Development of Microparticle Loaded Gel (MPLGs) for Prolong Ocular Drug Delivery Containing Ketorolac Tromethamine

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Abstract

Microparticles loaded gels (MPLGs) are colloidal drug carriers in the micro- and submicron range. These systems were developed to overcome solubility problems of poorly soluble drugs as well as for long acting injectable depot formulations and specific drug targeting options. The main objective for these systems was to improve the classical aqueous eye drop formulations which have major disadvantages like a rapid elimination of the drugs from the precorneal area. Consequently, colloidal carrier microparticles were designed to combine ophthalmic prolonged action with the ease of the application of liquid eye gels. The applications of ketorolac tromethamine microparticles loaded gels are presented with focus on reduction of ocular pain and burning/stinging following corneal refractive surgery. The viscosities of microparticles loaded gel were found to be in the range (1500 to 1900 Cps). The maximum gel strength and mucoadhesion was found to be up to (40.79 seconds) and (26.66 dynes/cm^2) respectively. The value of 'r' for Higuchi kinetics which ranged from 0.989 to 0.990. It was understood that F code formulation were following predominantly zero order release.

Keywords: Microparticles loaded gels, HPLC, SEM, and Controlled Release

INTRODUCTION

The recommended dose of ACULAR LS™ (ketorolac tromethamine ophthalmic solution) ophthalmic solution is one drop four times a day in the operated eye as needed for pain and burning/stinging for up to 4 days following corneal refractive surgery. Ketorolac tromethamine ophthalmic solution has been safely administered in conjunction with other ophthalmic medications such as antibiotics, beta blockers, carbonic anhydrase inhibitors, cycloplegics, and mydriatics.

Most conventional ophthalmic dosage forms are simplistic. It is usual that water-soluble drugs are delivered through topical administration in an aqueous solution [1], and water-insoluble drugs are administered topically as an ointment or aqueous suspension. The major deficiencies of these conventional dosage forms include poor ocular drug bioavailability, pulse-drug entry after topical administration, systemic exposure because of nasolacrimal duct drainage, and a lack of effective systems for drug delivery to the posterior segment of ocular tissue.

Poor ocular drug bioavailability is the result of ocular anatomical and physiological constraints, which include the relative impermeability of the corneal epithelial membrane, tear dynamics, nasolacrimal drainage [2], and the high efficiency of the blood–ocular barrier [3]. It is standard for only 1% or less of a topically applied dose to be absorbed across the cornea and thus reach the anterior segment of the eye [4,5]. Pulse entry is a common, and yet highly undesirable, pharmacokinetic characteristic associated with eye drops [6].The initial high drug concentration found in tears, followed by a rapid decline, poses a potential risk of toxicity, and suggests a requirement for frequent dosing.

Early attempts

A considerable amount of effort has been made in ophthalmic drug delivery since the 1970s.The various approaches attempted in the early stages can be divided into two main categories: bioavailability improvement and controlled release drug delivery. The former was attempted by the methods like Viscosity enhancers [4,7–9], Gels [10], Penetration enhancer [4,11], Prodrugs [4], Liposomes [12–17] and the latter was attempted by various types of inserts and nanoparticles. After initial investigations, some approaches were dropped quickly, whereas others were highly successful and led to marketed products.

Recent developments

An important lesson learned from earlier efforts in ophthalmic drug delivery is the necessity of balancing the technologies of sustained drug release or bioavailability improvement with patient comfort and ease of use. The preferred system would also provide improved bioavailability, site-specific delivery, and/or continuous drug release [2,4,18]. With this in mind, recent research efforts have focused largely on microparticle enriched gel (MEG) systems.

Microsponges are porous microspheres having myriad of interconnected voids of particle size ranging from 5-150 μm.

Microsponge drug delivery system is a unique technology which provides controlled release of active ingredients [19,20]. It offers numerous advantages over other technologies like reduced side effects, improved stability, increased elegance and enhanced formulation flexibility [21,22]. Microsponges are porous, polymeric microspheres that are used mostly for topical and recently for oral administration. They can be incorporated into conventional dosage forms such as creams, lotions, gels, ointment, tablet and powder and share a broad package of benefits & thus provides formulation flexibility [23-25].

MATERIALS AND METHODS

Preparation of Microparticle:
The microparticle containing drugs were prepared by quasi emulsion solvent diffusion method [26] using different polymer ratio as shown in Table 1. The inner phase, Ethyl cellulose was dissolved in dichloromethane and then added drug to solution under ultrasonication at 35°C and outer phase prepared by dissolving PVA in distilled water at 60ºC for 10 min. The inner phase is poured into PVA solution in water. The resultant mixture was stirred by magnetic stirrer for 60 min at 25ºC, and filtered to separate the microparticle. The microparticle were dried in an air heated oven at 40 °C for 12 hrs, and weighed to determine the yield [27].

Preparation of microparticle loaded gel (MPLGs):
Accurately weighed amount of gelling agents was taken and dissolved in water and soaked for overnight. Accurate amount of prepared gelling agent was dispersed slowly in appropriate of the drug containing microparticle with the help of overhead stirrer. Finally add few drops of triethanolamine to adjust the pH. The suitable gelling agent was selected on the basis of compatibility with microparticle structure, feel and ease of spreadability.

METHODS

Particle size studies:
Particle size analyses were performed on microparticle by optical microscopy (DN-117M, USA). The results are the average of three analyses. The values (d50) were expressed for all formulations as mean size range.

Scanning electron microscopy [28]:
The morphology and size of microparticle were observed by scanning electron microscopy. Prepared microparticle were coated with gold and studied by scanning electronmicroscopy (Phenoworld) under vacuum at room temperature.

Determination of loading efficiency:
The drug content in the microparticle was determined by HPLC method. A sample of drug containing microparticle (10 mg) was dissolved in 100 ml of methanol. The drug content was calculated from the calibration curve and expressed as loading efficiency [28].

$$\text{Loading Efficiency} = \frac{\text{Actual drug contect in microsponge}}{\text{Theoritical drug content}} \times 100$$

Determination of production yield:
The production yield of the microparticle was determined by calculating accurately the initial weight of the raw materials and the last weight of the microparticle obtained.

$$\text{Production Yield (PY)} = \frac{\text{Practical mass of microsponges}}{\text{Theoritical mass (Polymer + drug)}} \times 100$$

Diffusion studies
The diffusion medium used was simulated tear fluid pH 7.4. Assembly of diffusion cell for in–vitro diffusion studies the diffusion cell was designed as per the dimension given. Diffusion cell with an effective diffusion area of 3.14 cm^2 was used for in vitro permeation studies. The diffusion cells were placed on the magnetic stirrers. The donor compartment consisting of 1% w/w of microparticle loaded gel (MPLGs) containing ketorolac. The receptor compartment was filled with fluid. Then the cellophane membrane was mounted on the cell carefully so as to avoid the entrapment of air bubble under the chicken membrane. Intimate contact of cellophane membrane was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment. With the help of 1ml pipette 1ml of sample was withdrawn at a time intervals of 60 minutes from sampling port of receptor compartment and same volume was the replaced with receptor fluid solution in order to maintain sink condition. The samples were appropriately diluted and measured by using HPLC method.

Determination of pH
The pH of the microparticle loaded gel was determined using a calibrated pH meter. The readings were taken for average of 3 samples.

Viscosity Studies
The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of MPLGs was determined at different angular velocities (0.3, 0.6,12, 20, 30, 40….to 60 rpm) and average of two reading were used to calculate the viscosity.

Determination of mucoadhesive force
The mucoadhesive force of all the optimized batches was determined as follows, a section of the chicken mucosa fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. microparticle loaded gel was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The chicken mucosa was changed for each measurement [29].

Detachment stress (dynes/cm^2) = m g/A

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2; and A is the area of tissue exposed.

Measurement of Gel Strength

A sample of 50 gm of microparticle loaded gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37^0 C. The apparatus for measuring gel strength (apparatus as shown in figure 14 weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels at physiological stimuli was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [30].

Spreadability

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability [29].

S= ML/T

Where,

M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

RESULT AND DISCUSSION

Formulations of Ketorolac microparticle loaded gel are prepared by using hydroxypropyl cellulose (HPMC) polymers. Physicochemical properties such as viscosity, spreadability, gel strength, mucoadhesive force were performed and the results are recorded.

Viscosity

Viscosity is the resistance to gradual deformation by shear stress. Viscosity is an important parameter in application on skin. If the formulation is viscous enough, it may retain on skin for longer period of time which may help in the sustained release of drug delivery. The viscosity is measured by using Brookfield viscometer. When the rpm (0.3, 0.6, 1.5, 3.0, 12.0) increases, the viscosity decreases (16% HPMC). However in 18% HPMC, as the rpm (0.3, 0.6, 1.5, 3.0, 12.0) increases, the viscosity increases too. This shows that the 18% HPMC is much more viscous than 16% HPMC.
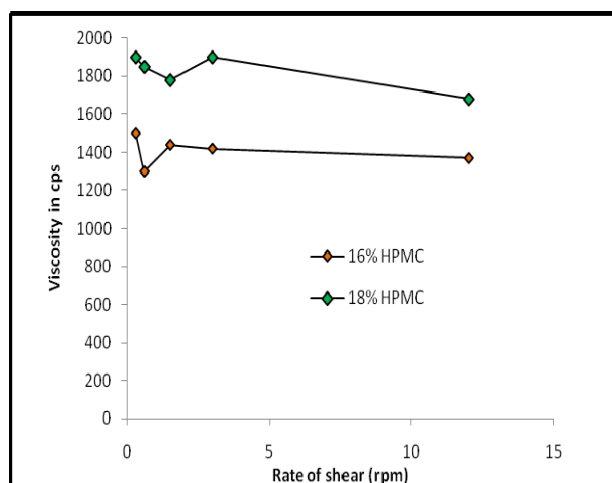

Figure 1: Showing the viscosity optimized formulation Spreadability

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of 16% HPMC is 2.39 whereas 4.23 (gm.cm/sec.) for 18% HPMC. This indicates that as the concentration of polymer increases, the spreadability is increases. The formulation should have good spreadibilty so that it is easily apply and spread on skin. Hence, 18% HPMC is sufficient to produce good spreadability properties.

Table 1: Composition of microparticle loaded gel containing Ketorolac: Ethylcellose

Formulation code	Drug : polymer ratio (w/w)	Theoretical drug Content (mg)	Actual drug content (mg)	Production yield (%)	Mean particle size (µm)
B1 (MS)	1 : 2	150	97	64.67	4.193
B2 (MS)	1 : 1	200	45.3	22.65	2.0344
B3 (MS)	1.5 : 1	250	108.2	43.28	4.939
B4 (MS)	1 : 2	300	177.9	59.3	10.228
B5 (MS)	1 : 3	400	334.3	83.58	-
B6 (SO)	1 : 2	300	153.2	68.55	-
B7 (SO)	1 : 3	400	108.9	36.3	-
B8 (MS)	1.5 : 1 (20ml solvent)	250	274.2	61.28	20.3523

(M.S- Magnetic stirrer, SO- Sonication)

Table 2: Characteristics of Various microparticle loaded gel Formulations

Formulation code	Viscosity (Cps)	% drug content (w/w)	Mucoadhesive force (dynes/cm^2)	Spread ability (gm.cm/sec.)	Gel Strength sec.
HPMC 16%	1900	92.10	26.66	2.39	25.6
HPMC 18%	1500	90.23	24.67	4.23	40.79

Gel Strength

Gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out of the site of administration. The average gel strength of 16% HPMC is 25.60 seconds whereas 40.79 seconds for 18% HPMC. Formulations HPMC 18% shows good gel strength than 16% HPMC. This is probably due to increased in concentration of polymer.

Mucoadhesive Force

Mucoadhesive drug delivery system to mucosal membrane leads to an increase in the drug concentration at the absorption site. Besides, if the mucoadhesive force of a formulation is good, it tends to improve the bioavailability of systemic delivery drugs. In addition, skin adhesive system has been used to target local disorders at the skin surface to reduce the overall dosage required and minimize unwanted side effects. The mucoadhesive force is an important physicochemical parameter for topical application on skin. The formulation 16% HPMC showed an average mucoadhesive force of 26.66 dynes/cm^2. On the other hand, 18% HPMC showed 24.67 dynes/cm^2. In this case, as the concentration of polymer increases, the mucoadhesive force decreases. The force that required by 16% HPMC to detached is stronger than 18% HPMC which showing an inversely proportional relationship.

In vitro Release Studies:

The *in vitro* diffusion profile of Ketorolac from microparticle loaded gel formulations F4 and F5 were conducted in diffusion medium (2.38 g Na_2HPO_4, 0.19g KH_2PO_4 and 8.0g Nacl in 1000 ml of distilled water adjusted to pH 7.4). The formulations F4 and F5 released 81.7% and 85.4% respectively at 8 hours.

Drug Release Kinetics:

The drug release from (MPLGs) formulation was followed diffusion controlled zero order mechanism from the gel, as the value of 'r' for zero order kinetics was 0.990 and also found to be more than that of first order which ranged from 0.983 to 0.986.The value of 'r' for Higuchi kinetics which ranged from 0.989 to 0.990. All the formulations were subjected to PCP DISSO software analysis. the formulations F5 and F6 exhibited good in vitro release kinetics with fickian type of diffusion mechanism. More over to comprehend the drug release mechanism the data were fitted in to korsmeyer -peppas exponential model where the 'n' values were in the range of 0.055 to 0.057. It was understood that F code formulation were following predominantly zero order and fickian diffusion mechanism of drug release from its formulations.
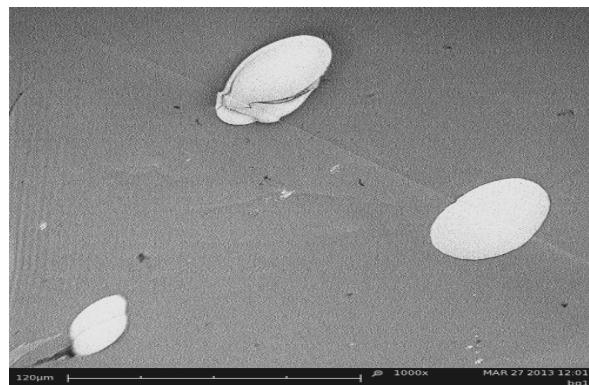

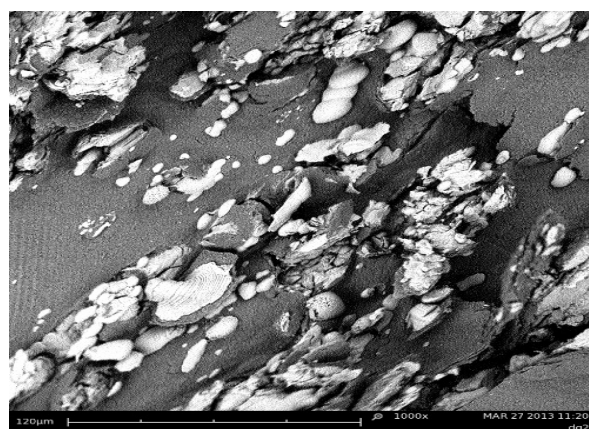**Figure 2: Showing the SEM Photography of optimized formulation (microparticle)**

Figure 3: Showing the SEM Photography of optimized formulation (microparticle loaded gel)
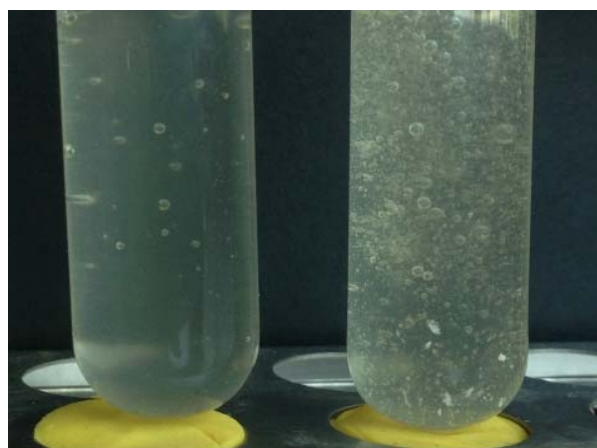

Figure 4: Showing the Photography of (plain gel and microparticle loaded gel)

Chromatographic conditions

HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50μL loop volume. LC solution version 1.25 was applied for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of methanol -10mM ammonium acetate buffer (pH 3.0 adjusted with orthophosphoric acid (30:70, v/v)), and detection was made at 298.0nm. The mobile phase was prepared daily, filtered through a 0.45μm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5μ) was used for the separation.
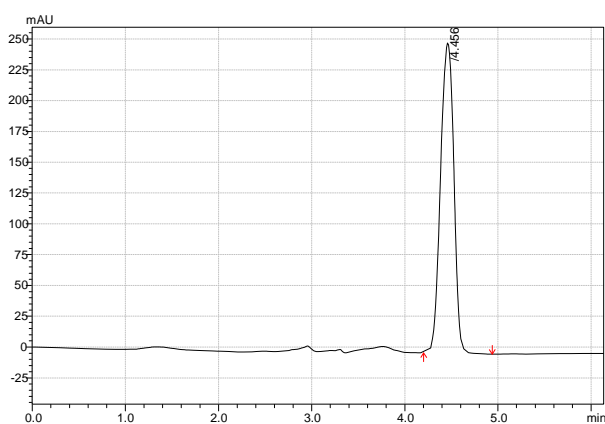

Figure 5. Typical chromatogram of drug sample

CONCLUSION

Microparticle loaded gel (MPLGs) novel delivery system has been developed to provide topical delivery of ketorolac tromethamine. The formulations showed controlled release of drug through skin, indicating better potential of delivery system as compared with ophthalmic solution. If this process can be scaled-up to manufacturing level; this technology has the potential to provide the topical ketorolac tromethamine microparticle loaded gel with better patient compliance. On the grounds of efficacy and improved patient compliance due to reduced frequency of application, microparticle based gel formulations will have significantly better role in topical treatment of pain and burning.

REFERENCES

1. Lang, J.C. (1995) Adv. Drug Deliv. Rev. 16, 39–43
2. Desai, S.D. and Blanchard, J. (1995) in Encyclopedia of Pharmaceutical Technology Swarbrick, J. and Boylan, J.C., eds), pp. 43–75, Marcel Dekker, New York, NY, USA
3. Peyman, G.A. and Ganiban, G.J. (1995) Adv. Drug Deliv. Rev. 16, 107–123
4. Lee,V.H.L. and Robinson, J.R. (1986) J. Ocul. Pharmacol. Ther.2, 67–108
5. Mezei, M. and Meisner, D. (1993) in Biopharmaceutics of Ocular Drug Delivery
6. (Edman, P., ed.), pp. 91–104, CRC Press, Boca Raton, FL, USA Shell, J.W. (1985) Drug Dev. Res. 6, 245–261
7. Chrai, S.S. et al. (1973) J. Pharm. Sci. 62, 1112–1121
8. Adler, C.A., Maurice, D.M. and Paterson, M.E. (1971) Exp. Eye Res. 11, 34–42
9. Patton,T.F. and Robinson, J.R. (1975) J. Pharm. Sci. 64, 1312–1315
10. Zignani, M.,Tabatabay, C. and Gurny, R. (1995) Adv. Drug Deliv. Rev. 16,51–60
11. Liaw, J. and Robinson, J.R. (1993) in Ophthalmic Drug Delivery Systems
12. (Mitra, A.K., ed.), pp. 369–381, Marcel Dekker, New York, NY, USA
13. Smolin, G. et al. (1981) Am. J. Ophthalmol. 91, 220–225
14. Singh, K. and Mezei, M. (1983)Int. J. Pharm. 16, 339–344
15. Stratford, R.E. et al. (1983) Int. J. Pharm. 13, 263
16. Benita, S. et al. (1984) J. Microencapsulation 1, 203–206
17. Singh, K. and Mezei, M. (1984) Int. J. Pharm. 19, 263–269
18. Lee,V.H.L.,Takemoto, K.A. and Iimoto, D.S. (1984) Curr. Eye Res. 3,585–591.
19. Le Bourlais, C. et al. (1998) Prog. Retinal Eye Res. 17, 33–58
20. Jain NK. Advances in controlled and novel drug delivery. New Delhi: CBS Publishers and Distributors; 2003: 89-91.
21. Kydonius AF, Controlled releas e technologies: Methods, Theory and Applications, Boca Raton: CRC; 1980 Press,: 2149.
22. Nacht S, Katz M. The microsponge ; a programmable delivery system. In Osborne , DW, Aman AH , Topical drug delivery formulations. New York: Marcel Dekker; 1990 : 299-325.
23. Embil K, Nacht S. The microsponge delivery system (MDS): a topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for release of actives. J. Microencapsul 1994; 13:575-588.
24. Kim W, Hwang S, Park J, Park H. Preparation and characterization of drug loaded polymethacrylate microspheres by an emulsion solvent evaporation method. J. Microencapsul200; 6: 811-822.
25. Kawashima Y, Niwa T, Hand T, Takeuchi H, Iwamoto T. Control of prolonged drug release and compression properties of ibuprofen microspheres with acrylic polymer by changing their intra-particle porosity. Chem. Pharm Bull 1992; 40: 196-201.
26. Dashora K, Saraf S. Effect of processing variables on micro particulate system of nimesulide. Chinese J. Pharm 2006; 58: 67-74.
27. Dsouza, J.I., Jagdish, K., Saboji, S.G. and Killedar, H.N.: *More "Design and Evaluation of Benzoyl Peroxide Microsponges to Enhance Therapeutic Efficacy in Acne Treatment",* Accepted for presentation in 20th fapa congress, Bangkok , Thailand , Nov30-Dec 3 (2004).
28. Jaya Raja Kumar, Selvadurai Muralidharan, Sokkalingam Arumugam Dhanaraj. Anti-Fungal Activity of microemulsion based fluconazole gel for onychomycosis against aspergillus niger, International Journal of Pharmacy and Pharmaceutical Sciences 11/2012; Vol 5(1):96-102.
29. Jaya raja Kumar, Selvadurai Muralidharan and Sanggetha Ramasamy, Microsponges Enriched Gel (MEGs): A Novel Strategy for Opthalmic Drug Delivery System Containing Ketotifen, , *Journal of Pharmacy Research 2013,5(4),97-102*
30. Deboroh Evelyn, Chuah Chong Wooi, Jaya Raja Kumar, Selvadurai Muralidharan. Development and evaluation of microemulsion based gel (MBGs) Containing econazole nitrate for nail fungal infection, Journal of Pharmacy Research 2012,5(4),2385-2390.