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pH-Induced *In Situ* Gelling Systems of Indomethacin for Sustained Ocular Delivery

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The low bioavailability and ocular residence time exhibited by the topical conventional liquid ophthalmic formulations because of spillage by overflow, dilution of drug by tear turn over, naso-lacrimal drainage and systemic absorption may be overcome by the use of *in situ* forming systems that are instilled as liquid drops into the cul-de-sac of the eye, where they transform into a gel or semisolid phase. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antiinflammatory drug, indomethacin for the treatment of uveitis based on the concept of pH induced *in situ* gelation. The carbopol solutions which are acidic and less viscous, transform into stiff gels upon increase in pH of eye as the gelling agents and its combination with hydroxypropylmethylcellulose-$K_{15}M$, a well known ocular viscosity enhancing agent. The enhanced therapeutic efficacy and sustained release of indomethacin over 8 hour period *in vitro* make them an excellent candidate for *in situ* gelling ocular delivery systems.

Topical administration of drugs is the treatment of choice for diseases of anterior segments of the eye. Physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs resulting in a short duration of therapeutic effect. When a drug solution is dropped into the eye, effective tear drainage and blinking results in a 10-fold reduction of the drug concentration in 4-20 minutes[1]. The limited permeability of the cornea contributes to the low absorption of ocular drugs. Due to tear drainage, most of the administered dose is absorbed via the naso-lacrimal duct to the GI tract, leading to side effects. The rapid elimination of the administered eye drops often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the pre-corneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use not only to prolong the contact time of the vehicle at ocular surface, but also to slow down the elimination of the drug[2,3]. Successful results were obtained with inserts[3] and collagen shields[4], although these preparations present some disadvantages, such as non-compliance, especially by elderly people and many patients loose the device sometimes without becoming aware of it. From the point of view of patient acceptability, a liquid dosage form is preferable.

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This problem can be overcome by using *in situ* gel forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions and pseudo-plastic behavior to minimize interference with blinking[5]. Such a system can be formulated as liquid dosage form suitable for administration by instillation into the eye, which upon exposure to physiological conditions shifts to the gel phase, thus increasing the pre-corneal residence of the delivery system and enhancing ocular bioavailability.

Depending on the method employed to cause sol to

gel phase transition on the ocular surface, the following three types of systems are recognized: pH triggered systems- cellulose acetate hydrogen phthalate latex (CAP)[6,7], Carbopol[8]; temperature-dependent systems such as Pluronics[9,10] and Tetronics[11,12], and ion-activated systems, Gelrite[13] and Gellan[14].

Ideally, an *in situ* gelling delivery system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops, and the gel formed following phase transition should be strong enough to withstand the shear forces in the cul-de-sac and demonstrate long residence times in the eye. The prolonged residence time of the gel formed *in situ* along with its ability to release drugs in a sustained manner will assist in enhancing the bioavailability of the instilled drug and improve patient compliance.

The objective of the present study was to develop an pH induced *in situ* gelling system of indomethacin, a NSAID used as an alternative to steroids in the treatment of uveitis[15,16] and other external inflammations of the eye. Carbopol and its combination with hydroxypropylmethylcellulose (HPMC) was investigated as a vehicle for the formulation of eye drops of indomethacin (1% w/v), which would undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug during the treatment of uveitis. Carbopol has been recently used as a gelling vehicle for the ocular delivery of ofloxacin[8].

MATERIALS AND METHODS

Indomethacin was generously gifted by Ranbaxy Research Laboratories, Gurgaon. Carbopol 974P and HPMC $K_{15}M$ were gift samples from B. F. Goodrich, North Carolina and Dow Chemical Company, Texas, respectively. Bovine serum albumin (BSA), lysozyme and gamma globulin were purchased from CDH Ltd, Mumbai. All other reagents used were of analytical grade.

Preparations of formulations:

Carbopol and its combination with HPMC (Table 1) were dissolved in respective buffers (prepared in fresh water for injection under laminar flow) such as, phosphate buffer (pH 7.4) and acetate buffer (pH 5.0).The solutions were rendered isotonic with 5% mannitol. Required quantity of indomethacin to give a final drug concentration of 1.0 % w/v was added to the polymeric solutions and stirred until

TABLE 1: COMPOSITION OF THE PREPARED *IN SITU* GELLING FORMULATIONS

Batch Code	Carbopol (%w/v)	HPMC (%w/v)	Drug (%w/v)	Mannitol (%w/v)
CH_1P	0.2	0.7	1.0	5.0
CH_2P	0.3	0.5	1.0	5.0
CH_3P	0.3	0.7	1.0	5.0
CH_4P	0.4	0.1	1.0	5.0
CH_5P	0.4	0.3	1.0	5.0
CH_6P	0.4	0.5	1.0	5.0
CH_7P	0.5	0.3	1.0	5.0
CH_8P	0.5	0.5	1.0	5.0
CH_1A	0.2	0.7	1.0	5.0
CH_2A	0.3	0.5	1.0	5.0
CH_3A	0.3	0.7	1.0	5.0
CH_4A	0.4	0.1	1.0	5.0
CH_5A	0.4	0.3	1.0	5.0
CH_6A	0.4	0.5	1.0	5.0
CH_7A	0.5	0.3	1.0	5.0
CH_8A	0.5	0.5	1.0	5.0

The suffixes P and A denote formulation prepared with phosphate and acetate buffers, respectively

dissolved. The formulations were filled in 10 ml amber colored glass vials, capped with rubber closures and sealed with aluminum caps. The formulations, in their final pack, were terminally sterilized by autoclaving at 121° and 15 psi. for 20 min. The sterilized formulations were stored in refrigerator (4-8°) until further use.

Drug content uniformity:

The vials (n=3) containing the formulations were shaken for 2-3 min and 100 ml of the formulation was transferred aseptically to sterile 25 ml volumetric flasks with a micropipette and the final volume made up with phosphate buffer (pH 7.4). The concentration of indomethacin was determined at 319.5 nm (Shimadzu, UV-1601, Japan).

***In vitro* gelation and rheological studies:**

The gelation studies were carried out by a method reported earlier by us[17] in gelation cells, fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (simulated tear fluid, STF). Within the cells located at the bottom was a 250-µl transparent plastic cup to hold the gel sample in place after its formation. The studies were carried out using STF consisting of composition 1[8], sodium chloride: 0.670 g, sodium bicarbonate: 0.20g, calcium chloride dihydrate: 0.008 g and purified water *qs* 100 g and of composition 2[18], BSA: 0.268 g, lysozyme: 0.268 g, D-glucose: 0.15 g, sodium chloride: 0.65 g and distilled water *qs* 100 g, which simulate the divalent cation content and both the protein and divalent cation content of the tear fluid, respectively. One hundred microlitres of the preparation was carefully placed into the cavity of the cup using a micropipette and 2 ml of the gelation solution (STF 1 and 2) were added slowly. Gelation was detected by visual examination. Viscosity determinations of the prepared formulations were carried out using modern adaptation of original Ostwald viscometer. The evaluations were conducted in triplicate.

***In vitro* release studies:**

Two millilitres of each of the formulations were filled into small circular plastic containers (30 mm id, 40 mm depth). Stainless steel mesh No. 100 was placed on the plastic containers containing the formulation in such a way so as to prevent the overflowing of the formulations upon contacting the release medium. The containers were suspended in 50 ml of STF 1, in 100 ml beakers and the whole set up was thermostated at 37±0.1°. The release medium was stirred at 500 rpm using magnetic stirrer. At pre-determined time intervals, the entire medium was replaced with fresh medium to ensure sink conditions. The drug content in each sample was analysed spectrophotometrically (Shimazdu, UV-1601, Japan) at 319.5 nm.

Pharmacodynamic studies:

Approval of the Institutional Animal Ethics Committee was obtained prior to the commencement of the study. A total of 6 Albino rabbits weighing 2-3 kg (2.68±0.84) were used for the present study. Prior to the commencement of the study the animals with observed ocular abnormalities were excluded after thorough examination. The animals were housed in individual cages, and the experiments were conducted in a sanitized room at a temperature maintained around 24°. Uveitis was induced in both eyes of each rabbit by an intra-vitreal injection (30 g needle) of a sterile solution of BSA (0.5 ml/eye of 50 µ/ml sterile solution). Two days after the intra-vitreal injection of BSA, the eyes of the individual rabbits were observed by slit-lamp examination for the induction of uveitis. The following clinical parameters – congestion, keratitis (keratopathy), flare, aqueous cells, clot and synechias were evaluated and scored as shown in Table 2. Based on the pre-treatment scores of the above descriptors, the eye (left or right) showing more severe uveitis was treated with test formulation CH_5P and a marketed formulation at a dose level of 1200 µg in appropriate numbers of drops (2 or 3 drops as the case may be), and other eye served as the control. At times 1, 4, 8 and 24 h post instillation, the change in inflammatory condition was assessed visually and by slit lamp technique and graded according to the method described by Hogan *et al.*[19]. The instillation of drops was done through a micropipette and the eyelid was held closed for 5 s after instillation. The rabbits were conditioned to the treatment protocol prior to start of experiments by instillation of respective drug free vehicle to avoid drug loss due to head movement of the animals. Further, the animals were subjected to the treatment protocol using the test formulation and the marketed formulation prior to induction of uveitis to observe for any visible signs of ocular irritation (by slit lamp). The improvement or otherwise of the uveitis condition was noted up to 24 h. Additionally, the tissues around the eye were examined for any evidence of redness, swelling and/or other pathological signs other than the clinical parameters were observed.

RESULTS AND DISCUSSION

The physicochemical properties of the prepared formulations are shown in Table 3. The drug content and clarity of the formulations were found to be satisfactory and the prepared formulations were liquid at both room tempera-

TABLE 2: SCORING OF THE VARIOUS CLINICAL PARAMETERS OF UVEITIS MONITORED

Grading	Congestion	Keratitis	Clot	Flare	Aqueous cells
0	No congestion	No inflammation	No clot	Complete absence	No cells
+	Slight to moderate circum-corneal congestion	Slight diffuse stromal edema	Small clot in lower angle of papillary area	Faint	5 to 10 cells per field
++	Marked circum-corneal ciliary congestion	Moderate epithelial and stromal edema with thickening and folds in Decemet's membrane	Clot occupying lower third of anterior chamber	Moderate	10 to 20 cells per field
+++	Marked circum-corneal, diffuse episcleral and conjuctival congestion	Diffuse epithelial and stromal edema; and folds in Descemet's membrane; Peripheral vascularisation.	Clot filling lower half of anterior chamber	Marked	20 to 50 cells per field
++++	Marked circum-corneal, diffuse episcleral and conjuctival congestion with edema	Severe edema of the stroma	Solid clot, filling almost the entire anterior chamber	Intense	More than 50 cells per field

ture and when refrigerated.

Gelation studies were conducted using STF 1, which simulates the calcium ion content of the tear fluid and STF 2, which simulates both the protein and cation content of the tear fluid. In the formulated systems, Carbopol acts as the *in situ* gelling agent (pH triggered), while HPMC acts as a viscosity enhancer. The optimum concentration for *in situ* gelation of Carbopol 974 P is reported to be 2%w/v[20], however, the concentration of Carbopol required to form stiff gels results in highly acidic solutions which are not easily neutralized by the buffering action of the tear fluid. A reduction in Carbopol concentration without compromising the gelling capacity and rheological properties of the delivery system may be achieved by the addition of viscosity enhancing polymers such as HPMC. The Carbopol being acidic in nature (pH of 1% solution is between 2.5-3), lowered the pH of the formulation prepared with pH 7.4 phosphate buffer to pH 6 0, but the pH of formulation prepared with acetate buffer (pH 5.0) was unaffected. All the formulations except CH_1P and CH_1A showed instantaneous gelation when contacted with both the galation fluids. However, the nature of the gel formed depended on the concentration of Carbopol used. The formation of instantaneous gels can be attributed to the buffering capacity of the simulated tear fluid, which provided an optimum pH for Carbopol to behave as an *in situ* gelling agent.

The system showed an increase in viscosity with increase in the polymer concentration (Carbopol or HPMC). The significant differences in viscosity were found between formulations prepared with acetate and phosphate buffers. The viscosities of CH_2A, CH_4A, CH_5A, CH_6A and CH_8A were 73.3, 20.0, 23.3, 153.3 and 253.3, respectively, while that of CH_2P, CH_4P, CH_5P, CH_6P and CH_8P were 93.3, 43.3, 108.3, 226.6 and 550 cps, respectively. This difference is solely due to Carbopol, which has a property of increasing the viscosity at elevated pH. Formulation with less than 0.3% Carbopol, and increased concentration of HPMC though highly viscous, were not identical because of lack of optimum gelation. Although both the polymers (Carbopol and HPMC) contributed to an increase in viscosity, HPMC seemed to be more efficient than Carbopol in both acetate and phosphate buffers.

TABLE 3: PHYSICOCHEMICAL PROPERTIES OF THE PREPARED GELLING SYSTEMS

Batch Code	Percent drug content	Gelation studies	
		STF I	STF II
CH_1P	98.7±2.06	-	-
CH_2P	102±1.86	+	+
CH_3P	101±3.11	+	+
CH_4P	99.9±2.23	++	++
CH_5P	97.5±4.12	++	++
CH_6P	103±3.12	+++	+++
CH_7P	102±4.43	+++	+++
CH_8P	100±1.47	+++	+++
CH_1A	97.5±1.59	-	-
CH_2A	99.9±1.98	+	+
CH_3A	104±4.86	+	+
CH_4A	96.6±2.36	++	++
CH_5A	99.6±2.12	++	++
CH_6A	101±3.24	+++	+++
CH_7A	103±0.27	+++	+++
CH_8A	97.7±1.30	+++	+++

- stands for no gelation, + indicates gels after a few minutes, ++ denotes gelation immediate but remains for a few hours (less stiffer) and +++ refers to gelation immediate and remains for extended periods and formed gels are stiffer

The gelling studies showed that the nature of gelation of the formulations with STF of either composition 1 or 2 was similar. But STF 1 was selected as the dissolution medium to avoid interference by the protein components used in STF 2 during spectrophotometric analysis of the release study samples for indomethacin content. The cumulative percent of indomethacin released as a function of time are shown in figs. 1 and 2. The results showed that the amount of drug released decreased with increasing polymer concentration. This could be attributed to the increase in viscosity and gel strength of the formulation with increase in HPMC and Carbopol concentration, respectively.

The *in vitro* drug release conditions may be very different from those likely to be encountered when instilled into the eye. However, the results clearly show that the gels have ability to retain the drug for prolonged periods (8 h) and premature drug release will not occur. In the cul-de-sac of the eye, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movements[8] or dissolution in the cul-de-sac will proceed more slowly than that seen in the *in vitro* experiments, as the normal resident volume of the lachrymal fluid in the human eye is 7.5-10 μl[21].

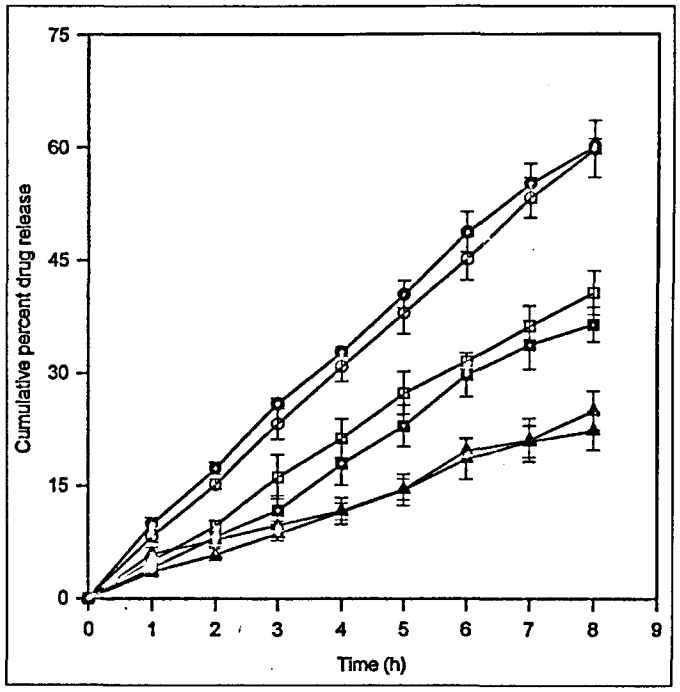

Fig. 2: Effect of HPMC on the indomethacin release from formulations prepared with phosphate and acetate buffers in STF 1.

The *in vitro* release profiles of (-●-) CH_4P, (-▫-) CH_5P, (-▲-) CH_6P (-○-) CH_4A (-□-) CH_5A (-△-) CH_6A conducted in STF 1 are shown. The compositions of these systems are shown in Table 1

No significant difference in the *in vitro* release of formulation prepared with phosphate (pH 7.4) and acetate buffer (pH 5.0) was noted, except for batches CH_3P/CH_3A and CH_4P/CH_4A ($P>0.05$; student t-test), which showed a significant difference. This again could be due to the similar gelation properties shown by the formulations prepared with two buffers in STF.

The gels on visual inspection at periodic intervals during the *in vitro* drug release experiments showed a gradual swelling after 6 h that resulted in an increase in the volume of most gels. No discernible relationship between the extent of swelling and gel composition could be established. Also, no apparent changes or disruptions in the integrity of the gels were noticed during the course of experiment. The only evidence to suggest a gradual dissolution of the polymers comprising the gels was that the filtration of the aliquot of release medium became increasingly difficult after each successive withdrawal. Moreover, the linearity of the relationship between the amount of indomethacin released

TABLE 4: PHARMACODYNAMIC EVALUATION IN UVEITIS INDUCED RABBIT EYE

Formulation	Inflammatory condition	Pre-treatment		Post-treatment			
		Left*	Right	1 h	4 h	8 h	24 h
Marketed formulation	Congestion	+++	++	+++	+++	++	++
	Keratitis	+++	++	+++	+++	+++	++
	Flare	+++	++	+++	+++	+++	++
	Aqueous cells	+++	++	+++	+++	++	++
	Clot	+++	+++	+++	+++	+++	+++
	Synechia	Present	Present	Present	Present	Present	Present
CH_5P	Congestion	+++	+++	+++	+++	+++	+
	Keratitis	+++	++	+++	++	0	0
	Flare	+++	++	+++	++	++	0
	Aqueous cells	+++	++	++	++	+	0
	Clot	+++	+++	+++	++	++	0
	Synechia	Present	Present	Present	Present	Absent	Absent

*- treated eye

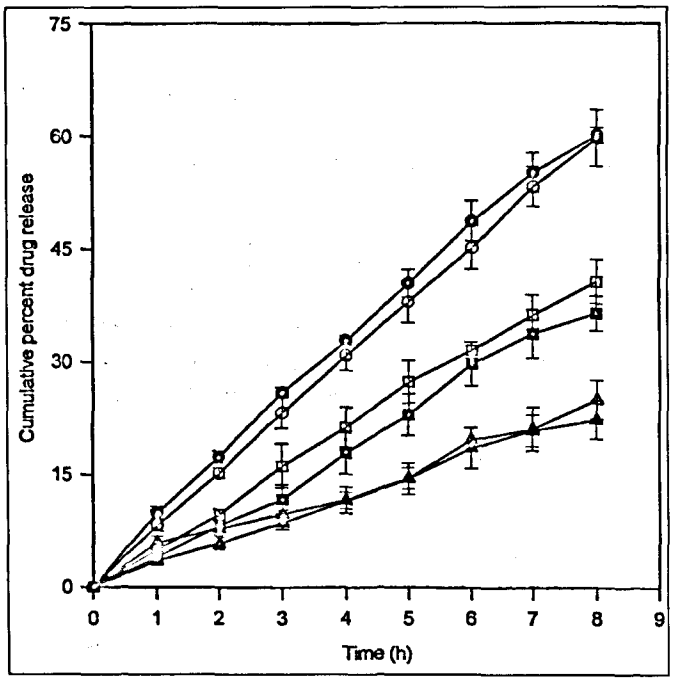

Fig. 1: Effect of Carbopol on the indomethacin release from formulations prepared with phosphate and acetate buffers in STF 1.

The *in vitro* release profiles of (-●-) CH_2P, (-▣-) CH_6P, (-▲-) CH_8P (-○-) CH_2A (-□-) CH_6A (-△-) CH_8A conducted in STF 1 are shown. The compositions of these systems are shown in Table 1

and the square root of time (r^2 values for Q Vs $t^{1/2}$ ranged between 0.992 to 0.999) in conjunction with the slow dissolution rate of the gels suggests that the *in vitro* release occurs primarily by diffusion.

The formulation CH_5P containing 0.4% Carbopol with 0.3% HPMC showed optimum gelation and *in vitro* release behaviour and consequently was selected for *in vivo* studies. The results of the pharmacodynamic evaluation of selected formulation (CHP_5) and marketed formulation of indomethacin (1%w/v) are shown in Table 4.

The pharmacodynamic studies revealed that the *in* situ gelling system was effective even after 24 h when compared to the marketed preparation. In the case of the marketed preparation, no improvement was noticed at the same dose level after 4 h indicating that a frequent dosing is needed to produce optimum therapeutic effect. Moreover, the results are indicative, but not conclusive, of the beneficial effect of Carbopol in the formulation vis-a-vis prolonged residence time, due to the mucoadhesive properties of carbopol[22].

The use of HPMC with Carbopol allowed for a reduction in the concentration of Carbopol (to 0.4%) for optimum gelation. The optimum concentration of HPMC ($K_{15}M$) for ideal gelation and release characteristics, in combination with 0.4% Carbopol was found to be 0.3%. This combina-

tion could serve as a suitable *in situ* gelling vehicle for ophthalmic use.

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