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In vitro Characterization of Physically Reinforced Ocular Inserts of Indomethacin

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Abstract

Physical reinforcements of polyvinyl alcohol (PVA; high - 125,000 and low - 14,000 molecular weights) based ocular inserts of indomethacin were conducted by subjecting to heat (80° C and 100° C for 24 and 48 hours) and freeze-thaw cycles (3 and 6 cycles). *In vitro* drug release was studied in a continous flow-through apparatus, and compared with the non-reinforced inserts.

The rate of indomethacin release was inversely proportional to the content of low molecular weight PVA (PVA 14,000). The duration of heating had more effect on release properties than the temperature and the release pattern of freeze thawed inserts showed lower drug release than the non-reinforced and heat treated inserts.

Key Words: Ocular inserts, Indomethacin; Heating and freeze-thawing

Introduction

Topically applied drops of ocularly used drugs are mostly eliminated rapidly from the pre-corneal area (Lee and Robinson, 1979), and only 1 to 2% of the instilled drug is bioavailable (Patton and Robinson,1976). To overcome the poor ocular bioavailability, many ocular drugs are frequently administered in large doses, resulting in an unusually high drug and preservative concentration and accumulation at the corneal epithelial surface. The present study was undertaken to design soluble ocular inserts of indomethacin using PVA, which has been extensively used in ocular drug delivery (Lai *et al.*, 1987, Saettone *et al.*, 1992, Suzuki,1994). The high aqueous solubility of PVA films is a major disadvantage, which reduces their apparent usefulness in controlled ocular delivery dosage forms. Since the usual chemical initiators for cross-linking of PVA gels cannot be removed easily, we adopted the physical methods of cross-linking-heating (Lim and Lucy, 1995) and freeze thawing (Peppas and Mongia, 1997) in our study, in an attempt to reduce the solubility of PVA and to control drug release.

Materials and Methods

Indomethacin and PVA (both high and low molecular weights) were obtained as gift samples from Jagsonpal Pharmaceuticals Ltd. (New Delhi, India) and S.D. Fine Chemicals Ltd. (Mumbai, India) respectively. All other reagents used were of analytical grade.

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Preparation of PVA inserts containing indomethacin: The inserts were prepared by solvent casting method. Solutions of PVA of different compositions of low and high molecular weights were prepared in hot distilled water. The mixture was stirred for 24 h to get a clear solution. This was then filtered through a 0.45μ membrane filter under vacuum. Then PEG 200 was added as the plasticizer to the cooled viscous polymer solution, and stirred for 6 h Weighed amounts of indomethacin were added after passing through sieve (mesh No 400) and stirred for 12 h to get a uniform dispersion. The dispersion was then degassed and casted on glass substrate and dried at 50° C for 36 h The dried films were carefully removed and elliptical shaped inserts of dimension 8x5 mm and average thickness of 0.2 mm were punched out, wrapped individually in aluminium foil and stored in well-closed vials till further use.

Preparation of physically reinforced PVA inserts by heating method: The drug loaded PVA films were prepared as described above. The dried films were then wrapped in aluminium foil and placed at 80° and 100° C for 24 h and 48h in a temperature controlled oven. Both the drug and PVA were reported to be stable under these conditions (Byron and Dalby, 1987). After cooling the films, inserts of the shape and size described above were punched out and wrapped in aluminium foil and stored at room temperature (30° C) till further use.

Fabrication of physically reinforced PVA inserts by freeze thaw method: Drug loaded films were prepared as described earlier and placed at -18° C for 18 h and then at 40° C for 6 h This was considered one freeze-thaw cycle. Likewise three and six cycles were performed for each composition. The films were taken out carefully and 8x5 mm inserts were punched out, wrapped in aluminium foil and stored in glass vials till further use.

Characterization of fabricated films

Thickness and weight variation: The thickness of the fabricated inserts was measured at 10 different randomly selected spots with a screw gauge and for weight variation 10 inserts were weighed individually.

Drug content uniformity: The inserts were weighed individually and dissolved in 50 ml of 0.2M phosphate buffer pH 7.4 by stirring for 6 h The solution was then filtered through G2 glass filter and an aliquot of the filtrate was diluted suitably and analyzed spectrophotometrically (Shimadzu, Japan) at 319.5 nm for indomethacin content.

Surface pH: The inserts were allowed to swell in closed petridishes at room temperature for 30 min. in 0.1 ml of bidistilled water. The swollen device was removed and placed on pH paper to determine the surface pH. After 60 sec. the colour developed was compared with the standard colour scale.

Water uptake studies: Swelling rate (water uptake) was determined by immersing the insert in a preweighed stainless steel basket in 20 ml of freshly boiled and cooled 0.2 M phosphate buffer (pH 7.4) at 37° C. The weights of the swelled inserts were determined at specified time intervals and the relative weight gain (water uptake) was calculated using the following relation (Bottenberg et al., 1992, Kim et al., 1996):

Relative weight gain (water uptake) = $(SW_2-Sw_1)/SW_0$ where Sw_1 is the weight of the stainless steel wire basket and insert, Sw_2 is the weight of the swollen insert and the basket, and Sw_0 is the initial (unswollen) weight of the insert. Equilibrium water uptake (EWU) of the insert was determined directly from the water uptake versus time curve (Vyavahare et al., 1990).

In vitro release studies: The inserts were evaluated for drug release kinetics by using a continuous flow-through apparatus, which mimics the continuous flow of tear to a certain extent, but the constant blinking action of the eye was not attempted to be simulated. The apparatus, fabricated locally, consisted of 2 circular plates of acrylic material (3.8 cm diameter and 1.2 cm thick) fitted together with the help of three screws. The bottom plate was provided with a circular groove of 1.7 cm diameter and 6 mm deep fitted with a mesh (No 80) mesh for supporting the insert and was also provided with an outlet tube for collecting the eluate. The top plate was also provided with a hole, for the inlet of the buffer (0.2 M phosphate buffer, pH

7.4). The whole unit was connected from the top 1-mm (i.d.) silicone tubing to a peristaltic pump for introducing the buffer maintained at 37 ± 0.2 °C. The pump was programmed to introduce the buffer through the top of the assembled cell at the rate of 0.80±0.5 ml / h.
For the drug release studies, one insert was weighed, covered with Whatman filter paper (1 cm^2, pre-equilibrated with the phosphate buffer) and the whole was covered with a stainless steel wire mesh (No 80). This set up was used to simulate the insert when kept in between the cornea and the eyelid as *in vivo*. This was placed in the center of the bottom plate and the components of the unit were screwed together. The peristaltic pump was stared, and the eluates were collected in amber coloured glass vials as a function of time. The eluates were analysed spectrophotometrically for indomethacin content as described earlier. After 10 h of release study, the residual drug from the insert was determined by stirring the residual inserts in 10 ml buffer for 6 h and the contents were filtered through G2 filter and analyzed by UV.

Results and discussion

The formulation variables of the various batches of inserts prepared and their physico-chemical properties are shown in Tables 1 (A and B) and 2. The surface pHs of prepared inserts were between 5.75 to 7.25. This showed that the prepared inserts would not alter the pH of the tear fluid in the eye.

Table 1A. Formulation variables of non-reinfoced inserts

Batch code	%PVA 14,000	%PVA 125,000
PA	0	100
PB	15	85
PC	25	75
PE	50	50
PF*	50	50
PH	75	25
PI	85	15
PJ	100	0

Table 1B. Physical reinforcement parameters with batc code

Composition (%PVA 125,000: %PVA 14,000)	Temp.(^{0}C)/Time (h)				Freeze thawing cyles numbers	
	80/24	80/48	100/24	100/48	3	6
100:0	PA 801	PA 802	PA 1001	PA 1002	AFT 3	AFT 6
85:15	PB 801	PB 802	PB 1001	PB 1002	BFT 3	BFT 6
75:25	PC 801	PC 802	PC 1001	PC 1002	CFT 3	CFT 6
50:50	PE 801	PE 802	PE 1001	PE 1002	EFT 3	
50:50	PF 801	PF 802	PF 1001	PF 1002		
25:75	PH 801	PH 802	PH 1001	PH 1002		
15:85	PI 801	PI 802	PI 1001	PI 1002		
0:100	PJ 801	PJ 802	PJ 1001	PJ 1002		

801,802,1001,1002 indicates that respective batches were subjected to heating temperatures of 80 for 24 and 48 h and 100 for 24 and 48 h.

Table 2 Physico-chemical properties of the prepared inserts.

Batch code	Weight uniformity (mg ± SD)	Thickness uniformity (mm ± SD)	Content uniformity (mg ± SD)	Surface pH	EWU *
PA	16.54 ± 0.75	0.232 ± 0.007	1.14 ± 0.085	6.83	2.492
PB	14.91 ± 0.75	0.238 ± 0.005	1.59 ± 0.125	6.76	2.580
PC	18.56 ± 0.73	0.265 ± 0.004	1.52 ± 0.102	6.56	2.340
PE	14.92 ± 1.4	0.251 ± 0.002	2.10 ± 0.85	6.13	2.750
PF	16.98 ± 1.64	0.244 ± 0.160	1.85 ± 0.004	7.13	1.620
PH	14.9 ± 0.80	0.216 ± 0.005	1.41 ± 0.136	6.60	NE
PI	16.87 ± 1.9	0.238 ± 0.008	1.24 ± 0.114	6.40	NE
PJ	16.6 ± 0.56	0232 ± 0.005	1.31 ± 0.028	6.50	NE
PA 801	13.90 ± 0.53	0.248 ± 0.064	1.43 ± 0.037	7.25	2.266
PA 802	17.80 ± 0.32	0.255 ± 0.038	1.28 ± 0.029	7.0	2.084
PA 1001	14.30 ± 0.43	0.252 ± 0.050	1.33 ± 0.081	6.90	2.236
PA 1002	13.50 ± 0.86	0.262 ± 0.043	1.33 ± 0.072	7.0	1.988
PB 801	15.73 ± 0.50	0.249 ± 0.007	1.53 ± 0.057	6.50	1.928
PB 802	12.14 ± 0.35	0.242 ± 0.005	1.37 ± 0.087	6.67	1.693
PB1001	16.02 ± 1.21	0.247 ± 0.010	1.26 ± 0.064	6.50	1.863
PB 1002	14.04 ± 0.32	0.221 ± 0.009	1.32 ± 0.088	6.50	1.673
PC 801	14.23 ± 0.96	0.280 ± 0.010	1.59 ± 0.115	6.25	1.963
PC 802	15.27 ± 0.60	0.259 ± 0.006	1.44 ± 0.080	6.50	1.717
PC 1001	17.19 ± 0.73	0.268 ± 0.007	1.50 ± 0.070	5.75	1.870
PC 1002	18.18 ± 0.58	0.270 ± 0.010	1.51 ± 0.022	5.50	1.648
PE 801	18.47 ± 0.92	0.260 ± 0.007	1.38 ± 0.078	7.0	2.488
PE 802	20.0 ± 0.98	0.259 ± 0.009	1.33 ± 0.031	6.50	2.168
PE 1001	19.70 ± 0.50	0.270 ± 0.010	1.14 ± 0.095	6.50	2.382
PE 1002	18.56 ± 1.23	0.275 ± 0.009	1.16 ± 0.099	6.0	2.262
PF 801	19.05 ± 1.15	0.264 ± 0.013	1.65 ± 0.029	5.90	NE †
PF 802	21.58 ± 1.26	0.303 ± 0.016	1.47 ± 0.169	5.90	NE
PF 1001	16.64 ± 0.41	0.263 ± 0.019	1.64 ± 0.130	6.0	NE
PF 1002	16.34 ± 0.84	0.230 ± 0.019	1.62 ± 0.023	5.90	NE
PH 801	18.40 ± 0.27	0.255 ± 0.017	1.53 ± 0.161	7.0	NE
PH 802	16.57 ± 0.54	0.250 ± 0.018	1.29 ± 0.102	6.50	NE
PH 1001	17.63 ± 0.88	0.274 ± 0.014	1.27 ± 0.007	6.25	NE
PH 1002	16.95 ± 0.89	0.228 ± 0.010	1.26 ± 0.057	6.50	NE
PI 801	17.47 ± 0.24	0.266 ± 0.018	1.15 ± 0.019	6.50	NE
PI 802	19.11 ± 0.59	0.251 ± 0.017	1.10 ± 0.082	6.50	NE
PI 1001	18.04 ± 0.78	0.247 ± 0.009	1.01 ± 0.016	6.50	NE
PI 1002	19.74 ± 0.76	0.283 ± 0.014	1.05 ± 0.045	6.50	NE
PJ 801	17.12 ± 0.06	0.205 ± 0.008	1.47 ± 0.078	6.50	NE
PJ 802	18.28 ± 0.81	0.273 ± 0.011	1.11 ± 0.011	6.50	NE
PJ 1001	19.90 ± 0.89	0.289 ± 0.018	1.16 ± 0.20	6.50	NE
PJ 1002	18.18 ± 0.95	0.268 ± 0.006	1.21 ± 0.047	6.50	NE
AFT3	16.81 ± 0.66	0.206 ± 0.010	1.48 ± 0.063	6.50	2.550
AFT6	19.34 ± 0.56	0.266 ± 0.081	1.44 ± 0.115	6.0	2.460
BFT3	20.93 ± 0.80	0.319 ± 0.007	1.60 ± 0.099	6.50	NE
BFT6	23.63 ± 0.78	0.316 ± 0.008	1.38 ± 0.086	6.0	NE
CFT3	22.27 ± 0.37	0.308 ± 0.006	1.98 ± 0.091	6.0	NE
CFT6	20.78 ± 0.99	0.254 ± 0.021	1.51 ± 0.113	6.25	NE
EFT3	18.6 ± 0.34	0.261 ± 0.010	1.53 ± 0.031	6.75	NE

NE – Not estimated because there was no declining phase within 75 minutes.
EWU - Equlibrium water uptake

Table 3 Effect of physical reinforcement on *in vitro* drug release.

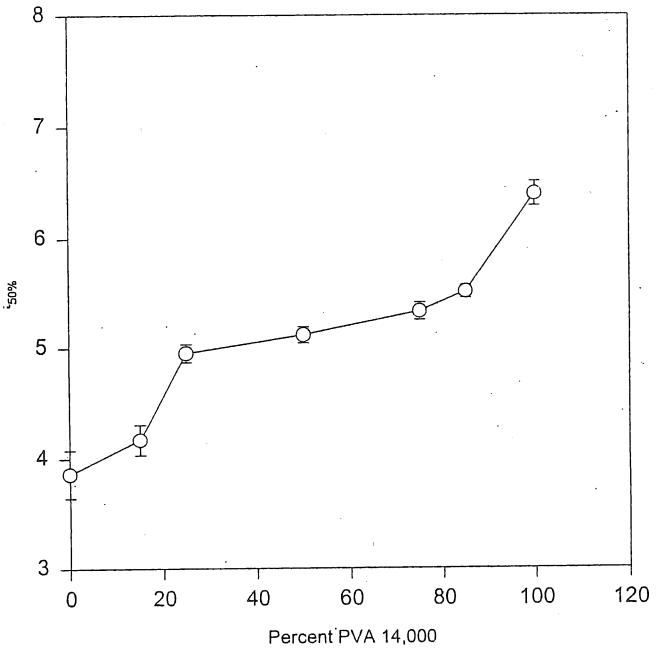
Batch code	% Drug release at the end of 10 hours	$t_{50\%}$(hrs)
PA 801	82.26 ± 0.071	4.375
PA 802	81.39 ± 2.86	5.250
PA 1001	82.06 ± 0.37	4.583
PA 1002	76.51 ± 1.74	6.083
PB 801	80.92 ± 0.24	4.291
PB 802	66.06 ± 1.19	6.833
PB1001	76.84 ± 2.50	5.0
PB 1002	60.17 ± 1.47	7.667
PC 801	76.77 ± 2.13	5.140
PC 802	68.28 ± 1.12	7.330
PC 1001	72.28 ± 1.53	6.580
PC 1002	57.10 ± 2.10	8.880
PE 801	74.67 ± 1.93	5.250
PE 802	66.0 ± 0.11	7.125
PE 1001	68.29 ± 1.04	5.250
PE 1002	59.09 ±2.41	7.330
PF 801	58.46 ± 2.52	7.500
PF 802	48.20 ± 2.50	-
PF 1001	55.74 ±1.80	8.917
PF 1002	44.38 ± 0.84	-
PH 801	67.05 ± 0.23	5.833
PH 802	61.95 ± 2.63	6.583
PH 1001	66.89 ± 2.45	5.917
PH 1002	58.53 ± 1.91	8.0
PI 801	63.21 ± 0.17	6.250
PI 802	61.36 ± 2.36	6.917
PI 1001	63.54 ± 0.20	6.417
PI 1002	61.02 ± 1.39	7.417
PJ 801	60.52 ± 1.12	7.333
PJ 802	56.52 ± 1.98	8.167
PJ 1001	57.10 ± 2.52	8.167
PJ 1002	55.84 ± 2.30	8.500
AFT3	83.56 ± 0.88	4.750
AFT6	75.03 ± 2.10	5.833
BFT3	77.78 ± 2.30	5.583
BFT6	39.04 ± 0.23	-
CFT3	71.20 ± 1.77	6.333
CFT6	38.02 ± 0.97	-
EFT3	54.91 ± 1.12	8.583

In vitro studies

Non-reinforced inserts: The studies indicated that the inserts containing high proportions of PVA 14.000 showed an initial burst effect and demonstrated a first order release with r^2 values of 0.9971, 0.9965, 0.9861 and 0.9943 for batches PE, PH, PI and PJ, respectively, while the release from batches containing high proportions of PVA (125.000) showed zero order release, with r^2 values for batches PA and PB being 0.9824 and 0.9826, respectively.

The drug release from the batches containing higher proportions of PVA 14,000 was comparatively less than the batches containing higher proportions of PVA 125.,000 (Fig.1)

Fig. 1. Effect of PVA 14.000 on drug release from the prepared inserts



The initial burst effect seen in batches PE, PH, and PI could be attributed to the more soluble nature of PVA 125.000, which was present in lesser quantities. At later stages, the release from these batches was controlled by the less soluble PVA 14.000 matrix.

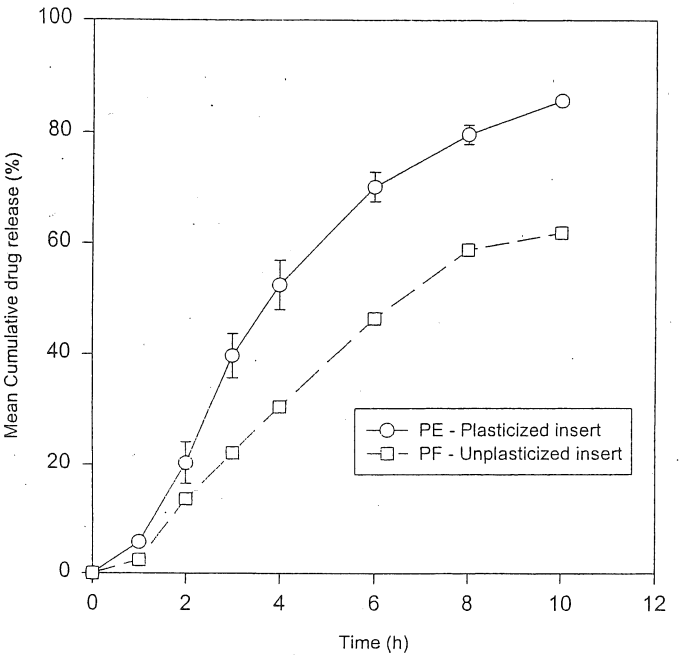
Inserts reinforced by heating: Heat reinforced inserts exhibited a slower release, as evidenced from the T_{50} values (Table 3) when compared to the corresponding non-reinforced inserts in all the cases. The decrease observed in the release rate might be due to the formation of crystallites, which increased the water resistance of the polymer. The results indicated that the duration of heating had more effect on release properties than the temperature in all the cases.

Inserts reinforced by freeze thawing: The inserts were subjected to 3 and 6 freeze-thaw cycles to study the extent of freeze-thawing on the drug release. The results indicated that the drug release from the freeze-thawed insert was slower than the corresponding heat treated and non-reinforced inserts. The drug release in the case of freeze-thawed inserts decreased with an increase in the proportions of PVA 14,000 present in the inserts. Freeze-thawing of PVA increased the contact angle, which in turn decreased the surface area, thus decreasing drug release (Peppas and Mongia, 1997).

Effect of plasticizer on drug release: The plasticizer is the most important formulation factor that may affect the mechanical properties of the films as it shifts the glass transition

temperature to lower levels. Thus, in the present study, drug releases from unplasticized inserts were compared to the corresponding plasticized inserts (PE). The results indicated that the plasticized inserts released 85.65% of the drug in comparison to 61.82 % by the unplasticized insert (PF) (Fig. 2).

Fig. 2. Effect of plasticizer on in vitro drug release from the inserts



In case of batches PE and PF, both PVA 14,000 and PVA 125.000 were present in equal proportions and the initial burst effect observed in both cases can be attributed to the more soluble PVA 125.000. The fact that the drug release from the batch PE was much higher than PF clearly indicated that the presence of PEG 200 in PE was responsible for increasing the hydrophilicity of PVA 14.000 matrix. The increase in the hydrophilicity resulted in enhanced swelling and the consequent increase in the porosity of the matrix, accounting for the higher drug release. Similar behavior was observed in the case of the corresponding heat-treated and freeze-thawed inserts.

Conclusion

The indomethacin insert provided an initial phase of high release followed by a phase of moderate release. Reinforcement by freeze-thawing was found to be more effective in sustaining the release of indomethacin. Thus the freeze thawed inserts could probably form the basis of once a day application of indomethacin and other probable ocular drugs.

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