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ORIGINAL ARTICLE

Nasal in situ gel containing hydroxy propyl β-cyclodextrin inclusion complex of artemether: development and in vitro evaluation

Hitendra S. Mahajan · Saurabh K. Shah · Sanjay J. Surana

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Abstract The objective of the present investigation was to explore the formulation and evaluation of in situ gel for the nasal delivery of artemether (ARM), a poorly water-soluble antimalarial agent using temperature induced gelation technique using Pluronic with mucoadhesive polymer Hydroxy Propyl Methyl Cellulose (HPMC) K4M in different ratios. Initially, due to low water solubility, an inclusion complex of the antimalarial artemether (ARM) in hydroxypropyl-β-cyclodextrin (HPβCD) was prepared and characterized. The in situ gels so prepared were characterized for its gelation properties, viscosity, gel strength, mucoadhesion, drug content, drug release rate and for its histopathological studies. Pluronic and HPMC based in situ gel (PLH2) showed the effective gelation, viscosity, gel strength and drug release properties along with good mucoadhesive strength, it is further subjected for stability studies carried out at 30 ± 2 °C and 60 ± 5% RH for 90 days in order to know the influence of temperature and relative humidity on drug content and on drug release profile. Histological examination of formulations did not show any remarkable damage to nasal mucosa. The formulation also retained the good stability at accelerated conditions over the period of 90 days. Owing to these properties it can be used as an effective delivery system for the nasal route. These in situ gelling systems would be definitely useful for cerebral malaria.

Keywords Phase solubility · Inclusion mode · In situ gel · Temperature induced gelation · Mucoadhesion

H. S. Mahajan (✉) · S. K. Shah · S. J. Surana
R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India
e-mail: hsmahajan@rediffmail.com

Introduction

Malaria (from the Italian *mala aria*—bad air; English—malaria, ague, marsh fever;) is the name of a group of closely related severely infectious diseases, which are caused by the appearance and multiplication in the body of blood parasites [2, 18]. Malaria parasitic diseases are of immense global significance as around 30% of world's population experiences parasitic infections [15]. Malaria has a devastating effect throughout tropical regions. There are approximately 300–500 million clinical cases each year resulting in 1.5–2.7 million deaths [23]. Nearly all fatal cases are caused by Plasmodium falciparum [21], the problem is compounded by the spread of drug resistant strains of the parasite [1]. As a result, traditional alkaloid drugs such as chloroquine and quinine are now largely ineffective. The spread of parasite resistance has led the World Health Organization (WHO) to predict that without new antimalarial drug intervention, the number of cases of malaria will have doubled by the year 2010 [13]. Treatment of CM, which is usually by i.v. injection, requires hospital admission. This represents an additional problem, since hospitals are not easily and immediately accessible in all affected areas. In the present study we propose a novel treatment approach—intranasal administration of antimalarial drugs. One particular advantage of the nasal cavity is provide a port of entry for the drug to the brain [14, 31].

Cyclodextrins (CDs) are truncated-cone polysaccharides mainly composed of six to eight D-glucose monomers linked by a-1,4-glucose bonds, usually enhances drug solubility in aqueous solution and affects the chemical characteristics of the encapsulated drug [24–26, 32]. Hydroxypropyl-β-cyclodextrin (HPβCD) is a hydroxyalkylated βCD derivative that combines relatively high

water solubility with low toxicity and satisfactory inclusion ability [8, 17].

In situ gelling systems are the aqueous polymeric solutions that are transformed into gels due to changes in environmental conditions, like temperature and pH [3].The in situ gels are fluids that can be introduced into the body in a minimally invasive manner prior to solidifying or gelling within the desired tissue, organ, or body cavity [22].

The most commonly used thermo-reversible gels are those prepared from poly (ethylene oxide)-*b*-poly (propylene oxide)-*b*-poly (ethylene oxide) popularly known as: Pluronics®, Tetronics®, Poloxamers etc. [7]. The primary aim of this study is to enhance the solubility of artemether using HPβCD. The specific objective of the present study is development and in vitro characterization of in situ gel system suitable for nasal administration.

Materials and method

Materials

ARM was received as a kind gift from Macleods Pharmaceuticals Ltd. (Daman, India). Pluronic F-127 were obtained from Sigma Chemicals (Germany), HPMC K4M were obtained as a gift sample from Dow Chemical Company (US). Benzalkonium Chloride were taken from Opthoremedies Ltd. (New Delhi), Mannitol were procured from Loba Chemie (Mumbai, India) and used as received. All other reagents used were of analytical grade.

Methodology

Development of inclusion complex of artemether/HPβCD

As artemether is practically insoluble in water, an inclusion complex of the antimalarial artemether (ARM) in hydroxypropyl-b-cyclodextrin (HPβCD) was prepared and characterized.

Preparation of ARM/HPβCD inclusion complex

It is prepared by Kneading method [19]. The mixture of artemether and HPβCD in 1:1 M ratio was triturated in a mortar with a small volume of water- methanol (1:2 v/v) solution. The thick slurry that formed was kneaded for 45 min and then dried at 45 °C. The dried mass was pulverized and sieved through sieve no. 60 [5].

Characterization of HPβCD inclusion complex

Differential scanning calorimetry (DSC)

DSC studies of plane artemether, plane HPβCD, ARM/HPβCD physical mixture and ARM/HPβCD inclusion complex were performed [10]. The samples were put on DSC reference pan and DSC curves were obtained by Differential Scanning Calorimeter (DSC 60, Shimadzu, Japan) at a heating rate of 10 °C/min from 0 to 300 °C in nitrogen atmosphere.

X-ray diffractometry (XRD)

The X-ray diffraction patterns of ARM, HPβCD, physical mixture and complex were recorded using Philips diffractometer (PW 1140) and Cu-Kα radiation; voltage, 40 kV; current, 20 mA. Diffractogram were run at a scanning speed of 2°/mm and a chart speed of 2°/2 cm per 2ø.

Phase solubility studies

The phase solubility technique permits the evaluation of the affinity between HPβCD and artemether in water. Phase solubility studies were performed according to the method reported by Higuchi and Connors. The apparent stability constant (K_c) of complexes was calculated from the phase solubility diagram using the following equation

$$K_c = \text{slope}/\ S_0\,(1 - \text{slope}) \quad (1)$$

The slope obtained from the initial straight line portion of the plot of artemether concentration against HPβCD concentration, and S_0 is the equilibrium solubility of Artemether in water (Table 1).

Preparation of Pluronic F-127 In situ gel

Pluronic F127 (PF127) and artemether were solubilized in ultra pure water containing 1% propylene glycol. PF127 vehicles with concentration varying from 16% w/v to

Table 1 Phase solubility study of artemether in distilled water

Sr. no.	Ingredients	Formulations composition (% w/v)				
		PLB	PLH1	PLH2	PLH3	PLH4
1	Artemether	2.0	2.0	2.0	2.0	2.0
2	Pluronic F-127	18	18	18	18	18
3	HPMC K_4M	–	0.5	1.0	1.5	2.0
4	Mannitol	4	4	4	4	4
5	Benzalkonium Chloride	0.001	0.001	0.001	0.001	0.001
6	Ultra pure water	q.s.	q.s.	q.s.	q.s.	q.s.

20% w/v were screened preliminarily to decide lowest possible concentration. PF127, 18% w/v, was found to be lowest concentration (when formulated in addition of artemether) that exhibited thermo reversible property below 34 °C (temperature of the nasal cavity). Hence, 18% w/v of PF127 was selected for further studies. The liquid was left at 4 °C until a clear solution was obtained. Thermoreversible gels were prepared using cold method [20]. Bioadhesive anionic polymer HPMC K_4M was slowly added to the solution with continuous agitation. HPMCK_4M was added in concentration range of 0.5% w/v to 2.0% w/v to PF127 solution (Table 2). Appropriate quantities of Mannitol and Benzalkonium chloride were also added simultaneously. pH of formulations was adjusted between 4.5 to 5.5; by using 0.1 N HCl. The formulations were filled in 10 mL amber colored glass vials, capped with rubber bungs and sealed with aluminium caps. Formulations were stored in a refrigerator (4–8 °C) until use [16].

Characterization of in situ gel

Gelation studies

Gelation is the process, by which the liquid phase makes a transition to gel. In brief, a 10 ml transparent vial containing a magnetic bar and each formulation were placed in a water bath. The temperature was maintained at 34 °C. The gelation point was determined when the magnetic bar stopped moving due to gelation. The consistency of formed gel was checked by visual inspection and graded.

Viscosity measurements

Viscosities of formulations before and after gelation were measured by using Brookfield DV-E viscometer using Spindle number-3 at 100 rpm shear rate.

Gel strength determination

It is expressed in terms of time (in seconds) required by a 35 g piston for penetration of 5 cm distance, through the 50 g gel formulation. Test was performed using 'Gel strength apparatus' modified at laboratory. Pluronic solution (50 g) was placed in a 100 mL measuring cylinder and gelation was induced by means of temperature. The piston (weight: 35 g) was then placed onto the gel. The gel strength was measured as the time (seconds) required moving the piston 5 cm down through the gel. The gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel. As shown in Fig. 1

Table 2 Composition of artemether in situ gelling systems containing Pluronic F-127 and HPMC K_4M

Sr no.	Formulation code	A1	A2	A3	A4
1	Drug (mg)	20	20	20	20
2	HPβCD (mg)	20	40	60	80
3	Dist. water (mL)	20	20	20	20

Evaluation of the mucoadhesive strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified balance method. In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after

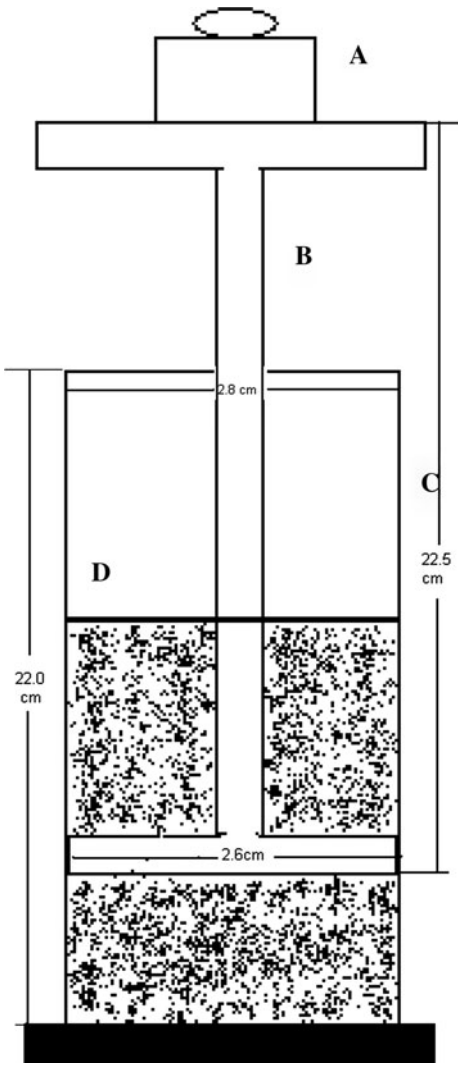

Fig. 1 Gel strength measuring apparatus. (*a*) Weight; (*b*) Shaft; (*c*) measuring cylinder; (*d*) Polymer gel

separation. At the time of testing, a section of nasal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The upper probe was attached to precalibrated force displacement transducer SS12LA; (BIOPAC Systems) connected to the Student's Physiograph apparatus. The surface area of each exposed mucosal membrane was 4.2 cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe. The probes were equilibrated and gelation was induced by means of temperature. Probe with nasal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a slight force was applied for 2 min to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation.

$$\text{Detachment Stress}\left(\text{dyne}/\text{cm}^2\right) = \frac{\text{m} \times \text{g}}{\text{A}} \quad (2)$$

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 Surface area of sheep nasal mucosa.

Drug content uniformity [30]

The vials ($n = 3$) containing the preparation were shaken for 2–3 min manually and 100 µl of the preparation was transferred to 25 mL volumetric flasks with a micropipette and the final volume was made up with Phosphate buffer pH 6.6. Artemether concentration was determined at 254 nm (UV-1700, Shimadzu).

In vitro diffusion studies

In vitro diffusion study of formulated in situ gels was carried out on Franz diffusion cell having 2.0 cm diameter and 16 mL capacity. Dialysis membrane (Himedia) having molecular weight cut off range 12000–14000 kDa was used as diffusion membrane. Pieces of dialysis membrane were soaked in phosphate buffer (PB) pH 6.6 for 24 h prior to experiment. Diffusion cell was filled with phosphate buffer pH 6.6; dialysis membrane was mounted on cell. The temperature was maintained at 34 °C. After a pre-incubation time of 20 min, pure drug solution and formulation equivalent to 8 mg of artemether was placed in the donor chamber. Gelation was induced using temperature. At predetermined time points, 0.5 mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with PB pH 6.6 after each sampling, for a period of 210 min.

Ex vivo permeation studies

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 3.14 cm^2. 16 mL of PB pH 6.6 was added to the acceptor chamber. The temperature was maintained at 34 °C. After a pre-incubation time of 20 min, formulation equivalent to 8 mg of Artemether was placed in the donor chamber. At predetermined time points, 1 mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.6 after each sampling, for a period of 5 h [4].

Permeability coefficient (P) was calculated by following formula

$$\text{P} = \frac{\text{dQ}/\text{dt}}{\text{C}_\text{o} \times \text{A}} \quad (3)$$

where dQ/dt is the flux or permeability rate (mg/h), C_o is the initial concentration in donor compartment, A is the effective surface area of nasal mucosa

Histopathological evaluation of mucosa

Histopathological evaluation of tissue incubated in Phosphate buffer saline (PBS pH 6.4) after collection it was compared with tissue incubated in the diffusion chamber with gel formulations (PLH2).Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with haematoxylin and eosin. Sections were examined under a light microscope, to detect any damage to the tissue during ex vivo permeation study.

Stability study

Formulations showing optimum gelation, gel strength, mucoadhesive force and drug release rate were selected for stability studies. Stability studies were carried out on gel formulation according to ICH (International Conference on Harmonization) guidelines. The stability chamber was placed at room temperature at 30 ± 2 °C, and samples were withdrawn at 0, 30, 60, 90 days interval. The physical stability of gel was observed periodically for the occurrence of turbidity and gelation

Results and discussion

Development of Inclusion complex of artemether and HPβCD

Cyclodextrins (CDs) are truncated-cone polysaccharides mainly composed of six to eight D-glucose monomers

linked by a-1,4-glucose bonds [11]. They have a hydrophobic central cavity and hydrophilic outer surface and can encapsulate model substrates to form host–guest complexes or supramolecular species. This usually enhances drug solubility in aqueous solution and affects the chemical characteristics of the encapsulated drug [9]. Hydroxypropyl-b-cyclodextrin (HPβCD) is a hydroxyalkylated βCD derivative that combines relatively high water solubility with low toxicity and satisfactory inclusion ability [6]. The binding behaviours of hydroxypropyl b-cyclodextrin with ARM and the solubilisation effect of HPβCD toward ARM, may provide a useful approach to produce a novel ARM formulations with improved bioavailability.

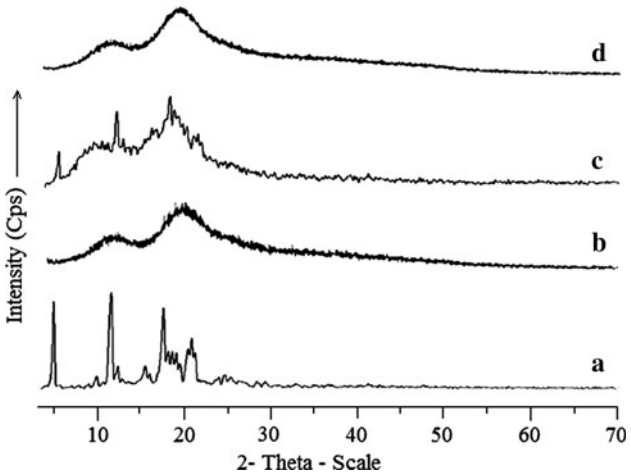

Fig. 2 DSC spectra of (*a*) Artemether, (*b*) HPβCD, (*c*) ARM/HPβCD Physical mixture, (*d*) ARM/HPβCD inclusion complex

Characterization of inclusion complex

DSC study

The thermal properties of the HPβCD/ARM complex were investigated by differential scanning calorimetry (DSC). The differential scanning calorimetry (DSC) thermogram provides further information about the thermal properties of the HPβCD/ARM complex. The DSC curve of ARM displays an endothermic peak at 86.42 °C and exothermic peak at 189.58 °C. In contrast, the DSC curve of pure HPβCD shows broad endothermic peaks at 91.20 and 286.45 °C indicating HPβCD loses water at temperatures slightly above 91 °C and decomposes above 286 °C. DSC curve of ARM/HPβCD physical mixture shows less intense endothermic peak at 86.73 and 335.43 °C. However, in the DSC curve of the HPβCD/ARM complex, the exothermic peak at about 189 °C corresponding to the free ARM disappears, while two new endothermic peaks appear at 86.34 and 301.24 °C which is less intense than drug peak. This suggests that the HPβCD/ARM complex is more stable than ARM. It proposes that this result may be related to the complexation of HPβCD with ARM. As shown in Fig. 2

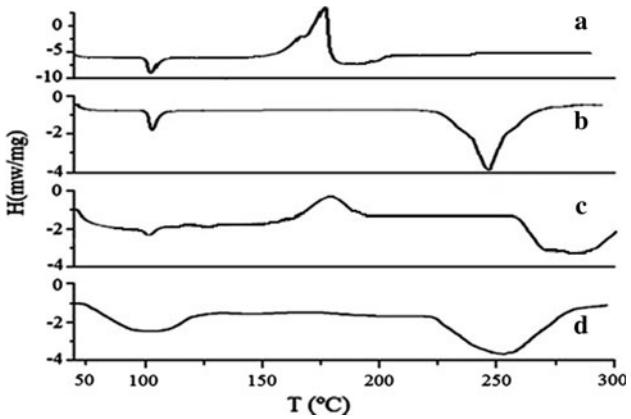

Fig. 3 X-Ray diffractogram of (*a*) ARM, (*b*) HPβCD, (*c*) ARM/HPβCD physical mixture, (*d*) ARM/HPβCD inclusion complex

X-Ray diffraction study

The X-ray diffraction pattern were recorded for pure ARM, HPβCD, ARM/HPβCD physical mixture and ARM/HPβCD inclusion complex. The X-ray diffractogram of ARM has sharp peaks at diffraction angle 4.834°, 12.540°, 13.422°, 17.056°, 19.512°, 21.223°, 23.264° and 27.771° which shows a typical crystalline pattern. Plane HPβCD showed less intense peaks due to amorphous character; however X-ray diffraction pattern of the physical mixture confirmed the presence of both species as isolated solids, as the diffractogram showed both ARM peaks and the HPβCD amorphous form. ARM/HPβCD inclusion complex shows peaks but of low intensity indicating that some amount of ARM converts to amorphous form. As shown in Fig. 3

Phase solubility study

Phase solubility diagram for the complex formation between artemether and HPβCD. This plot showed that the aqueous solubility of the drug increases linearly as a function of HPβCD concentration. The apparent solubility constant K_c, obtained from the slope of the linear phase solubility diagram was found to be 0.1045 mol^{-1}. As shown in Fig. 4

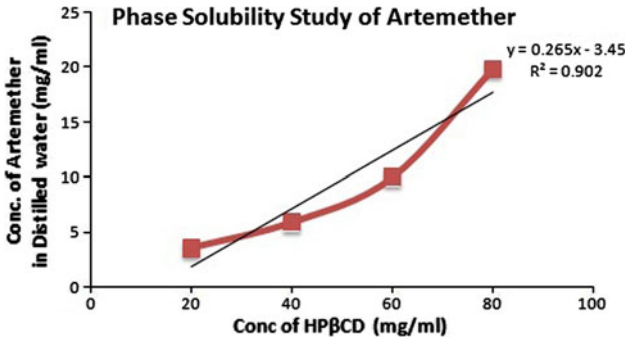

Fig. 4 Phase solubility diagram of artemether

Evaluation of in situ gel

Gelation study

Gelation studies were carried out using temperature change. In these studies the gelling capacity (speed and extent of gelation) for all formulations were determined. After easy instillation into nasal cavity the liquid polymeric solutions should undergo rapid sol to gel transition by means of thermo sensitivity [12]. Thus the in situ formed gel should preserve its integrity without dissolving or eroding so as to localize the drug at absorption site for extended duration. As per the visual inspection the pre-formed gels were graded.

The combination of thermosensitive polymer Pluronic F127 with HPMC K_4M is also the dual stimuli responsive in situ gelling system ideal or nasal drug delivery [29]. From the results of gelation study the optimum concentration of HPMC K_4M to be used as mucoadhesive agent was found to be 0.5% to 2.0% w/v in combination with Pluronic F127. In the preliminary studies, the minimum concentration of Pluronic F127 that formed gel below 34 °C was found to be 18% w/v. In general, the gelation temperatures have been considered to be suitable if they are in the range of 25 to 34 °C. If the gelation temperature of a thermoreversible formulation is lower than 25 °C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 34 °C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. As the temperature of the nasal cavity is 34 °C, this study aimed at preparing the liquid formulations of PF127 that may gel below 34 °C.

Viscosity study

The apparent viscosity values were measured for liquid formulations and gel using Brookfield viscometer DV-E with spindle no. 3 at 100 rpm. The marked increase in viscosity of the formulations in solution form of Pluronic F127 was observed. The viscosity of formulations in gel state; was found to be proportionate with the increasing polymer concentration. In PLB and PLH1 to PLH4 series of formulations there was slight difference in viscosities of solutions but the large difference was observed in gel state of same formulation series.

Gel strength

In the development of nasal in situ gelling system, the gel strength is important in finding the condition, which can delay the post nasal drip or anterior leakage. The gel strength was found to be affected by concentrations of gelling and bioadhesive polymers. Optimal in situ gel must have suitable gel strength so as to be administered easily and can be retained at nasal mucosa without leakage after administration. In Pluronic gels it was found to reduce the gel strength with increasing concentration. The gel strength values between 25 to 50 s were considered sufficient. The gel strength less than 25 s may not retain its integrity and may erode rapidly while gels having strength greater than 50 s are too stiff and may cause discomfort to the mucosal surfaces [33].

Evaluation of the mucoadhesive strength

All the formulations were subjected to mucoadhesion studies by the method reported by Murthy et al. [27]. The mucoadhesion force is an important parameter for in situ gelling nasal formulations since it prolongs the nasal clearance of gels and increase its residence time in nasal cavity. In case of Pluronic gels the mucoadhesion force proportionally increased with increase in HPMC K_4M concentration. At 1.0% w/v concentration of HPMC K_4M with 18% w/v concentration of Pluronic showed the significant mucoadhesion. Mucoadhesion in Pluronic gels was very high. The reinforcement of the mucoadhesive forces in the nasal in situ gels by the use of mucoadhesive polymers could be explained by the fact that secondary bond forming groups (hydroxy, ethoxy and amine) are the principle source of mucoadhesion. The bioadhesive force is known to be dependent on the nature and concentration of bioadhesive polymers. The stronger the bioadhesive force more is the nasal residence time. But if the mucoadhesion is too strong the gel can damage to the mucosal membrane.

Drug content

The drug content of the formulations was ranging from 98.2 to 99.52%. Drug content of simple Pluronic gel was

found to be 99.23% and that drug content of selected formulation PLH2 was found to be 99.52%.

In vitro drug release study

In vitro release studies of formulations were performed using the Franz diffusion cell with dialysis membrane. Phosphate buffer of pH 6.6 was used as diffusion media. The initial rates of drug release were very rapid due to incomplete gel formation, but as the time progresses the release rate decreases due to complete gel formation. With increase in concentration of HPMC K_4M the release rates were found to decrease gradually. The release profiles exhibited an inflection point, which indicated the gel formation in the donor compartment of diffusion cell. During gel formation, a portion of drug might be loaded into the gel matrix, thus the cross linking of polymer reduces the drug release rate. The initial rapid release of artemether was may be due to formation of prehydrated matrix containing water filled pores due to presence of aqueous vehicle. The results showed that the formed gels had the ability to extend the release of artemether for the duration of about 420 min. In vitro release study indicated that the release of drug varied according to the type and concentration of polymers. The results further showed that the amount of the drug released in first hour decreased with the increasing polymer concentration and this pattern continued till the entire duration of study. PF127 gels are viscous, isotropic liquid crystals consisting of micelles, it was hypothesized that the drug is released by mixed ordered initial rapid release i.e. first order followed by zero order diffusion through the extramicellar water channels of the gel matrix. As shown in Fig. 5

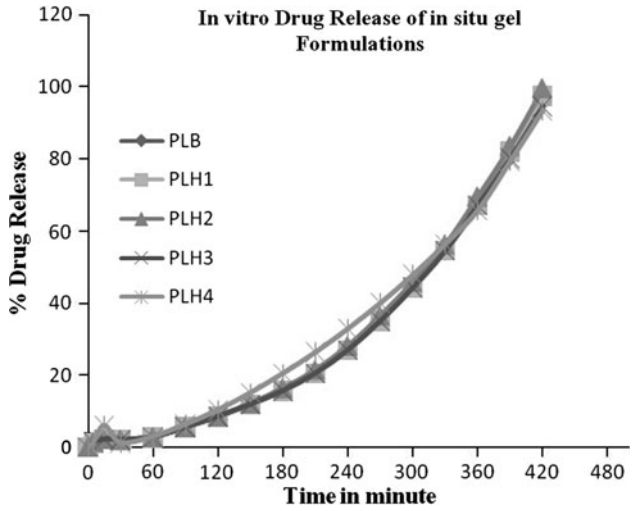

Fig. 5 In vitro drug release profile of in situ gel formulations

Drug release kinetics

To precisely know the drug release mechanisms from in situ gelling systems, the in vitro release data of formulation PLH2 were treated with Higuchi's diffusion equation ($Q = kt^{1/2}$). The graph between percentage cumulative drug release and square root of time showed almost linear relationship after the initial period. It was not possible to correlate the release in the early stages of drug release study, due to incomplete gel formation. After the complete gel formation, the release profiles were found to be linear with square root of time and followed the Higuchi's equation. The drug transport mechanism of the same formulations was determined by using the Korsmeyer- Peppas exponential equation. [$(Mt/M) = kt^n$]. Form the plot of log (Mt/M); fraction of drug released at time 't' versus log of time. The kinetic parameters '*n*' and 'k' were calculated from Korsmeyer-Peppas exponential equation. The slope of the plot of PLH2 formulations was observed to be 0.8987 and 0.6659 respectively, indicating the anomalous transport mechanism [28]. On the basis of gelation properties, gel strength, viscosity, mucoadhesion, percent drug content, in vitro diffusion profile the optimum in situ

Table 3 Evaluation parameters of the in situ gel

Sr no.	Formulation code	Degree of gelation	Viscosity study (cP)		Gel strength (s)	Mucoadhesion force (dyne/cm^2)	Drug content (%)	In vitro drug release (%)
			Solution	Gel				
1	PLB	+++	91 ± 1.73	1050 ± 2.51	100 ± 2.00	4666.67	99.23 ± 0.12	96.29%
2	PLH1	+++	111 ± 1.00	1800 ± 1.73	67 ± 1.00	5600	98.2 ± 0.13	97.56%
3	PLH2	++++	112 ± 2.30	4200 ± 2.08	47.33 ± 1.52	6300	99.52 ± 0.04	99.59%
4	PLH3	++++	139 ± 2.88	4970 ± 1.15	43.67 ± 2.08	6766.67	98.45 ± 0.12	94.58%
5	PLH4	++++	196 ± 0.57	5470 ± 1.16	42.67 ± 1.15	7583.33	98.55 ± 0.12	93.53%

(++) Immediate gelation remains for few hrs (less stiff gel), (+++) Immediate gelation remains for extended period (stiff gel)

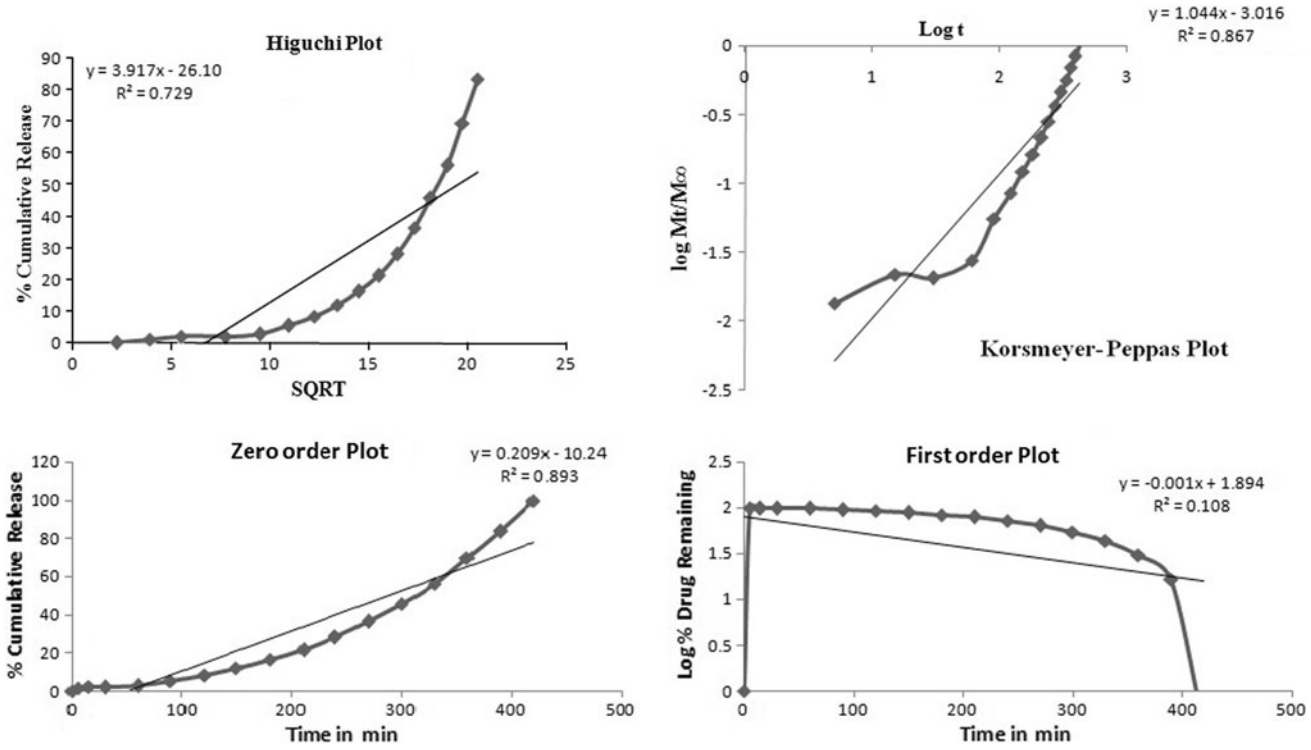

Fig. 6 Drug release kinetics of PLH2 in situ gel

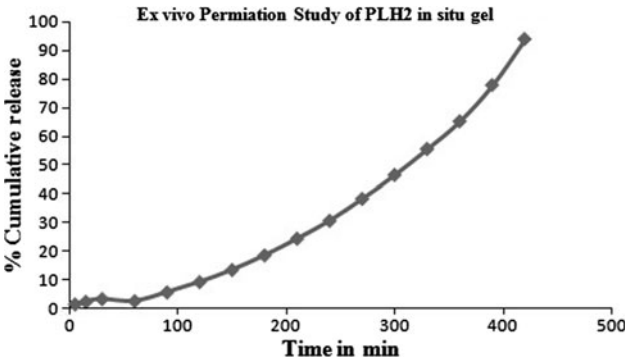

Fig. 7 Ex vivo permeation study of PLH2 in situ gel

gelling formulation (PLH2) were selected and subjected for further studies (Table 3). As shown in Fig. 6.

Ex vivo permeation study

Formulation PLH2 was further subjected to ex vivo permeation studies using the sheep nasal mucosa. The percent drug permeated after 7 h was found to be 93.79%. The permeability coefficient (P) was also calculated and found to be 0.031033 cm^2/h for PLH2. Slight increase in permeability of drug from Pluronic formulation can be correlated with the surfactant nature of Pluronic F127. As shown in Fig. 7.

Table 4 Histopathological inference of Photomicrographs

Sr. no.	Histological findings	Formulations	
		PBS treated (A)	PLH2 (C)
1	Degeneration of mucosa	–	+
2	Erosion of mucosa	–	–

Histopathological study

Photomicrographs of sheep nasal mucosa after the permeation studies were observed for histopathological changes in comparison with the PBS treated mucosa. The section of mucosa treated with formulation PLH2 showed very slight degeneration of nasal epithelium along with no erosion. There was increased vascularity in basal membrane and superficial part of sub mucosa as compared with PBS-treated mucosa. This might be the result of mucoadhesive and permeability enhancing property of HPMC in the formulation. None of the severe sings such as appearance of epithelial necrosis, sloughing of epithelial cells was detected (Table 4). A shown in Fig. 8.

Table 5 Stability study data of PLH2 formulation

Sr. no.	Parameters	Storage period (days) at 30 ± 2 °C temperature and 60 ± 5% RH			
		0	30	60	90
1	Appearance	Clear white	Clear white	Clear white	Clear white
2	Drug content (%)	99.52 ± 0.04	99.60 ± 1.41	98.74 ± 1.27	98.92 ± 1.34
3	Gelation study	+++	+++	++	+++
4	Gel strength (s)	47.23 ± 1.52	47.12 ± 2.14	47.76 ± 1.75	47.43 ± 1.07
5	In vitro drug release (%)	99.59	98.41	98.24	98.47

(++) Immediate gelation remains for few hrs (less stiff gel), (+++) Immediate gelation remains for extended period (stiff gel)

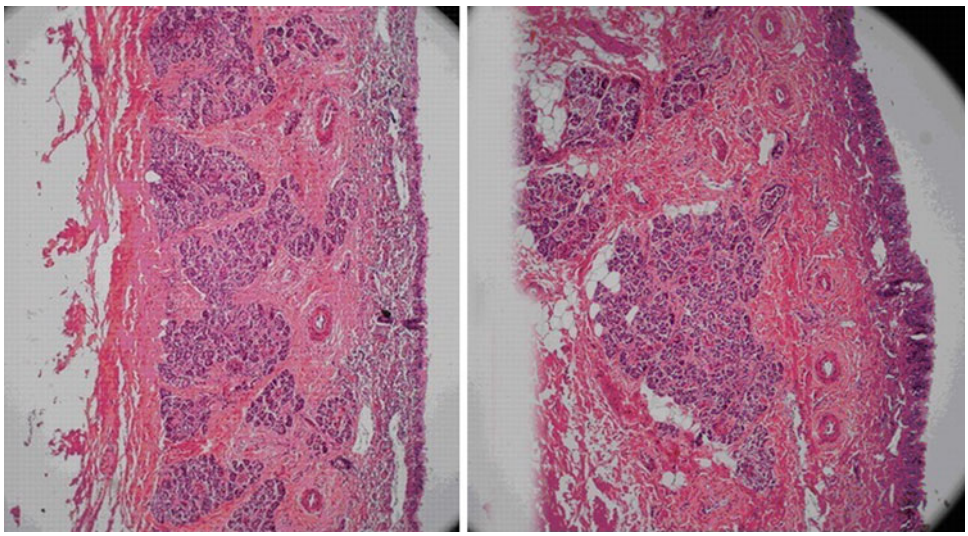

Fig. 8 Photomicrograph of sheep nasal mucosa used in mucosal toxicity study of in situ gel formulations (PLH2)

Stability study

The stability studies carried out on optimized formulation PLH2 at 30 ± 2 °C temperature and 60 ± 5% RH for 90 days. The formulation was showing good stability with no remarkable change in drug content, gelation property, gel strength and in vitro drug release profile (Table 5).

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