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FORMULATION AND EVALUATION OF THERMOREVERSIBLE *IN SITU* GELLING AND MUCOADHESIVE DILTIAZEM HYDROCHLORIDE LIQUID SUPPOSITORY

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ABSTRACT

Objective of the present study was to develop and evaluate thermoreversible in situ gelling and mucoadhesive liquid suppositories to improve patient compliance and systemic absorption of diltiazem hydrochloride. Thermoreversible mucoadhesive liquid suppositories were prepared by adding mucoadhesive polymers to a formulation that contained pluronic F-127, pluronic F-68 and diltiazem hydrochloride. Mucoadhesive polymers such as Carbopol 974P, Polyox WSR-301, hydroxypropyl methylcellulose, polycarbophil and polyvinylpyrrolidone were investigated to modulate the gel strength and mucoadhesive force for diltiazem hydrochloride liquid suppository. Addition of these polymers reinforced the gel strength and the mucooadhesive force of the prepared liquid suppository formulation. Increasing the concentration of mucoadhesive polymers retarded the release of diltiazem hydrochloride from the pluronic gel. Carbopol and polycarbophil showed highest retardation of drug release than other polymers investigated. Diltiazem hydrochloride formulated as mucoadhesive thermoreversible poloxamer solution for rectal administration can have potential to avoid first-pass effect through oral route, improve the bioavailability of drug and it can be used as a safe and sustained release rectal delivery system to control severe post-operative pain.

KEYWORDS

Diltiazem hydrochloride, thermoreversible, mucoadhesive, pluronics liquid suppository.

INTRODUCTION

Diltiazem hydrochloride (diltiazem HCl) is a calcium channel blocker widely prescribed for treatment of arrhythmia, angina pectoris and hypertension[1]. It undergoes an extensive biotransformation, mainly through cytochrome P-450 CYP3A which results in less than 4% of its oral dose being excreted unchanged in urine. Its bioavailability is around 30% to 40% owing to an important first pass metabolism and has an elimination half-life of 3.5 hours[2, 3]. Recently it reported, further, that although liver is considered to

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be the major organ of diltiazem biotransformation the extra hepatic organs such as intestine and lungs contribute to the first pass uptake and systemic elimination of diltiazem[4, 5].Nowadays one of the basic tasks of drug formulation is to develop an already existing dosage form in a way which makes drug release the best possible under the given circumstances, that is to enhance bioavailability in this way[6, 7, 8]. The other important aim is to widen the choice of products with respect to dosage that is to make a given drug available in as many dosage forms as possible[9].

In a previous study by Diwan et al[10], compressed rectal suppositories of diltiazem HCl were formulated, wherein it was found that the relative bioavailability of diltiazem HCl was about 75% greater after rectal administration compared to oral route. In addition, by virtue of its high lipid solubility and high partition coefficient, it represents an excellent model for rectal administration. The formulation of this dosage form would add to the choice of existing treatment methods and would also improve the possibilities of individual cure in cases when the oral administration of diltiazem HCl should be avoided (vomiting, shock, patient with bad compliance, patient with parenteral nutrition and elderly patients). Other calcium channel blockers which have been studied through rectal route are nifedipine[11], and verapamil[12].

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[13]. 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 solve 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 mucoadhesive force so as not to reach the end of the colon. As a base of liquid suppositories, poloxamer as a surfactant, copolymer of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene), has been studied. Poloxamer solutions (pluronics) are known to exhibit the phenomenon of reverse thermal gelation; remaining as solutions at low temperature and gelling when temperature increases. The temperature –dependent gelling process is micellar in nature, being constructed from cubic orientation of micellar subunits[14]. The micellar mode of association has been useful as drug delivery systems[15]. The reversal thermal gelation exhibited by pluronic aqueous solutions has been used as drug delivery system for ophthalmic[16], parenteral[17], rectal[18], and percutaneous use. Furthermore, pluronics were reported not to cause any damage on mucosal membranes[19], possessed good drug release characteristics and were compatible with other chemicals. There have been several attempts to modulate the gelation temperature of poloxamer-based liquids. The gelation temperature of these poloxamer solutions was adjusted by modifying cross-linking agents and monomers, by mixing the different series of poloxamers, by changing the weight of poloxamers, or by changing the pH and

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the ionic strength. However, most 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 end of the colon after administration[19].The previous studies[20, 21, 22], has also reported the mucoadhesive drug delivery systems of diltiazem HCl in the form of tablets for oral route and transdermal patches; however there is no report available on mucoadhesive liquid suppositories.Thus in this study, we developed not only temperature-sensitive but also mucoadhesive liquid suppositories containing diltiazem HCl using poloxamers and mucooadhesive polymers. To modulate the gel strength and mucoadhesive force of the liquid suppository bases mucoadhesive polymers such as carbopol 974P (CP), polyox WSR 301, hydroxypropyl methylcellulose (HPMC K4M), polycarbophil and polyvinyl pyrrolidone (PVP K30) were studied. The physicochemical properties and the release of diltiazem HCl from the prepared poloxamer gel formulations were evaluated.

MATERIALS

Diltiazem hydrochloride (USP) was a gift sample from Ranbaxy Pvt. Ltd. Goa, India. Pluronic F-127 (PF-127) and pluronic F-68 (PF-68) of extra pure grade were supplied by Signet Chemical Corporation, Mumbai, India. Carbopol 974P NF and Noveon Polycarbophil AA-1 were gifted by Lubrizol Advanced Materials Pvt. Ltd. Mumbai, India. Hydroxypropyl methylcellulose and Polyox WSR-301 were generously donated by Colorcon Asia Pvt. Ltd. Goa, India. Polyvinyl pyrrolidone (PVP K30) was obtained from BASF Corporation, Mumbai, India. All other chemicals were of research grade.

METHODS

Preparation of DTZ liquid suppositories:

Aqueous diltiazem HCl liquid suppositories using different concentration of PF-127 and PF-68 and various formulation additives as shown in Table 1 were prepared by cold method described by described by Choi et al[19]. 90 mg of diltiazem HCl was dissolved in the calculated amount of distilled water at room temperature. The method involved slow addition of polymer, drug and other additive in cold water with continuous agitation. The formed mixtures were stored overnight at 4^{o}.The diltiazem HCl liquid suppository formulation showing satisfactory gelation temperature (30^{o}- 37^{o}) was selected as an optimized formulation. Further study on this optimized formulation was carried out using additional amount of mucoadhesive polymers namely CP, Polyox WSR 301, HPMC K4M, Polycarbophil and PVP K30 in the concentrations 0.5 and 1.0%. The mixtures were left at 4° in refrigerator until clear solutions were obtained.

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Ingredients (%w/v)	F0	F1 A	F1 B	F2 A	F2 B	F3 A	F3 B	F4 A	F4 B	F5 A	F5 B
*Diltiazem Hydrochloride (mg)	90	90	90	90	90	90	90	90	90	90	90
PF - 127	20	20	20	20	20	20	20	20	20	20	20
PF-68	10	10	10	10	10	10	10	10	10	10	10
Carbopol 974P	-	0.5	1.0	-	-	-	-	-	-	-	-
Polyox WSR-301	-	-		0.5	1.0	-	-	-	-	-	-
HPMC K4M	-	-	-	-	-	0.5	1.0	-	-	-	-
Polycarbophil	-	-	-	-	-	-	-	0.5	1.0	-	-
PVP K30	-	-	-	-	-	-	-	-	-	0.5	1.0
Propylene Glycol	10	10	10	10	10	10	10	10	10	10	10
Sodium chloride	1	1	1	1	1	1	1	1	1	1	1
Distilled water	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml	q.s to 5ml

F0 to F 5B represents the various formulations of Diltiazem HCl.
**Each formulation contained 90 mg of Diltiazem HCl.*

TABLE NO 1

FORMULATION AND EVALUATION OF THERMOREVERSIBLE *IN SITU* GELLING AND MUCOADHESIVE DILTIAZEM HYDROCHLORIDE LIQUID SUPPOSITORY

Composition of thermally reversible in situ gelling and mucoadhesive diltiazem hydrochloride liquid suppositories

EVALUATION OF DILTIAZEM HCl LIQUID SUPPOSITORIES

Measurement of gelation temperature:

Gelation temperature was assessed using the tube tilting method[23]. 2 ml aliquot of gel was transferred to test tubes, immersed in a water bath at 4° and sealed with aluminum foil. The temperature of water bath was increased in increments of 1° and left to equilibrate for 5 min at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90°.

Measurement of gel strength:

The gel strength was determined according to the method adopted by Choi et al[19]. 50 g of liquid suppository was put in a 100 ml graduated cylinder and gelled in a thermostat at 37°. The apparatus for measuring the gel strength (weight 35 g) was then placed into the liquid suppository. The gel strength was determined by the time in seconds the apparatus (Fig 1.) took to penetrate 5 cm down through the gel.

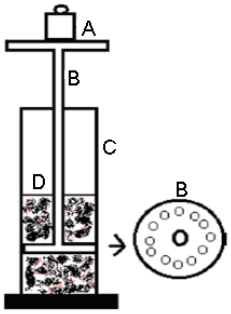

Fig. 1 ***Gel strength-measuring device***

Figure represents gel strength measuring device and labeled as; (A) weights; (B) device; (C) mess cylinder; (D) poloxamer gel

Determination of the mucoadhesive force:

The mucoadhesive force, the detachment stress of the liquid suppositories was determined using a modification of the mucoadhesive force-measuring device used by Choi et al[24]. A section was cut from the fundus of sheep rectum and instantly secured with the mucosal side out into each glass vial. The vials were stored at 36.5°C for 10 min. 1 vial connected to the balance and the other fixed with the pluronic gel (0.5ml) added and the height adjusted so that the gel is placed between the mucosal sides of both vials (Fig 2.). Water from a burette was allowed to fall in a beaker at a constant rate of 10 mg/sec. Increasing weight of water added gradually would detach the two vials. Mucoadhesive force, the detachment stress (dyne/cm^2), is determined from the minimal weights of water that detached the 2 vials.

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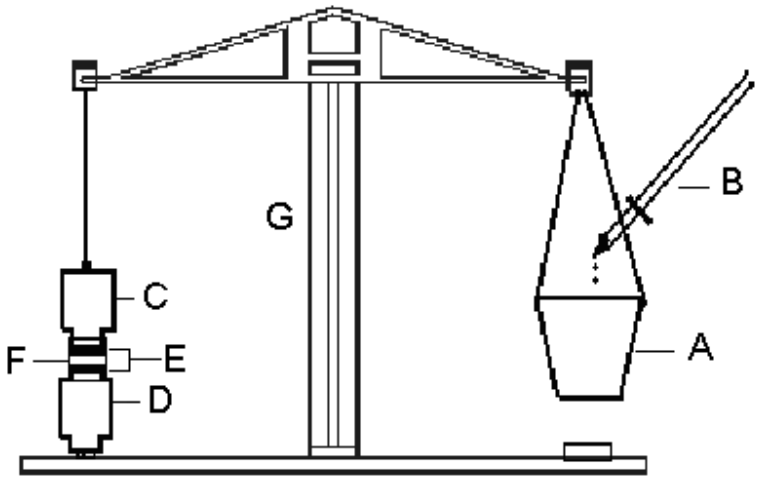

Fig. 2 ***Mucoadhesive force measuring device***

Figure represents mucoadhesive force measuring device and labeled as; (A) Light plastic glass; (B) Burette; (C) Upper glass vial; (D) Lower glass vial; (E) Rectal membrane; (F) liquid suppository; (G) Modified balance

pH:

The pH values of the liquid suppository formulations were determined by using calibrated pH meter (Equip Tronics, Mumbai)

Drug content estimation:

One ml of the solution was pipetted and dissolved in about 70 ml of phosphate buffer (pH 7.4) in a 100 ml volumetric flask. The flask was shaken for 10 min and volume made up to the 100 ml mark. After shaking for 2 min, the solution was filtered; rejecting first few ml of the filtrate. From the above solution, 1 ml was transferred to a 25 ml volumetric flask and diluted up to the mark with phosphate buffer. The absorbance of this solution was recorded at 237 nm against blank reagent using UV/Vis spectrophotometer (Perkin Elmer, Lambda 25, Mumbai). Drug content studies were carried out in triplicate for each formulation batch. The concentration of the drug present in formulation was computed from the equation obtained from calibration curve.

***In vitro* release of DILTIAZEM HCl from the rectal solutions:**

The *in vitro* drug release studies from the prepared rectal solutions were monitored by using USP II dissolution apparatus[25, 26] (Labindia, Mumbai). An amount of rectal solution (5 ml) equivalent to 90 mg of diltiazem HCl was inserted into a semi permeable cellulose membrane bag tied from both ends. The

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membrane bag was then connected to the paddle of the dissolution tester and immersed in 500 ml of phosphate buffer pH 7.4 maintained at 37 ± 0.5°. The speed of rotation of the paddle was adjusted at 100 rpm. Aliquots of 5ml were withdrawn from the release medium at 1h time intervals upto 8 h and analyzed by UV/Vis spectrophotometer at 237 nm. The experiments were conducted in triplicates. The results are shown in Figs. 3 to 4.

Data treatment:

To analyze the mechanism of drug release from the liquid suppository formulations, the release data were fitted according to various kinetic equations namely, the zero-order[27], first-order[28], Higuchi-square root[29] and Korsmeyer-Peppas equation[30].

Statistical analysis:

The data were analyzed by using two-way analysis of variance (ANOVA). P values lower than 0.05 were considered statistically significant.

Stability study:

Liquid suppository formulations containing 1.0% mucoadhesive polymers were tested for stability under the actual condition of storage (refrigeration condition). Gels were stored in clean, dry, airtight moisture proof bottles, kept away from light. The gel samples were withdrawn after 30 days and evaluated for gelation temperature, gel strength, mucoadhesive strength, pH and drug content.

RESULTS AND DISCUSSION

Liquid suppository development:

Liquid suppository formulations of diltiazem HCl were prepared by using PF-127 and PF-68 as thermoreversible polymers in a mixture which gels at a body temperature at a specific concentration. Propylene glycol was used as a humectant to maintain the consistency for a long period of time and sodium chloride was used to adjust the tonicity and to lower the gelation temperature. CP, Polyox WSR 301, HPMC K4M, Polycarbophil and PVP K30 in the concentrations 0.5 and 1.0% were used as mucoadhesive polymers required to modulate the gel strength and increase the residence time of the gel by adhering to rectal mucosa (Table 1).

Evaluation of DILTIAZEM HCl liquid suppositories:

Gelation temperature

Gelation temperature is the temperature at which the liquid phase makes a transition to gel. A gelation temperature range suitable for liquid suppository would be 30–36°. If the gelation temperature of liquid suppository is lower than 30°, gelation occurs at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than 36°, the suppository still stays as a liquid at body temperature, resulting in leakage from the anus. Therefore, liquid suppository must have the suitable gelation temperature between 30–36°, to be in a liquid form at room temperature and to form a gel phase instantly in the rectum[19].

During preliminary work the rectal gel containing less than 20 % w/w PF-127 did not gel over the temperature range tested (up to 50°) and that increasing PF-127 concentration, by an increments of 2-3%, the gelation temperature of solution is decreased. On the other hand, PF-68 in the tested concentrations (0-7%) failed to give the suitable range of gelation temperature, where all the recorded temperature values were >50° and a gelation started to be observed at 48° with a 10% solution of PF-68. Increase in the PF-68

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concentration decreases the gelation temperature. The previous finding indicates that neither PF-127 nor PF-68 alone could provide gelation at the physiological temperature. A modulation of the gelation temperature to reach the desired range (30-37^{o}) could be achieved through the use of a combination of the two poloxamer grades. At concentration of PF-127 (20%), it was found that the gradual increase in PF-68 concentration was accompanied by a concomitant decrease in the gelation temperature of the prepared rectal gel. Thus during preliminary studies formulation containing PF-127/PF-68 in the ratios of 20/10%w/v was selected as an optimized formulation for further study since it showed a gelation temperature in the range of 30-36^{o}C (Table 2). This revealed that PF-127 is the main polymer determining the gelation temperature of the solution and might be explained on the basis of its higher molecular weight (average molecular weight 12,500) compared to that of PF-68 (8,600) and its higher amount in the formulation.

Table No 2

Gelation temperature of medicated liquid suppositories.

Pluronics	Concentrations of pluronics	*Gelation temperature (^{o}) ± S.D
PF-127	(15%)	>50
	(17%)	>50
	(20%)	44±1.20
	(21%)	32±0.34
	(22%)	23.5±0.96
PF-68	(5%)	>50
	(7 %)	>50
	(10%)	48±1.10
	(15%)	41±1.50
(PF-127/PF-68)	(15%/ 5%)	>50
	(15%/ 7%)	>50
	(15%/ 10%)	>50
	(17%/ 5%)	50.5±0.50
	(17%/ 7%)	47±0.20
	(17%/ 10%)	44±1.0
	(20%/ 5%)	42±1.0
	(20%/ 7%)	39±0.75
	(20%/ 10%)	35.5±1.20
	(21%/ 5%)	29±1.0
	(21%/ 7%)	26.5±0.83
	(21%/ 10%)	21.7±0.25

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**Denotes average of three determinations*

Table No. 3

Effect of mucoadhesive polymers on the drug release, gelation temperature, mucoadhesive force and gel strength on liquid suppository formulations

Code	*Drug Release after 8h (%) ±S.D	*Gelation temperature (^{o}) ±S.D	*Gel strength (s) ± S.D	*Mucoadhesive force (dynes/cm^2*10^2) ±S.D	pH	*%Drug Content ±S.D
F0	#95.83±1.9	35.67±0.3	8.33±2.52	24.04±11.27	6.57	101.83±1.23
F1A	84.76±1.6	32.0±1.0	67.0±3.61	175.89±9.44	6.98	99.16±0.85
F1B	72.28±1.83	28.5±0.5	114.0±3.0	319.03±13.33	6.70	98.67±1.15
F2A	86.55±1.36	33.5±0.5	31.33±2.8	109.82±11.89	6.83	99.33±1.61
F2B	76.63±1.70	31.5±0.71	46.67±1.5	210.15±15.27	6.28	99.57±1.12
F3A	91.03±1.24	34.0±0.62	39.67±1.5	77.28 ±16.85	6.42	99.75±0.99
F3B	80.34±1.94	32.0±1.41	75.0±2.65	141.08±7.87	6.29	98.25±0.72
F4A	77.71±1.39	30.67±0.58	57.67±3.1	150.90 ±15.55	7.01	99.96±0.84
F4B	60.58±2.30	27.75±1.06	93.33±3.2	243.85±9.75	6.89	98.98±1.24
F5A	94.46±1.80	35.25±0.35	14.33±1.5	47.14 ±10.66	6.98	100.74±0.49
F5B	87.32±1.83	34.0±0.78	24.0±1.73	55.58 ±8.33	6.95	99.79±1.21

F0 to F 5B represents the various formulations of Diltiazem HCl.
**Denotes average of three determinations*
#Cumulative percent drug release of formulation F0 after 5 h.

To reinforce the gel strength and mucoadhesive force of the rectal solutions, mucoadhesive polymers were further incorporated[31]. The different mucoadhesive polymers used in this study are either swellable or water soluble but differ in their nature and charge. The addition of mucoadhesive polymers lowered the gelation temperature of all rectal gel formulations (Table 3). The impact of mucoadhesive polymers on the gelation temperature was found to depend on their nature and

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concentrations. Increasing the concentration of any of the used bioadhesive polymer from 0.5% to 1.0% produced a gradual decrease in the gelation temperature of rectal gel.

In comparison to formulation containing no mucoadhesive polymers, the mean average decrease in the gelation temperature noticed with all the rectal gel formulations, containing 0.5 and 1.0% of different mucoadhesive polymers, was found to be as follows: Polycarbophil> CP> Polyox> HPMC K4M> PVP K30.

This order of arrangement correlated well with the viscosity of polymers present in all the formulations. The direct relation between gelation temperature and viscosity of polymer solution has been reported by Jeong et al[32]. The gelation temperature lowering effect of mucoadhesive polymer could be explained by their ability to bind to the polyoxyethylene chains present in the pluronic molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature[33, 34].

Measurement of gel strength

In the development of liquid suppository, the gel strength is important in finding the condition which allows the easy insertion of the suppositories and no leakage from the anus. At high gel strength, it is difficult to insert the suppositories. On the other hand, at low gel strength the suppositories leaked from the anus[26]. It has been previously reported that the optimal *in situ* gelling and mucoadhesive liquid suppositories must have suitable gel strength, in the range of 10 to 50 seconds.

In the present study it was found that, the addition of mucoadhesive polymers increased the gel strength of poloxamer mixture in a concentration-dependent manner. Out of the five mucoadhesive polymers, CP exhibited higher gel strength. In comparison of formulation containing no bioadhesives the average increase gel strength noticed with all the bioadhesives used can be arranged in the following order, PVP K30< Polyox WSR-301< HPMC K4M< Polycarbophil< CP (Table 3).The large increase in gel strength caused by the addition of CP might be attributed to the strong cross-linking bonding of CP with the cross-linking reticular poloxamer gel forming more closely packed micelles[35]. The mechanism of the increase incase of other mucoadhesive polymers might be related to hydrogen bonding between pluronic and bioadhesive polymers in the rectal gel[31].

Measurement of the mucoadhesive force

Mucoadhesive force means the force with which liquid suppositories bind to rectal mucous lining at 36.5°. Since rectal mucous lining consists of oligosaccharide chains with sialic acid, the polymers with hydrophilic groups such as the carboxyl and hydroxyl groups can bind strongly to oligosaccharide chains, resulting in strong bioadhesive force. The stronger the Mucoadhesive force is, the more it can prevent the gelled suppositories from reaching the end of the colon, the pathway for the first-pass effect. But if the mucoadhesive force is too excessive, the gel can damage the rectal mucous membrane[19]. Therefore, liquid suppositories must have the balanced mucoadhesive force. The force, with which diltiazem HCl rectal gel is bound to sheep rectal mucosa, is obtained by modified pan balance method (Fig. 2).

In the present investigation, the addition of different bioadhesive polymers reinforced the mucoadhesive force of the rectal gel and the mucoadhesive force

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significantly increased as the concentration of mucoadhesive polymers increased from 0.5 to 1.0% (Table 3). The mucoadhesive polymers could be arranged according to their mucoadhesive force enhancing effect at 1.0% concentration of rectal gel as, CP> Polycarbophil> Polyox WSR-301> HPMC K4M> PVP K30.The mucoadhesive force of the liquid suppository formulations was found to be function of nature and concentration of polymers; the findings are in consonance with the literature. Increasing the polymer amount may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting consequently in aggrandization of mucoadhesive strength.The mechanism of the mucoadhesion enhancing effect of different polymers might be related to hydrogen bonding between the liquid suppository base and the mucosal membrane (glycoprotein) via carboxyl groups in the mucoadhesive polymers[36]. The mucoadhesive force-enhancing effect of HPMC could be explained based on the theory postulated by Liu and Chu[37] that, cellulose derivatives having many hydroxyl groups promote dehydration of poloxamers and consequently the hydrophobic interactions between the poly(oxypropylene)blocks[38].

pH

The maximum pH of all the formulations was found to be 7.01 and the minimum was 6.28 (Table 3). The results reveal that all formulations had pH values close to rectal pH range. Hence, they may not produce any irritation of the rectal mucosa.

Drug content uniformity

The drug content uniformity was calculated for all formulations, the results of which are shown in Table 3. For the various formulations the drug content uniformity varies from 98.25 to 101.83%. The cumulative percentage drug released and the percentage drug retained by the formulation in the in the in vitro release studies were based on the mean content of the drug presenting the respective formulations. The tests were done in triplicates and the content uniformity was found to be uniform in all the formulations.

***In vitro* release of *DILTIAZEM HCl* from liquid suppositories**

The effect of mucoadhesive polymers on the release of diltiazem HCl from liquid suppository was illustrated in Fig. 3 and 4. In the present study it was found that the plain gel (F0) containing no mucoadhesive polymer, released around 95% of the drug within 5h. The addition of mucoadhesive polymers retarded the drug release from all other formulations. The retardation of drug release also occurred with an increase in the concentrations of the various mucoadhesive polymers (Table 3). Among the five different mucoadhesive polymers studied, polycarbophil showed the highest retardation of drug release, whereas PVP produced the least retardation. These various mucoadhesives can be arranged according to their release-retarding effect as follows: Polycarbophil >CP >Polyox WSR-301> HPMC K4M> PVP K30. This order of arrangement correlated well with the gel strength and the gelation temperature-lowering effect of these polymers as previously mentioned.

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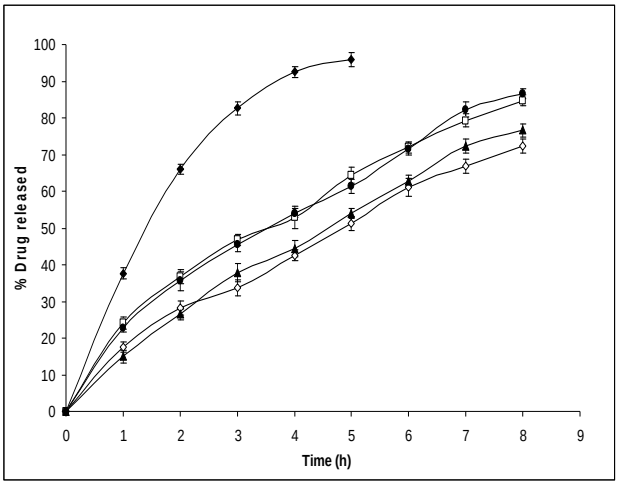

Fig. 3

The in vitro release profiles of DILTIAZEM HCl from F0, F1A, F1B, F2A and F2B.*Release profiles of DILTIAZEM HCl from liquid suppository formulations, F0 [plain gel](-♦-), F1A [Carbopol, 0.5%] (- -), F1B [Carbopol, 1.0%] (-◊-), F2A [Polyox, 0.5%] (-●-) and F2B [Polyox, 1.0%] (-▲-). Each data point represents mean ±SD (n = 3).*

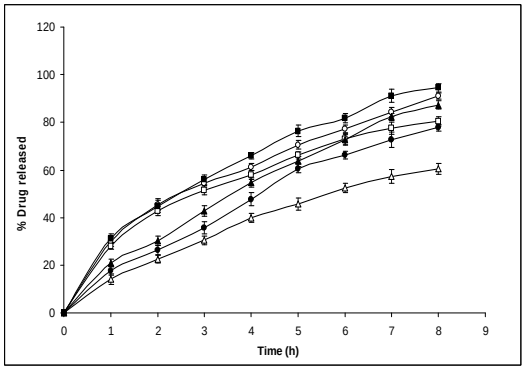

Fig 4

The in vitro release profiles of DILTIAZEM HCl from F3A, F3B, F4A, F4B, F5A and F5B.
Release profiles of DTZ from liquid suppository formulations; F3A [HPMC, 0.5%] (-◊-), F3B [HPMC, 1.0%] (-o-), F4A [Polycarbophil, 0.5%] (-●-), F4B [Polycarbophil, 1.0%] (-Δ-), F5A [PVP, 0.5%] (-■-), and F5B [PVP, 1.0%] (-▲-). Each data point represents mean ±SD (n = 3).

The retarding effect of these mucoadhesive polymers could be attributed to their ability to increase the overall product viscosity[39] as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses thereby delaying the release process[19].The null effect of PVP on diltiazem HCl release may be attributed partly to the low viscosity of the K-30 grade used in this study, and partly due to its water soluble nature which allowed more rapid penetration of dissolution fluid into the product initiating product surface dissolution/erosion[25]. The increase in gel strength and/ or molecular interaction between diltiazem and polymers also appeared to be one of the factors involved in the retarded release of the drug by the addition of mucoadhesive polymers. According to the release data, it was thus possible to modulate the release of diltiazem by adjusting the concentration of the polymer to obtain a sustained drug release profile for 8h. It could be concluded that, the addition of different mucoadhesive polymers in different concentrations retarded the release of diltiazem HCl from liquid suppositories. This mainly depended on the viscosity of the polymer added and its concentration.

Analysis of drug release data

The kinetic parameters are shown in Table 4. It is evident from the table that the drug release process is not zero-order in nature. This indicates that the dissolution rate of the drug is independent of the amount of drug available for dissolution and diffusion from the matrix (Fig. 3). The dissolution data of all formulations when fitted in accordance with the first-order equation it is evident that a linear relationship was obtained with 'r' (correlation coefficient) value

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close to unity and higher than 'r' obtained from the zero-order equation for all formulations, showing that the release is an apparent first-order process. This indicates that the amount of drug released is dependent on the matrix drug load with formulation F0 best fitting according to the first order kinetics (Fig. 4). As concentration reduces on drug release, the diffusional path increases resulting in drug release at a comparatively slower rate in the later phase, thus fitting into Higuchi's kinetics. All the formulations in this investigation could be best expressed by Higuchi's classical diffusion equation, as the plots showed high linearity (r: 0.9705 to 0.9983). The linearity of the plots indicates that the release process is diffusion-controlled. To confirm the diffusion mechanism, the data were fit into Korsmeyer-Peppas model. All the formulations showed high linearity (r: 0.9795 to 0.9992), with slope (n) values ranging from 0.5059 to 0.7500. The 'n' values for all formulations (except F3B) were greater than 0.5 indicating an anomalous or non-fickian release suggesting a coupled erosion – diffusion mechanism. Formulation F3B(HPMC K4M, 1.0%), showed 'n' value close to 0.50 indicating that diltiazem HCl might be released by Fickian diffusion (Higuchi model) through extracellular aqueous channel of the gel matrix.. This was in agreement with the results reported by Suh and Jun[40] who studied the release profile of naproxen from PF-127 gels. The release of diltiazem HCl was found to be through extra-micellar aqueous channels of the gel matrix which means the outer layer of poloxamer cross-linking system (poloxamer micelle)[41].

Table No. 4

Kinetic values obtained from different plots of the liquid suppository formulations

Code	Zero order plots	First order plots	Higuchi's plots	Korsmeyer –Peppas plot	
	r^a	r^a	r^a	n^b	r^a
F0	0.9120	0.9949	0.9919	0.5931	0.9974
F1A	0.9483	0.9930	0.9925	0.6083	0.9988
F1B	0.9762	0.9951	0.9784	0.6882	0.9965
F2A	0.9628	0.9824	0.9778	0.7180	0.9988
F2B	0.9780	0.9826	0.9705	0.7906	0.9991
F3A	0.9024	0.9853	0.9992	0.5235	0.9985
F3B	0.8769	0.9905	0.9989	0.5059	0.9976
F4A	0.9796	0.9945	0.9716	0.7500	0.9948
F4B	0.9711	0.9978	0.9811	0.7196	0.9984
F5A	0.9131	0.9808	0.9983	0.5442	0.9992
F5B	0.9859	0.9914	0.9869	0.6438	0.9795

F0 to F5B represents the various formulations of Diltiazem HCl

[a]*Correlation coefficient*

[b]*The diffusional exponent is based on Korsmeyer-Peppas equation,* $M_t/M_\infty = kt^n$.

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Statistical analysis

Statistical analysis (ANOVA) revealed that the five different polymers and their two different concentrations (0.5% and 1.0%) used in the present study had a significant ($p < 0.05$) effect on the *in vitro* drug release, bioadhesive strength and gel strength.

Stability study

Formualtions containing 1% of mucoadhesive polymers were subjected to stability studies at 2 – 8°. The pH and drug content results indicated that there was no significant change in the diltiazem HCl liquid suppository formulations after 30 days when compared with the initial values. There was slight decrease (0.5 – 1.5°) in the gelation temperature of the formulation studied. In the rectal drug delivery through a liquid suppository formulation, gel strength and mucoadhesive force is also required to retain the formulation at the point of application. The results indicated that the formulations did not show any major changes in gel strength and mucoadhesive force when stored under cool condition (2 to 8°). The gel strength and mucoadhesive force studies were done after pre-warming the formulation at 37°. Thus above results indicated that refrigeration condition (2 to 8°) was suitable for the storage of the liquid suppository formulations (Table 5).

Table No. 5.

Stability study data of formulations containing 1.0% of mucoadhesive polymers at refrigeration condition (2 – 8°)

Code	*Gelation temperature (°) ±S.D	*Gel strength (s) ± S.D	*Mucoadhesive force (dynes/cm^2*10^2) ±S.D	pH	*%Drug Content ±S.D
F1B	27.5±1.21	123.0±2.4	59.27±3.0	6.30	98.21±1.12
F2B	31.0±0.29	52.67±1.03	42.09±1.53	6.23	97.67±0.92
F3B	31.0±1.10	77.0±1.35	26.92±1.39	6.86	99.75±0.85
F4B	27.20±1.46	101.47±2.90	49.63±1.15	6.54	98.10±1.23
F5B	33.50±1.52	31.0±0.47	12.01±2.4	6.16	98.73±1.62

F1B to F5B represents liquid suppository formulations of diltiazem HCl containing 1.0% of mucoadhesive polymer

**Denotes average of three determinations*

CONCLUSION

The designed formulation exhibited excellent physiochemical properties, dissolution profile and performance efficacy with adequate drug stability. Overall formulation F3B (1.0 % HPMC) showed satisfactory results for the parameters evaluated and produced a drug release of 80% at the end of 8 h. This work needs to be proved effective by its bioavailability, preclinical

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and clinical studies thus providing platform for further development and optimization.

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