

Original Article

DEVELOPMENT AND CHARACTERIZATION OF DICLOFENAC SODIUM LOADED EUDRAGIT RS100 POLYMERIC MICROSPONGE INCORPORATED INTO *IN SITU* GEL FOR OPHTHALMIC DRUG DELIVERY SYSTEM

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ABSTRACT

Objective: Purpose of the study to design and formulate Diclofenac sodium (DIC) microsponges.

Methods: With varied polymer: drug ratio DIC loaded microsponges were prepared with Eudragit RS100 polymer by quasi solvent diffusion method. Microsponges evaluated for particle size, entrapment efficiency, drug content, *in vitro* drug release, Fourier Transform Infrared Spectroscopy (FTIR), Differential scanning calorimetry (DSC) and Scanning electron microscopy (SEM). DIC loaded microsponges incorporated into ocular *in situ* gel to attained controlled release by microsp sponge and improved residence time by gelling system. Ocular *in situ* gel evaluated for pH, drug content determination, gelling capacity, *in vitro* drug release and sterility study.

Results: DSER4 microsp sponge formulation having polymer to drug ratio 1:7 showed satisfactory production yield (68.13%), entrapment efficiency (62.86%), drug content (80.73%), requisite particle size (less than 10 μm) (7.52 μm) and *in vitro* release 87.94% after 6 h. Selected DSER4 formulation was incorporate into *in situ* gel. Carbopol 940 forms stiff gel at higher pH so used as a gelling agent, whereas Hydroxypropyl Methylcellulose E4M was used as a viscosity-enhancing agent for the formulation of *in situ* gel in varied compositions. *In situ* gel formulation IG4 showed sustained release of 76.92% till the end of 8 h and satisfactory gelling capacity so IG4 further evaluated for sterility test. Rheological studies reveal the sol-gel transition of *in situ* gel occur at the physiological condition to form stiff gel.

Conclusion: Prepared *in situ* gel formulations showed sustained drug release for a period of 8 h, which is satisfactory for management of ocular pain.

Keywords: Microsp sponge, Ocular *in situ* gel, Controlled release, Ophthalmic drug delivery, Kinetic release

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INTRODUCTION

Microsponges are polymeric particulate delivery systems having 5 to 300 μm diameter with a porous spherical structure formed with polymer enclosing active drug ingredients and thereby control drugs release [1]. Microsponges are drug-contained polymeric sponge-like structures with large porous surface and non-collapsible structure made through numerous of interconnecting voids. Microsponges display good stability for extreme acidic (pH 1) to extreme basic (pH 11) conditions also stable at high temperatures (up to 130 °C). This entrapment technologies offer high drug payload than others [2].

Several different research works of microsponges explored on various areas like colonic delivery [3-5], topical delivery [6, 7], gastroretentive delivery [8] and percutaneous delivery [9] for controlled and sustained drug release. Fewer attempts have been made for ophthalmic drug delivery [10-12] of microsponges.

Ophthalmic drug delivery systems have the main problem of fast and substantial precorneal loss, caused by high turnover of tear fluid and further draining of tear fluid from eye [13]. Particulate system suffers from this nasolacrimal drainage. This possibility of short residence time is eliminated by the incorporation of particulate system *in situ* gel and increases its retention in eye. A lot of attempts have been made on the incorporation of particulate system in ocular *in situ* gel [14, 15]. But lesser studies are carried out on microsp sponge-loaded ocular *in situ* gel.

In situ gels, upon installation in ocular cul-de-sac, go through a sol-gel phase transition to form a stiff gel. Phase transition occurs from droppable low viscous solution to stiff gel by the influence of physicochemical changes i.e. pH, temperature and ions composition, caused by tear fluid. This system allows administration of accurate

doses in the form of drop and augmented residence time which in turn improves bioavailability at ocular area [16].

Carbopol is a polyacrylic acid (PAA) polymer, which go through a sol to gel conversion in an aqueous solution with a change in pH above its pKa of about 5.5. However, the high amount of carbopol required to get stiff gel results in highly acidic solutions. Such acidic polymeric solution is unable to neutralize with the buffering capacity of the tear fluid. So to good gelling capacity could be achieved with no further increment in the amount of carbopol in polymer solution; by adding HPMC polymer as viscosity enhancing polymer [17]. HPMC E4M was selected for *in situ* gel formulation as its frequent use in ophthalmic drug delivery, reliability, superior transparency and less incompatibility [18].

Diclofenac sodium is effectively used for pain management in cases of corneal epithelial defects followed by surgery or accidental trauma, and prevention of intraoperative miosis during cataract surgery, treatment of postoperative ocular inflammations, chronic non-infectious inflammations and for symptomatic relief of seasonal allergic conjunctivitis [19].

The purpose of this study is to attain controlled release through microsp sponge and improve its residence time by incorporating microsponges in *in situ* gel. Eudragit RS100 is used as a polymer for the preparation of microsponges. Further microsponges were added to HPMC E4M and carbopol *in situ* gel.

MATERIALS AND METHODS

Materials

Diclofenac Sodium (DIC) was gifted by Nulife Pharmaceuticals, Pune, India. Eudragit RS 100 (ERS) and Hydroxypropyl Methylcellulose E4M (HPMC E4M) was gifted from Evonik India Pvt. Ltd., Mumbai and

Colorcon Asia Pvt. Lmt, Goa, India respectively. Carbopol 940, Dichloromethane (DMC), Triethylcitrate, Polyvinylalcohol 30,000–70,000 (PVA) were procured from Research lab fine chem. Industries, Mumbai. All other chemicals used for analysis were analytical grade.

Preparation of DIC loaded microsponges

For the preparation of DIC-loaded microsponges with ERS polymer; the quasi-emulsion solvent diffusion method was considered. To prepare internal phase ERS polymer and triethylcitrate (1% w/v) dissolved in dichloromethane. To the prepared solution of polymer, DIC was added and homogeneously dispersed by ultrasonication to achieve particle size reduction. Polymer: drug ratio was varied as 1:1 to 1:13. The internal phase was added dropwise to 100 ml of 1% w/v PVA solution. This emulsion was stirred for 4hr using magnetic stirrer at 1000 rpm. The DIC loaded microsponges were filtered, washed with double distilled water and then dried for 48 h at room temperature. DIC-loaded microsponges stored for further characterization [5, 20].

Characterization of DIC loaded microsponge

Determination of Percentage Yield

The percentage yield of obtained microsponges was determined by calculating the percentage of the weight of the DIC loaded microsponges and the collective weight of DIC and ERS polymer.

$$\text{Production yield} = \frac{W_{Pr}}{W_{Th}} \times 100 \dots (\text{Equation 1})$$

Where,

W_{Pr} = Practical mass of DIC loaded microsponges

W_{Th} = Theoretical mass (Total weight of DIC and ERS polymer added to formulation)

Drug content

DIC-loaded microsponge formulation equivalent to 10 mg of DIC of each batch was finely crushed in a glass mortar. Further to this add 60 ml of phosphate buffer (pH 7.4) and the solution was shaken for 4 h. to dissolve DIC and, filtered. Filtered solution was diluted to 100 ml with 7.4 phosphate buffer. The resultant solution was analyzed on UV spectrophotometer (Jasco V-530) at 275.5 nm. The DIC content of the microsponges (D_{At}) was calculated.

Entrapment Efficiency (%)

Entrapment Efficiency (%) of DIC contained microsponge was calculated with an equation,

$$\text{Entrapment Efficiency (\%)} = \frac{D_{At}}{D_{Th}} \times 100 \dots (\text{Equation 2})$$

Where,

D_{At} = Actual drug content in Dried microsponges

D_{Th} = Practical drug content (Weight of DIC added in microsponges)

Particle size

Optical microscopy was used to measure particle size of microsponge by using digital microscope (Motic CV5-2), calibrated with ocular micrometer (AmScope MR400 Microscope calibration slide). Selected microsponge formulation was dispersed in water and few drops were placed on a glass slide. The drop of dispersion was observed under a digital microscope. The mean of particle size for 300 particles was calculated [21].

In vitro drug release from DIC loaded microsponges

Franz diffusion cell was used to carry out *in vitro* drug release of DIC-loaded microsponges. Freshly prepared STF of pH 7.4 was filled in the receptor compartment of the Franz diffusion cell and with a

small magnetic bar STF was constantly stirred at 100 rpm. A dialysis membrane (12 000–14 000 Dalton MW, Hi-media, India) soaked for 12 h in the STF and stretched in between donor and receptor chambers. DIC loaded microsponge dispersed in distilled water and placed in donor compartment; and covered with a coverslip. Samples each of 1 ml, were withdrawn for at predetermined time intervals from the receptor chambers and replaced with fresh STF and sink conditions. *In vitro* drug release was performed thrice for each formulation; further samples were analyzed by measuring the absorbance at 275.5 nm using UV spectrophotometer [22].

In vitro drug release kinetic study of DIC loaded microsponges

Drug Kinetic model studies of *in vitro* release data of microsponge formulations were performed to conclude the DIC release mechanism. *In vitro* release data was fitted to the following models and regression coefficient values (r^2) were calculated.

- Zero-order kinetic model ($d_0 - d = Kt$)
- First order kinetic model ($\log d = \log d_0 - Kt/2.303$)
- Hixson-crowell kinetic model ($d_0^{1/3} - d^{1/3} = Kt$)
- Higuchi kinetic model ($d_0 - d = Kt^{1/2}$)

Where d is the quantity of the DIC remaining in the microsponge at time t and d_0 is the initial amount of the DIC in the microsponge. Diffusion mechanism was explained by the diffusion exponent (n) value of Korsmeyer–Peppas equation ($d_0 - d/d_0 = Kt^n$). The diffusion exponent value $n < 0.45$ signifies the release follows fickian mechanism. If exponent value indicates $0.5 < n < 0.8$ signifies the release follows the non-fickian mechanism [23].

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of DIC, physical mixture of DIC and ERS polymer and; selected microsponge formulations were recorded in ATR mode with a Perkin Elmer (Model: SPECTRUM BX).

Differential scanning calorimetry (DSC)

Thermal analysis of DIC and the selected microsponge were scanned at a rate of 20 °C/min on a Differential scanning calorimetry (Mettler Toledo, Model: StareSW920, Mumbai) between 40 °C and 320 °C under dynamic nitrogen atmosphere DSC studies were carried out intend to find out any incompatibility.

Scanning electron microscopy (SEM)

A Field Emission Scanning Electron Microscope (FEI Nova NanoSEM 450) operated at 3.00 kV and magnification of $\times 500$ and $\times 5000$; was used to study the morphology of microsponges. Selected microsponge need to coat with gold-palladium alloy for 45s under an argon atmosphere before observation.

Formulation of DIC loaded microsponges containing ocular *in situ* gel

Ocular *in situ* gel formulations containing varied concentrations of Carbopol 940 and HPMC E4M were prepared as per the composition summarized in table 1. Accurately weighed HPMC E4M was dispersed in 50 ml of distilled water and carbopol 940 was dispersed over this solution and kept to hydrate 12h. The solution was stirred with an overhead stirrer. DIC-loaded microsponges (equivalent to 0.1 gm DIC) were dispersed in distilled water and added to the polymeric solution. Benzalkonium chloride was mixed to the prepared solution. Required amount of distilled water was added to make up the volume to 100 ml. Prepared *in situ* gel formulation thoroughly agitated and sterilized in an autoclave at 121 °C for 20 min [24, 25].

Table 1: Composition of DIC loaded microsponge containing ocular *in situ* gel formulations

Ingredient (%W/V)	IG1	IG2	IG3	IG4
Carbopol 940	0.1	0.1	0.1	0.1
HPMC E4M	0.2	0.4	0.6	0.8
Benzalkonium chloride	0.02	0.02	0.02	0.02
Distilled water qs	100	100	100	100

Characterization of DIC loaded microsponges containing ocular *in situ* gel

Determination of visual appearance and clarity

The appearance and clarity ocular *in situ* gel formulations were evaluated by observing gel in front of black and white background in fluorescent light inside a bright, lightened cabinet.

Determination of pH

The pH of *in situ* gel formulation was determined using a pH meter (Labindia, Mumbai, Model: GMPH) which was calibrated before use with the buffered solution at pH 4 and 7. Three readings were taken for every sample and the average of three samples was calculated.

Drug content determination

In situ gel formulation was added to 100 ml volumetric flask. To this add 60 ml of pH 7.4 phosphate buffer was added and shaken for 4 h. and filtered. Filtered solution was further diluted to 100 ml with 7.4 phosphate buffer. Analyzed using UV spectrophotometer (UV1Jasco, Japan, Model: V-530) with phosphate buffer (pH 7.4) as blank. The drug content of the microsponges was calculated.

Determination of gelling capacity

To vial containing 2 ml of freshly prepared STF (maintained at 37 °C), drop of the *in situ* gel was added. The drop of *in situ* gel was observed and the time taken by drop for gelation was noted also the time taken for the gel formed to dissolve was noted [12]. Gelling capacity of formed *in situ* gel was noted as follows.

- (-) No gel formation
- (+) The gel formation occur after few minutes and dissolves quickly
- (++) Instantaneous gel formation and resides for few hours
- (+++) Instantaneous stiff gel formation which resides for long period of time

Rheological studies

Brookfield digital viscometer (DVII+PRO) was used to measure the viscosity (in cps) of the prepared *in situ* gel formulations. The spindle (T-D) was rotated at 20 rpm. Three readings were reported 30 sec after putting the motor on and an average calculated.

In vitro drug release from *In situ* ocular gel containing DIC loaded microsponges

The *in vitro* drug release from the *in situ* ocular gel was carried out as per procedure described above under '*In vitro drug release from DIC loaded microsponges*'. The *in situ* ocular gel was placed on donor compartment. To the top of the *in situ* gel 250 μ l of the STF was added to simulate the small amount of tear secreted on the eye surface and covered with a coverslip. 1 ml of samples were taken out at time intervals 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h with timely maintenance of sink condition. Samples were analyzed by using UV

spectrophotometer [22]. *In vitro* drug release studies performed thrice for each *in situ* gel formulation.

In vitro drug release kinetic study of *in situ* ocular gel containing DIC loaded microsponges

Kinetic study of drug release data was performed ocular *in situ gel* as described above under '*In vitro drug release kinetic study of DIC loaded microsponges*'.

Sterility study

The sterility study was performed according to Indian Pharmacopoeia [26]. *In situ* ocular gel formulation was directly inoculated aseptically into fluid thioglycolate medium (20 ml), and incubated for 14 d at 30 °C to 35 °C. Separately, *In situ* ocular gel formulation was directly inoculated aseptically into soyabean-casein digest medium (20 ml) and incubated for 14 d 20 °C to 25 °C. Microbial contamination was tested with a Fluid thioglycolate medium and the presence of fungi tested with soybean-casein digest medium with incubation under aerobic conditions. The tests were performed in triplicate under aseptic conditions to avoid accidental contamination. The positive and negative controls were performed for the examination of growth promotion and sterility, respectively.

RESULTS

Characterization DIC loaded microsphere formulations

Production yield (%), drug content (%) and entrapment efficiency (%)

With increase in polymer: drug ratio there is increment in production yield, drug content and entrapment efficiency. Production yield varied between 27.50% and 72.50%. At low DIC concentration production yield was found extremely low (27.50%). Drug content varied from 27.27% to 90.15% (table 2).

Particle size analysis

Particle size is an important parameter in ocular administration of particular system. Particle size range from 20.39 \pm 4.21 μ m and 0.78 \pm 0.11 μ m. Particle size decreased as an increase in Polymer: drug ratio. This finding is in accordance with literature [2].

In vitro drug release of DIC loaded microsponges

In vitro drug release is graphically presented in fig. 1. Formulation DERS4 (87.94%) and DERS5 (90.73%) showed relatively comparable drug release as compared to DERS3 (79.41%) at the end of 6 h. Study revealed that the highest cumulative drug release after 6 h is 90.73%.

Kinetic analysis of the *in vitro* release data of DIC loaded microsphere formulations

The release of DIC in both DIC-loaded microsponges formulations DERS3 and DERS4 showed more adherence to Higuchi's diffusion kinetic model, with regression coefficient (r^2) value 0.9730 and 0.9655, respectively (table 3).

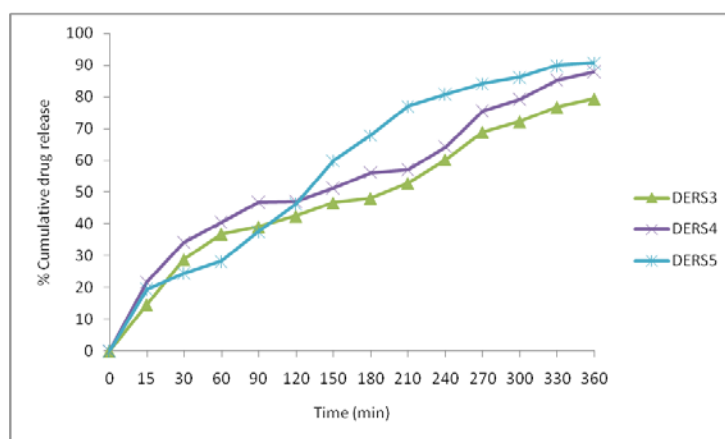


Fig. 1: *In vitro* release profiles of DIC loaded microsphere formulations (DERS3, DERS4 and DERS5). The data is expressed as mean \pm SD, n=3

Table 2: Characterization data of DIC loaded microsponges

S. No.	Formulation code	Polymer: drug ratio	Particle size (μm)	Production yield (%)	Drug content (%)	Entrapment efficiency (%)
1	DERS1	1: 1	20.39 \pm 4.21	27.50 \pm 3.54	27.27 \pm 5.43	15.00 \pm 3.15
2	DERS2	1:3	13.14 \pm 2.63	43.75 \pm 2.51	45.71 \pm 4.92	26.67 \pm 4.84
3	DERS3	1: 5	8.32 \pm 1.94	57.50 \pm 4.13	73.91 \pm 3.46	51.00 \pm 3.61
4	DERS4	1:7	7.52 \pm 2.29	68.13 \pm 3.98	80.73 \pm 3.51	62.86 \pm 5.49
5	DERS5	1: 9	6.98 \pm 1.45	64.00 \pm 4.56	86.72 \pm 1.36	61.67 \pm 2.27
6	DERS6	1:11	1.64 \pm 0.82	68.75 \pm 1.32	87.88 \pm 2.40	65.91 \pm 3.65
7	DERS7	1: 13	0.78 \pm 0.11	72.50 \pm 2.12	90.15 \pm 4.22	70.38 \pm 1.74

*n=3, mean \pm SD

Table 3: Release kinetics study of DIC loaded microspoon formulations

Formulation code	Zero-order model (r^2)	First-order model (r^2)	Higuchi model (r^2)	Hixson-crowell model (r^2)	Korsmeyer-peppas model (n)
DSER3	0.9271	0.9602	0.9730	0.9625	0.67
DSER4	0.9139	0.9301	0.9655	0.9483	0.67
DSER5	0.9373	0.9883	0.9772	0.9861	0.72

DERS5 followed first-order drug release kinetic, with regression coefficient (r^2) value 0.9883. The diffusion exponents (n) of all microspoon formulations have a value more than 0.5 but less than 0.8. This indicates that the DIC release mechanism from microsponges is non-Fickian diffusion.

Microspoon formulation DERS4 was selected as having requisite particle size, sustained release and satisfactory Production yield, Entrapment efficiency and Drug content. DERS4 was further studied for FTIR, DSC and SEM.

Fourier transform infrared spectroscopy (FTIR)

Drug-polymer compatibility in the selected microsponges DERS4 was evaluated by FTIR. The principle IR peaks of DIC appeared at wavenumbers of 3361.34 cm^{-1} (NH stretching of secondary amine groups), 1302.12 cm^{-1} (C-N stretching), 1503.46 cm^{-1} (C=C ring stretching) and 1548.76 cm^{-1} (C=O stretching of the carboxyl ion) and 757.12 cm^{-1} (C-Cl stretching) [27]. The characteristic DIC peaks were present in the physical mixture as well as in the DIC-loaded microsponges DERS4 (fig. 2) with no significant changes in frequencies.

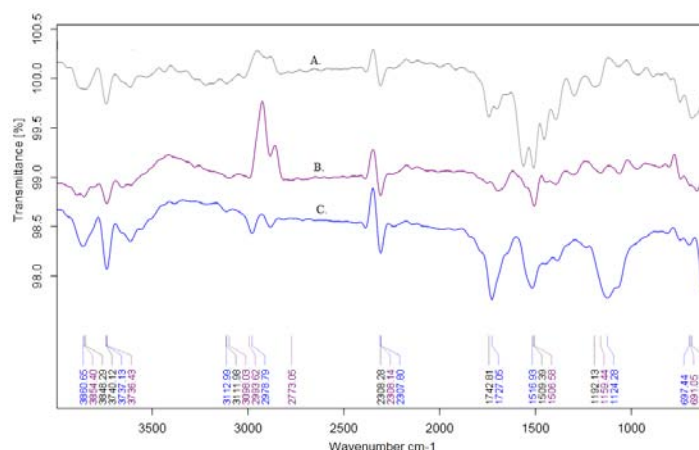


Fig. 2: FTIR spectrum of (a) DIC (b) Physical mixture of DIC and ERS polymer (c) Selected microspoon formulation DERS4

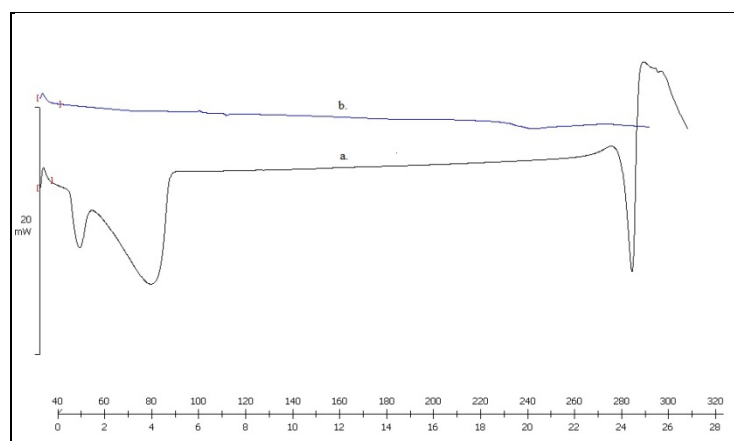


Fig. 3 DSC thermogram of (a) Pure DIC and (b) Selected microspoon DERS4

Differential scanning calorimetry (DSC)

The thermal behavior of the DIC, and selected microsponge DERS4 are presented in (fig. 3).

DSC thermogram of DIC exhibited endothermic peaks, initial peak due to loss of water followed by the other sharp endothermic peak at 291.97 °C, indicating to melting point of DIC in crystalline form; reflecting DIC purity [9]. The DSC curves of DERS4 formulation exhibited suppressed peak of the DIC at 280.40 °C.

Scanning electron microscopy (SEM)

Morphology DIC loaded microsponges were studied by SEM analysis. The representative SEM images of microsponge (DERS4) are shown in (fig. 4 and 5).

Formulation and characterization of in situ ocular gel containing DIC loaded Microsponges

In situ ocular gel found clear with suspended microsponge particles. The pH of in situ gel solution was found in range 6.03 to 6.13.

The formulation have an optimum viscosity at Non-Physiological conditions that will give ease in instillation to eye in the form of drop, which go through a quick sol to gel transition (triggered by pH) at physiological conditions (37 °C and 7.4 pH) as shown in table 4.

The *in vitro* drug release from *in situ* ocular gel showed sustained release. The % cumulative drug release from formulations was found to be IG1 (89.25%), IG2 (84.23%), IG3 (76.92%), and IG4 (69.64%) after 8 h as shown in fig. 6.

The drug release kinetic of all *in situ* gel formulations suggests Higuchi kinetic model (table 5) and diffusion exponent showed non-fickian diffusion mechanism.

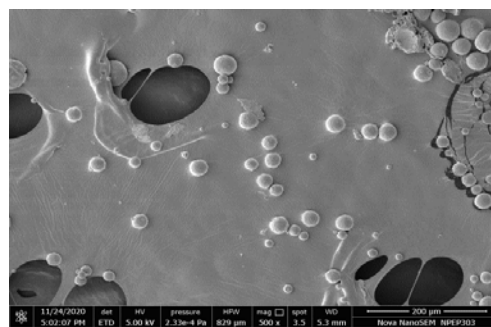


Fig. 4: SEM of selected microsponge DERS4 (Magnification x 500)

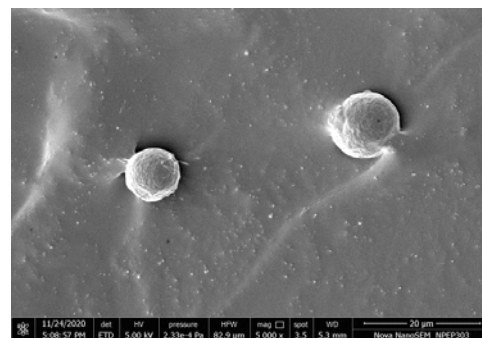


Fig. 5: SEM of selected microsponge DERS4 (Magnification x 5000)

Table 4: Characterization of DIC loaded microsponge containing ocular *in situ* gel

Formulation code	IG1	IG2	IG3	IG4
Gelling capacity	+	++	++	+++
pH	6.04	6.03	6.13	6.10
Viscosity At Non-Physiological conditions (cP)	860±120	1008±142	1843±176	2308±241
At Physiological conditions (pH 7.4 and 37 °C)	1852±194	4340±241	6651±186	7035±108
Drug content (%)	86.54±1.74	90.73±1.40	94.26±1.61	89.83±2.24

*n=3, mean±SD

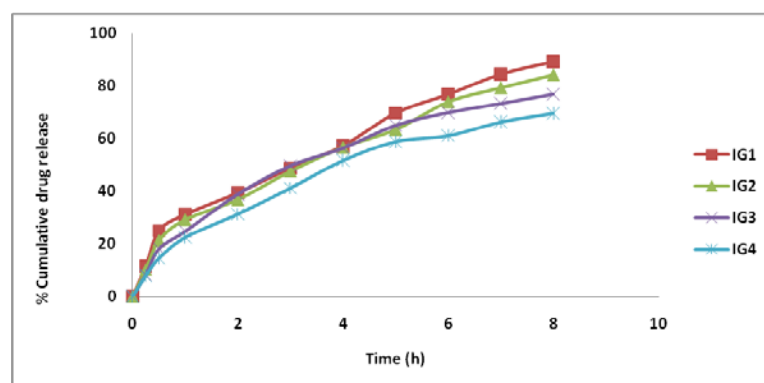


Fig. 6: *In vitro* release profiles of DIC loaded microsponge containing ocular *in situ* gel. The data is expressed as mean±SD, n=3

Table 5: Release kinetics study of DIC loaded microsponge containing ocular *in situ* gel

Formulation code	Zero order model (r ²)	First order model (r ²)	Higuchi model (r ²)	Hixson-crowell model (r ²)	Korsmeyer-peppas model (n)
IG1	0.9536	0.9755	0.9900	0.9880	0.68
IG2	0.9536	0.9884	0.9936	0.9904	0.68
IG3	0.9260	0.9904	0.9941	0.9753	0.69
IG4	0.9410	0.9877	0.9931	0.9763	0.68

Sterility study

The study results of the sterility study are summarized in table 6, the prepared ocular *in situ* gel formulation passed the sterility test. There was zero growth found in fluid thioglycollate medium and soyabean casein digest medium.

Table 6: Sterility test of ocular *in situ* gel formulation IG3

Organism	Bacteria	Fungi
+ve Control	Turbid	Turbid
-ve Control	Clear	Clear
IG3	Clear	Clear

*n=3, mean±SD

DISCUSSION

DIC-loaded microsponges were prepared by the quasi emulsion solvent diffusion method using ERS polymer. In literature, this method is considered to be simple, replicable, rapid and solvent toxicity also got eliminated [3]. With the increase in drug concentration in polymer: drug ratio; production yield, drug content and entrapment efficiency increases. As more drug are available for entrapment leading to high amount of drugs in microsponges leading to increased values of production yield, drug content and entrapment efficiency. The lower values of entrapment efficiency (15.00%) could be due to dissolution of some fraction of DIC in PVA solution during the emulsification process [20]. With an increase in level of DIC (besides drug dissolution), more drugs available for entrapment in ERS polymer matrix. As a result entrapment efficiency increases with increment in polymer: drug ratio.

High PVA concentration and high stirring speed yield small particle size which is requisite for this study. In the quasi emulsion solvent diffusion method, organic phase i.e. dichloromethane diffuses out to yield particles in which drug particle surrounded by polymer. At high polymer: drug ratio less polymer amounts surrounds the drug causing decreased particle sizes of microsponges [2].

As in the polymer: drug ratio the concentration of drug increased, polymer units surrounding drug units is less than at low. During phase transition process less polymer got bound to the drug giving smaller size microsphere. Particle size of less than 10 µm is required for ocular administration [11]. Hence microsphere formulations above 10 µm were not further investigated. Microsponges have particle size range from 5-300 µm. So microsponges below 5 µm were also excluded from further studies.

Sustained release of drug was as a result of incorporation of drug in polymer Matrix [28]. Drug release is inversely related to the Polymer: drug ratio. High Polymer: drug ratio yield smaller particles having more active surface area which coming in contact with diffusion media. Also smaller particle exhibit shorter path length drug need to travel through microsphere pores into the diffusion media [8]. Drug release rates were increased with an increasing amount of DIC in microsphere. At a high Polymer: drug ratio, the corresponding polymer amount is lesser. As more drug got released more void channels formed which allows more diffusion media to enter and in turn increases release rate.

FTIR studies confirms that no interaction between drug and ERS polymer. The thermogram of microsphere formulation showed suppressed peak signify uniform dispersion of DIC in polymeric matrix and altered crystallinity of DIC. Absence of any other significant endothermic peak, in the melting point range of DIC (melting point found as 286.26 °C) indicates that absence of any interaction between the DIC and ERS polymer, and verify the aptness of microsphere preparation technique [27].

SEM results indicated that DERS4 microsponges were tiny, porous and spherical. Surface integrity of microsphere got retained. Microsponges particles are free from aggregates. This might be due to aqueous solution of PVA which is used during the quasi emulsion solvent diffusion method. PVA reduces surface charge on the particle giving aggregate free microsponges [29].

In the present investigation, incorporation of DIC-loaded ERS polymeric microsponges was performed into *in situ* gel formulated using polymers Carbopol 940 and HPMC E4M. Carbopol 940 and HPMC E4M were used in combination as alone high concentration of carbopol 940 yield a highly acidic solution, which is not suitable for ocular administration (pH of lacrimal fluid 7.4) [30]. So moderate concentration of Carbopol 940 (0.1% w/v) along with viscosity-enhancing polymer HPMC E4M (0.2-0.8 % w/v) [31] was used to formulate *in situ* gel. The pH of formulation would raises instantly to pH 7.4 (an ideal ocular pH) after instillation into eye due to pH of lacrimal secretion. Thus, the formulations were considered to be suitable for ocular administration and may not cause any irritation to ocular tissues upon instillation [25].

At Physiological conditions formulation form stiff gel which increases residence time of microsponges incorporated in gel. In *in situ* gel formulation contains HPMC E4M and carbopol 940 which are known to gel reversibly on elevation of temperature and pH respectively [31]. Hence at physiological conditions viscosity of formulation increases to form a stiff gel, as both pH and temperature increases.

Formed stiff gel should display good integrity and not to get dissolve and erode for extended period of time. Formulations IG2 and IG3 exhibited satisfactory “++” grade of gelling capacity [12].

In situ gel formulation IG3 showed sustained release with a satisfactory “++” grade of gelling capacity. Hence, IG3 formulation was taken for further Sterility test. Sterility study results proved the efficiency of autoclaving for sterilization of ocular *in situ* gel.

CONCLUSION

With the present study, the potential of DIC loaded microsponges as drug carriers for ocular delivery was investigated. Microsponges showed good practical yield, entrapment efficiency, drug content and suitable particle size for ocular administration. Controlled release of up to 6 h observed through DIC loaded microsponges. DIC loaded microsponges successfully incorporated in *in situ* gels. The clarity, pH, drug content, gelling capacity and rheological studies of DIC loaded microsponges containing ocular *in situ* gel formulations were found to be acceptable. Further, *in situ* gel formulations showed sustained drug release for a period of 8 h, which is satisfactory for management of ocular pain.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

ABBREVIATIONS

DIC: Diclofenac Sodium; ERS: Eudragit RS 100; HPMC E4M: Hydroxypropyl Methylcellulose E4M; DMC: Dichloromethane; PVA: Polyvinylalcohol; FTIR: Fourier Transform Infrared Spectroscopy; DSC: Differential scanning calorimetry; SEM: Scanning electron microscopy; STF: Simulated tear fluid.

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AUTHORS CONTRIBUTIONS

All authors have read and approved the manuscript. Rajashri B. Ambikar designed the study, carried out experimental work, Analyzed data and Drafted the manuscript. Dr. Ashok V. Bhosale contributed in designing of study, checked the paper content, helped in data interpretation and critical revision of the manuscript for intellectual content.

CONFLICT OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Osmani RA, Aloorkar NH, Kulkarni AS, Thaware BU, Kulkarni PK. Microsponge based drug delivery system for augmented gastroparesis therapy: formulation development and evaluation. *Asian J Pharm Sci* 2015;10:442-51.
- Nokhodchi A, Jelvehgari M, Siah MR, Mozafari MR. Factors affecting the morphology of benzoyl peroxide microsponges. *Micron* 2007;38:834-40.
- Kumari A, Jain A, Hurkat P, Tiwari A, Jain SK. Eudragit S100 coated microsponges for Colon targeting of prednisolone. *Drug Dev Ind Pharm* 2018;44:902-13.
- Sareen R, Nath K, Jain N, Dhar KL. Curcumin loaded microsponges for colon targeting in inflammatory bowel disease: fabrication, optimization, and *in vitro* and pharmacodynamic evaluation. *J Biomed Biotechnol* 2014. DOI:10.1155/2014/340701.
- Srivastava R, Kumar D, Pathak K. Colonic luminal surface retentive Meloxi-cam microsponges delivered by erosion based colon targeted matrix tablet. *Int J Pharm* 2012;427:156-62.
- Bhatia M, Saini M. Formulation and evaluation of curcumin microsponges for oral and topical drug delivery. *Prog Biomater* 2018;7:239-48.
- Abdelmalak NS, El-Menshawe SF. A new topical fluconazole microsphere loaded hydrogel: preparation and characterization. *Int J Pharm Pharm Sci* 2012;4:460-9.
- Arya P, Pathak K. Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *Int J Pharm* 2014;460:1-12.
- Maiti S, Kaity S, Ray S, Sa B. Development and evaluation of xanthan gum-facilitated ethyl cellulose microsponges for controlled percutaneous delivery of diclofenac sodium. *Acta Pharm* 2011;61:257-70.
- Ambikar RB, Bhosale AV. Formulation and evaluation of eudragit RL100 polymeric drug loaded microsphere for ophthalmic use. *J Pharm Res Int* 2021;33:45-51.
- Abd-Elal RMA, Elosaily GH, Gad S. Full factorial design, optimization, *in vitro* and *ex vivo* studies of ocular timolol-loaded microsponges. *J Pharm Innov* 2020;15:651-63.
- Obiedallah MM, Abdel-Mageed AM, Elfaham TH. Ocular administration of acetazolamide microsponges in situ gel formulations. *Saudi Pharm J* 2018;26:909-20.
- Ali J, Fazil M, Qumbar M, Khan N, Ali A. Colloidal drug delivery system: amplify the ocular delivery. *Drug Delivery* 2016;23:710-26.
- Kesarla R, Tank T, Vora PA, Shah T, Parmar S, Omri A. Preparation and evaluation of nanoparticles loaded ophthalmic *in situ* gel. *Drug Delivery* 2016;23:2363-70.
- Upadhyay P, Kumar M, Pathak K. Norfloxacin loaded pH triggered nanoparticulate *in-situ* gel for extraocular bacterial infections: optimization, ocular irritancy and corneal toxicity. *Iran J Pharm Sci* 2016;15:3-22.
- Al-Kinani A, Zidan G, Elsaid N, Seyfoddin A, Alani AW, Alany R. Ophthalmic gels: past, present and future. *Adv Drug Delivery Rev* 2018;126:113-26.
- Wu Y, Liu Y, Li X, Kebebe D, Zhang B, Ren J, *et al*. Research progress of in-situ gelling ophthalmic drug delivery system. *Asian J Pharm Sci* 2019;14:1-15.
- Khangtragool A. Methocel E4M: preparation and properties as a vehicle for the ocular drug delivery of vancomycin. *Chiang Mai J Sci* 2014;41:166-73.
- Adelli GR, Balguri SP, Bhagav P, Raman V, Majumdar S. Diclofenac sodium ion exchange resin complex loaded melt cast films for sustained release ocular delivery. *Drug Delivery* 2017;24:370-9.
- Jelvehgari M, Siah Shadbad MR, Azarmi S, Martin GP, Nokhodchi A. The microsphere delivery system of benzoyl peroxide: preparation, characterization and release studies. *Int J Pharm* 2006;308:124-32.
- Martin A, Bustamante P, Chun A. Micromeritics. In: *Physical pharmacy-physical chemical principles in the pharmaceutical science*. 5th ed. B I Waverly Pvt. Ltd; 2002. p. 446-8.
- Nair AB, Shah J, Jacob S, Al-Dhubiab BE, Sreeharsha N. Experimental design, formulation and *in vivo* evaluation of a novel topical in situ gel system to treat ocular infections. *PLoS One* 2021;16:e0248857.
- Son GH, Lee BJ, Cho CW. Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles. *Int J Pharm Investig* 2017;47:287-96.
- Li SS, Li GF, Liu L, Jiang X, Zhang B, Liu ZG, *et al*. Evaluation of paeonol skin-target delivery from its microsphere formulation: *in vitro* skin permeation and *in vivo* microdialysis. *PLoS One* 2013;20;8:e79881.
- Song J, Bi H, Xie X, Guo J, Wang X, Liu D. Preparation and evaluation of sinomenine hydrochloride in situ gel for uveitis treatment. *Int. Immunopharmacol* 2013;17:99-107.
- Indian Pharmacopoeia. The controller of publication, New Delhi; Ministry of health and family welfare. India. 6th Ed. Volume II; 2010. p. 59-66.
- Kucukturkmen B, Oz UC, Bozkir A. *In situ* hydrogel formulation for intra-articular application of diclofenac sodium-loaded polymeric nanoparticles. *Turk J Pharm Sci* 2017;14:56-64.
- Kumar PM, Ghosh A. Development and evaluation of metronidazole loaded microsphere based gel for superficial surgical wound infections. *J Drug Delivery Sci Technol* 2015;30:15-29.
- Deshmukh K, Poddar SS. Tyrosinase inhibitor-loaded microsphere drug delivery system: new approach for hyperpigmentation disorders. *J Microencapsul* 2012; 29:559-68.
- Charoo NA, Kohli K, Ali A. Preparation of in situ-forming ophthalmic gels of ciprofloxacin hydrochloride for the treatment of bacterial conjunctivitis: *in vitro* and *in vivo* studies. *J Pharm Sci* 2003;92:407-13.
- Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. *Results Pharma Sci* 2016;6:1-6.
- Sheshala R, Ming NJ, Kok YY, Singh TRR, Dua K. Formulation and characterization of pH induced in situ gels containing sulfacetamide sodium for ocular drug delivery: a combination of carbopol@/HPMC polymer. *Indian J Pharm Educ Res* 2019;53:654-62.