram-negative bacteria, causing ocular surface infections such as conjunctivitis and keratitis, as well as against less common pathogen agents as *chlamydia trachomatis* and *neisseria gonorrhoeae*.

Materials and Methods

Materials

Oxytetracycline HCl (OXT) was kindly given by Emcure Pharmaceuticals, Pune, India. Polymethylmethacrylate (PMMA) and curing agent (CA) were purchased from HiMedia, (Bombay, India). Chitosan (CS) was obtained as a gift sample from Central Institute of Fisheries Technology (Cochin, India). Sodium chloride was purchased from S. D. Fine Chem. (Bombay, India). Porcine stomach mucin (PSM) was purchased from Sigma Aldrich India. Buffer substances and all other chemicals or solvents were of reagent grade.

Preparation of Inserts

Polymethylmethacrylate/Chitosan rod-shaped inserts (PCRsI) were prepared using appropriate amounts of PMMA/CS, CA, OXT and NaCl (as release modifier) (Table 1). A crosslinked gel is prepared to form an IPN using a dialdehydic crosslinking agent and the mixture was then injected into aluminium moulds (diameter 0.8 mm, length 22.0 mm), and were allowed to cure at 45°C for 24 h. The resulting rubbery cylinders (diameter 0.9 mm, length 22 mm) were appropriately cut to give a OXT content of 0.8 mg. The final lengths and weights were in the range (diameter 0.8 mm, length 8–10 mm, weight 3–8 mg) depending on insert type (Table 2). The PCRsI were used for hydration tests and for in vitro drug release studies. Flat, disk-shaped inserts for in vitro mucoadhesion tests were prepared by accurately spreading the same mixture of on a Polypropylene mould (diameter 50 mm). After curing at 45°C for 24 h, the resulting rubbery films were cut in the shape of disks (average thickness 1.0 mm, diameter 12.0 mm).

Table 1. Compositions and Characteristics of Inserts

Insert Type	PMMA (%w/w)	Chitosan (%w/w)	NaCl (%w/w)	Dia. (mm)	Wt(mg) /l(mm)
PP I	60	40	10	0.9	5.3/8.0
PP II	60	40	20	0.9	5.3/8.0
PP III	40	60	10	0.9	7.6/9.0
PP IV	40	60	20	0.9	7.6/9.0

Hydration tests

Hydration studies on PCRsI were performed by maintaining the inserts in 66.7 mM pH 7.38 So¨rensen phosphate buffer at 30°C. At appropriate intervals, the inserts were withdrawn from the solution, superficially dried and weighed. The hydration was calculated as percent weight increase and plotted vs. time.

In vitro mucoadhesion tests

The mucoadhesive properties of PCDs disks were evaluated by measuring their work of adhesion (W) on a mucin substrate [11]. The latter consisted of a 25% w/w dispersion of PSM in water, spread uniformly on wet filter-paper [12]. The apparatus consisted of a testing cell connected to a custom-made tensile apparatus. For testing, the PCDs were placed between the upper and lower mucous surface of the testing cell in the absence of external bathing fluid; the overall applied load (upper cell weight) was 8.2 g. The detachment tests were performed after 20 min of contact, at 30 ±0.5 °C. The resulting force vs. elongation curves were analyzed and the reported W values, corresponding to the areas under the curves, are the average of three determinations.

In vitro release of oxytetracycline HCl (OXT)

For in vitro release tests, glass vials containing one insert in 10.0 ml of pH 7.4, 66.7 mM phosphate buffer were placed in a thermostated (30 ±0.5°C) shaking water bath. At appropriate intervals the solution was completely withdrawn for analysis, and replaced with fresh buffer. Sampling was discontinued after 2 weeks.

In vivo retention study

One PCRsI was introduced into the lower conjunctival sac of one eye of the rabbit; the eyelids were then gently kept closed for 30 s. At different times after administration (10, 30, 60, 120 min, 4.5 and 7 h) the PCRsI was checked for its retention in the eye. The protocol of the in vivo study was approved by animal ethics committee of the department and the experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India.

Results and Discussion

Characteristics of Rod Shaped Insert

The characteristics of the different types of PCRsI, all containing 0.8 mg OXT, are illustrated in Table I.

Hydration Tests

The results of hydration tests carried out on PCRsIs are reported in Fig. 1 as percent water absorbed vs. time. After 8 h (approximately at equilibrium) the weight increases for the PP II and PP I were 45.0 and 70.0% w/w, respectively, while the PP III and PP IV showed lower weight increases (15.0 and 34.0% w/ w, respectively). Thus, the data indicate an increase of the hydration degree, and hence of the hydrophilic character, with increasing thickness of the IPN layer. The type of IPN layer appeared also to exert a significant effect on hydration: the more hydrophilic layer(s) favored absorption of greater amounts of water when compared with the less hydrophilic ones.

Mucoadhesion Tests

The results of the mucoadhesion tests, carried out on PCD, are reported in Fig. 2 as the work (W; J/cm2) required for detachment of two hydrated mucin surfaces between which was placed one insert. The first bar in the graph (Self) indicates the W value of the two mucin surfaces in absence of interposed insert, and represents the work of cohesion of hydrated mucin. The W values for PP III and PP IV were in the range 8.0-9.5 x 10^{-5} J/cm2, close to 10.75 10^{-5} J/cm2, the W value obtained with a reference polyacrilate disk. Since the mucoadhesive performances of polyacrylate are considered excellent, the data are indicative of the good mucoadhesive properties conferred to inserts by PMMA/Chitosan IPN. The mucoadhesive properties of the inserts increased significantly with increasing thickness of their IPN layers: the W values were 8.19 and 9.22 10^{-5} J/cm2, respectively for and PP III and PP IV, while they were 4.95 and 6.17 10^{-5} J/cm2, respectively for PP I and PP II. Such a dependence of mucoadhesion on thickness of IPN layer is probably related to the different composition gradients of IPN layers of different thickness. Statistically significant differences (P, 0.05) were observed among the mucoadhesion values measured for the different PCDs.

Oxytetracycline HCl (OXT) release in vitro

The in vitro release profiles of OXT from the different rod-shaped inserts are illustrated in Fig. 3 and 4. The release kinetics were analyzed using the semi empirical relationship $M_t/M_\infty = Kt^n$, where Mt/M_∞ is the fraction of drug released at time t, K is a constant, characteristic of the system and the exponent n is indicative of the release kinetics [13,14]. A value of $n = 0.5$ indicates the occurrence of Fickian diffusion, while $n = 1$ corresponds to zero-order kinetics. Values of n between 0.5 and 1 indicate anomalous (non-Fickian) transport. The main in vitro release parameters are reported in Table 2. Table 2 reports for each insert, in addition to the n and K values, the $t_{20\%}$ values (times required for release of 20% OXT) and the R20% values (instantaneous release rates at 20% released drug). Even if some calculated interpolation curves appeared to indicate a non-ideal fit, the relevant correlation coefficients (r) were in the range 0.967–0.996. The n values of the PP III and I, ranging from 0.440 to 0.482, are indicative of a 'quasi-fickian' release mechanism while in case of PP IV and PP II, n values ranging from 0.949 and 0.955, are indicative of near zero order release. Further inspection of the release data shows a dependence of the release rate on the NaCl content. A comparison, e.g., of inserts PP I and PP II, containing 10 and 20% NaCl respectively, indicates a faster release in the case of the latter insert. This effect is probably due to greater porosity induced in by the higher release modifier content. It can be speculated that NaCl in the matrices may exert two opposing actions: (i) a release-promoting effect, due to osmotically-activated formation of water-filled pores; (ii) a release-reducing effect resulting from the presence of the common Cl̃ion. Either effect might prevail depending on the amount (10 or 20%) of NaCl in the matrix. The presence of a PMMA IPN layer in general increased the drug release rate, as evidenced by the release profiles and the release data in Table 2.

In vivo retention study

Preliminary experiments showed that retention in rabbit eyes of inserts was significantly higher in case of PP I and PP II. Retention times longer then 4 days were observed for PP III and PP IV inserts in 70 and 80% of the cases, respectively. The reduced occurrence of expulsion from the conjunctival sac of PP I and PP II devices with respect to PP III and PP IV ones was presumably due to their inferior degree of swelling, as confirmed by the hydration profiles (Fig. 1), and consequently to a reduced increase in size.

In vitro minimum inhibitory concentration (MIC) activity

The MIC values (90%) of OXT observed in vitro against the micro-organisms responsible of common ocular infections range from 0.8 to 2.0 mg/ml, while MIC 90% values in the range 14-50 mg/ml have been indicated for *Pseudomonas aeruginosa* [15]. All of the presently described inserts would ensure a prolonged OXT release in tear fluid with 'plateau' concentration values 10/30-fold higher with respect to the MIC 90% for common micro-organisms. Based on analogous assumptions in the literature these concentrations should be sufficient to reach a therapeutic level [16,17].

Conclusion

An ideal chemotherapy for ocular bacterial infections, such as trachoma (a major cause of blindness in the rural populations of developing countries) would require a sustained, constant-rate delivery of antibiotics to produce concentrations significantly above the MIC of ocular pathogens. Indeed, in contrast to bacteria, *chlamydia trachomatis* has a long life cycle of about 3–4 days. The presently described mucoadhesive silicone inserts might likewise prove efficient platforms for delivery of antibiotics to the eye. As shown in this report, sustained release of OXT, zero-order release kinetics and prolonged retention in the conjunctival sac of rabbits could be obtained by suitably adjusting the matrix components and type of IPN layer.

Table 2: Oxytetracycline release parameters in vitro

PCRsI	n	K ($days^{-n}$)	$t_{20\%}$ (days)	$R_{20\%}$ ($days^{-n}$)
PP I	0.482	5.62	0.048	15.16
PP II	0.955	7.09	1.43	13.33
PP III	0.440	10.27	0.31	14.32
PP IV	0.949	6.69	1.69	11.16

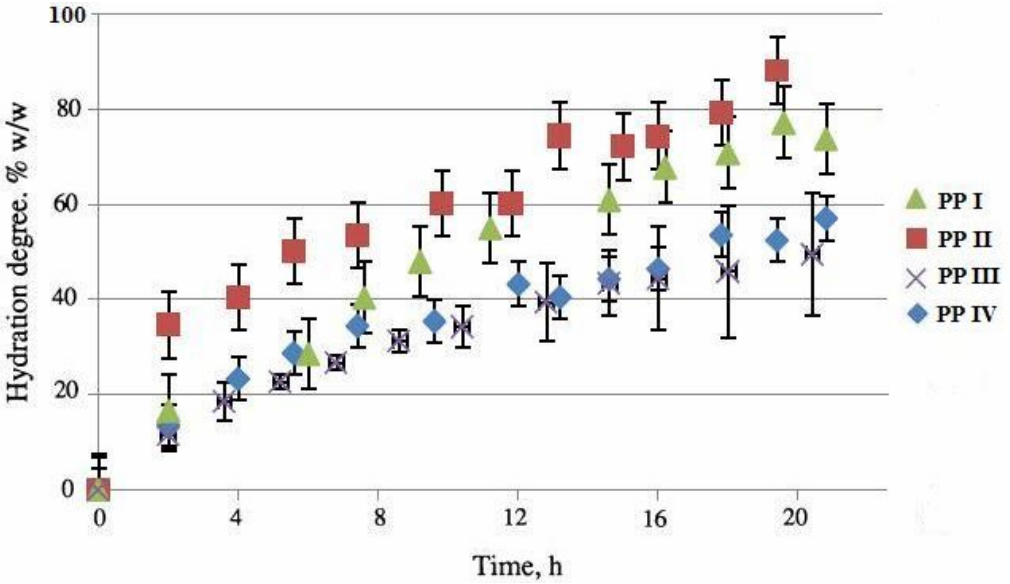

Fig. 1. Hydration vs. time profiles of various PCRsIs.

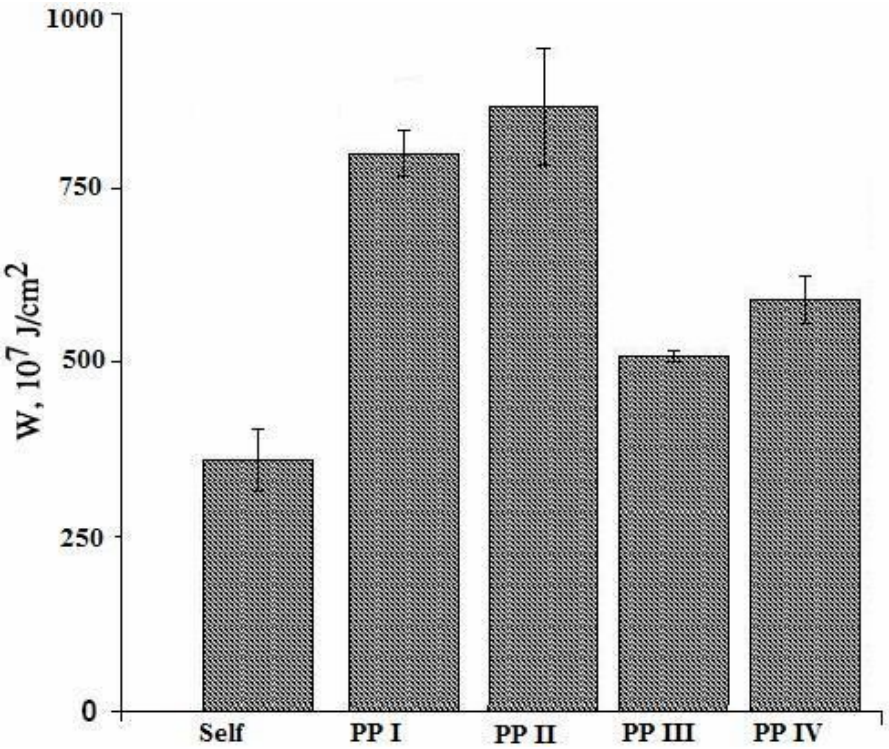

Fig. 2. Work of adhesion (W) of different disk-shaped inserts (PCDs) on a mucin substrate. The first bar (Self) indicates the work of self-adhesion of the substrate. Vertical bars represent SE (*n* = 6).

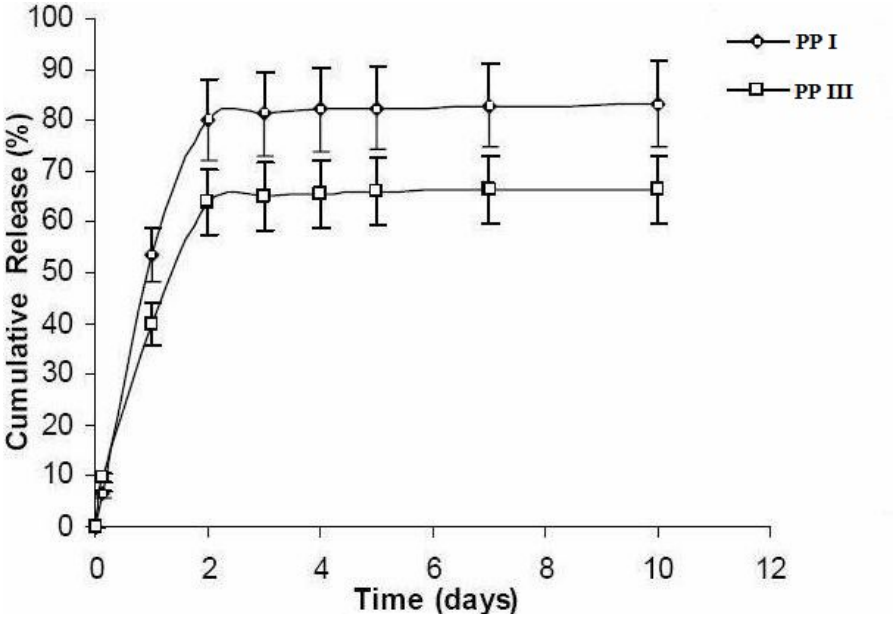

Figure. 3. In Vitro Release Kinetics of Oxytetracycline from PP I and PP III Rods.

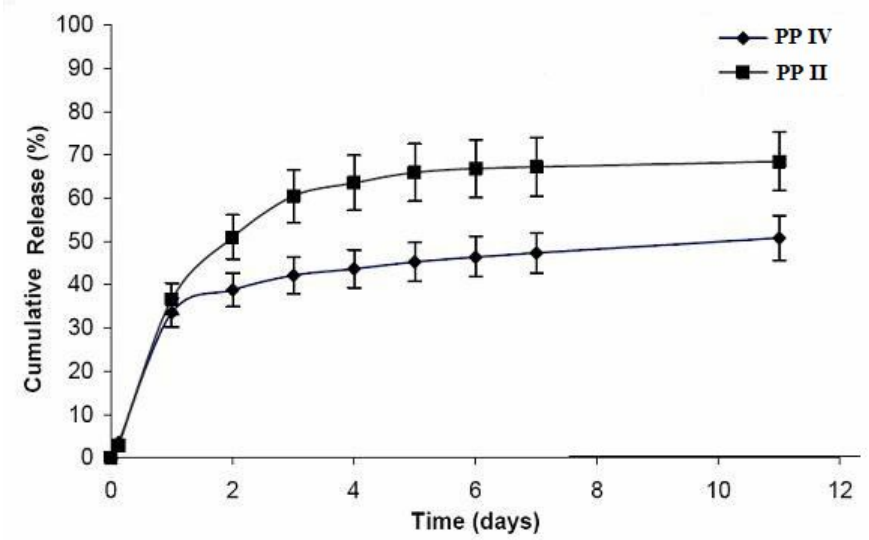

Figure 4. In Vitro Release Kinetics of Oxytetracycline from PP IV and PP II Rods

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