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In vitro and *in vivo* characterization of scleral implants of indomethacin

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Scleral implants of indomethacin with sodium alginate as carrier were fabricated and evaluated for various physico-chemical properties such as uniformity of thickness, weight, drug content, surface pH, percent dissolution and water up-take capacity (swelling index). The effect of drug particle size, polymer concentration, drug loading, plasticizer concentration, and effects of physical reinforcement (freeze-thawing for 3 and 6 cycles) and chemical cross-linking with calcium chloride, on the *in vitro* drug release characteristics were evaluated. Selected batches of the implants were subjected to pharmacodynamic studies, after scleral placement, in uveitis induced (intravitreal injection of Bovine Serum Albumin (BSA)−50 μg/ml) rabbit eyes. The release of indomethacin from the prepared implants followed predominantly matrix diffusion kinetics. Swelling and moisture absorption/loss studies correlated well with the *in vitro* release studies. The pharmacodynamic studies showed a marked improvement in the various clinical parameters (congestion, keratitis, flare, clot, aqueous cells and synechias), in the implanted eye when compared to the control eye in the rabbits.

1. Introduction

A major problem encountered with the topical delivery of ophthalmic drugs is the rapid pre-corneal loss caused by drainage and tear turnover. Hence, typically less than 5% of the instilled drug penetrates the cornea and reaches the intraocular tissues, while a major fraction of the instilled dose is often absorbed systemically via the conjunctiva and nasolacrimal duct [1].

Potent immuno-suppresant therapy in transplant patients and the developing epidemic of AIDS have generated an entirely new population of patients suffering from virulent uveitis and retinopathies.

Uveitis can occur as an ocular manifestation of a variety of auto-immune diseases such as juvenile rheumatoid arthritis, Reiter's syndrome, inflammatory bowel diseases [2] and sarcoidosis [3], frequently leading to blindness [4]. It can be treated with topical or systemic steroids, but frequently recurs after discontinuation of therapy [4, 5]. Complications of topical steroids include cataract formation, poor wound healing, toxicity to corneal epithelium and increased intra ocular pressure [6]. Complications arising from systemic administration of steroids are varied and often extremely unpleasant [7]. To overcome the disadvantages of steroid administration, NSAIDs such as indomethacin have been investigated. Historically, most of the research has been aimed at drug delivery to the anterior tissues of the eye. Only recently, research has been directed at delivery to the tissues of the posterior globe (uveal tract, vitreous, choroid and retina) [8–10].

The conventional ophthalmic dosage forms are no longer sufficient to combat these diseases. Barriers presented by the cornea, lens and rapid aqueous turnover make it very difficult to achieve therapeutic drug concentration in the vitreous after topical administration. After systemic administration, the tight junctions between epithelial cells reduce drug availability to the aqueous, and vitreous availability is reduced by tight junctions of retinal pigmented epithelial cells and between endothelial cells of retinal capillaries [11]. Plasma binding of many drugs further lowers their penetration from systemic circulation to the eye [12].

The use of implants, to be placed sclerally by minor surgery, represents a possibility to increase residence time. Thus the present study was undertaken to develop scleral implants of indomethacin using a bio-degradable carrier (sodium alginate) and to characterize their *in vitro* behavior and pharmacodynamic efficacy in uveitis induced rabbit eyes.

2. Investigations, results and discussion

The formulation variables and the physico-chemical characteristics of the prepared implants are shown in Tables 1 and 2 respectively.

Table 1: Formulation variables of the prepared implants

Batch Code	Drug/implant (mg)	Glycerol concentration (% w/w)	PEG 200 concentration (% w/w)	Freeze-thaw cycles	Calcium chloride concentration (% w/v)
MT_1*	1	10	NIL	–	–
MT_2	1	10	NIL	–	–
MT_3	1	10	NIL	–	–
MT_4	1	12.5	NIL	–	–
MT_5	1	15	NIL	–	–
MT_6	1	NIL	8	–	–
MT_7	1	NIL	10	–	–
MT_8	1	NIL	5	–	–
MT_9**	1	10	NIL	–	–
MT_{10}***	1	10	NIL	–	–
MT_{11}	0.75	10	NIL	–	–
MT_{12}	1.25	10	NIL	–	–
MT_{13}	1.5	10	NIL	–	–
PMT_1	1	10	NIL	3	–
PMT_2	1	10	NIL	6	–
PMT_3	1	NIL	8	3	–
PMT_4	1	NIL	8	6	–
PMT_5**	1	10	NIL	3	–
PMT_6**	1	10	NIL	6	–
PMT_7	1.5	10	NIL	3	–
PMT_8	1.5	10	NIL	6	–
CMT_1	1	10	NIL	–	10
CMT_2	1	10	NIL	–	20
CMT_3	1	10	NIL	–	25
CMT_4	1	10	NIL	–	30
CMT_5	1	10	NIL	–	35
CMT_6	1	10	NIL	–	40
CMT_7	1	NIL	8	–	20
CMT_8	0.75	10	NIL	–	20
CMT_9	1.5	10	NIL	–	20

* unsieved drug was added (MT_1); in all other cases drug was sieved through #100
** Sodium Alginate was used at the concentration of 7.5% w/w (in all other cases 5%w/w was used), *** contains 10% w/v sodium alginate and MT_2 contains 20% overage

Table 2: Physico chemical properties of the prepared implants

Batch code	Thickness uniformity (mm ± sd)	Weight uniformity (mg ± sd)	Drug content uniformity (%)	Surface pH	Percentage moisture absorbed/lost	
					24 h	48 h
MT_1	0.440 ± 0.05	2.34 ± 0.38	91*	7.4 ± 0.002	12 ± 0.5	9 ± 0.6
MT_2*	0.376 ± 0.02	2.68 ± 0.44	115*	7.4 ± 0.005	12 ± 0.2	8 ± 0.5
MT_3	0.386 ± 0.02	2.91 ± 0.25	97	7.4 ± 0.001	12 ± 1.2	9 ± 0.8
MT_4	0.330 ± 0.01	2.93 ± 0.25	105	7.2 ± 0.122	11 ± 0.6	7 ± 1.1
MT_5	0.436 ± 0.05	2.69 ± 0.26	100	7.2 ± 0.004	13 ± 0.6	9 ± 1.02
MT_6	0.446 ± 0.04	3.03 ± 0.35	105	7.2 ± 0.002	9 ± 0.3	7 ± 0.5
MT_7	0.443 ± 0.04	3.05 ± 0.44	97	7.2 ± 0.001	8 ± 1.2	6 ± 0.8
MT_8	0.437 ± 0.04	2.87 ± 0.24	100	7.2 ± 0.122	6 ± 1.1	3 ± 1.1
MT_9	0.432 ± 0.03	2.87 ± 0.09	92	7.2 ± 0.005	16 ± 0.8	16 ± 1.2
MT_{10}	0.487 ± 0.03	3.68 ± 0.52	93	7.0 ± 0.001	20 ± 1.8	19 ± 0.9
MT_{11}	0.300 ± 0.06	2.72 ± 0.25	97	7.0 ± 0.004	10 ± 1.9	5 ± 0.3
MT_{12}	0.355 ± 0.01	2.30 ± 0.34	101	7.2 ± 0.005	7 ± 0.6	6 ± 0.4
MT_{13}	0.389 ± 0.05	2.99 ± 0.28	96	7.4 ± 0.002	8 ± 1.2	6 ± 1.6
PMT_1	0.326 ± 0.03	2.04 ± 0.54	94	7.2 ± 0.003	16 ± 1.2	17 ± 1.4
PMT_2	0.356 ± 0.03	3.36 ± 0.14	101	7.2 ± 0.003	17 ± 1.8	18 ± 1.6
PMT_3	0.500 ± 0.03	3.17 ± 0.11	101	7.4 ± 0.004	16 ± 1.4	16 ± 1.4
PMT_4	0.460 ± 0.02	2.50 ± 0.12	93	7.4 ± 0.002	17 ± 2.1	18 ± 2.2
PMT_5	0.454 ± 0.01	3.35 ± 0.06	93	7.2 ± 0.003	19 ± 1.6	20 ± 1.3
PMT_6	0.480 ± 0.04	3.00 ± 0.08	99	7.4 ± 0.006	20 ± 1.5	21 ± 0.8
PMT_7	0.420 ± 0.03	2.76 ± 0.01	103	7.2 ± 0.003	14 ± 1.6	15 ± 1.1
PMT_8	0.410 ± 0.02	2.79 ± 0.12	92	7.2 ± 0.002	14 ± 0.6	14 ± 1.2
CMT_1	0.438 ± 0.02	3.21 ± 0.44	99	7.0 ± 0.004	20 ± 4.2	21 ± 5.4
CMT_2	0.418 ± 0.01	2.93 ± 0.14	94	7.2 ± 0.006	31 ± 7.8	31 ± 7.6
CMT_3	0.437 ± 0.03	2.61 ± 0.11	97	7.4 ± 0.002	15 ± 1.4	16 ± 1.4
CMT_4	0.465 ± 0.02	2.70 ± 0.12	98	7.4 ± 0.004	14 ± 2.8	17 ± 1.2
CMT_5	0.392 ± 0.04	2.64 ± 0.06	95	7.2 ± 0.006	25 ± 8.6	28 ± 7.3
CMT_6	0.354 ± 0.01	2.49 ± 0.08	93	7.2 ± 0.003	36 ± 9.5	38 ± 9.8
CMT_7	0.478 ± 0.08	3.65 ± 0.08	97	7.0 ± 0.002	5 ± 1.6	5 ± 1.15
CMT_8	0.378 ± 0.42	3.02 ± 0.12	99	7.4 ± 0.007	2 ± 1.6	4 ± 1.2
CMT_9	0.473 ± 0.82	3.72 ± 0.14	93	7.2 ± 0.004	3 ± 1.2	4 ± 0.5

* 20% overage was added to observe the effect of sieving (#100 mesh) on drug loading

The addition of plasticizers was necessary to obtain drug-loaded films with sufficient pliability and to allow subdivision of the films into implants of uniform dimensions. For this reason drug release studies from implants without plasticizer could not be undertaken. Films prepared with less than 10% glycerol were brittle, whereas at the same concentration of PEG 200, the resultant films were so very soft that they could not be handled conveniently. However, PEG 200 at 5% and 8% concentrations produced films with sufficient pliability to allow easy handling.

The surface pHs of the prepared implants ranged from 7 to 7.4 for all batches. This indicates that the prepared implants did not have an irritation potential, as it is identical with the pH of normal tears.

There was a considerable reduction in the percentage of moisture absorbed with an increase in the time of exposure to humidity conditions in the case of the non-reinforced implants. The chemically cross-linked batches showed a comparatively higher difference in the percentage of moisture absorbed between 48 and 24 h than the corresponding physically reinforced implants. The result obtained could be explained on the following lines.

In the case of non-reinforced batches, sodium alginate, being hydrophilic in nature, absorbs moisture from the environment at a rapid rate and, upon prolonged exposure, the excess of moisture dissolves the sodium alginate, which explains the rapid loss in weight and hence the reduction in the percentage of moisture absorbed. When reinforced, the matrix integrity of the implants improves and furthermore there is a reduction in the rate of absorption of moisture, which explains the comparatively lesser weight loss and increased percentage of moisture absorbed. Of the two modes of reinforcement, the chemical method was found to be more effective than the physical method, as evidenced by the percent of moisture absorbed (Table 2).

2.1. In vitro release studies

2.1.1. Effect of particle size

Drug release from MT_2 (#100) is significantly ($P < 0.01$) higher than MT_1 (unsieved). The release of drug from both MT_1 and MT_2 followed Higuchi type kinetics with K values of 0.056 mg/mm^2/h$^{1/2}$ and 0.079 mg/mm^2/h$^{1/2}$, respectively (Table 4). This increase in the release rate and consequently in the amount of drug released (64.12% for MT_1 and 84.61% for MT_2) is due to the decreased particle size of indomethacin.

2.1.2. Effect of polymer concentration

Drug release seemed to increase with an increase in sodium alginate concentration. The release was significantly higher ($P < 0.001$) when the polymer concentration was increased from 5% to 7.5%, while there was not much difference between the release patterns (Table 4) of MT_9 and MT_{10} containing 7.5% and 10% w/v of sodium alginate, respectively.

The increase in drug release with an increase in polymer concentration may be due to the hydrophilic and swellable nature of sodium alginate [13]. When present in higher concentrations, sodium alginate gels to a greater extent in the presence of dissolution medium (phosphate buffer

Table 3: Swelling characteristics of the prepared implants

Batch code	Swelling index (mean ± S.D.) at various time intervals					
	0 h	1 h	2 h	3 h	5 h	6 h
MT_1	0	3.69* ± 0.12	3.50 ± 0.22	3.15 ± 0.12	2.70 ± 0.23	2.33 ± 0.26
MT_2	0	1.01* ± 0.56	0.46 ± 0.02	0.26 ± 0.01	1.04 ± 0.11	0.48 ±0.02
MT_3	0	3.6* ± 0.32	2.65 ± 0.34	1.22 ± 0.36	0.76 ± 0.54	0.36 ± 0.02
MT_4	0	4.75* ± 0.23	4.14 ± 0.22	3.22 ± 0.11	2.49 ± 0.14	3.27 ± 0.26
MT_5	0	4.09* ± 0.23	2.73 ± 0.12	1.08 ± 0.12	0.67 ± 0.03	0.44 ± 0.01
MT_6	0	3.07 ± 0.14	3.29* ± 0.44	2.85 ± 0.45	2.41 ± 0.23	2.68 ± 0.32
MT_7	0	3.05* ± 0.22	2.46 ± 0.25	1.22 ± 0.08	0.67 ± 0.01	0.21 ± 0.02
MT_8	0	2.18* ± 0.44	1.53 ± 0.22	1.02 ± 0.11	0.81 ± 0.04	0.80 ± 0.06
MT_9	0	3.75* ± 0.12	3.58 ± 0.58	3.22 ± 0.24	2.98 ± 0.18	3.11 ± 0.22
MT_{10}	0	1.13* ± 0.03	0.9 ± 0.04	0.46 ± 0.01	0.12 ± 0.001	0.24 ± 0.02
MT_{11}	0	4.57 ± 0.69	6.51* ± 0.88	2.73 ± 0.45	1.64 ± 0.22	2.05 ± 0.14
MT_{12}	0	2.9* ± 0.29	1.57 ± 0.3	0.99 ± 0.05	0.08 ± 0.001	0.06 ± 0.002
MT_{13}	0	3.22* ± 0.54	2.62 ± 0.34	2.22 ± 0.41	2.25 ± 0.12	1.61 ± 0.21
PMT_1	0	4.95* ± 0.65	1.79 ± 0.36	1.61 ± 0.25	1.29 ± 0.14	0.72 ± 0.06
PMT_2	0	4.22* ± 0.88	2.35 ± 0.58	2.03 ± 0.15	1.72 ± 0.32	2.06 ± 0.21
PMT_3	0	2.26* ± 0.32	2.1 ± 0.12	2.17 ± 0.15	1.80 ± 0.36	1.84 ± 0.32
PMT_4	0	2.95* ± 0.65	1.86 ± 0.32	1.31 ± 0.14	1.53 ± 0.32	0.43 ± 0.19
PMT_5	0	3.58* ± 0.69	2.6 ± 0.58	2.66 ± 0.52	2.2 ± 0.41	2.19 ± 0.15
PMT_6	0	3.42* ± 0.21	2.8 ± 0.28	2.93 ± 0.47	2.66 ± 0.46	2.42 ± 0.38
PMT_7	0	3.69* ± 0.54	3.54 ± 0.68	3.55 ± 0.45	2.96 ± 0.36	2.43 ± 0.31
PMT_8	0	1.87* ± 0.25	0.11 ± 0.01	0.11 ± 0.01	0.4 ± 0.002	0.56 ± 0.008
CMT_1	0	1.57 ± 0.12	3.72* ± 0.65	2.54 ± 0.21	2.97 ± 0.23	2.73 ± 0.25
CMT_2	0	1.32 ± 0.11	2.04 ± 0.17	2.37 ± 0.22	2.46 ± 0.23	2.62 ± 0.31
CMT_3	0	1.24 ± 0.33	2.04 ± 0.12	2.37 ± 0.12	2.46 ± 0.31	2.62 ± 0.21
CMT_4	0	1.9 ± 0.56	2.09 ± 0.46	2.37 ± 0.36	2.30 ± 0.12	2.24 ± 0.34
CMT_5	0	4.03* ± 0.81	2.87 ± 0.16	2.76 ± 0.22	2.0 ± 0.17	2.04 ± 0.09
CMT_6	0	4.54* ± 0.65	4.12 ± 0.38	2.92 ± 0.008	3.30 ± 0.13	2.06 ± 0.004
CMT_7	0	1.48 ± 0.21	1.31 ± 0.14	1.63 ± 0.28	2.06 ± 0.27	2.45 ± 0.19
CMT_8	0	1.28 ± 0.14	1.38 ± 0.008	1.75 ± 0.009	1.75 ± 0.12	2.30 ± 0.26
CMT_9	0	1.43 ± 0.47	1.62 ± 0.32	2.16 ± 0.31	2.70 ± 0.48	2.63 ± 0.22

* Equilibrium water uptake (EWU) values

Table 4: Effect of particle size, polymer concentration, drug loading and plasticizer concentrations on *in vitro* release characteristics in phosphate buffer pH 7.4

Batch Code	Cumulative % drug release at the end of 8 h	r^2 (for Q Vs $t^{1/2}$)	K ($mg/mm^2/h^{1/2}$)	t_{50} (h)
MT_1	64.12 ± 3.98	0.989	0.056	0.83
MT^*_2	84.61 ± 3.46	0.978	0.079	0.61
MT_3	74.44 ± 1.66	0.941	0.046	1.03
MT_4	86.54 ± 0.76	0.947	0.061	0.79
MT_5	84.34 ± 1.35	0.947	0.059	0.92
MT_6	72.57 ± 1.66	0.992	0.034	2.97
MT_7	93.81 ± 1.41	0.973	0.052	0.96
MT_8	65.68 ± 1.66	0.998	0.021	4.44
MT_9	88.82 ± 1.10	0.956	0.059	0.78
MT_{10}	88.51 ± 1.74	0.971	0.056	0.76
MT_{11}	72.12 ± 1.15	0.953	0.043	2.03
MT_{12}	73.59 ± 1.13	0.960	0.040	1.94
MT_{13}	77.76 ± 1.02	0.977	0.045	1.92

* 20% overage was added to observe the effect of sieving (#100 mesh) on drug release

pH 7.4). This swelling results in an increase in the pore size of the matrix film, ultimately resulting in rapid leaching of the drug molecules.

2.1.3. Effect of plasticizer type and concentration

The plasticizer is the most important formulation factor that may affect the mechanical properties of the films as it lowers the glass-transition temperature [14].

The effect of the plasticizing capacities of glycerol and PEG 200 on drug release was studied at concentrations allowing for convenient handling of the films and implants. Some general trends appear, which are clearer for PEG 200 than for glycerol. For example, the cumulative drug release for batches MT_6, MT_7 and MT_8 increases linearly with PEG 200 concentration (Table 4). No such clear relationship is observed with glycerol; although drug release is slowest at 10% glycerol concentration, it peaks at 12.5% concentration, with no change at 15% concentration, while K values are also similar at each polymer concentration. Nonetheless, the t_{50} values for glycerol showed an initial decline at 12.5% concentration, and then increased (at 15% glycerol concentration) to almost the same value as at 10% plasticizer concentration (Table 4). No definite reason could be attributed to this observation, but some possible reasons are discussed. First, the drug may have become partly solubilized in the presence of the plasticizer. This would result in the release of more drug as a function of plasticizer type and concentration. Moreover, the presence of solubilized drug in the device would cause the formation of pores due to higher local release of the drug and subsequent speedier penetration of the drug release medium into the device. To examine this possibility we studied the saturation solubility of indomethacin in distilled water and in 5 and 10% aqueous PEG 200 and glycerol solutions. Saturation solubility in distilled water was found to be 5.8 mg/ml, while in PEG 200 5 and 10% the values were 6.8 and 7.6 mg/ml, respectively, and in glycerol the values were 6.4 and 7.3 mg/ml respectively. The times to reach saturation were 7 h for distilled water and PEG 200 and 6 h for glycerol. Thus the plasticizers did not affect drug solubility. Second, the compatibility of the plasticizer with the polymer is a manifestation of the

solubility of the polymer in the plasticizer. PEG 200 has been reported to decrease the glass transition temperature of HPMC [15] to a greater extent than glycerol, and of the various PEGs studied, PEG 200 showed maximum intrinsic viscosity in combination with HPMC, indicating its greater potential plasticizing effect on HPMC [16]. Although no comparable information is available for the PEG-sodium alginate system, an analogy to our studies can be drawn. Essentially, the very soft and pliable implants containing 10% of PEG 200 compared with the firmer implants containing 10% of glycerol imply that the former has better plasticizing efficiency than the latter, presumably because of softening of the polymer matrix by increasing polymer chain mobility. Moreover, the shape of the plasticizer molecule apparently plays an important role in its interposition between the polymer chains for eventual polymer chain relaxation, and cylindrical plasticizer molecules would fit better than, for example, spherical or other shapes.

2.1.4. Effect of drug loading

The results indicated that drug loading had no significant effect on drug release ($p > 0.05$).

2.1.5. Effect of reinforcement on in-vitro drug release

Watase [17] has reported a technique of freeze-thaw processing of PVA solution. PVA gels obtained by this procedure were reported to be very stiff and resistant to swelling when immersed in water [18].

The above observation with PVA pointed to the possibility of physically reinforcing sodium alginate by freeze-thaw cycles. We selected two numbers of freeze-thaw cycles to study its effect on *in vitro* drug release.

Freeze-thawing was done for 3 and 6 cycles to study the effect of the number of cycles on drug release. Freeze-thaw cycles were chosen such that the effect on *in vitro* drug release could be studied on implants containing sodium alginate 5% and glycerol (PMT_1 and PMT_2), sodium alginate 5% and PEG 200 8% as plasticizer (PMT_3 and PMT_4), sodium alginate 7.5% (PMT_5 and PMT_6) and indomethacin 1.5 mg/implant (PMT_7 and PMT_8).

The results showed that freeze-thawing retarded drug release in almost all cases (Fig. 1), except in batches PMT_1 and PMT_2. In most of the cases freeze-thawing for 6 cycles was more successful in retarding drug release than freeze-thawing for 3 cycles; however, significant retardation in drug release was observed in the case of batch PMT_3, which was subjected to 3 freeze-thaw cycles, in comparison to the corresponding batch subjected to 6 freeze-thaw cycles.

Surface cross-linking was achieved with the parent batch by a procedure reported in the literature [19]. Calcium chloride solutions (10 and 20 to 40% w/v in 5% increments) were used in the present study. The results indicated that drug release was markedly reduced in comparison to the corresponding non-reinforced and physically reinforced batches (Fig. 2).

As the concentration of the cross-linking agent increased there was a reduction in drug release, as evidenced by the K values; 0.014, 0.011 and 0.008 mg/mm/h for batches CMT_1, CMT_2 and CMT_3, respectively. However, this was true only with 10, 20 and 25% w/v of calcium chloride. Further increase in the concentration of calcium chloride resulted in an increase in drug release. The kinetic analysis of the cross-linked batches showed zero order drug

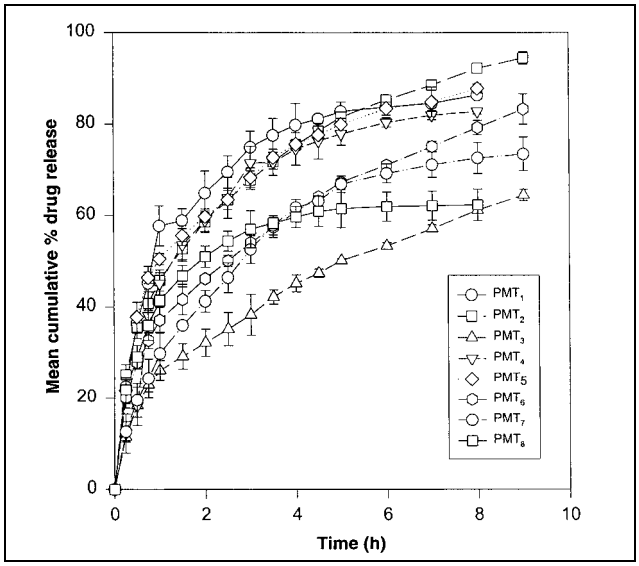

Fig. 1: *In vitro* release profiles of physically reinforced implants in phosphate buffer pH 7.4

release for all the batches (r^2 values for CMT_1 to CMT_5 were 0.998, 0.991, 0.993, 0.999 and 0.951, respectively for Q Vs t plot) except for the batch cross-linked with 40% w/v solution of calcium chloride (r^2 values for CMT_6 were 0.915 for Q Vs t and 0.989 for Q Vs $t^{1/2}$ plots), which is rather surprising, since the formation of insoluble calcium alginate is mainly responsible for the reduction in drug release [20]. The method of cross-linking employed in the present study seems to be responsible to a large extent for the observed results. In this study, the films

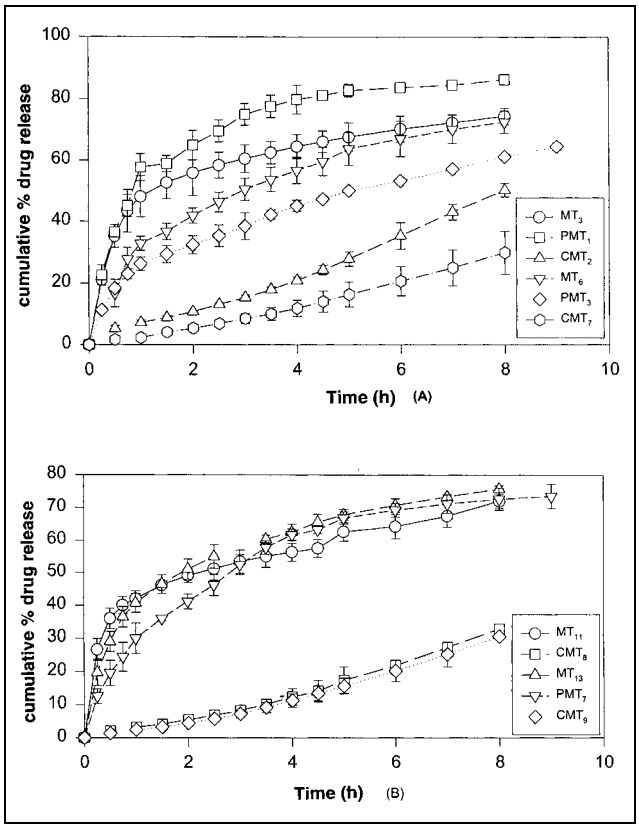

Fig. 2: Comparison of chemically cross-linked implant (20% calcium chloride) with the corresponding non-reinforced and freeze-thawed (3 cycles) implants for drug release in phosphate buffer pH 7.4

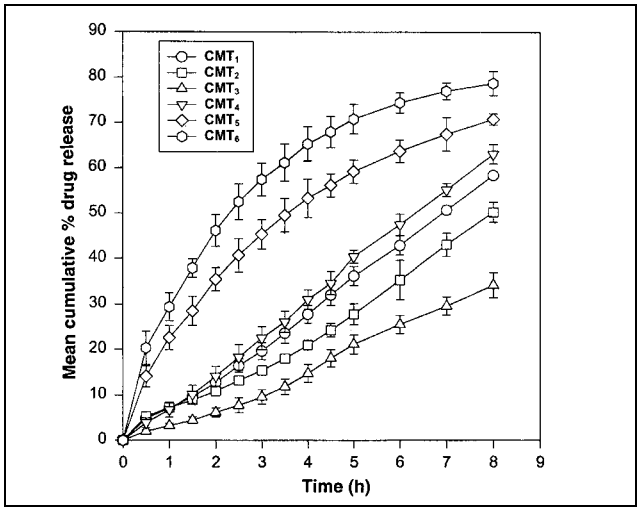

Fig. 3: Effect of chemical cross-linking on *in vitro* drug release from the implants in phosphate buffer pH 7.4

were exposed to the cross-linking solution from both surfaces. This could result in the termination of the cross-linking process before the cross-linking ions could travel from one surface of the film to the other. Thus it is likely that cross-linking starts at the exposed surface, yielding a nearly completely cross-linked surface, and resulting in decreased size and number of voids. Consequently, there will be a lesser chance of the remaining cross-linking ions diffusing further into the body of the film. Moreover, any cross-linking ions that succeed in penetrating the surface to cross-link additional sites in the adjacent deeper layers lead to further hindrance of ionic movement. This process continues until no further penetration is possible, leading to incomplete cross-linking of the remaining layers. The formation of a film of rigid calcium alginate would result in incomplete cross-linking. Our findings are in accordance with those of Kwon et al. [21], who have reported an increase in drug release from similar systems, attributing this to the low molecular weight of the drug. The drug used in our study, indomethacin, with a molecular weight of 357.81 could very well be regarded as a drug of low molecular weight.

2.1.6. Swelling index

All nonreinforced batches showed maximum swelling in one hour. This is in accordance with the in vitro release study, where considerable burst effect was observed within the first hour of the study (Table 3).

The batches containing different concentrations of glycerol showed that the Equilibrium Water Uptake (EWU) increased with an increase in glycerol concentration from 10 to 12.5%, but there was not much difference between the EWU of the batches containing 12.5% and 15% w/w. Similar results were obtained with batches containing different concentration of PEG-200 as plasticizer.

The results of the swelling studies indicated an increase in the swelling index with an increase in the number of freeze-thaw cycles in the case of batches containing sodium alginate 5% and glycerol 10%, but in the case of other batches the EWU decreased with physical reinforcement. However, the number of freeze thaw cycles did not have a great effect.

2.2. Pharmacodynamic studies

Five batches of the fabricated implants were selected to study the influence of physical and chemical reinforcement (PMT_8, CMT_9) in comparison with non-reinforced batches (MT_3; MT_9 and MT_{13}), on the resolution of induced uveitis in rabbit eys. Additionally, the effects of drug loading (MT_3 Vs MT_{13}) and polymer concentration (MT_3 Vs MT_9) were also studied.

Six characteristics of uveitis were evaluated pre-and post-treatment with the fabricated implants, but the results are discussed here with reference to those characteristics which are more primary manifestations of uveitis, viz., congestion, keratitis and aqueous cells. Comparison of PMT_8 and CMT_9 (physical and chemical reinforcement) reveals that the latter batch resolves these three parameters to a greater extent than the former. For example, CMT_9 reduced congestion to level zero in two eyes out of three, but PMT_8, although showing better improvement than non-reinforced batches, did not reduce congestion to zero in any treated eye. No noticeable difference was observed in the effect of either drug loading (MT_3 Vs MT_{13}) or polymer concentration (MT_3 Vs MT_9) on the level of congestion. The resolution of keratitis and aqueous cells also showed that – a) the chemically reinforced implant produced better effects than the physically reinforced implant, b) both the reinforced implants showed more marked effects on these characteristics of uveitis than the non-reinforced implants, and c) drug loading and polymer concentration of the implants had no effect on resolution of the parameters, the results obtained with MT_9 and MT_{13} being similar to MT_3.

The implanted sites in the eyes were exposed after 6 days of placement. No implant was visible in any eye. Thus, the actual difference in residence time of the reinforced and non-reinforced implants could not be determined. Nonetheless, the total drug loading was apparently released within 6 days. A shorter retrieval time such as the third day post implantation would probably have revealed the state of the implant between implantation and total disappearance. Thus at this juncture it can only be argued that chemically reinforced implants were retained for a longer time than physically reinforced implants and retention was shorter still for non-reinforced implants. Thus, by implication the rate of disappearance of indomethacin from the implanted site is dependent on the rate of disappearance of the implant, and drug loading or the polymer concentration had no effect since the non-reinforced implants dissolved/degraded at apparently similar rates *in vivo*. Further investigation is in progress to determine the *in vivo* rate of disappearance of the implants.

In conclusion, the indomethacin implant provided an initial phase of high release, followed by a phase of moderate release. Chemical cross-linking with calcium chloride was more effective in sustaining the release of Indomethacin *in vitro* than physical cross-linking. Even though the implants were studied in a small group of animals and the survival time and distribution kinetics to the various ophthalmic tissues were not monitored, the results indicate the potential effectiveness of the implants, as was evidenced by the results of the pharmacodynamic studies. On the basis of the results of this study we infer that the scleral implant with maximum indomethacin load of 1.5 mg/implant appears to have a high potential for development as a superior mode of therapy for uveitis than the conventional modes. Thus in our opinion this sclerally implantable ocular system could serve as a better alternative in the long term treatment of uveitis in immuno-compromised patients. We are currently examining some unexplored issues involved in the scleral implantation of indomethacin.

Table 5: Pharmacodynamic evaluation of the implants

Batch implanted	Animal No	Implanted eye*/control	Clinical parameters											
			Congestion		Keratitis		Flare		Aqueous cells		Clot		Sinekese	
			Pr-T	Po-T	Pr-T	Po-T	Pr-T	Po-T	Pr-T	Po-T	Pr-T	Po-T	Pr-T	Po-T
MT_{13}	1	R*	++++	+	+++	+	+++	++	+++	+	+++	0	P	P
		L	+++	++	++	+	+++	++	+++	+	0	0	P	P
	2	R*	++++	+	++	0	++	+	+++	+	++	0	P	P
		L	++	+	++	0	++	+	++	++	0	0	P	P
	3	R*	++++	+++	++	++	+++	++	++	+	+	0	P	P
		L	+++	+++	+	+	++	+	+	0	0	0	P	P
CMT_9	4	R*	++++	+	++	0	++	0	+++	+	++	0	P	P
		L	++	++	++	+	+	+	++	++	+	0	P	P
	5	R	+	+	0	0	+	+	+	0	0	0	P	P
		L*	++++	0	+++	0	+++	+	+++	0	+++	0	P	P
	6	R*	++++	0	++++	0	+++	0	+++	0	+++	0	P	P
		L	+++	+	+	+	+	+	+	+	0	0	P	P
MT_3	7	R	++	++	+	+	+	+	+	+	0	0	P	P
		L*	++++	++	++++	+	ND	++	ND	+	ND	+	P	P
	8	R*	+++	+	++	+	+	0	++	0	++	+	P	P
		L	++	+	++	+	+	0	+	+	+	+	P	P
	9	R	++	++	+	0	++	+	+	+	++	+	P	P
		L*	++++	+	++++	+	ND	+	ND	0	ND	+	ND	P
MT_9	10	R*	+++	+	++	+	++	+	+++	+	+++	0	P	P
		L	+	+	+	+	+	+	+	+	+	0	P	P
	11	R*	+++	+	+++	+	+++	++	++	+	++	0	P	P
		L	++	+	++	++	++	++	+	+	++	0	P	P
	12	R	++	+	+	+	+	+	++	+	+	0	A	A
		L*	++++	++	++++	+++	+++	+	++	+	++	0	P	P
PMT_8	13	R*	+++	+	+++	+	++	+	+++	++	+	0	P	P
		L	++	++	+	+	++	+	+	+	+	0	P	P
	14	R	++	+	+	+	+	+	++	+	++	+	P	P
		L*	++++	+	++++	++	ND	++	ND	+	ND	+	P	P
	15	R*	+++	+	++	+	++	+	+++	+	++	0	P	P
		L	+	+	+	0	0	0	0	0	+	0	P	P

* Implanted eye
ND – Could Not be Determined due to severe inflammation score > ++++
P – Present
A – Absent
Pr-T – Pre treatment scores
Po-T – Post treatment scores

3. Experimental

3.1. Materials

Indomethacin was generously donated by Jagsonpal Pharmaceuticals Ltd, New Delhi, India. Sodium alginate was obtained commercially from Loba Chemie (India) Ltd. All other reagents used were of analytical grade.

3.2. Methods

The fabrication and the *in vitro* evaluation of the implants were carried out under clean room conditions.

3.2.1. Fabrication of implants

Implants with different concentrations of sodium alginate (5, 7.5, 10% w/v), different drug loadings (0.75, 1.0, 1.5 mg/implant) and different concentrations of glycerol (10, 12.5, 15% w/w, w.r.t sodium alginate) and/or PEG 200 (5, 8, 10% w/w of sodium alginate) as plasticizers were fabricated using distilled water as the solvent.
Sodium alginate was dissolved in distilled water and the calculated quantity of indomethacin was incorporated into it, followed by further stirring and degassing. The resultant dispersion was cast on levelled glass moulds ($6.5 \times 6.5 \times 0.8$ cm) and dried in an oven at 50 °C for 24–26 h. The films were removed and cut into implants of 1×5 mm (0.3 - 0.487 mm) using a surgical scalpel. The implants were stored in amber coloured glass vials in a desiccator until further use.

3.2.1.1. Fabrication of physically reinforced implants by the freeze-thaw method

The dispersion of indomethacin in sodium alginate with the plasticizer was prepared and cast on glass moulds as described above and the moulds were placed at −18 °C for 2 h and then thawed at 50 °C for 1 h. This gave one full freeze-thaw cycle. Three and six such cycles were repeated for selected batches. After completion of the desired number of cycles, they were dried at 50 °C for 24–26 h. The implants were cut and stored as described earlier.

3.2.1.2. Fabrication of chemically cross-linked implants

The parent batch (prepared by casting as described earlier) was placed in petri dishes of 90 cm diameter and 25 ml of calcium chloride solution of different concentrations (10, 20, 25, 30, 35, 40% w/v) was poured separately over each parent batch, such that the film remained immersed in the solution. After 30 min, the film was inverted in the cross-linking solution and left for a further 30 min [19]. The film was then removed, dried at 40 °C in an oven and cut into implants for further studies.

3.2.2. Evaluation of the implants

3.2.2.1. Thickness, weight and uniformity of drug content

The thickness of the implant was measured at 5 different randomly selected spots with a screw gauge. For uniformity of weight, ten implants from each batch were weighed individually and their average determined. For determination of uniformity of drug content, 6 implants from each batch were weighed individually and dissolved in 50 ml of phosphate buffer pH 7.4. The resultant solution was filtered through a G2 glass filter. An aliquot of the filtrate was suitably diluted and analyzed for indomethacin content at 319.5 nm (Shimadzu, UV-1601, Japan).

3.2.2.2. Swelling studies

Weighed implants were placed in a stainless steel wire mesh holder of dimensions $2 \times 8 \times 8$ mm and the system was accurately weighed and placed inside vials containing 10 ml of phosphate buffer pH 7.4. The holder was removed at pre-determined time intervals, dried and weighed.

The relative water uptake was then calculated using the formula [22]

$$\text{Relative water gain} = SW_2 - SW_1/SW_0$$

where SW_1 is the weight of the holder, SW_2 is the weight of the swollen implant and holder and SW_0 is the initial weight of the implant.
All the implants remained intact at the end of the studies (6 h).

3.2.2.3. In vitro release studies

Weighed implants were placed in a stainless steel wire mesh holder of dimensions $2 \times 4 \times 6$mm and suspended in amber coloured vials containing 3 ml of phosphate buffer pH 7.4, as the dissolution medium. The vials were stoppered and placed in the vial holder (to prevent dislodging) in a water bath thermostated at 37 ± 1 °C. At pre-determined times the dissolution medium was completely withdrawn and replaced with a fresh 3 ml portion of the pre-warmed buffer, to ensure sink conditions. The samples were analyzed for indomethacin content at 319.5 nm, after appropriate dilution.

3.2.2.4. Moisture absorption/loss of implants

A modification of the American Standard Test Method, test no. D570-59T, was used for the testing of moisture absorption/loss of implants.
The implants were conditioned by placing them in an oven at the temperature and for the time that had been used originally in drying the wet patches. This step was carried out to ensure uniformity of the patches within each group before testing. The conditioned samples were accurately weighed, and kept in a constant humidity chamber (humidity of 80.5% at 20–30 °C). At the end of 24 and 48 h the implants were removed and weighed again. Percent moisture absorption was calculated by means of the following formula:

$$\text{Percent Moisture Absorption} = \frac{\text{Wt. of exposed film} - \text{Wt. of conditioned film}}{\text{Wt. of conditioned film}} \times 100$$

All the processes were carried out under clean room (class 100) conditions.

3.2.3. Pharmacodynamic evaluation

A total of 15 albino rabbits weighing 2–3 kg (2.75 ± 0.75) were used for the present study. Animals with observed ocular abnormalities were excluded after thorough ocular examination, prior to the commencement of the study.
Uveitis was induced in both eyes of each rabbit by an intra-vitreal injection (30 g needle) of a sterile solution of Bovine Serum Albumin (BSA-0.5 ml/eye of 50 µg/ml sterile solution). The induction and resolution of uveitis were observed by slit-lamp examination.
Three days after the intra-vitreal injection of BSA, the eyes of the individual rabbits were examined for the induction and resolution of uveitis and the following clinical parameters – Congestion, Keratitis (Keratopathy), Flare, Aqueous Cells, Clot and Synechias [23] were evaluated and scored as follows.
Congestion:
0 – No congestion
+ – Slight to moderate circum-corneal congestion
++ – Marked circum-corneal ciliary congestion
+++ – Marked circum-corneal, diffuse episcleral and conjunctival congestion
++++ – Marked circum corneal, diffuse episcleral and conjuctival congestion with edema
Keratitis (Keratopathy):
0 – No inflammation
+ – Slight diffuse stromal edema
++ – Moderate epithelial and stromal edema with thickening and folds in Descemet's membrane
+++ – Diffuse epithelial and stromal edema; folds in Descemet's membrane; peripheral vascularisation
++++ – Severe edema of the stroma
Flare:
0 – Complete absence
+ – Faint flare
++ – Moderate flare
+++ – Marked flare
++++ – Intense flare
Aqueous cells:
0 – No cell
+ – 5 to 10 cells per field
++ – 10 to 20 cells per field
+++ – 20 to 50 cells per field
++++ – more than 50 cells per field
Clot:
0 – No clot
+ – Small clot in lower angle or pupillary area
++ – Clot occupying lower third of anterior chamber
+++ – Clot filling lower half of anterior chamber
++++ – Solid clot, filling almost the entire anterior chamber
The eye (left or right) showing more severe uveitis, based on the pre-treatment scores of the various descriptors, was selected for placing the implant.
The animals were lightly anaesthetized with ether. The eye into which the implant was to be surgically implanted was anaesthetized by instillation of one drop of proparacaine hydrochloride (0.5% w/v) solution.
A fornix-based conjunctival flap was raised. After hemostasis was achieved, a partial thickness scleral pocket was made by a crescent knife 4 mm behind the limbus and the implant was placed. The scleral pocket was then closed with 6.0′ silk suture.
After 6 days the animals were examined for improvements in the clinical parameters and the suture was opened to retrieve the remaining implant, if any, to assay the remaining drug in the implant. However, no remaining implant was observed in any eye.

3.2.4. Statistical analysis of the data

Experimental results are expressed as mean ± standard deviation (S.D.). In case of multiple comparisons of groups, analysis of variance (ANOVA) was performed. The Student 't' test was also performed to determine the level of significance. Differences were considered to be statistically significant at $P < 0.05$.

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