MICROSPHERIC *IN-SITU* GEL FOR OCULAR DRUG DELIVERY SYSTEM OF BROMFENAC SODIUM

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ABSTRACT

Purpose: The aim of the study was to develop a sustained release microspheric in-situ gel for bromfenac sodium (BNa) to be administered through ocular route. It was prepared for the treatment of post-operative cataract surgery. It increases the therapeutic efficiency, reduces dosing frequency and prolonged duration of action. Methods: Albumin microspheres were prepared by protein gelation process and dispersed into in-situ gel, which was prepared by pH triggered method. Result and conclusion: The particle size analysis revealed that all formulations gave particles size in the range of 1-10 µm which is suitable for ocular administration of formulation. The entrapment efficiency of microspheres was found to be 84.2% and drug release from microspheres were found to be 80.558 % at 24 h. The microspheric *in-situ* gel of optimized formulation shows drug release of 77.989% at the end of 24 hours. Stability studies were conducted. Hence, it can be concluded that microsphere in-situ gels were a viable alternative to conventional eye drops by providing sustained release of medicaments in the eye.

Keywords: Bromfenac sodium (BNa), micsrospheric *in-situ* gelling system, egg albumin.

INTRODCUTION

In last few decades, significant attention has been focused on development of controlled and sustained drug delivery systems. New drug delivery system development is largely based on promoting the therapeutic effects of a drug and minimizing its toxic effects by increasing the amount and persistence of a drug in the vicinity of target cell and reducing the drug exposure to non target cells. There are various approaches in delivering therapeutic substance to the target site in a sustained /controlled release fashion. One such approach is using microspheres as carriers for drugs.

Eye is a unique and very valuable organ. This is considered a window hinge. There are many eye diseases that can affect loss of vision. The bioavailability of ophthalmic drugs is, however, very poor due to efficient protective mechanisms of the eye. Blinking, baseline and reflex lachrymation, and drainage remove rapidly foreign substances, including drugs; from the surface of the eye.[1] The conventional drug delivery systems like solutions, suspensions and ointments are no longer sufficient to fulfill the present day requirements of providing a constant rate delivery for prolonged period of time. The main reason for this is poor residence time of drug at the site of action, hence poor bioavailability.[2]

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions are due to rapid pre-corneal elimination of the drug which can be overcome by the use of a gel system. Gels can be instilled as drops into the eyes which undergo a sol-gel transition from the instilled dose.

Bromfenac sodium is non-steroidal anti-inflammatory drug (NSAID) and is widely used to treat severe inflammations and related diseases of the ocular posterior segment, such as retinal and choroidal neovascularization and cystoid macular edema.[3, 4, 5]

Albumin microspheres were prepared by protein gelation process[6]. During the preparation numerous variables were found influencing the size, shape and entrapment efficiency of the microspheres[7-10]. The data obtained from *in vitro* release was fit into various kinetic models to study the release mechanism and release kinetics.

MATERIALS AND METHODS

Bromfenac sodium was a gift sample from Ajanta Pharma, Kandivali, Mumbai, India. Egg albumin was purchased from Hi-Media; Mumbai, cottonseed oil was purchased from local market, Mumbai, and used as obtained. Ether was procured from Thomas Baker Ltd., Mumbai, HPMC K4M, Potassium di-hydrogen orthophosphate; dihydrogen phosphate and citric acid were obtained from Microlabs Ltd, Mumbai, India. Carbopol 940 was obtained from SD Fine Chem. Ltd, Mumbai, India and all other chemicals and reagents were used of analytical grade.

Preparation of Microspheres

Albumin microspheres were prepared by protein gelation process. Egg albumin was dissolved in distilled water. This solution was added drop wise in cottonseed oil to make an emulsion. From the dropping funnel, emulsion was added drop wise in the preheated cottonseed oil (140±5°), kept in a beaker, which was continuously stirred at 1500 rpm. After heat stabilization time of 15 min the preparation was cooled to 25°C, centrifuged at 2500 rpm and supernatant was decanted. Microspheres thus obtained were washed twice with ether to get a free flowing and discreet product. The microspheres were then suspended in anhydrous ether and stored at 4°C in an airtight container. A number of variables were studied affecting size, shape and entrapment ability of microspheres. Separate batches were prepared and minimum of 50 particles were observed under optical microscope using oil immersion lens to optimize the variables.

Optimization of process variables

Various processes such as preparation of emulsion, effect of temperature on stability of microspheres, effect of stirring rate on particle size and drug polymer ratio were studied by preparing series of batches. Results are summarized in Table 1-4. For further studies M5 batch was selected. The data obtained from *in vitro* release was fit into various kinetic models to study the release mechanism and release kinetics.

RESULT AND DISCUSSION

Morphological study of Microspheres

Shape and surface morphology of microspheres were studied using scanning electron microscopy (SEM). To detect the surface morphology of the Microspheres, sample was subjected to SEM. The SEM of sample was done by using model JSM-7600F, under resolution at 1.0nm (15kv), 1.5nm (1kv) and accerating voltage at 0.1 to 30kv. From above analysis, the best batch of microspheres was selected to formulate an in-situ gel. It indicates that the microspheres were discrete, uniform and spherical in shape with a smooth surface area. (Figure 1)

Encapsulation efficiency

An accurately weighed quantity of the microparticles were extracted with pH 7.4 phosphate buffer for 24h, centrifuged at 6000 rpm for 15 min and filtered (Micro-Lab, India) and the absorbance of the filtrate was measured at 268 nm after appropriate dilution in a UV-visible spectrophotometer (Systronic-Model-2203). The drug content was estimated in triplicate using a calibration curve constructed in the same solvent.

Formula = (Practical yield / Theoretical yield) X 100

The encapsulation efficiency of microspheres was found to be 84.2%.

In vitro drug release study of microspheres

Drug release was determined with the help of modified USP XXII dissolution rate model. A 250 ml beaker was placed in the vessel. A glass cylinder of diameter 2.5 cm opened from both the ends was tied at one end with treated cellophane membrane Albumin microspheres were suspended in 1ml of phosphate buffer (pH 7.4) in to this assembly and dipped into the beaker containing dissolution media. The cylinder was then attached to the metallic device shaft attached to the motor. The beaker was filled with 90 ml phosphate buffer (pH 7.4) and temperature was maintained at 37±1°. Speed was maintained at 50 rpm. Samples were withdrawn. Concentration was determined spectrophotometrically at 268 nm. Drug releases from microspheres were found to be 80.558 % at the end of 24 h.

Preparation of *in-situ* gel

The *in-situ* gels of BNa microspheres were prepared in aseptic area. The *in-situ* gel forming system of BNa was prepared by pH-triggered system. The buffer salts were dissolved in 75 ml of purified water, HPMC K4M and carbopol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer. BNa microspheres were dispersed in small quantity of water and benzylkonium chloride (BKC) was added to *in-situ* gel. The drug solution was then added to the polymer solution and volume was made up to 100ml. The preparation was then filtered through muslin cloth. Formula of in-situ gel is given in (Table 5)

Appearance

All developed formulations were evaluated for clarity by visual observation against a black and white background.

Autoclaving

The prepared formulations were filled in 5 ml capacity glass vials, closed with rubber closures, and sealed with aluminum caps. The sealed vials were subjected to sterilization by autoclaving at 121°C and 15 psi for 20 min. The preparation was cooled down to room temperature and was evaluated for their physical properties.

Gelling Capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared phosphate buffer pH (7.4) and visually observed. The time taken for its gelling was noted.

pH

The pH of each formulation was measured using digital pH meter (Euip-Tronics Model EQ-610) which was calibrated using buffers of pH 4.0 and pH 7.0 before measurements. Each recording was made in triplicates.

Viscosity Studies

The viscosities of all prepared formulations were measured using Digital Brookfield Viscometer (DV-II+Pro). The measurements were carried out using spindle no.62. The viscosity of the sample solutions was measured at different speeds at a temperature of 25 ± 1°C. The developed ophthalmic formulation with pH 6.0 was determined and angular velocity increased gradually from 10 to 100 rpm. The pH of the formulation was then raised by 0.5 N sodium hydroxide solutions and the viscosity and shear stress was determined.

Drug content

The drug content was determined by diluting 1 ml of the formulation to 100 ml with phosphate buffer solution pH 7.4 and digested in 10 ml of 0.5N HCl. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with phosphate buffer pH 7.4. Absorption of this solution was measured at 268 nm on systronic 2203 UV spectrophotometer against phosphate buffer as a blank. Drug content was estimated using calibration curve of BNa in phosphate buffer. Result of above mention tests were given in (Table 6).

Sterility Testing

Sterility testing was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate and soybean casein digest medium respectively as per the Indian Pharmacopoeia. The method used for sterility testing was direct inoculation method.10 ml culture was added to 100 ml of culture medium. Both media were kept for incubation at 32^0C for 7 days and observed for any microbial growth. The sterility test results were compared with positive and negative controls. The sterility test results were compared with positive and negative controls. The number of microbial growth was found to be negative.

Eye Irritancy test

Ocular irritation study was performed on optimized formulation on three albino rabbits, each weighing about 2 to 3 kg, and 0.1 ml of the optimized sterile Bromfenac Sodium formulation was instilled in to over cul-de-sac twice a day for a period of 14 days. The rabbits were monitored periodically for redness, swelling and watering of the eye. Registration no: OCP/CPCSEA/2013-14/OCP/11. The results of the ocular irritation studies indicate that formulation was non-irritant.

***In-vitro* studies of microspherc *in-situ* gel**

Drug release was determined with the help of modified USP XXII dissolution rate model. A 250 ml beaker was placed in the vessel. A glass cylinder of diameter 2.5 cm opened from both the ends was tied at one end with treated cellophane membrane. 1ml of microspheric in-situ gel was suspended in of phosphate buffer (pH 7.4) in to this assembly and dipped into the beaker containing dissolution media. The cylinder was then attached to the metallic device shaft attached to the motor. The beaker was filled with 90 ml phosphate buffer (pH 7.4) and temperature was maintained at 37±1°. Speed was maintained at 50 rpm. Samples were withdrawn. Concentration was determined spectrophotometrically at 268 nm. The microspheric *in-situ* gel of optimized formulation shows drug release of 77.989% at the end of 24 hours.

Comparative studies of release of drug from microspheres and microspheric *in-situ* gel were shown in (Table 7) and (Figure 2).

Stability Studies

Accelerated stability studies were carried out according to the ICH guidelines. Optimized formulations were sealed in amber colored bottles with cap which was further covered by aluminum foil. These packed formulations were stored at two different temperatures. According to ICH guidelines formulations were stored at room temperature 28^0 C $\pm1^0$ and 40^0 $\pm2^0$ C. Results were given in (Table 8).

CONCLUSION

The objective of the current study was to develop sustained release microsphere In-situ gel for the drug to be administered through ocular route. BNa is used to prepare a sustained release ocular dosage form for the treatment of post-operative cataract surgery. It increases the therapeutic efficiency, reduces dosing frequency and prolonged duration of action. The binding of drugs depends on the physicochemical properties of the drugs, microparticle polymer and also on the formulation process of these particles. After microspheric gel formation

the drug absorption in the eye is enhanced significantly in comparison to conventional ophthalmic delivery systems like eye drops solution.

The particle size analysis revealed that all formulations gave particles size in the range of 1-10 µm which is suitable for ocular administration of formulation. The entrapment efficiency of microspheres was found to be 84.2% and drug release from microspheres were found to be 80.558 % at 24 h. pH dependent *in-situ* gelling system was prepared by using carbopol 934 and HPMC K4 M. The microspheric *in-situ* gel of optimized formulation shows drug release of 77.989 % at the end of 24 hours. Eye irritancy test was performed on albino rabbits. The results of the ocular irritation studies indicate that formulation was non-irritant.

The stability data recorded over a 3 months period according to ICH guidelines it was observed that formulations were stable.

The in-situ gel forming will have good patient acceptance because it is easy to instill and gradually erodes by dissolution of the gel avoiding the need for removal.

The long residence time of the gel formed in-situ along with its ability to release drugs in a sustained manner would enhance bioavailability

Hence, it can be concluded that microsphere in-situ gels were a viable alternative to conventional eye drops by providing sustained release of medicaments in the eye.

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Table 1: Preparation of emulsion (W/O) using cottonseed oil

Batches	**Water (ml)**	**Oil (ml)**	**Stability of Emulsion**
E1	2	20	Unstable (breaking)
E2	2	25	Breaking
E3	2	30	Stable then broke within few mins.
E4	3	20	Stable then broke within few mins
E5	3	25	Breaking
E6	3	30	Stable then broke within few mins.
E7	4	20	Breaking
E8	4	25	Stable then broke within few mins
E9	**4**	**30**	**Stable**

Table 2: Effect of Temperature on Stability of Microspheres

Emulsion batch	**Preheated Oil Temp (^{0} C)**	**Stability of Microspheres**
E9	110	Unstable (agglomeration)
E9	120	Unstable (agglomeration)
E9	130	Stable then agglomerated
E9	**140**	**Stable**
E9	150	Charring

Table 3: Effect of Stirring Rate on Particle Size of Microspheres

Emulsion batch	**Stirring Rate (rpm)**	**Average Particle Size (µm)**
E9	800	26.0±1
E9	1000	24.02±0.2
E9	1200	17.5±0.5
E9	1400	11.7±0.2
E9	**1500**	**7.5±0.1**

Table 4: Selection of Drug Polymer Ratio

Microspheres code	**Drug : Polymer ratio**	**Percent Entrapment Efficiency (%) SD ±**
M1	1:2	30±0.22
M2	1:4	35.2±.071
M3	1:6	42.1±0.1
M4	1:8	50.5±0.2
M5	**1:10**	**84.2±0.5**
M6	1:12	76.7±0.27

Table 5: Formulation of in-*situ* gel

Sr. No.	**Ingredients**	**F1 (gm)**	**F2 (gm)**	**F3 (gm)**	**F4 (gm)**
1	BNa Microspheres of M5 batch (equivalent to 0.09 mg of drug)	0.1	0.1	0.1	0.1
2	Carbolpol 940	0.25	0.3	0.35	0.4
3	HPMC K4M	0.8	1.0	1.25	1.5
4	Citric acid IP	0.407	0.407	0.407	0.407
5	Disodium Hydrogen Phosphate IP	1.125	1.125	1.125	1.125
6	Benzalkonium Chloride (BKC)	0.02	0.02	0.02	0.02
7	Purified water	100ml	100ml	100ml	100ml

Table 6: Characteristics of formulation after autoclaving microspheric *in-situ* gel

Formulation	**pH (SD±)**	**Clarity**	**Gelling Capacity**	**Viscosity (cp) of sol**	**Viscosity (cp) of gel**	**Drug Content(%)**
F1	6.05 ± 0.7	Clear	++	550	1320	76.75±0.3
F2	**6.01 ± 0.3**	**Clear**	**++**	**825**	**1404**	**78.65±0.5**
F3	6.03 ±0.1	Clear	++	1121	2020	74.23±0.2
F4	6.01 ± 0.3	Clear	+	1380	2601	72.55±0.1

Table 7: Values of Drug Release of M5 Batch and F2 Formulation

	% Drug Release of Microspheres (M5) and Formulation (F2)	
Time (Hrs)	**M5 (SD±)**	**F2 (SD±)**
0.5	5.968±0.1	5.332±0.1
1	9.271±0.3	8.478±0.2
2	17.694±0.25	15.606±0.6
4	24.575±0.1	19.196±0.2
6	37.732±0.57	29.804±0.6
8	55.511±0.68	48.479±0.5
10	69.218±0.71	64.293±0.3
24	80.558±0.11	**77.989±0.2**

Table No 8: Stability Studies Data of F2 batch

Temp / R.H.	Time (days)	Appearance	pH	Drug Content (%)
(R.T) 28±0.1^{0}C/60±5%	30	Clear	6.03±0.3	77±0.2
(R.T) 28±0.1^{0}C/60±5%	60	Clear	6.04±0.2	77±0.5
(R.T) 28±0.1^{0}C/60±5%	90	Clear	6.03±0.4	77±0.4
40 ± 2^{0}C/75 ± 5%	30	Clear	6.07±0.3	77±0.3
40 ± 2^{0}C/75 ± 5%	60	Clear	6.05±0.2	77±0.5
40 ± 2^{0}C/75 ± 5%	90	Clear	6.04±0.2	77±0.2

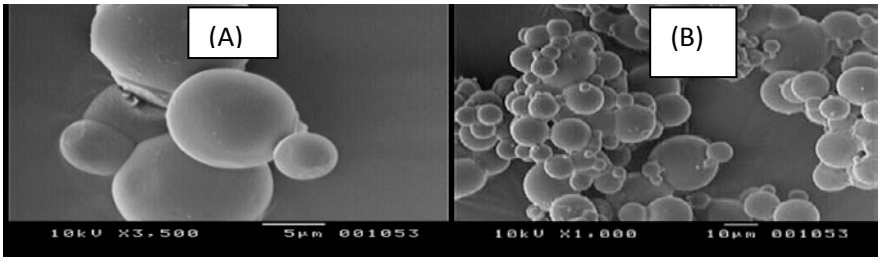

Figure 1: (A) and (B) SEM of Microspheres

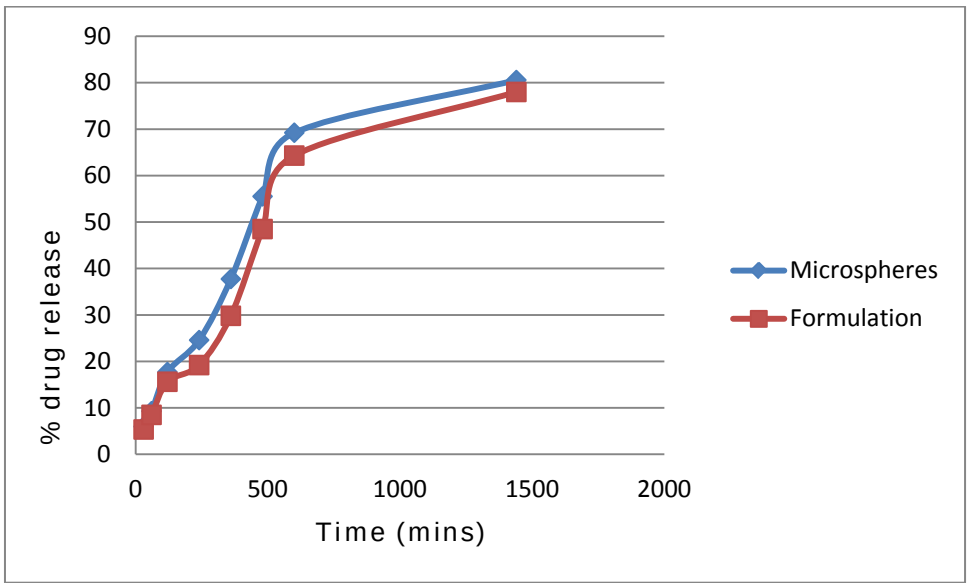

Figure 2: Comparison of % drug release of microspheres and in-situ gel of M5 and F2 batch