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Effect of Physical Cross-linking on *In vitro* and *Ex vivo* Permeation of Indomethacin from Polyvinyl Alcohol Ocular Inserts

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Polymeric ophthalmic inserts containing indomethacin were formulated with combinations of two different types of polyvinyl alcohol (high-1, 25,000 and low-14, 000 molecular weights) and physically reinforced by heating (80° and 100° for 24 and 48 h) and freeze-thawing (3 and 6 cycles). *In vitro* drug release and permeation kinetics across goat cornea was studied in a continuous flow-through apparatus and a modified Keshary-Chien cell, respectively, and compared with the non-reinforced inserts. The rate of indomethacin release was inversely proportional to low molecular weight polyvinyl alcohol content. The duration of heating had more effect on drug release than the temperature and freeze thawing was more successful in retarding the drug release. The permeation of indomethacin correlated well with the *in vitro* release.

Liquid and semi solid ocular medications are mostly rapidly removed from the absorption area[1], and only 1-2% of the administered dose is bioavailable[2]. Improved bioavailability is possible through ocular inserts but these suffer from poor patient acceptability. Soluble inserts provide somewhat longer duration of drug release and are more patient friendly due to their unique physical nature. In a search for ensuring a prolonged time of residence of the medication in the eye and, possibly, a controlled release, the present study was undertaken to design polyvinyl alcohol based soluble ocular inserts of indomethacin. Polyvinyl alcohol has been extensively used in ocular drug delivery[3,4]. However, 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 two physical methods of cross-linking - heating[5] and freeze thawing[6] in our study, in an attempt to control drug release from soluble polyvinyl alcohol inserts containing indomethacin.

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MATRIALS AND METHODS

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

Fabrication of inserts:

The inserts were fabricated by solvent casting method. Solutions of PVH and PVL of different compositions (Table 1) were prepared in hot distilled water. The mixture was stirred for 24 h to get a clear solution, and then filtered through a 0.45 μ membrane filter under vacuum. PEG 200 was added as plasticizer[7] to the cooled viscous polymer solution, and stirred for further 6 h. Weighed amounts of indomethacin (passed through sieve #400) was added and stirred for 12 h to get a uniform dispersion. The dispersion was then degassed and casted on glass substrate and dried at 50° for 36 h. The dried films were carefully removed and elliptically shaped inserts of dimension 8x5 mm and average thickness of 0.2 mm were punched out, wrapped individually in aluminium foil and stored at room temperature in

TABLE 1: COMPOSITION OF NON-REINFORCED INSERTS.

Batch code	% PVL	% PVH
PA	0	100
PB	15	85
PC	25	75
PE	50	50
PF*	50	50
PH	75	25
PI	85	15
PJ	100	0

* Does not contain any plasticizer, PVL and PVH represent the low and high molecular weight polyvinyl alcohol, respectively.

well-closed amber vials till further use.

Physical reinforcement of inserts by heating (Method I):

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° for 24 h and 48 h in a forced circulation oven. Both indomethacin and polyvinyl alcohol[8] are reported to be stable under these conditions. After cooling, inserts of the shape and size described above were punched out and wrapped in aluminium foil and stored at room temperature (30°) till further use.

Physical reinforcement of inserts by freeze-thawing (Method II):

Drug loaded films were prepared as described earlier and placed first at −18° for 18 h and then at 40° for 6 h. This was considered one freeze-thaw cycle. Inserts of each composition were subjected to 3 and 6 such cycles. Afterwards, the films were taken out carefully and 8x5 mm inserts were punched out, wrapped individually in aluminium foil and stored in amber glass vials at room temperature till further use.

Characterization of fabricated films:

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. To determine drug content uniformity the inserts were weighed individually and dissolved in 50 ml of Phosphate buffer pH 7.4 (0.2 M) 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 of the inserts were determined by allowing them to swell in closed petridish at room temperature for 30 min in 0.1 ml of double distilled water. The swollen devices were removed and placed on pH paper to determine the surface pH. After 60 s the colour developed was compared with the standard colour scale. Swelling rate (water uptake) was determined by immersing the insert in a preweighed stainless steel basket in 20 ml of freshly boiled and cooled phosphate buffer pH 7.4 (0.2 M) at 37°. The weights of the swelled inserts were determined at specified time intervals and the relative weight gain (water uptake) was calculated using the following relationship[9,10]. Relative weight gain (water uptake)= Sw_2-Sw_1/Sw_0, Where, Sw_1 is weight of the stainless steel wire basket and insert, Sw_2 is weight of the swollen insert and the basket, and Sw_0 is the initial weight of the insert. Equilibrium water uptake (EWU) of the insert was determined directly from the water uptake versus time curve[11].

***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 designed by us consisted of 2 circular plates of acrylic material (3.8 cm diameter and 1.2 cm thickness) fitted together with the help of three screws. A circular bottom plate (1.7 cm diameter and 6 mm deep) was fitted with a #80 mesh for supporting the insert and was also provided with an outlet tube for collecting the eluate. The top plate was provided with a hole, for introducing the buffer (phosphate buffer pH 7.4-0.2 M). The whole unit was connected from the top through 1-mm i.d. silicone tubing to a peristaltic pump and the buffer container maintained at 37±0.2° (fig. 1). The pump was programmed to introduce the buffer at a rate of 0.80 ml/h.

In the drug release study, one insert was weighed, and placed on the wire mesh support of the bottom plate. A Whatman filter paper circle (1 cm^2 area) was then placed over the insert, and the components of the unit were screwed together. The peristaltic pump, connected with the thermostated buffer (37±0.2°) was started, and the eluate was collected in amber coloured glass vials as a function of time. The eluates were analyzed spectrophotometrically for

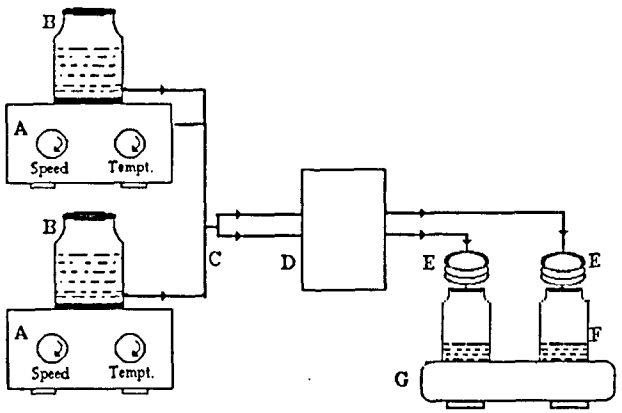

Fig. 1: Schematic representation of *in vitro* continuous 'flow through' apparatus.

The flow through apparatus consisted of two stirrers with thermostatic control (A) on which the buffer reservoirs (B) are placed. The reservoirs are connected with silicone tubing (C) to the Miclins peristaltic pump (D), which in turn is connected to the diffusion cells (E). The diffusion cells are linked to the Receptor vials (F), which are placed on a platform (G).

indomethacin content as described earlier. After 10 h of release study, the residual drug in 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 spectrophotometrically.

Preparation of cornea from excised goat eye for permeation studies:

The entire eye bulbus from freshly slaughtered goat (within 30 min) were removed, rinsed in fresh saline and kept in the same fluid at 4°. The conjuctiva and eye muscles were removed with fine iris scissors. A small transverse incision was made posteriorly from the limbus and subsequently, the cornea with 2-mm radius scleral ring was cut out. Care was taken to avoid contamination of the epithelial surface with blood and physical trauma to the tissue. The lens and the iris were carefully removed, leaving the cornea as a transparent film. The cornea was then placed in phosphate buffer pH 7.4 containing 0.1 % sodium azide as preservative.

Drug permeation studies:

Immediately after preparation of the cornea the permeation studies were started on a modified vertical Keshary-Chien cell. The cornea was fixed between the two halves of the cell in a wrinkle free manner, so that the endothelial side faced the buffer in the receptor chamber. A small star headed magnetic bead was used to maintain the hydrodynamics of the medium. An insert was gently placed on the cornea and above that a small circular aluminum film of 2.5-cm diameter was placed to avoid rolling of the insert. The two half-cells were held together with the help of low-tension soft rubber bands to maintain the normal curvature of the cornea throughout the experiment. The fitted permeation cell was placed in a thermostatic water bath (37±0.2°). At predetermined time intervals the whole buffer solution from the receptor cell was collected and replaced with the same volume of fresh pre-warmed buffer. Drug release was measured spectrophotometrically as described earlier.

Determination of apparent corneal permeability:

The apparent corneal permeability coefficient was determined according to the formula[12,13] $P_{app}=\delta Q/\delta t.60.60.A.C_0$, where, Q is the total amount permeated at time t, $\delta Q/\delta t$ (slope of the linear portion of the graph) is the steady state flux (permeation rate) of indomethacin to the receiver side (μg/h), 60x60 is the conversion of hours to seconds, A is the corneal surface area (1.259±0.0123 cm^2, in the study) and C_0 is the amount of the drug present in the insert (μg). The surface area of the cornea was determined by making an incision on the cornea and flattening it on a plastic sheet and drawing a continuous line along the limbus. Thereafter, the area was determined from the plastic sheet by cut and weigh method. The permeation parameters were calculated from the mean values (n=3).

RESULTS AND DISCUSSION

The formulation variables of the various batches of prepared inserts are shown in Tables 1 and 2. The variation in thickness, weight uniformity and drug content of the prepared inserts were within accepted limits. The surface pH of the prepared inserts were between 5.75 to 7.25. This shows that the prepared inserts would not alter the pH of the tear fluid in the eye.

The release studies indicated that the inserts containing high proportions of PVL 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 PVH showed zero order release, with r^2 values for batches PA and PB being 0.9824 and 0.9826, respectively.

Previously Takamura *et al.*[14] have reported matrix diffusion kinetics from polyvinyl alcohol gels containing

TABLE 2: PHYSICAL REINFORCEMENT CONDITIONS WITH BATCH CODES.

Composition	Method I (Heating)				Method II (Freeze-thawing)	
(% PVH: %PVL	80°/24	80°/48	100°/24	100°/48	3 cycles	6 cycles
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	PA 801	PA 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		

* The digit sequence represents the temperature and duration of heating: thus, 80 and 100 represent the temperatures and the last digit (1 and 2) represents the duration (24 and 48 h, respectively) of heating.

flurbiprofen. The reason attributed by them for such behaviour was due to the presence of sodium alginate and Pluronic L-62 used by them to retard drug release from the gels. Moreover, the type of dissolution set up used by us in this study allows the dissolution medium to flow through the device at a constant rate, unlike the dissolution set up used by Takamura *et al.* and other investigators where the device was placed in contact with a large volume of the dissolution medium which favours a diffusion controlled mechanism as a result of swelling/erosion of the polymeric matrix.

The drug release from the batches containing higher proportions of PVL was comparatively less than the batches containing higher proportions of PVH (Table 3). The initial burst effect seen in batches PE, PH and PI could be attributed to the more soluble nature of PVH, which was present in lesser quantities. At later stages, the release from these batches was controlled by the less soluble PVL matrix.

Heat reinforced inserts exhibited a slower release, as evidenced from the T_{50} values (Table 4) 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 increases the water resistance of the polymer[5]. The results indicate that the duration of heating had more effect on release properties than the temperature in all the cases.

Drug release from the freeze-thawed inserts was slower than the corresponding heat treated and non-reinforced inserts, and decreased with an increase in the proportions of PVL in the inserts. Freeze-thawing of polyvinyl alcohol increases the contact angle, which in turn decreases the surface area, thus decreasing drug release[6].

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 release from unplasticized insert was compared to the corresponding plasticized insert (PE). The results indicated that the plasticized insert released 85.7% of the drug in comparison to 61.8% by the

TABLE 3: EFFECT OF CONCENTRATION OF PVL AND PLASTICIZER ON DRUG RELEASE.

Batch code	% released at the end of 10 h (mean±SD)	$t_{50\%}$, h
PA	92.4±3.66	3.85
PB	85.2±2.48	3.85
PC	79.8±3.76	5.00
PE	85.7±0.77	3.83
PF	61.8±0.18	6.58
PH	70.6±0.23	4.95
PI	66.2±0.07	5.50
PJ	61.4±3.78	6.41

The compositions of the batches (non reinforced inserts) are shown in Table 1.

unplasticized insert (Table 3).

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

In case of non-reinforced inserts the permeation rate of the inserts containing high proportions of PVH were higher and the rate decreased with an increase in the proportion of PVL (Table 5). The effect of PEG 200 on the permeation

TABLE 4 : EFFECT OF PHYSICAL REINFORCEMENT ON *IN VITRO* DRUG RELEASE.

Batch code	% drug release at end of 10 h (mean±SD)	$t_{50\%}$(h)
PB 1002	60.2±1.47	7.67
PC 802	68.3±1.12	7.33
PC 1002	57.1±2.10	8.88
PE 802	66.0±0.11	7.13
PE 1002	59.1±2.41	7.33
PF 801	58.5±2.52	7.50
PF 802	48.2±2.50	-
PF 1001	55.7±1.80	8.92
PF 1002	44.4±0.84	-
PH 1002	58.5±1.91	8.00
PI 1002	61.0±1.39	7.42
PJ 801	60.5±1.12	7.33
PJ 802	56.5±1.98	8.17
PJ 1001	57.1±2.52	8.17
PJ 1002	55.8±2.30	8.50
AFT3	83.6±0.88	4.75
BFT6	39.0±0.23	-
CFT6	38.0±0.97	-
EFT3	54.9±1.12	8.58

- indicates that 50% drug release was not attained during the course of the release studies. The inserts having $t_{50\%}$ of less than 7 hours are not shown in the table (batches PA801-PA1002, PB801-PB1001, PC801, PC 1001, PE801, PE1001, PH801-PH1001, PI801-PI1001, AFT3, AFT6, BFT3, CFT3).

TABLE 5: *EX VIVO* PERMEATION DATA OF THE INSERTS.

Batch code	Steady State flux (permeability rate μg / ml)	P_{app} (x 10^{-6} cm / sec)
PA	111.8	16.48
PB	112.5	16.57
PC	111.3	16.39
PE	120.0	17.68
PF	97.4	14.34
PH	97.6	14.37
PI	90.7	13.36
PJ	87.5	12.89
PA 1002	94.7	13.95
PB 1002	100.7	14.83
PC 1002	92.3	13.60
PE 1002	87.7	12.91
PF 1002	57.5	8.46
PH 1002	86.6	12.75
PI 1002	89.1	13.13
PJ 1002	85.8	12.63
AFT3	94.8	13.96
BFT3	75.0	11.05
CFT3	50.2	7.43
EFT3	48.2	7.10
AFT6	81.6	12.01
BFT6	58.9	8.67
CFT6	39.8	5.80

P_{app} is the permeation co-efficient. The composition of the inserts are given in Tables 1 and 2.

rate across excised goat cornea was evaluated using batches PE and PF. The permeation of the drug from the unplasticized insert was lower than the plasticized insert. This may be due to the leaching out of the plasticizer and formation of pores through which the drug diffuses out. The insert that was subjected to heating at 100° for 48 h was selected for permeation studies in order to know the effect of heat reinforcement on permeation. The permeation of heat reinforced inserts was lower than the corresponding non-reinforced inserts. It also further ascertained the fact that permeation decreased with increase of PVL in the inserts. In case of freeze-thawed inserts similar results were obtained and the permeation rate decreased with an increase in the number of freeze-thaw cycles.

Drug migration from the polyvinyl alcohol matrix to the surrounding medium is a composite process of slow dissolving of the matrix and diffusion of the drug through it. The process of matrix dissolution can be slowed down by cross-linking. The surface pH along with the dissolution characteristics of the inserts was monitored on a monthly basis for six months upon storing the prepared inserts at room temperature. The results showed that there was no significant change either in the surface pH or in the drug release profiles of the prepared inserts (data not shown).

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 form the basis of once a day application of indomethacin and other ocular drugs.

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