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Nanoparticles for Post Cataract Treatment

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ABSTRACT

Dexamethasone sodium phosphate (DSP) nanoparticles were prepared by solvent evaporation process. The prepared nanoparticles were evaluated for size, drug content uniformity, viscosity and release studies. The prepared particulates ranged from 1 to 3 µm. The highest drug content uniformity was high in 0.2% methyl cellulose (MC) in 1% sodium alginate (SA) gel and Poly (DL-lactide-co-glycolic acid) (PLGA) in 3% SA gel. *In vitro* release from a formulation containing 0.2% MC in 1% SA gel and PLGA in 3% SA gel showed a 2-3–fold increase in drug release when compared to a drug in solution. The release from 0.2% MC in 1% SA gel and PLGA in 3% SA gel formulation followed a zero order release. The 0.2% MC in 1% SA gel formulation follows a non-Fickian release mechanism whereas PLGA in 3% sodium alginate gel follows an analomous type mechanism. Hence these ophthalmic gels may be a viable alternative to conventional eye drops which will help to improve patient compliance.

Keywords: dexamethasone sodium phosphate (DSP), methyl cellulose, PLGA, sodium alginate gel

INTRODUCTION

Most ocular diseases are treated with topical applications of solutions administered as eye drops. The major deficiencies of this conventional dosage form include poor ocular drug bioavailability, pulse drug entry, systemic exposure due to the nasolacrimal duct drainage, and poor entrance to the posterior segments of the eye due to the lens diaphragm (Kimura and Ogura 2001).

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constrains, which protect the eye and maintain visual functions. After instillation of an ophthalmic drug, most of it is rapidly eliminated from the pre-corneal area due to drainage by the nasolacrimal duct, blinking and dilution by tear turnover (approximately 1 µl/min). It has been determined that as much as 90% of the 50 ml dose administered as eye drops is cleared within 2 min and only 1-5% of the administered dose permeates to the eye (Bourlais *et al*. 1998).

After instillation, the flow of lacrimal fluid removes instilled compounds from the surface of the eye. Even though the lacrimal turnover rate is only about 1 µl/min the excess volume of the instilled fluid flows to the nasolacrimal duct rapidly in a couple of minutes. Another source of non-productive drug removal is its systemic absorption instead of ocular absorption. Systemic absorption may take place either directly from the conjunctival sac via local blood capillaries or after the solution flow to the nasal cavity (Arto *et al*. 2006). Most small molecular weight drug doses are absorbed into systemic circulation rapidly in a few minutes. This contrasts the low ocular bioavailability of less than 5%. Drug absorption into the systemic circulation decreases the drug concentration in lacrimal fluid extensively (Barbu 2006).

Cataract is a degradation of the optical quality of the crystalline lens. The development of cataract is therefore a continuum, extending from minimal changes of original transparency in the crystalline lens to the extreme stage of total opacity (Loganathan 2007).

Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment. At present no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through novel approaches in drug delivery systems (Biju *et al.* 2006*)*.

In the treatment of post-operative cases of cataract, instillation of eye drops containing corticosteroids with antibiotics, for every hour installation of medicaments is one of the major draw back for getting compliance from the patient (Maria *et al.* 2001). It needs nursing care.

To overcome the above drawbacks we wish to formulate an ophthalmic preparation containing dexamethasone sodium phosphate (DSP) nanoparticles for the post-cataract treatment with the objectives to enhance contact time of drug in eye, to enhance the bioavailability to corneal epithelium, to provide a sustained action, to reduce the dosing frequency, to improve the patient compliance and to study the effect of bioadhesive property of DSP nanoparticles gel in post cataract surgery (Bourlais *et al.* 1997).

DSP is a crystalline corticoid that has been used for the treatment of post cataract treatment administered as eye drops (Eroglu *et al*. 2000). The goal of this work was to formulate and optimize DSP nanoparticles in gel form by using (1 and 3%) sodium alginate and 0.1% carbopol for post-cataract treatment.

MATERIALS AND METHODS

Materials

Methyl cellulose (MC), carboxy methyl cellulose (CMC), poly (vinyl alcohol) (PVA), sodium alginate (SA), carbopol(C) were purchased from Loba-Chemie, Mumbai. poly (D,L-lactide-co-glycolic acid) (PLGA) (85:15), phosphotidyl choline were purchased from Sigma–Aldrich, Hyderabad. Dialysis membrane (M.wt. 14,000) was purchased in Hi-Media, Mumbai. Dexamethasone sodium phosphate (DSP) was provided as a gift sample by Appasamy formulations Ltd, Pondicherry. Dichloromethane, benzalkonium chloride, polysorbate 80, EDTA in analytical grade were purchased from Loba-Chemie.

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Original Research Paper

Preparation of particulate solution

The particulate solution was prepared by solvent evaporation method. Dexamethasone sodium phosphate (0.1% by weight) was added to dichloromethane and sonicated for 3 min (PCI, 50 Hz, Chennai). The organic phase was added to corresponding aqueous phase like Methyl cellulose (0.1-0.4%), or carboxy methyl cellulose (0.1-0.4%), or PVA (0.25%), then magnetically stirred (Remi Instruments, Mumbai) at 1200 rpm at room temperature to evaporate dichloro methane (about 4 h). The particulate solution was obtained (Kumar *et al*. 2006; Kim and Martin 2006).

Particle size determination

The particulate solution was taken in a glass slide and particle size was determined by optical microscopy using a pre calibrated eye piece. The size of 50 particles was measured randomly using ocular and stage micrometer (Shekunov *et al*. 2007; Fatal *et al*. 2007). Eye piece was calibrated using stage micrometer at 40 X magnification. Size of each division for calibration of eye piece micrometer was determined using the formula

Size of each division = (Number of divisions of stage micrometer/Number of divisions of eye piece micrometer) X 10

Each division of eye piece micrometer was found to be 2 µm at 40 X magnification.

Photo micrographs

A drop of ophthalmic particulate solution was placed on the microscopic glass slide. Photographs of formulations were taken at 40 X magnification using the digital camera (Olympus, 8 mega pixels) attached to the eye piece of the microscope (**Fig. 1**).

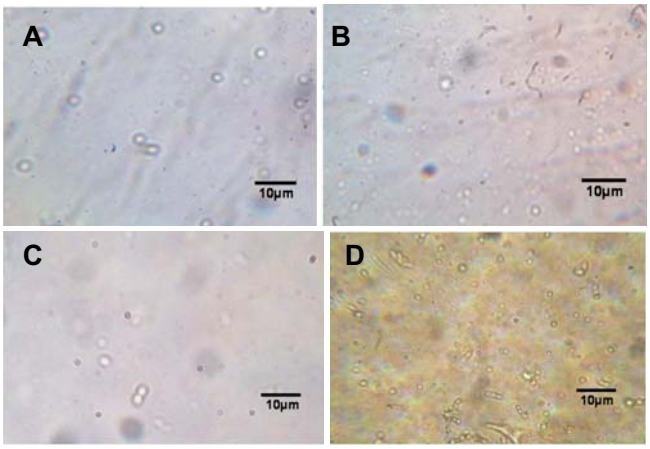

Fig. 1 Optical microscopical image of DSP particulates in ophthalmic particulate solution. Formulation with (**A**) 0.2% MC, (**B**) 0.2% CMC, (**C**) 0.4% CMC, (**D**) PLGA.

Dispersion of particulate solution in gels

The ophthalmic particulate solutions were taken and mixed with the specified quantity of sodium Alginate (1 or 3%) or carbopol (0.1%) correspondingly, and triturating was continued for 1 hr till the particulate solution was dispersed and to get a gel consistency. The ophthalmic gels were sterilized by autoclave at 121°C for 20 min (Kristinsson *et al*. 1996).

Drug content uniformity

The vials containing the preparations were shaken for a few minutes and 100 µl of the preparations were transferred to 25 ml volumetric flasks using a Micro Pipette. Phosphate buffer (pH 7.4) was added in small portions (5 ml), shaken to dissolve the contents, volume was adjusted to 25 ml, and the solutions were assayed for Dexamethasone sodium phosphate content at 242 nm (Pandit *et al*. 2003, 2007).

Drug Content Uniformity = ((Concentration x Dilution factor)/1000).

pH of ophthalmic gels

2.5 g of gel was accurately weighed and dispersed in 25 ml of purified water. The pH of dispersions was measured using pH meter (Jain *et al*. 2007).

Determination of viscosity

Viscosity of formulated gels was determined using Brookfield Viscometer. Gels were tested for their rheological characteristics at 27 ± 2°C using Brookfield Viscometer (LVDV-E Brookfield engineering Lab) spindle no 62. The measurement was made at the speed of 30 rpm (Doijad *et al*. 2006).

In vitro drug release

In vitro release from formulation was carried out by dialysis membrane diffusion technique (Hi-Media dialysis membrane 12000–14000 M.wt cut off). The membrane used was transparent and regenerated cellulose type, which was permeable to low molecular weight substances. The release study of the gel was performed with 1 ml ophthalmic gel in a dialysis bag and sealed with closure clips, then immersed in phosphate buffer (pH 7.4). The receptor compartment was continuously stirred (50 rpm) using a magnetic stirrer. The temperature was maintained at 37 ± 1°C. Samples were withdrawn at predetermined time intervals and the same volume was replaced with fresh buffer medium to maintain sink condition. The absorbance of the withdrawn sample was measured after suitable dilution. At various time intervals, aliquot samples was withdrawn and assayed for drug content by UV spectrophotometer method. Drug release profiles were plotted by taking time on X axis and percentage release on the Y axis (Zhang *et al*. 1996).

RESULTS AND DISCUSSION

Formulation of ophthalmic particulate solution

The ophthalmic particulate formulation was formulated using solvent evaporation method. The particles in the particulate solutions were found to be good and visible in the formulation prepared with 0.2% MC, 0.2% CMC, 0.4% CMC and PLGA. Hence these formulations were selected for the further investigations (**Tables 1-3**).

Table 1 Formulation of nanoparticle solution with methyl cellulose.

Ingredients	0.1%	0.2%	0.3%	0.4%
Dexamethasone sodium phosphate	0.01 g	0.01 g	0.01 g	0.01 g
Methyl cellulose	0.01 g	0.02 g	0.03 g	0.04 g
Carboxy methyl cellulose	-	-	-	-
Sodium edetate	0.001 g	0.001 g	0.001 g	0.001 g
Benzalkonium chloride	0.001 g	0.001 g	0.001 g	0.001 g
Polysorbate 80	0.005 g	0.005 g	0.005 g	0.005 g
Phosphate buffer (qs)	10 ml	10 ml	10 ml	10 ml

Table 2 Formulation of nanoparticle solution with carboxy methyl cellulose.

Ingredients	0.1%	0.2%	0.3%	0.4%
Dexamethasonesodium phosphate	0.01 g	0.01 g	0.01 g	0.01 g
Methyl cellulose	-	-	-	-
Carboxy methyl cellulose	0.01 g	0.02 g	0.03 g	0.04 g
Sodium edetate	0.001 g	0.001 g	0.001 g	0.001 g
Benzalkonium chloride	0.001 g	0.001 g	0.001 g	0.001 g
Polysorbate 80	0.005 g	0.005 g	0.005 g	0.005 g
Phosphate buffer (qs)	10 ml	10 ml	10 ml	10 ml

Table 3 Formulation of nanoparticle solution with PLGA.

Ingredients	PLGA
Dexamethasone sodium phosphate	0.01 g
PLGA	0.025 g
Phosphotidyl choline	0.2 g
Poly vinyl alcohol	0.025 g
Sodium eetate	0.001 g
Benzalkonium chloride	0.001 g
Polysorbate 80	0.005 g
Phosphate buffer (qs)	10 ml

Table 4 Average particle size in formulation.

Formulation	Particle sie (μm)
0.1 % MC	3.88
0.2 % MC	2.4
0.3 % MC	3.63
0.4 % MC	5.26
0.1 % CMC	4.12
0.2 % CMC	2.28
0.3 % CMC	3.56
0.4 % CMC	2.34
PLGA	2.22

Table 5 Drug content uniformity and pH of formulated gels

Gel formulation	Content	% Uniformity	pH
1% sodium aliginate gel	0.2% MC	77.36	7.2
	0.2% CMC	41.8	7.34
	0.4% CMC	60.56	7.12
	PLGA	87.57	7.42
3% sodium aliginate gel	0.2% MC	79.17	7.18
	0.2% CMC	64.4	7.32
	0.4% CMC	14.2	7.16
	PLGA	75.27	7.38
0.1% Carbopol gel	0.2% MC	18.8	7.22
	0.2% CMC	13.0	7.3
	0.4% CMC	14.9	7.1
	PLGA	24.75	7.4

Table 6 Viscosity of ophthalmic gels.

Gel formulation	Content	Viscosity[a]
1% sodium aliginate gel	0.2% MC	52
	0.2% CMC	198
	0.4% CMC	493
3% sodium aliginate gel	0.2% MC	189
	0.2% CMC	588
	0.4% CMC	994

[a] (cps) at 30 rpm

Particle size

The particle size in the prepared formulation was found to be small in PLGA (2.22 μm) and bigger in 0.4% MC (5.26 μm) (**Table 4**). Formulation with 0.2% MC and 0.2 CMC were also found to be smaller. Micro particles (mean diameter 1-3 μm) may be better suited for controlled release, but the presence of coarse particle above 25 μm makes them less tolerable and cause irritation to the eye (Shekunov *et al*. 2005). This confirms formulated particulate ophthalmic solutions were within the limit.

Drug content uniformity and pH

The drug content uniformity in 1% sodium alginate gel with PLGA and 0.2% MC was seemed to be high as 87.57 and 77.36%, whereas 3% sodium alginate gel with PLGA and 0.2% MC was 75.27 and 79.17%. Even though drug content uniformity in PLGA and 0.2% MC were alone seemed to be higher in carbopol gel, the other drug content uniformity (0.2% MC, 0.2% CMC, 0.4% CMC and PLGA) in Carbopol gel was less when compared with sodium alginate formulations (1 and 3%) (**Table 5**).

The pH of all ophthalmic formulated gels (1 and 3% sodium alginate gel and 0.1% carbopol gel) was found to be in limit (pH 7-7.5) (**Table 5**).

Viscosity of gels

When the nanoparticulate solutions dispersed in lower polymer concentrations, 1% sodium alginate the viscosity was found to be low. When the polymeric concentration increased (3% sodium alginate) the viscosity seems to be increased.

Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye. The viscosity range of 3% sodium alginate gels is 3-4 times higher than that of 1% sodium alginate gel. Viscosity values of 0.2% MC in 1% SA gel (52 cps) are promising; hence it may help to increase the contact time in conjunctival sac during application (**Table 6**).

In vitro release studies

Drug release from 1% sodium alginate gel, with the formulation 0.2% MC was 90.08% at 120 mins, where as in 0.2% CMC it was 26.08%. In 0.2% CMC, sodium alginate may interact with different oxygen atoms in 0.2% CMC and may form intrachem binding which may block the drug release. The higher polymeric concentration of 0.4% CMC shows 98.53% at 120 mins. The release from 0.2% MC and 0.4% CMC shows the maximum percentage release of about 90% at 120 mins. The release profile from PLGA formulation was found to be 33.65% at 300 mins; it may be due to the higher concentration of lactic acid which degrades slowly in the medium (**Fig. 2**).

Drug release from 3% sodium alginate gel shows 69.52% of release in lowest polymeric concentration of 0.2% MC at 120 mins; whereas the drug release was not found from the 0.2% CMC in the initial hours, it may be due to formation of egg box model through intrachem binding of two or more alginate chains in gel. Formulation containing 0.4% CMC concentration dispersed in 3% sodium alginate gel shows 76.47% of drug in 60 mins. PLGA shows the release of 82.80% at 120 mins, and it extends up to 210 mins where as release of drug was 99.46%. From the release study of 3% sodium alginate gel 0.2% MC and PLGA shows the maximum percentage release (**Fig. 3**).

Formulation was dispersed in 1 and 0.5% carbopol gel and the drug was not released from this vinyl polymer even after 2 hour (results not shown). The formulation 0.2% MC was dispersed in 0.1% carbopol and this release alone was found to be 78.48% in 270 min. Formulations 0.2% CMC, 0.4% CMC and PLGA were dispersed in 0.1% carbopol gel found that the release was less than 40% of drug in 270 min. The lower drug release may be due to the higher concentration of carbomer in carbopol which in turn causes poor swelling. The decreased drug release may also be due to the

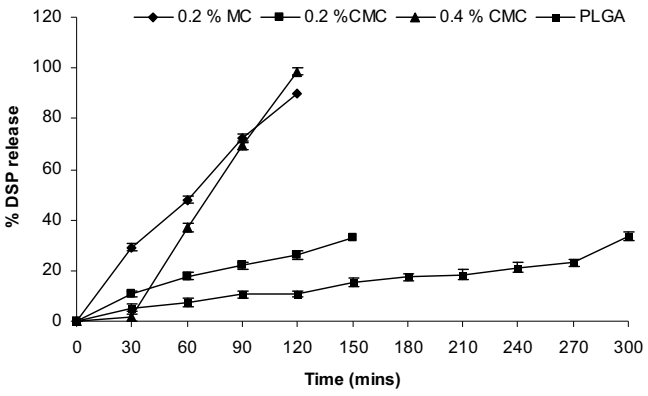

Fig. 2 ***In vitro*** **release of DSP from 1% sodium alginate gels.**

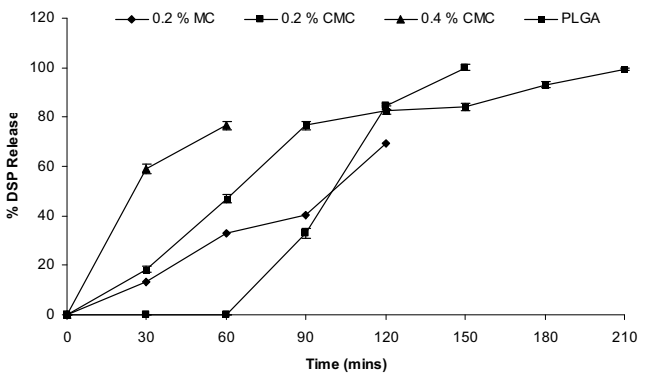

Fig. 3 ***In vitro*** **release of 3% sodium alginate gels.**

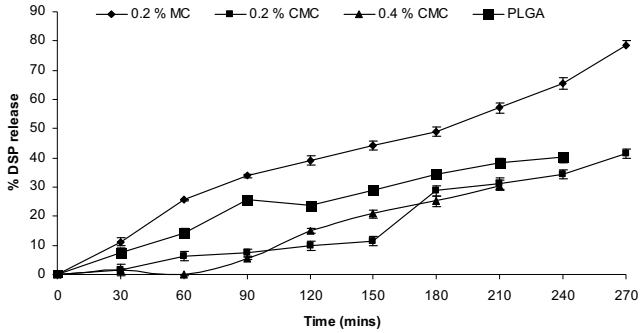

Fig. 4 ***In vitro*** **release of 0.1% carbopol gel.**

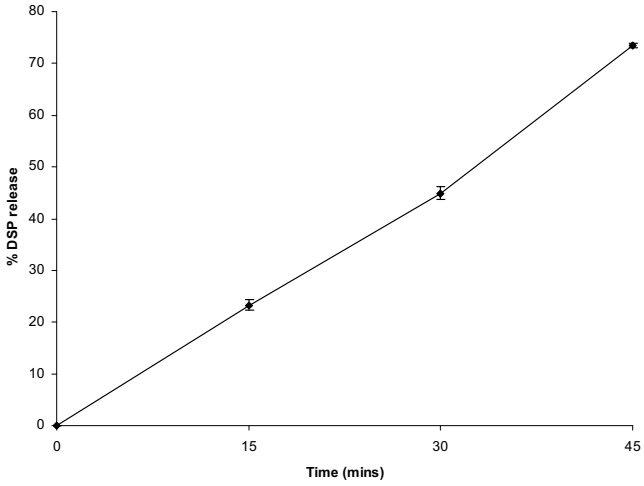

Fig. 5 ***In vitro*** **release of dxamethasone sodium phosphate solution (drug in solution).**

fillers in formulation. Hence these formulations were not taken for further studies (**Fig. 4**).

The release data of drug in solution shows 73.85% of drug released in 45 mins (**Fig. 5**).

The drug released from 0.2% MC in 1% sodium alginate gel and 3% sodium alginate was 90.08 and 69.52% at 120 min, where as PLGA in 1% sodium alginate gel and 3% sodium alginate was 33.65 and 99.46% in 210 min. The formulations with higher drug release and drug content uniformity, 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gels were selected for further studies.

The *in vitro* drug release from 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gel was about 20% in the initial 30 min.

The difference in drug release from the formulation may be due to the polymeric concentration in the particulate formulation, permeation of gel through the dialysis membrane and diffusion of drug particulates from the formulation into the gel.

From the kinetics data, the regression value for Higuchi plot shows 0.2% MC in 1% sodium alginate gel and PLGA in 3% sodium alginate gel follows diffusion.

From the slope value of the Korsmeyer Peppas plot 0.2% MC in 1% sodium alginate gel follows non fickian diffusion type of release and PLGA in 3% sodium alginate gel follows analomous type diffusion (diffusion and erosion).

CONCLUSION

In conclusion, ophthalmic gel formulation prepared with 0.2% MC in 1% sodium alginate and PLGA in 3% sodium alginate gives promising results in viscosity, drug content uniformity and *in vitro* studies which may be a viable alternative to ophthalmic eye drops.

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