Formulation and Evaluation of Diazepam as a Rectal Preparation

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Abstract

Diazepam has become a commonly used drug for treatment of acute repetitive epileptic seizures and febrile convulsions in children. Considering the advantages of rectal administration of diazepam, the objective of this study was to formulate and evaluate rectal hydrogels containing diazepam as a drug substance in combination with suitable co – solvents and preservatives.Prepared HPMC (hydroxypropyl methylcellulose) hydrogels containing different concentrations of diazepam (2, 4 and 6 mg / ml) manifested good quality in respect to physicochemical parameters (pH value, drug content, ingredients content and viscosity), antimicrobial efficiency and microbiological quality. Under the proposed HPLC conditions, satisfactory separation of diazepam and the preservatives used was achieved. *In vitro* release studies have shown that the total amount of diazepam was released in a period of 3 hours. Prepared formulations were stable for four months at $26°C$ (ambient temperature characteristic of the second climate zone).

دراسة و تقييم الصيغة التركيبية للديازيبام كمستحضر يعطى عن طريق المستقيم

علي أحمد ياس

المستخلص

الديازيبام أصبح العقار الشائع لعلاج نوبات الصرع الحادة المتكررة و التشنجات عند ألاطفال. مع ألاخذ بالاعتبار الفائدة من أعطاء الديازيبام عن طريق المستقيم، الهدف من هذه الدراسة هو تحضير و تقييم محاليل غروانية مائية تعطى موضعيا عن طريق المستقيم تحوي على الديازيبام كمادة فعالة مع مذيبات مختلطة مناسبة ومواد حافظة.تحضير المحاليل الغروانية المائية من هايدروكسي بروبيل مثيل سيليلوز والتي تحوي تراكيز مختلفة من الديازيبام (٢، ٤ و ٦ ملغم \ مل) ذات نوعية جيدة بالنسبة للعوامل الكيميفيزيائية (قيمة ألاس الهيدروجيني، تركيز الديازيبام و اللزوجة)، كفاءة المواد الحافظة و تأثيرها على ألاحياء المجهرية. تحت عوامل التحليل السائل الكروماتوغرافي ذو الكفاءة العالية و المقترحة، تم الحصول على فصل جيد للديازيبام و المواد الحافظة. أظهرت دراسات التحرر المخبرية ان الكمية الكلية للديازيبام تم تحررها خلال ثلاث ساعات. الصيغ التي تم تحضيرها أظهرت ثباتية لمدة اربعة أشهر في درجة حرارة ٢٦° سليزية.

Introduction

Treatment of acute seizures in children requires prompt medical attention, ventilation support and appropriate oxygenation until they either stop spontaneously or are controlled by drugs. Benzodiazepines are considered to be the treatment of choice for acute management of severe seizures. Benzodiazepines are active against a wide range of seizure types, have a rapid onset of action once delivered into the central nervous system, and are safe [1, 2]. Diazepam is a long – acting benzodiazepine with anticonvulsant, anxiolytic, sedative, muscle relaxant and amnesic properties and it is the most widely used drug for treatment of insomnia, febrile convulsions, status epilepticus and alcohol withdrawal symptoms [3]. However, it has a short duration of action, and should be given intravenously or rectally (since its absorption is slow if given intramuscularly). Oral and sublingual administration is frequently difficult, impossible, or hazardous when the patient is actively having a seizure or is in the postictal state. Also, absorption after oral administration of tablets is usually slower than after parenteral or rectal administration. Introduction of an intravenous line may also be difficult, particularly in children with generalized tonic – clonic febrile seizures [4, 5]. Rectal administration of diazepam is now widely employed and could be as effective as the intravenous route [6]. Also, nonmedical personnel, irrespective of the patient's ability to cooperate, can administer rectal preparations easily and safely [7, 8]. Rectal diazepam solutions have many characteristics sought in the ideal drug to treat acute repetitive seizures [9]. Their high lipid solubility permits both prompt absorption and rapid penetration into the central nervous system. In children, the peak serum plasma concentration was reached within 3 to 30 minutes, with bioavailability averaging between 80 and 100% [10]. In contrast, diazepam suppositories have slow, erratic absorption, which limits their use in the management of acute seizures [11]. In general, dosage forms designed for rectal administration should not cause irritation, should show good retention in the lower region of the large intestine, and should be suitable enough to be accepted by the patient. These requirements can be met by using hydrogels, having in mind that rectal solutions tend to leak out of the rectum, leading to inaccurate dosing and treatment failure [12]. A hydrogel is a three – dimensional network of hydrophilic polymer chains that could be cross – linked through either chemical or physical bonding. Because of the hydrophilic nature of polymer chains, hydrogels are capable of swelling when placed in aqueous media, i.e., they retain a significant amount of water but remain water insoluble [13, 14]. When a drug substance is loaded into such a hydrogel, the diffusion rate of the drug depends on the physical structure of the polymer network and its chemical nature. If the hydrogel is highly hydrated, diffusion occurs through the pores within the gel network. In gels of lower hydration, the drug dissolves in the polymer and is transported between the chains. When the polymers are cross – linked, the hydrophobicity of a gel is increased and the diffusion rate of the drug is diminished [15]. These characteristics of hydrogels, as well as their biocompatibility, increased duration of action with increased therapeutical efficiency due to the viscosity of the gel matrix and soft consistency (easy and safe administration at home by nonmedical persons) could be considered a potentially beneficial approach in the formulation of rectal forms of diazepam. Considering all the above mentioned facts, diazepam has been formulated as a rectal hydrogel using a contemporary approach in designing hydrogel preparations.

Experimental

Materials

Diazepam [FIS – SpA (Italy)]. The gelling agent hydroxypropyl methylcellulose (HPMC – E5) [Hercules (Methocel E5, USA)]. The antimicrobial preservatives (benzyl alcohol, benzoic acid and sodium benzoate), ethanol and propylene glycol were supplied by [SDI]. All other materials were of analytical grade.

Methods

Preparation of diazepam rectal gels – Rectal hydrogels containing different concentrations

of diazepam: 2 mg / ml (sample HG1), 4 mg / ml (sample HG2) and 6 mg / ml (sample HG3) were prepared by incorporation of the diazepam solution into a structured vehicle of HPMC – E5 (15%, *m / V*).The basic solvent in the formulation was deionized water but the solubility of diazepam in water is 0.05 mg / ml. For this reason, the mixture of co – solvents (ethanol / propylene glycol 1:3, *V / V*) was used in concentrations allowed for parenteral solutions [16] (Table I).In short, the antimicrobial preservatives (benzyl alcohol, benzoic acid and sodium benzoate, the combination of preservatives commonly used into commercial rectal solutions of diazepam – Stesolit®, Valium®, Desitin®) were dissolved in co – solvents present in the formulation according to their solubility, *i.e.*, benzyl alcohol were dissolved in ethanol and then the benzoic acid was added. Sodium benzoate was dissolved in deionized water. The drug substance was dissolved in the prepared mixture of co – solvents and antimicrobial preservatives. Afterwards, it was incorporated into the structured vehicle of HPMC – E5 by gentle mechanical stirring (25 rpm, 2 min), so the final concentration of diazepam in hydrogels was 2, 4, and 6 mg / ml respectively. The gel base, the structured vehicle of HPMC – E5, was prepared by the >> hot / cold << technique [17].Prepared formulations were packed into 2.5 – ml polyethylene containers provided with a suitable applicator for rectal administration and were stored in the stability chamber(Kottermann 2391, Kottermann, Germany) for 4 months at 26°C protected from light. ***Evaluation of the formulations***.pH value was determined according to the method proposed by ***European Pharmacopoeia*** (pH meter Jenway 3310, UK) [18].***Drug content in rectal hydrogels*** – The diazepam and preservatives content was determined using the reverse – phase HPLC technique by the external standard method [19]. Analyses were performed on a Waters HPLC system, equipped with Waters 660 E pump, sample injector Rheodyne 7725i with 20 – ml loop and Waters 996 photodiode array detector. The column used was LiChrospher® 60, RP Selected B, 125 X 4 mm i.d., 5 - μm particles

(Merck, Germany).The chromatographic conditions were: mobile phase – methanol and phosphate buffer pH 2.5 in a ratio 44:56 (*V / V*), flow rate – 1 ml / min and UV detection at 254 nm.Prepared diazepam rectal hydrogel (1 ml) was transferred to a volumetric flask of 20 ml, 10 ml of methanol was added, and the flask was placed in the ultrasonic bath for 30 min. After cooling at ambient temperature, the flask was filled with methanol to the volume. The sample was centrifuged on 3000 rpm for 15 min. Clear centrifuge (5 ml) was transferred to a 10 – ml volumetric flask and filled with mobile phase to the volume. After filtration through 0.45 - μm membrane filter, an aliquot of sample solution was injected into the HPLC system. Empty hydrogel – blank solution (without diazepam and preservatives) was also prepared and analyzed by the same procedure.The content of other ingredients, such as benzyl alcohol, benzoic acid / sodium benzoate were determined under the same chromatographic conditions.***Rheological studies*** – Viscosity determinations of the prepared formulations were carried out using a rotational viscometer with coaxial cylinders (Haake RV3, Sensor system MVI, Haake, Germany). Viscosity of the gels was measured at different angular velocities at a temperature of $23 \pm 1°C$ and then the temperature was raised to $37 \pm 1°C$. A typical run comprised changing of the angular velocity from 0.5 to 110 rpm at a controlled ramp speed. The hierarchy of angular velocity was reversed. The average of three readings was used to calculate the viscosity. Flow behavior was analyzed by regression analysis of the log – log plots of shear stress *vs.* shear rate.***In vitro release studies*** – A known quantity of the prepared rectal hydrogel (1 g) was poured in a glass tube in the presence of phosphate buffer pH 7.4 (10 ml). ***In vivo*** conditions were simulated in a horizontal shaker bath ($37 \pm 1°C$, 75 strikes / min; Haake SWB 20, Haake, Germany). Aliquots were taken at regular time intervals and were replaced by an equal volume of prewarmed phosphate buffer. Withdrawn aliquots were analyzed spectrophotometrically at 316 nm (Lambda 16, Perkin Elmer, USA). All experiments were carried out in triplicate and

average values are presented.*Antimicrobial preservation and microbiological quality of hydrogels* – The efficacy of antimicrobial preservation by preservatives was assessed according to the method given in *European Pharmacopoeia* [18]. Test microorganisms were *Candida albicans* (NCPF 3179), *Pseudomonas aeruginosa* (CIP 82.118) and *Escherichia coli* (CIP 53.126). Ten ml of the preparation were inoculated with 0.1 ml bacterial suspension and the number of microorganisms was counted in a blood – agar medium over a period of 7, 14 and 28 days. The following log values are adequate for the microorganisms of interest according to the *European Pharmacopoeia* [18], criteria B for topical preparations: 3 for bacteria and 1 for fungus during 14 days without growth increase for 28 days.The microbiological quality of the prepared rectal hydrogels was tested according to the method stated in *European Pharmacopoeia* (category 3) [18]. Sampling plan was carried out using the procedure for microbiological examination of non – sterile (water – soluble) products. The total count of viable microorganisms (*Candida albicans* and *Escherichia coli*) was determined by the plate – count method.*Stability of diazepam rectal hydrogels* – Stability was evaluated by maintaining the formulations at a temperature of $2 - 8°C$ and $37 \pm 0.5°C$ over a period of 1 month. The preparations were maintained over a period of 4 months at a temperature of $26 \pm 0.5°C$, ambient temperature characteristic of the second climate zone [20]. Physicochemical parameters such as pH value, drug and ingredients content, viscosity and microbiological quality were determined periodically after the 1st, 2nd, 3rd and 4th month after hydrogels preparation.

Results and discussion

Evaluation of diazepam rectal hydrogels

The prepared diazepam rectal hydrogel formulations were clear, light – yellowish homogenous gels. The physico – chemical parameters of freshly prepared formulations are presented in (Table II). Concerning the pH value, viscosity and preservatives content, no significant changes between the prepared hydrogels (HG1, HG2 and HG3) were observed.*Drug content in rectal hydrogels* – Although many HPLC methods have been developed for quantitative determination of diazepam in various materials and dosage forms, such as blood, plasma, urine, tablets and injections [21 – 24], no HPLC method for determination of diazepam in HPMC hydrogels has been reported in literature.From the chromatograms of standard solution, blank solution (empty hydrogel solution) and sample solution (for formulation HG1) presented in (Figure 1), it is evident that under the proposed chromatographic conditions complete separation of diazepam, benzyl alcohol and benzoic acid / sodium benzoate was obtained. Retention times were 2.16, 2.66 and 8.30 min for benzyl alcohol, benzoic acid / sodium benzoate and diazepam, respectively.The obtained values of capacity factor k' ($1 < k' < 10$), selectively factor α ($\alpha > 1$) and resolution factor Rs ($Rs > 2$) showed that the proposed chromatographic conditions are suitable for quantitative separation and determination of the analyzed components. Further, in the chromatogram of blank solution there are no interfering peaks at the retention times of investigated peaks, which indicate that the method is selective and could be used for identification and simultaneous quantification of diazepam, benzyl alcohol and benzoic acid / sodium benzoate.Validation data of the HPLC method demonstrated that the proposed method was selective, linear, precise and accurate [19]. Calibration curves fitted for diazepam, benzyl alcohol, benzoic acid / sodium benzoate gave correlation coefficients above 0.9996. The relative standard deviation (RSD) for inter – day analysis was 0.9, 0.4 and 1.7% for diazepam, benzyl alcohol and benzoic acid / sodium benzoate, respectively. The accuracy of the method was confirmed by the standard addition method and the obtained analytical recoveries were 99.6 – 100.9% for diazepam, 98.6 – 100.7% for benzyl alcohol, and 98.4 – 99.5% for benzoic acid / sodium benzoate.*Rheological studies* – Viscosity of rectal gels is an important factor, which affects the rate of drug release, distribution of the gel in the distal portion of the large

intestine as well as its retention time. Literature data suggest that the viscosity of rectal gels should be in a range of 1 – 2 Pa s [25].The prepared formulations exhibited pseudoplastic rheology, as evidenced by shear thinning and an increase in the shear stress with increased angular velocity. The gel structure is composed of random oriented molecules in the form of long chains. Dynamic viscosity of the prepared formulations was 2 Pa s. No change in the viscosity was observed by measuring the samples at different temperatures ($23°C$ and $37°C$). These results were important for the formulation stability in terms of observing the changes in the rheological behavior of the formulations during the stress conditions and to determine the rheological status of the prepared delivery systems after application in the rectum. In (Figure 2 and 3) flow curves and viscosity curves for sample HG3, measured at temperature of $37°C$, are presented.*In vitro drug release studies* – Profiles of drug release from rectal hydrogel formulations are presented in (Figure 4). Diazepam was fully released from the prepared formulations within a period of 3 hours. As can be seen, formulation HG3 initially showed a faster release rate ($\approx 50\%$ in the first half hour) than formulations HG2 and HG1 ($\approx 50\%$ after the first hour). The main reason for the observed effect might be the higher concentration gradient being responsible for a more efficient diffusion of the drug molecules through the hydrogel phase, while all other conditions were the same. Hence, changing the drug loading offers a real possibility of controlling the drug release [13].To study the drug release mechanism, the release data were fitted to the general exponential function: $M_t / M_0 = kt^n$ where M_0 is the initial amount of diazepam (amount of diazepam released at time zero) and M_t is the amount of diazepam released at time t, n is a diffusion exponent characteristic of the release mechanism, and k denotes the properties of the polymer and the drug [15]. According to *Brazel* and *Peppas* [25], this equation has been frequently used in the literature owing to its utility in describing the relative importance of **Fickian** ($n = 0.5$) and **non – Fickian**, anomalous diffusion ($n \leq$

1.0). If the exponent n is around 0.5, the drug release is represented by a square root equation; if $n = 1$, the fractional release is of zero – order. Values of n greater than 0.5 indicate anomalous diffusion, generally due to the swelling of the system in the solvent before the release take place.Exponential dependence of the amount of diazepam released on time was evidenced; the diffusion exponents were o.5, 0.55 and 0.47 for formulations HG1, HG2 and HG3, respectively, confirming that the mechanism of drug release from HPMC hydrogels involves diffusion through the elastic gel matrix. Good linear relationship was obtained between the cumulative percentage of released diazepam and the square root of time [26]. Drug release constants k and correlation coefficients $\mathbf{R}$ for the mathematical model of diffusion are presented in (Table III).

Preservatives efficacy and microbiological analysis – Considering the diazepam rectal gel formulations, the success criterion of the preservatives used (combination of sodium benzoate, benzyl alcohol and benzoic acid) was satisfied [18]. Regarding the microbiological quality, the prepared formulations complied with the standards proposed by the *European Pharmacopoeia* [18].*Stability* – After 4 months of storage at 26 $\pm$ $0.5°C$, no significant changes in the physicochemical properties of the prepared formulations were observed (for pH value of the gels F was 0.65, for diazepam content F was 2.67 and for preservatives content F was 5.31; F *crit.* $= 7.70$, $p < 0.05$).By maintaining the preparations at a temperature of $37°C$, after 1 month storage, significant changes in diazepam content and viscosity (Figure 2, 3) were observed ($p < 0.05$). These results are in agreement with the earlier findings, that in storage conditions above $30°C$, the commercially available diazepam rectal gel (Diastat®) was stable for 30 to 60 days [27]. When storing the formulations at a temperature of $2 – 8°C$, the physical status of the formulations changed (the drug substance precipitated) after one week. These findings point to the necessity of further investigations to obtain formulations of better stability for a broaden application, especially in regions where the temperature deviates from the

mean temperature characteristic of the second climate zone.Prepared diazepam delivery systems should be stored at room temperature of 15 – 30°C, protected from light and moisture, and should be used within a period of 4 months.

Conclusions

The HPMC hydrogels containing various concentrations of diazepam intended for rectal administration have been prepared and evaluated. It can be summarized that the prepared formulations showed satisfactory solubility of diazepam, adequate rheological characteristic, release behavior and microbiological quality. Under the conditions characteristic of the second climate zone, the dosage forms were stable for a period of 4 months. Further investigations will be focused on modification for the purpose of achieving better stability of the diazepam rectal delivery system.

Table I. Diazepam hydrogel preparation

Drug	Hydrogel code	Concentration (mg / ml)
Diazepam	HG1	2
	HG2	4
	HG3	6
Other excipients		(%, m / V)
Propylene glycol		30.0
Ethanol		10.0
HPMC – E5		15.0
Sodium benzoate		0.2
Benzyl alcohol		1.5
Benzoic acid		0.1
Deionized water		43.0

Table II. Physicochemical parameters of freshly prepared diazepam rectal hydrogels[a]

Hydrogel	pH[b]	Viscosity (Pa s)[b]	Diazepam (%)[c]	Preservative (%)[c]
HG1	7.16 ± 0.04	2.10 ± 0.05	110.50 ± 0.06	99.90 ± 0.06
HG2	7.12 ± 0.06	2.10 ± 0.07	106.80 ± 0.05	99.30 ± 0.08
HG3	7.21 ± 0.02	2.05 ± 0.09	103.83 ± 0.08	99.80 ± 0.05

[a] Mean $\pm$ SD.
[b] $n = 3$.
[c] $n = 5$.

Table III. Kinetic parameters for diazepam hydrogel formulations ($n = 3$)

Hydrogel	$n \pm SD$[a]	Higuchi diffusion model	
		R	$k \pm SD$ (mg / h$^{1/2}$)
HG1	0.50 ± 0.07	0.990	1.34 ± 0.20
HG2	0.55 ± 0.04	0.997	2.67 ± 0.12
HG3	0.47 ± 0.05	0.990	2.90 ± 0.19

[a]The release exponent n obtained from equation $M_t / M_0 = kt^n$.

a)

b)

c)
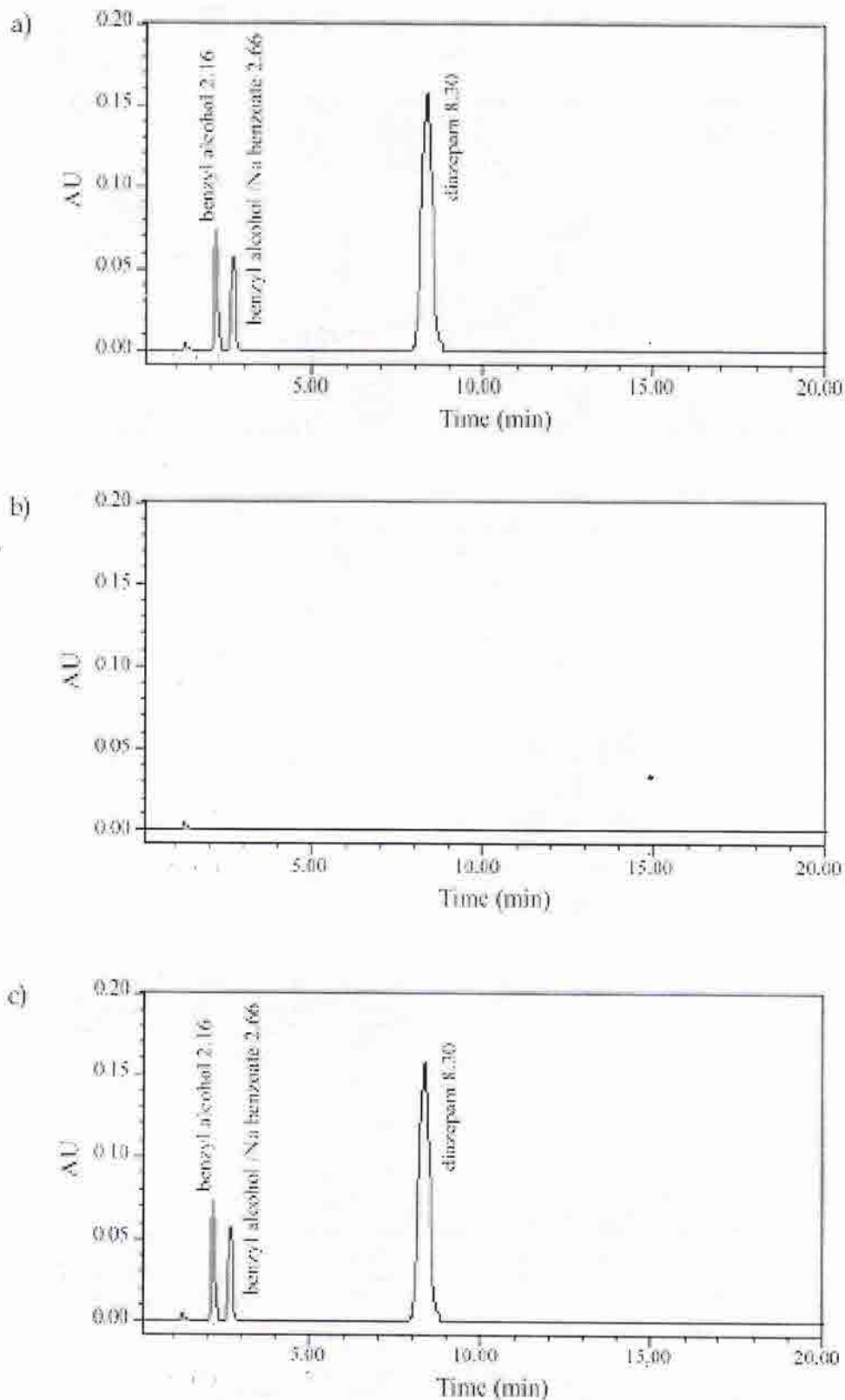

Fig. 1. Chromatograms of: a) standard solution of DZP (0.05 mg mL⁻¹), benzyl alcohol (0.375 µg mL⁻¹), benzoic acid (0.025 µg mL⁻¹) and sodium benzoate (0.05 µg mL⁻¹); b) blank solution without DZP, benzyl alcohol, benzoic acid and sodium benzoate, and c) sample solution of DZP (0.05 mg mL⁻¹), benzyl alcohol (0.375 µg mL⁻¹), benzoic acid (0.025 µg mL⁻¹) and sodium benzoate (0.05 µg mL⁻¹).
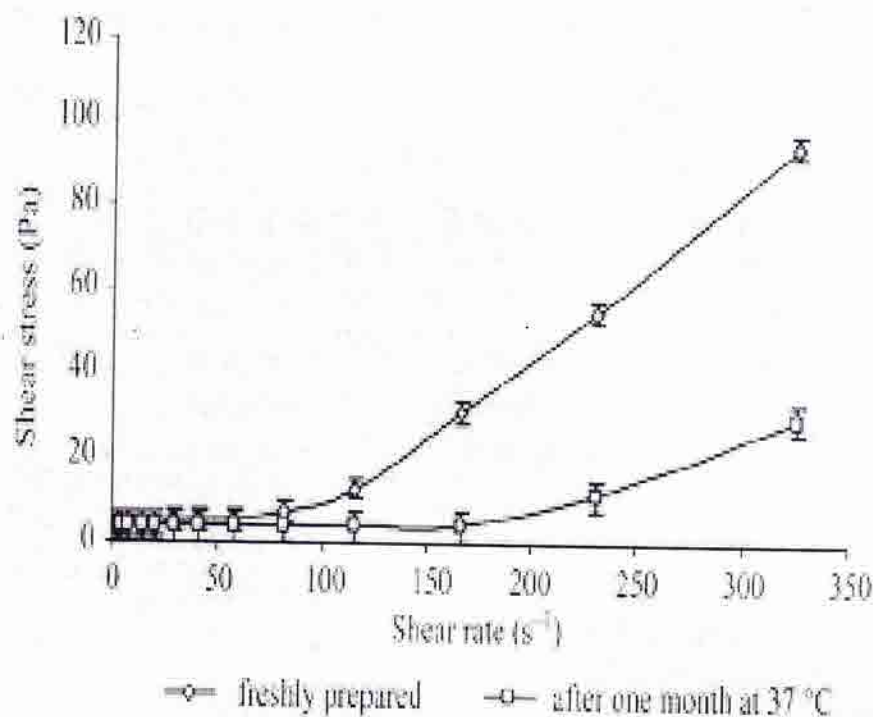
Fig. 2. Comparative flow curves for sample HG3, freshly prepared and after 1 month storage at 37 °C (mean ± SD, $n = 3$).
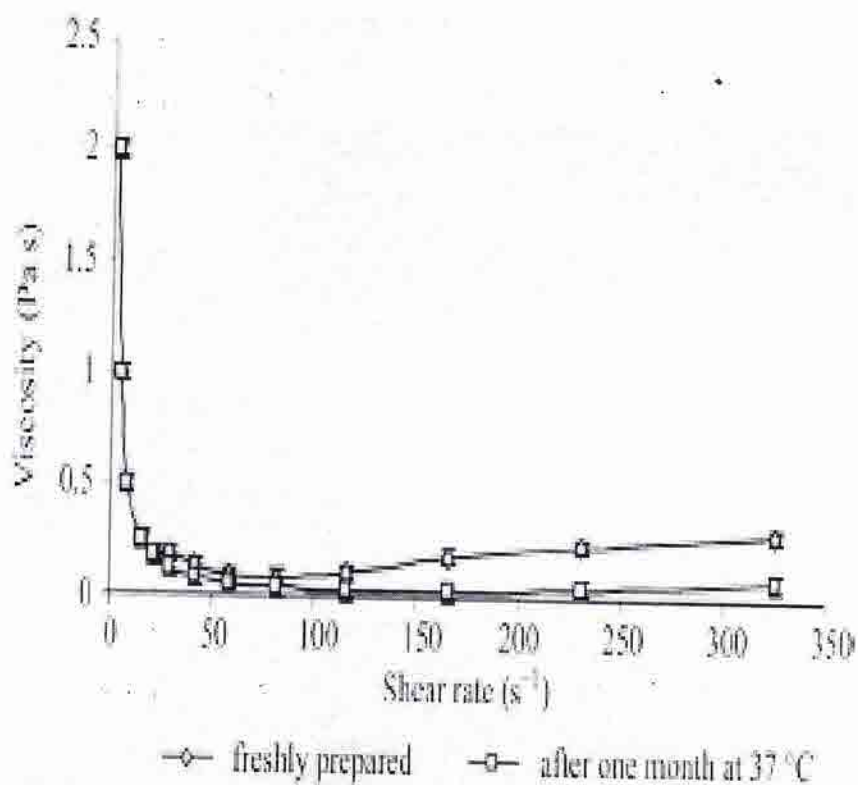

Fig. 3. Comparative viscosity curves for sample HG3, freshly prepared and after 1 month storage at 37 °C (mean ± SD, $n = 3$).
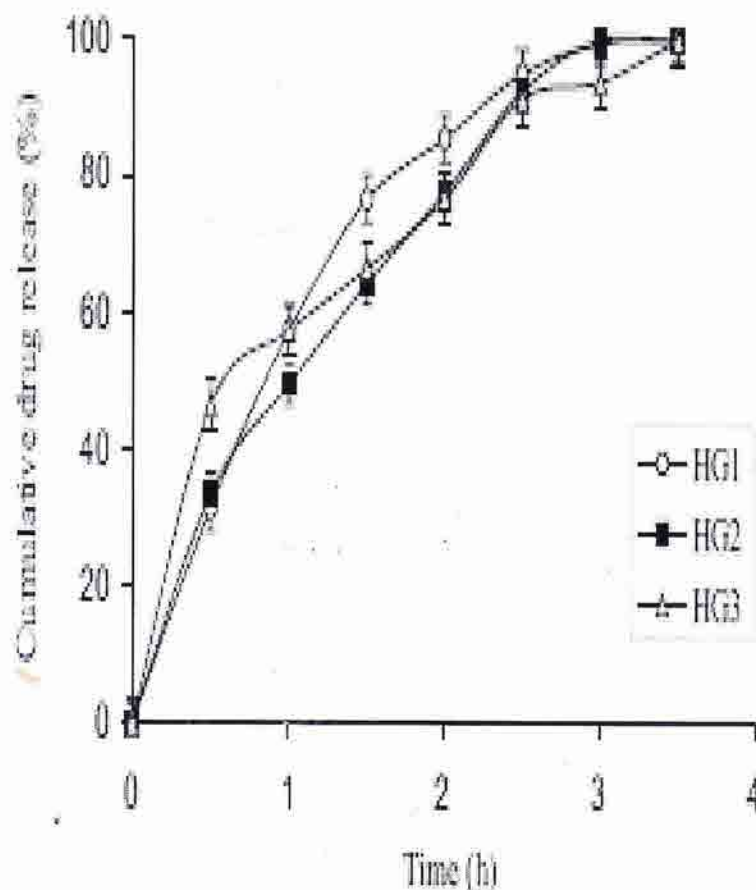
Fig. 4. Release profiles of DZP from rectal hydrogels HG1, HG2 and HG3; each point represent the mean ± SD, n = 3.

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