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Fabrication and *in vitro* Evaluation of Mucoadhesive, Thermoreversible, *in situ* Gelling Liquid Suppository of Chloroquine Phosphate

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ABSTRACT

Chloroquine Phosphate (CP), indicated for the treatment of infections caused by some sensitive strains of malarial protozoa is not a drug candidate feasible to be administered via oral route in unconsciousness state and nausea with vomiting symptoms. Parenteral administration is associated with numerous toxic effects. This obviates an alternative dosage form. Rectal delivery of this drug is a good substitute to parenteral administration. Conventional dosage forms like suppositories can cause patient discomfort and may reach end of the colon; causing the drug to undergo first-pass effect. In the present work, Rectal Chloroquine–Poloxamer gel systems composed of Poloxamer and bioadhesive polymers such as Polyvinyl pyrrolidone K30, Carbopol 934P and Polycarbophil were developed and evaluated. The physicochemical properties such as physical appearance, clarity, gelation temperature, gel strength, rheological studies and mucoadhesive force of various formulations were investigated. The gelation temperature for the formulations varied between 32.4 – 36.5^{0}C, the mucoadhesive force was found to be in the range of 37.34 – 321.05 dynes/cm^2 x 10^2 and rheological investigation revealed distinct shear thinning behaviour. Polycarbophil and Carbopol 934 P showed higher mucoadhesive strength, retardation in drug release from Pluronic F – 127 gels and significantly reduced the gelation temperature by about 6^{0}C. The drug release was found to be matrix diffusion controlled and the release mechanism was found to be Fickian. These results prove that Pluronic F – 127 liquid suppositories containing either Carbopol 934 P or Polycarbophil are suitable alternative formulations to the conventional suppositories for being physically safe, convenient, and effective rectal dosage forms to deliver anti-malarial drugs.

INTRODUCTION

Man in his quest to improve the quality of his life has always tried to find cure to various ailments that affect him. Over a period of time, this has led to development of many drugs and various routes for drug delivery. Of the many routes available, by and large, the oral route is the most preferred and convenient route for administration of drugs but not all the drugs are suitable for delivery through the oral route as they undergo first-pass metabolism in the liver; hence are effectively administered by alternate routes.

Malaria is one of the world's most devastating human infections with 300 to 500 million clinical cases and nearly 3 million deaths each year [1].

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Amongst the various drugs available to treat malaria, Chloroquine phosphate (CP) still remains the drug of choice which is effective against infections caused by *Plasmodium Vivax*, *Plasmodium Ovale*, *Plasmodium Malariae* and *Plasmodium Falciparum*. In cases where oral administration is not feasible as in case of severe and complicated cases of Malaria, parenteral administration of CP is recommended that can lead to many life threatening toxic effects. Therefore, in such cases, there is a need to develop an alternative drug delivery route which may be used to administer CP [2].

Amongst the various non invasive routes available, rectal route is a safe alternative route to deliver drugs when oral route is not feasible owing to unconsciousness, nausea/vomiting, and also when parenteral administration can lead to toxic effects. Rectal route is an ideal, promising alternative to parenteral route for administration

of drugs in paediatric and geriatric patients. Rectal route offers potential advantages for drug delivery which include rapid absorption of many low molecular weight drugs, partial avoidance of first-pass metabolism, potential for absorption into the lymphatic system, retention of large volumes, possibility of rate controlled drug delivery, absorption enhancement, amenable and relatively painless [3-5].

Conventionally solid suppositories are the most common dosage forms used for rectal drug administration and represent greater than 98% of all the rectal dosage forms. Other dosage forms include – rectal enemas, rectal solutions and rectal creams [6]. Typically the suppositories are torpedo-shaped solid dosage forms which melt or soften at body temperature. It is a favourable dosage form for infants, children and unconscious patients. One major advantage of suppositories over other oral dosage forms is that the drugs given by suppositories do not undergo the first-pass effect in the gastrointestinal tract and the liver. Moreover, the suppositories are less painful and more acceptable than injection forms. Due to these merits, suppositories have been widely applied to anti-inflammatory analgesics, anti-hemorrhoids and analgesics. However, the conventional solid type suppositories often give the patients a feeling of alien, discomfort and refusal. Furthermore, if the solid suppositories without mucoadhesivity reach the end of colon, the drugs delivered by the suppositories might undergo the first-pass effect [7]. From an industrial viewpoint, solid suppositories are inconvenient to manufacture and handle since a heating process is required for melting the suppositories and filling them in a vessel. The vessel needs to be packaged together to maintain the shape of suppositories until administration.

To overcome the problems of conventional solid suppositories, it would be desirable to develop a liquid suppository which: (1) forms a gel at body temperature; (2) has a suitable gel strength not to be leaked out from the anus after administration; and (3) has a suitable bioadhesive force so as not to reach the upper end of the colon. As a base for liquid suppositories, poloxamer, a copolymer of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene), has been investigated. Poloxamer solutions are known to exhibit the phenomenon of reverse thermal gelation; remaining as solutions at low temperature and gelling when temperature increases. Furthermore, poloxamers have been reported not to cause any damage on mucosal membranes [8, 9]. There have been several attempts to modulate the gelation temperature of poloxamer-based liquids. The gelation temperature of poloxamer solutions was modified by incorporation of cross-linking agents and monomers, by mixing the different series of poloxamers, by changing the weight of poloxamers, or by changing the pH and the ionic strength. However, majority of the previous studies have been focused on modulating only the gelation temperatures of poloxamer solutions. There has been a lack of knowledge on the strength and the bioadhesive force of gelled poloxamers, although these two factors are crucial in designing desirable liquid suppositories which do not leak out from the anus and do not reach the upper end of the colon after administration [10]. To improve the residence time of the formulation in the rectum, mucoadhesive polymers like polyvinyl pyrrolidone K 30 (PVP), Carbopol 934P and Polycarbophil were incorporated into the formulations.

Conventional suppositories of CP have been formulated using polyethylene glycol bases, but they are associated with problems like patient noncompliance due to feel of foreign matter in the rectum, unpredictable drug release pattern, leakage from the rectum and inter individual variability in plasma drug concentration [11]. To overcome these drawbacks, an attempt is being made to fabricate and examine the potential use of *in situ* gel formulations for rectal administration of CP. We developed not only thermosensitive but also mucoadhesive liquid suppositories containing CP using poloxamers in combination with various mucoadhesive polymers. The formulated suppositories were investigated for gelation temperature, gel strength, rheological behaviour, mucoadhesive forces and *in vitro* drug release characteristics.

MATERIALS AND METHODS

Chloroquine phosphate was obtained as a generous gift sample from Microlabs Limited Bangalore and Poloxamer (Pluronic F-127) was gifted to us from BASF Corporation, Germany. Carbopol 934P and Polycarbophil were purchased from Noveon, Mumbai. Polyvinyl Pyrrolidone K 30 (PVP) was purchased from SD fine chemicals, Mumbai. Benzalkonium chloride and Citric Acid were obtained from Rolex chemical Industries, Mumbai and Nice Chemicals (P) Limited, Kerala, respectively. All other reagents used were of analytical grade.

Table 1: Influence of the concentration of PF-127 and other excipients on gelation Temperature

Formulation Code	Concentration of PF-127 (%w/w)	Excipient concentration	GT (°C)	GT of 20% w/w PF-127 gels	Difference in GT (^{0}C)
F1	18	-	36.7	-	-
F2	20	-	32.0	-	-
F3	22	-	28.7	-	-
F4	24	-	25.8	-	-
F5	20	CP 1.5% w/w	29.9	32.0	2.1
F6	20	PVP K 30 1% w/w	31.8	32.0	0.2
F7	20	Carbopol 934 P 1% w/w	25.8	32.0	6.2
F8	20	Polycarbophil 1% w/w	25.9	32.0	6.1
F9	20	Citric acid 0.1% w/w	34.2	32.0	2.2
F10	20	BKC 0.001% w/v	29.8	32.0	2.2

GT - Gelation temperature, BKC – Benzalkonium chloride

Table 2: Composition of Chloroquine phosphate loaded PF-127 *in situ* gels

Formulation Code	Chloroquine Phosphate (mg)	Concentration of PF-127 (%w/w)	PVP K 30 (% w/w)	Carbopol 934P (% w/w)	Polycarbophil (% w/w)
A1	150	20	0.3	-	-
A2	150	20	0.6	-	-
A3	150	20	0.9	-	-
B1	150	20	-	0.3	-
B2	150	20	-	0.6	-
B3	150	20	-	0.9	-
C1	150	20	-	-	0.3
C2	150	20	-	-	0.6
C3	150	20	-	-	0.9

In all the formulations Citric acid concentration of 0.1% w/v, Benzalkonium chloride concentration of 0.001% w/v was kept constant.

PRELIMINARY INVESTIGATION

Determination of gelation temperature of Pluronic F-127

Pluronic F – 127 (PF-127) dispersions in water can undergo sol-gel transformation at varying temperatures. The temperature at which they undergo sol – gel transformation depends on the concentration of PF-127 in water [12]. Higher the concentration of PF-127, lower will be the temperature at which they undergo sol – gel transformation. It was found that, 18 % w/w of PF-127 in water would form a gel at 37^{0}C which is near to body temperature. Further, addition of other ingredients can as well alter the gelation temperature of PF-127 gels [13].

Taking into account the effect of all the ingredients on gelation temperature, four different concentrations viz. 18, 20, 22 and 24% w/w of PF-127 were employed to determine the gelation temperature. The gelation temperatures of these concentrations were found to be 36.7^{0}C, 32.0^{0}C, 28.7^{0}C and 25.8^{0}C respectively. Further to study the influence of excipients on the gelation temperature, 20%w/w of PF-127 solution was used.

Influence of excipients on gelation temperature of Pluronic F-127

The effect of each excipient on the gelation temperature was studied by adding the excipient separately to PF-127 solution and measuring the gelation temperature as discussed earlier. The excipients studied were CP (0.15% w/w), PVP (1% w/w), Carbopol 934P (1% w/w), Polycarbophil (1% w/w), Citric acid (0.1% w/w) and Benzalkonium chloride (0.001% w/v). The effect of drug on gelation temperature was also studied. The composition and the findings of the preliminary studies are complied in Table 1.

Preparation of Liquid Suppositories

Liquid suppositories of CP employing PF-127 were prepared by cold technique [12] method. Briefly, 150 mg of CP was dissolved in distilled water by agitation at room temperature. To the drug solution, mucoadhesive polymer was added which caused the precipitation of the mucoadhesive polymer. PVP, Carbopol and Polycarbophil were evaluated as mucoadhesive polymers. To re-disperse the precipitated mucoadhesive polymer citric acid was used as the stabilizer. After cooling the solution to 5^0C, PF-127 was added gradually with agitation. The mixture was then kept overnight at 5^0C until a viscous and clear transparent solution was obtained. 0.001% w/v of Benzalkonium chloride was included as a preservative for these gels. The prepared gels were packed into glass vials and sealed till further evaluation. The composition of the prepared *in situ* gel formulations is displayed in Table 2.

Evaluation of the fabricated liquid suppositories

Physical Appearance and Clarity:

The prepared formulations were visually checked for their physical appearance and clarity. Scores were given based on the clarity of the formulations [14].

Scanning electron microscopy (SEM)

The surface morphology of the *in situ* gels was examined by scanning electron microscopy (SEM JSM 840A JEOL, Tokyo, Japan) to study the structure and arrangement of the formulation [15]. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Gold – palladium alloy of 120 Å was coated on the sample using sputter coating unit (E5, 100 Polaron UK) in Argon at ambient of 8 -10 pascals with the plasma voltage of about 20 mA to render them electrically conductive. The SEM was operated at low accelerating voltage of about 20 KV with load current of about 80 mA.

Measurement of Gelation Temperature

The gelation temperature was measured according to the method reported by Yuan et al [16]. Briefly, 10 g of each of the different formulation was placed in a 20-ml transparent glass vial with a magnetic bar (15×6 mm). The preparation was placed in a low-temperature thermostat water bath (GFL, Germany) and the formulation was gradually heated, from 20°C, with an increase in temperature of 1°C/min with constant stirring at 50 rpm. The temperature at which the magnetic bar stopped moving, owing to sol-gel transformation of the formulation was recorded as the gelation temperature.

Measurement of Gel Strength

Liquid suppository base (50 g) was put in a 100 ml graduated cylinder and gelled in a water bath kept at 36 ± 0.5°C for 30 min. A disk for measuring gel strength (1.5 cm diameter, weight 35 g) was placed on the surface of the gelled base in the cylinder. The time (s) required for the disk to move 5 cm down through the cylinder was measured and taken as an arbitrary index of gel strength. In cases that required more than 5 min to drop the apparatus into the gel, various weights were placed on top of the apparatus and gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel [17].

Rheological Studies

Viscosity of the prepared formulations was measured by using Brookfield Viscometer L-model (Anton Paar DV-2P). For determination of viscosity before gelation, about 50 ml of sample was placed in a beaker and cool water (10^0C) was circulated through the water jacket. Once the formulation attained a temperature of 10^0C, the viscosity values were recorded using spindle L2 at different rpm of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 per min, To measure the viscosity values after gelation, the same procedure as mentioned above was followed, water at 37^0C was circulated through the water jacket. Spindles PF (T-Spindle) were used to measure the viscosity after gelation [18, 19]. The viscosity measurements after gelation were done at following rpm - 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 and 12.0 per min. Rheograms were constructed by plotting RPM values against corresponding viscosity values in cps units.

Measurement of Mucoadhesive Force

The mucoadhesive force of the liquid suppositories was measured by modified balance method [20, 21] employing a section of tissue that was cut from the fundus of the rat rectum from the discards of Pharmacology Laboratory. Two pieces of the tissue were secured by means of rubber bands and aluminium capping onto the surfaces of two glass vials, with the mucosal sides facing one another. The diameter of each exposed mucosal membrane was 6.3 mm. The vials were kept as 36.5^0C for 10 min before measuring the mucoadhesive force. One vial was connected to the balance and the other vial was placed on a height-adjustable pan. A 0.15g of sample was then spread between the mucosal membranes on the vials to attach them. The

weights on the other side of the balance required to separate the vials membranes was read. The mucoadhesive force of the liquid suppository per unit area (dyne/cm2) of mucosa was calculated using the following equation:

$$MF = 0.98 \times m/\pi r^2$$

where MF is the Mucoadhesive Force of liquid suppositories per unit area (dyne/cm^2) of mucosa, m and r represent the balance weight (g) and radius of the vial (i.e., 6.3 mm), respectively.

Estimation of Drug Content

10 ml of the formulation was added to 900 ml of 6.8 pH Phosphate buffer and stirred for 1 h on magnetic stirrer. The obtained solution was filtered and suitably diluted. Absorbance of this solution was measured at 343 nm in UV-visible spectrophotometer (Elico SL-159) against standard blank [11, 22]. The Drug content was calculated using the formula;

% Drug content = (Practical Drug content / Label claim of the product) x 100

In vitro drug release studies:

The *in vitro* drug release studies[23] from the *in situ* gel preparations was carried out using USP dissolution test apparatus II with a paddle type stirrer at 100 rpm using dialysis membrane (DM001 Dialysis Membrane 50). The dialysis membrane with an average flat width of 24.26 mm, average diameter of 14.3 mm and capacity of approximately 1.61 ml/cm was soaked in pH 6.8 phosphate buffer overnight. This hydrated membrane was used for diffusion study. 5 ml of the liquid suppository formulation was placed in this hydrated dialysis membrane and it was sealed at both ends. This sealed dialysis membrane containing the formulation was then placed into the jar of USP dissolution apparatus containing 500 ml of pH 6.8 phosphate buffer maintained at 37 ± 0.5^{0}C. The medium was stirred at 100 rpm and the diffusion studies were performed for a period of 8 hours. 1 ml of sample was withdrawn at hourly intervals and same volume of pre – warmed pH 6.8 phosphate buffer was replaced into the jar. The withdrawn sample was analyzed after suitable dilution for the CP content by recording absorbance at 343 nm employing a UV – visible spectrophotometer (Elico SL-159).

Data analysis

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the obtained data was fitted into software (PCP- Disso-V2) with zero order, First order, Higuchi matrix, Hixson-Crowell, Korsmeyer and Peppas model. By analyzing the R values, the best fit model was arrived at [24, 25].

Stability studies

Stability studies of the selected formulations were carried out as per ICH guidelines [26]. The selected formulations were stored at 25^0 ± 2^{0}C / 60 ± 5% RH, 30^0 ± 2^{0}C / 65 ± 5% RH and 40^0 ± 2^{0}C / 75 ± 5% RH for 3 months and were evaluated for their physical appearance, clarity, gelation temperature and drug content.

RESULTS AND DISCUSSION

Chloroquine phosphate loaded PF-127 gels were successfully prepared by cold technique. A sufficient drug-loading is needed in a liquid formulation to compensate the leakage after rectal administration resulting from the max volume limitation. CP is freely soluble in water; therefore, CP was dissolved and mucoadhesive polymers were dispersed, which caused the precipitation of the polymer. However, the unstable dispersion was stabilized by addition of an organic acid such as Citric acid, after considering its effect on gelation temperature. As a consequence, the mucoadhesive *in situ* gels so obtained exhibited good compatibility, which could form a stable and homogeneous gel at a suitable temperature after mixing with the other ingredients.

Influence of the amount of PF-127 and other excipients on the Gelation temperature

Gelation temperature is defined as the temperature at which a polymeric system undergoes sol-gel transition and is converted from a flowable liquid to a non – flowable gel mass. It has been reported [27] that the gelation temperature range suitable for liquid suppository would be 30–36^{0}C. If the gelation temperature of liquid suppository is lower than 30^{0}C, gelation occurs at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 36^{0}C, the suppository still stays as a liquid at body temperature, resulting in leakage from the anus.

The preliminary studies indicated that the minimum concentration of PF-127 that formed gel below 35°C was 20%w/w. The gelation temperature of different amounts of PF-127 is shown in Table 1. It was observed that as the concentration of PF-127 increased, the gelation temperature decreased, which could be attributed to the micelle formation, followed by micellar aggregation as the gel phase can only

occur when the concentration is above the critical micellar concentration [28]. When the material is in cold water, hydrogen bonding between polyoxypropylene chains and water keeps the hydrophobic portions of the pluronic separate. When the temperature is increased, the hydrogen bonding is disrupted, and hydrophobic interactions cause a gel to be formed. Therefore, the gelling properties of the poloxamers are dependent on percentage of hydrophobic portion. As the concentration of PF-127 increases, the hydrophobic portion also increases resulting in formation of gel at lower temperature.

Addition of excipients can influence the gelation temperature of PF-127 gels [29]. It has been found that while addition of Benzalkonium Chloride, Carbopol, and Polycarbophil decrease the sol-gel transition temperature, addition of Poloxamer 188, Propylene Glycol, and Ethanol increase the sol-gel transition temperature which means that the sol form of PF- 127 gets converted to gel form at higher temperatures in comparison to plain PF-127 solutions to which excipients have not been added [30].

Table 1 displays gelation temperature changes in reference to 20% w/w PF-127 solution indicating that CP, Benzalkonium Chloride, Carbopol 934P and Polycarbophil decreased the gelation temperature of the PF-127 solutions. Of the polymers, Polycarbophil and Carbopol 934P caused significant decrease in gelation temperature (up to 6^0C for 1% w/w polymer concentration) when compared to other adjuvants. Benzalkonium Chloride and CP decreased the gelation temperature by about 2^0C. It was interesting to note that, PVP did not have any significant effect on gelation temperature of PF-127[23]. Citric acid, which was used to adjust the pH of the formulation, caused a decrease in gelation temperature by about 2.5^0C.

Physical appearance and Clarity

Formulations containing PVP as a mucoadhesive polymer (A1, A2 and A3) had highest transparency and clarity while formulations with Carbopol 934 P (B1, B2 and B3) showed turbidity. The formulations with Polycarbophil (C1, C2, and C3) showed intermediate clarity and transparency. Plain PF-127 gels (F1, F2, F3 and F4) were clear and transparent.

Scanning Electron Microscopy

The SEM photographs of the formulations A2, B2 and C2 are presented in Fig. 1. The photographs reveal that the formulations containing PVP have a plate-like arrangement while the formulations with Polycarbophil and Carbopol 934 show greater folding in the polymer structure.

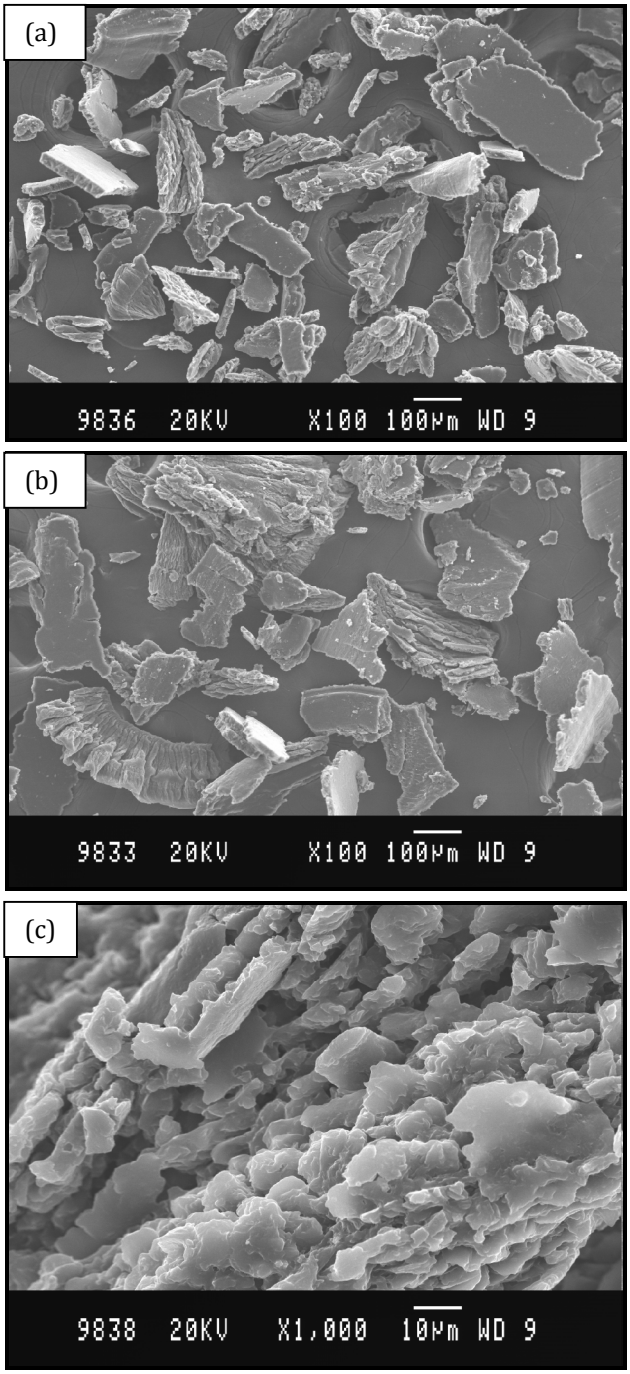

Figure 1: SEM photographs of Formulation A2 (a), Formulation B2 (b) and Formulation C2 (c)

Gelation Temperature of Formulation

The gelation temperature of formulations is listed in Table 3. All the formulations revealed a gelation temperature in the range of 32.4 ± 1.36^0C to 36.3 ± 0.73^0C which is well within the body temperature of 37^0C. This is highly desirable as it helps the formulation to be in liquid form during administration (at room temperature) and in gel form at body temperature.

Table 3: Gelation temperature, gel strength, mucoadhesive force and % drug content of Chloroquine phosphate loaded PF-127 *in situ* gels

Formulation Code	Gelation Temperature (^{0}C)*	Gel strength (Sec)*	Mucoadhesive Force(dynes/cm^2)x10^{2*}	% Drug content*
A1	34.1 ± 0.82	35 ± 3	37.34 ± 6.20	98.00 ± 1.12
A2	34.4 ± 1.21	50 ± 5	44.94 ± 9.65	101.91 ± 0.79
A3	34.3 ± 0.93	67 ± 4	51.36 ± 1.72	99.94 ± 1.34
B1	32.4 ± 1.36	55 ± 4	56.72 ± 5.41	101.20 ± 0.64
B2	32.5 ± 1.09	69 ± 5	79.57 ± 6.22	102.05 ± 0.51
B3	32.6 ± 0.98	80 ± 6	108.53 ± 8.54	99.14 ± 0.61
C1	36.4 ± 0.86	65 ± 2	66.64 ± 7.16	100.20 ± 0.72
C2	36.3 ± 0.73	82 ± 8	185.83 ± 5.74	101.77 ± 0.80
C3	36.5 ± 1.26	110 ± 5	321.05 ± 6.21	97.02 ± 1.08

*Data are expressed as mean ±SD. n = 3

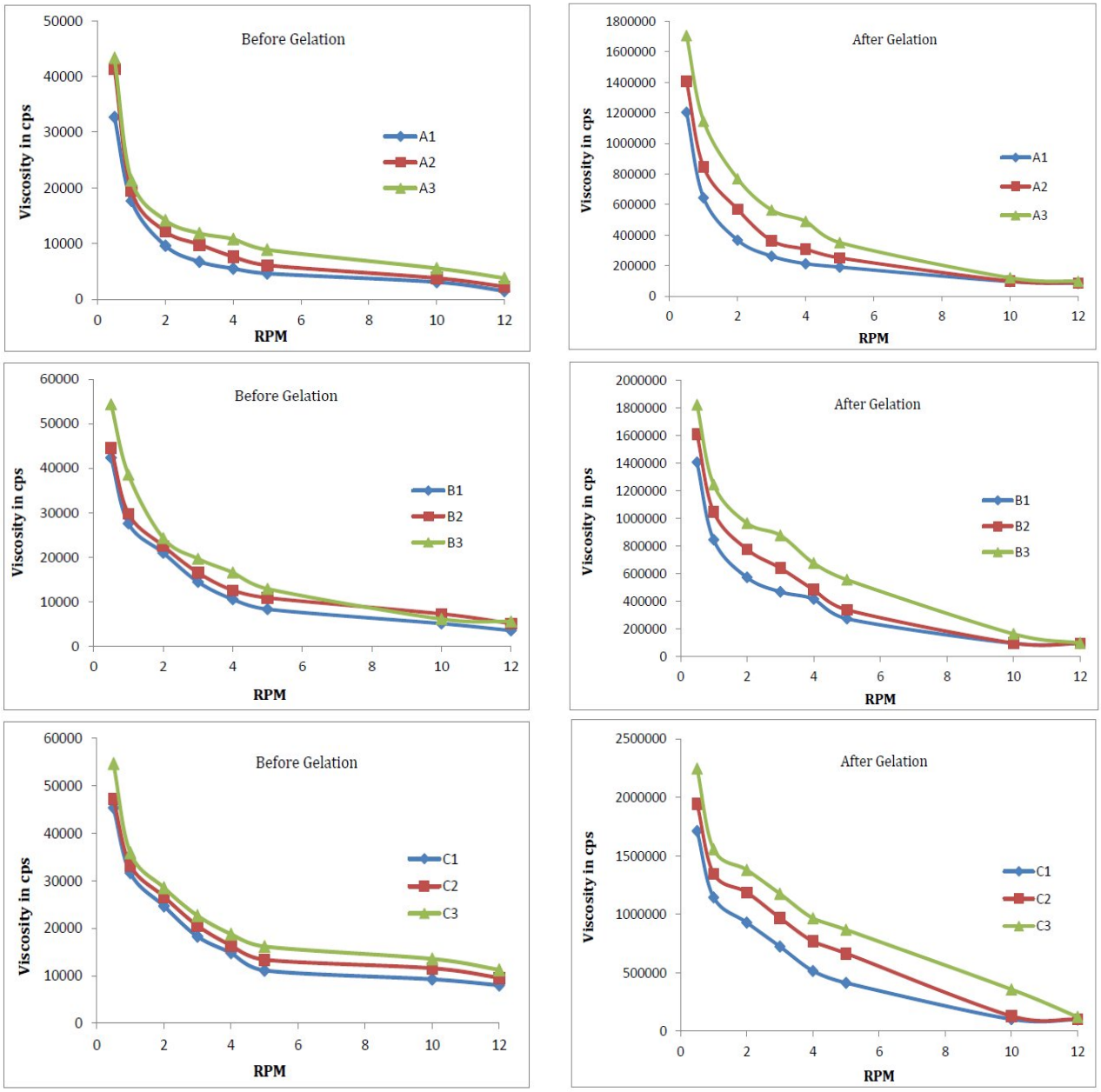

Figure 2: Rheograms of Liquid suppository formulations before and after gelation

Gel strength

Gel strength is an important parameter in finding the suitable condition which allows the easy insertion of the suppositories into the rectum and further preventing leakage from the anus. It was previously reported that the optimal liquid suppository must have suitable gel strength of approximately 10–50 s [12]. The gel strength of CP–Poloxamer gels was affected by the nature and composition of mucoadhesive polymers. All mucoadhesive polymers abruptly increased the gel strength as the concentration increased from 0.3 – 0.9%. The greater the increase in the concentration of the mucoadhesive polymer, the greater will be the gel strength of Poloxamer gels. This observation was in agreement with the findings of Yong et al [31]. It was speculated that the mucoadhesive polymer could bind strongly with the cross-linked Poloxamer gel by placing the polymer in the gel matrix. Polycarbophil exhibited the largest increase in the gel strength (65 –110 s) upon increasing the concentration from 0.3% to 0.9%, while, PVP showed the least effect on gel strength (35 - 67 s) in the same concentration range. The increment of strength might be related to hydrogen bonding between Poloxamers and mucoadhesive polymers in the suppository. Carbopol formulations showed the suitable gel strength of (55 - 80 s). The gel strength data of the formulations is presented in Table 3.

Rheological Studies

The samples showed marked difference in viscosity values (cps) before and after gelation, there was nearly 30 fold increase in viscosity after gelation in all the cases. All the formulations showed evidence of shear thinning behaviour which was indicated by the lower viscosity values with increasing RPM. Amongst the mucoadhesive polymers tested, Polycarbophil was found to affect the viscosity of PF-127 gels to the greatest extent. The rheograms of the liquid suppository formulations before gelation and after gelation are presented in Fig. 2.

Mucoadhesive force of Liquid Suppositories

Mucoadhesive force means the force with which liquid suppositories bind to rectal mucus lining at 36.5^{0}C. The stronger the mucoadhesive force, the more it can prevent the gelled suppositories from reaching the end of the colon, the pathway for the first-pass effect. Polycarbophil showed highest mucoadhesive force, PVP exhibited the lowest mucoadhesive force while Carbopol 934P showed an intermediate value. Polycarbophil which has a number of -OH and COOH groups undergoes interaction with the oligosaccharide chains of rectal mucosa and hence results in strong binding and greater mucoadhesive force, while, PVP which has less number of -OH and COOH groups resulting in poorer binding and hence low mucoadhesive force at the same concentration. At the same time, a greater magnitude of mucoadhesive strength can cause damage to the delicate rectal mucosa. Hence, it is necessary to choose appropriate concentration of mucoadhesive polymer. The mucoadhesive force of the formulated *in situ* gels is depicted in Table 3.

Drug Content

The drug content of each formulation was analyzed by UV – Spectrophotometer and it was found that all the formulations contained CP within the IP permissible limits of 95 – 105%.

The % drug content of the formulated *in situ* gelling formulations is compiled in Table 3.

In-vitro Drug release studies

The drug release from the prepared formulations through the rectal mucosa is shown in Fig. 3. As the concentration of mucoadhesive polymer increased from 0.3% w/w to 0.9% w/w, the drug diffusion decreased from 100.49 to 98.52% for PVP, 96.52 to 90.45% for Carbopol 934P and 95.20% to 89.06% for Polycarbophil.

In case of formulations containing Carbopol and Polycarbophil the drug release proportionally decreased with increasing concentration of these polymers, but in case of PVP, no such decrease in drug release with increasing concentration was seen. Polycarbophil exhibited highest retardation, which could be attributed to high mucoadhesive strength and viscosity. The gel structure is more closely packed and acts as a resistant barrier for drug release. Carbopol 934P formulations exhibited moderate drug release over a period of 8 hours.

The drug release kinetic data is compiled in Table 4. In all the cases, the R values of Higuchi matrix model were close to 1. The diffusion coefficient (n) values ranged between 0.339 and 0.671. Since the R values of Higuchi matrix were close to 1, the drug release follows matrix diffusion kinetics and the plot shown in Fig. 4 revealed linearity; hence it was concluded that diffusion was the main mechanism of drug release from the liquisolid suppositories. Further, the observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows Fickian transport mechanism.

Table 4: *In vitro* release data fitting into various mathematical models

	Zero order		First order		Matrix		Hixson-Crowell		Peppas	
	R	k	R	k	R	k	R	k	n	k
A_1	0.805	17.164	0.988	42.039	0.991	45.511	0.959	-0.181	0.454	45.511
A_2	0.821	17.150	0.985	41.944	0.992	43.624	0.958	-0.180	0.479	43.624
A_3	0.824	17.632	0.921	40.994	0.996	42.502	0.950	-0.180	0.482	42.502
B_1	0.700	19.651	0.977	48.469	0.999	62.950	0.958	-0.238	0.339	62.950
B_2	0.729	18.368	0.982	45.22	0.999	56.973	0.968	-0.211	0.358	56.973
B_3	0.742	17.917	0.984	44.084	0.998	54.248	0.972	-0.202	0.373	54.248
C_1	0.840	19.189	0.992	46.837	0.994	47.772	0.960	-0.232	0.491	47.772
C_2	0.871	17.924	0.976	43.463	0.985	45.454	0.961	-0.207	0.611	45.454
C_3	0.880	16.561	0.992	40.231	0.996	37.355	0.950	-0.172	0.547	37.355

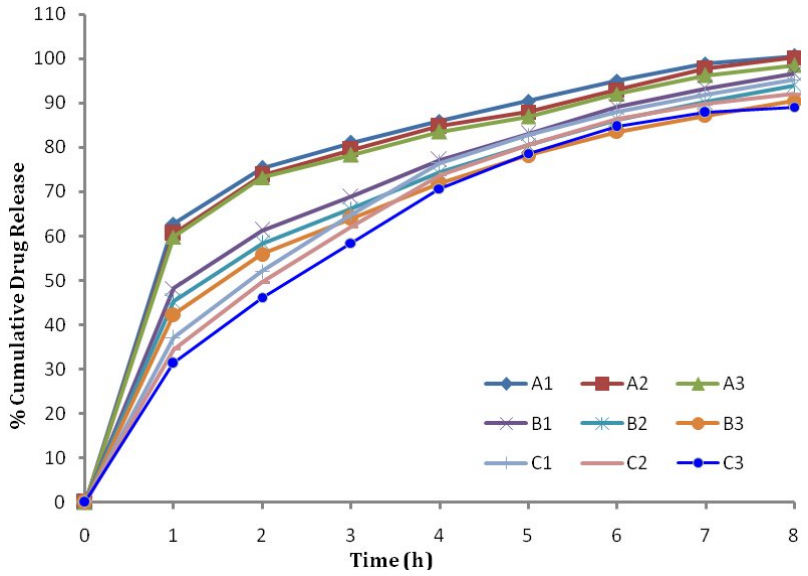

Figure 3: *In vitro* drug release profile from Liquid suppositories

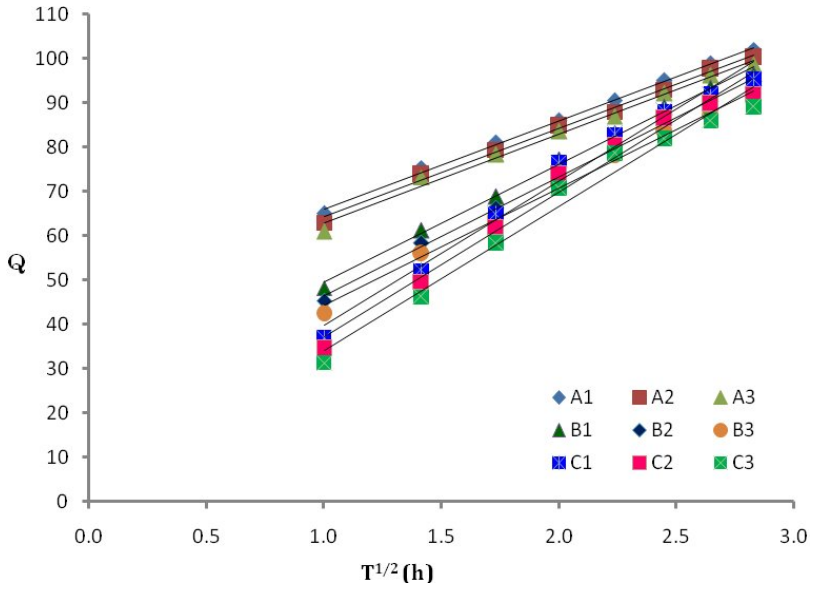

Figure 4: Higuchi Plot

Two formulations coded B3 and C2 were chosen for stability studies based on their Mucoadhesive strength and *in vitro* drug release characteristics. The stability study results indicate that there was no evident change in the physical appearance and clarity of formulations after subjecting them to stability studies. The gelation temperature also did not vary for formulations after subjecting them to stability studies.

The viscosity of the formulations was affected to certain extent after subjecting them to stability studies. It was found that the viscosity of the formulations increased with increasing temperature and humidity conditions (both before and after gelation). This may be attributed to loss/gain of water by the formulations during stability studies. The drug content was not altered after stability studies. Thus, we may conclude that, the drug does not undergo degradation on storage.

CONCLUSION

Modulation of the physicochemical and adhesive properties of Pluronic F-127 by incorporation of mucoadhesive polymers showed a prolonged *in vitro* release of Chloroquine phosphate. It is concluded that liquid suppository formulation B3 and C2, which remained at the administered sites due to strong gel strength and mucoadhesive force, could improve absorption of Chloroquine phosphate without damaging the rectum. The gelation temperatures of Chloroquine phosphate rectal formulations could be easily modulated to adjust the gelation just below the body temperature. This allows obtaining liquid systems at room temperature which can be conveniently handled and administered that develop an intimate contact with the rectal mucosa. Therefore, these results revealed the potential usefulness of the CP rectal *in situ* gel, which could alleviate the feeling of alien, discomfort and refusal during application to patients.

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