

PREPARATION OF DIAZEPAM RECTAL GEL USING CELLULOSE POLYMERS

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Abstract

Diazepam is a long-acting benzodiazepine with anticonvulsant, anxiolytic, sedative muscle relaxant properties and it is the most widely used drug for treatment of insomnia, convulsions, and status epilepticus. Considering the advantages of rectal administration of diazepam, the objective of our study was to formulate and evaluate rectal hydrogels containing diazepam as a drug substance in combination with suitable co-solvents and preservatives. Hydroxypropylmethyl cellulose (HPMC) gels containing 5 mg diazepam in a 2.5 mL rectal gel, manifested good quality in respect to physico-chemical parameters (pH value, drug content and viscosity). Three types of formulation containing benzoate buffer, phosphate buffer and without buffer were prepared and evaluated. Different physico-chemical parameters were investigated. Diazepam content in different formulations was in the range of 96 to 103 % which had less than 5% error. pH determination from all formulations did not change significantly during 60 days of preparation. Prepared gels showed good antimicrobial efficiency. Viscosity determination showed that viscosity of formulation No. 3 (without buffer) was highest among all formulations. Formulation containing water (without buffer) was the best formulation, because its shelf-life was 458 days at refrigerator condition and its pH was 6.5 which is in the acceptable range.

Keywords:

Diazepam, Rectal gel, HPMC, Seizure.

Introduction

Epilepsy is among the most common neurologic disorders, affecting approximately two million people in the USA (1). Seizures in children usually cease spontaneously within 5-10 min and are rarely associated with significant sequelae. Convulsive seizures lasting longer than 30 min constitute status epilepticus and may be complicated by cardiorespiratory depression and brain injury (2).

Benzodiazepines are the treatment of choice for management of acute seizures. Diazepam is a long-acting benzodiazepine

with anticonvulsant, anxiolytic, sedative, muscle relaxant, and amnesic properties. It is active against many types of seizures, has a rapid onset of action and is safe (3). Its actions are mediated by enhancement of the activity of aminobutyric acid (GABA), a major inhibitory neurotransmitter in the brain (4). It is used in the treatment of severe disabling anxiety disorders, as a hypnotic in the short-term management of insomnia, as a sedative and premedicant, as an anticonvulsant particularly in the management of status epilepticus.

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epiticus and febrile convulsions, in the control of muscle spasm as in tetanus, and in the management of alcohol withdrawal symptoms (5).

Diazepam is administered orally, rectally, and parenterally with the risk of dependence very much influencing the dose and duration of treatment (4).

When status epilepticus occurs outside the hospital or when IV access is not possible, alternative routes for drug administration must be used. It has been very difficult to obtain IV access, because of muscular contractures and atrophy, seizure movements and obesity. Oral administration is appropriate for many indications. Rectal administration may be by suppository or rectal solution; the rectal solution may have better absorption characteristics. Its lipid solubility permits both prompt absorption and rapid penetration into the central nervous system (6). Diazepam, given intravenously, is the drug of choice for the emergency treatment of convulsive status epilepticus (4). In some cases, intravenous injection of diazepam, especially in children with seizures, is not ideal since rapid treatment which necessitate the presence of professional to administer the medicine is required, therefore, rectal administration of diazepam is the suitable substitute for intravenous route. The aim of the present work was to formulate an effective diazepam rectal gel, in combination with suitable co-solvents and preservatives, which can be administered rectally in emergency situation, instead of diazepam injection.

Materials and methods

Instruments

A Jasco UV-VIS spectrophotometer (Japan); Sibata melting point apparatus (Japan); Brookfield viscometer (USA) and other apparatus such as pH-meter, shaker, balance, were used in this research.

Materials

Diazepam (Fabbrica-Italy) was obtained as a donation from Abidi Pharmaceutical, Iran; hydroxypropylmethyl cellulose (Methocel K100 M) was obtained as a gift from Colorcon Ltd. (U.K.); benzoic acid, sodium benzoate, propylene glycol, culture media (Nutrient agar, Nutrient broth, saboraud 2% dextrose broth and saborud 4% dextrose agar), Merck, (Germany) were all of analytical grade.

Methods

Identification of diazepam powder:

Three different methods were used to identify diazepam powder.

A: The IR spectra (7):

A small amount of diazepam powder was mixed with liquid paraffin and scanned over the range from 3800 to 650 cm^{-1} .

B: Determination of melting point (8)

C: UV-VIS scan (8).

Assay

The purity of diazepam was calculated taking 151 as the value of A (1%, 1cm) at the maximum of 368 nm. (8).

Preparation of base gel

Gels (25 g portions) were prepared by heating two thirds of the total amount of freshly prepared distilled water to 80 °C and then adding the required amount of polymer (HPMC) to disperse. Cold water was then added to make the gels up to weight. The gels were triturated to a uniform consistency and left overnight to equilibrate.

Diazepam gel formulation

Three different rectal formulations containing diazepam were made and evaluated. The solubility of diazepam in water is 0.05 mg/mL (4), therefore, mixture of co-solvents (ethanol/propylene glycol) was used to enhance diazepam solubility. Table 1 shows the materials used in the formulations. Formulation 1 contained benzoate buffer, formulation 2 contained phosphate buffer and third formulation had

no buffer. Each formulation contained 5 mg diazepam as active ingredient in a 2.5 mL dose of rectal gel. Required amount of diazepam powder was mixed with solvents (benzoate buffer, phosphate buffer or distilled water), then, propylene glycol (as co-solvent) was added and thoroughly mixed (9). After addition of preservative (benzyl alcohol 2% V/V), the required amount of gel base was added to make the gels up to weight and was mixed. The prepared formulations were packed into 3 mL syringe without needle for rectal administration and were stored at refrigerator.

Evaluation of the formulations

Drug Content in rectal gels

2.5 mL samples taken from each formulation were dissolved in HCl 0.1 N., separately. Each solution was poured into a 25 mL volumetric flask and then brought to volume with HCl 0.1 N. One mL of each solution was diluted to 50 mL with HCl 0.1 N. The amount of diazepam in each sample was determined by spectrophotometry at 240 nm against the blank solution (exactly the same formulation without active ingredient).

pH determination

Some drugs may be quite stable in the pure form, but may undergo degradation rapidly when combined with certain excipients. Excipients affect the stability of drugs by acting as surface catalysts, altering the pH of the moisture layer and/or undergoing

direct chemical reactions with the drug. The normal pH of rectum is 7 to 8 and diazepam is stable in the pH range of 6.2 to 6.9.

If the pH of rectal preparation, falls outside a fairly narrow range, the rate and extent of absorption may be affected by changes in the dissolution rates of drug or it may result in altered bioavailability, or patient discomfort. Since pH has a pronounced effect on the rate of degradation of the active ingredients (9), pH of prepared formulations during 60 days after preparation was investigated. For determination of pH, first, pH meter was calibrated by standard pH solutions of 4 and 9. Then, samples of diazepam rectal gels were examined at room temperature at day 1, 15, 30 and 60 following preparation. The mean of three pH determinations was used for each formulation.

Viscosity determination

Viscosity determinations of the prepared formulations were carried out using Brookfield viscometer. Viscosity of the gels (150 mL portions) was measured using spindle # 3RV/H at rotational speed of 10 to 100 rpm, and a temperature of 23 ± 1 °C. Gels were kept still for 5 min before the first viscosity test and also 3 min after each test, to reform their structure. The viscosity of gel formulations was determined at 72h following preparation and at day 15, 30 and 60. The average of three readings was used to calculate the viscosity.

Table 1: Materials used in the diazepam gel formulations

Name of used materials	application	%
Diazepam	Active agent	0.2 W/V
Ethanol	Solvent	10 V/V
Propylene glycol	Co-solvent	50 V/V
Buffer solution	Buffering agent	5 V/V
- Benzoate buffer (formulation 1)		
- Phosphate buffer (formulation 2)		
- Water (formulation 3)		
Benzyl alcohol	Preservative	2 V/V
HPMC gel (6%w/w)	Base gel	To volume

Antimicrobial preservative effectiveness

The antimicrobial preservative capacity of the formulations was conducted according to the standard procedure outlined in the United States Pharmacopoeia (USP 25 / NF 20) (10). Individual 20 mL portions of the formulations were inoculated each with 0.1 mL of bacterial suspension containing 10^8 cfu/mL of the following standard microorganisms:

- *Pseudomonas aeruginosa* (ATCC No. 9027),
- *Staphylococcus aureus* (ATCC No. 6538),
- *Escherichia coli* (ATCC No. 8739),
- *Candida albicans* (ATCC No.10231)and
- *Aspergillus niger* (ATCC No.16404).

Suspensions were incubated at $22.5 \pm 2.5^\circ\text{C}$ for 28 days. At specified intervals (7, 14, 21 and 28 days), samples were examined for levels of growth by the pour plate method (10).

Stability testing

Physical stability

Physical stability, the difference of pH in different preparation, at day 1, 15, 30 and 60 after preparations were investigated. Also, the difference in viscosity of products after above mentioned days were investigated. Multiple samples of 3 formulations were placed in the incubator at various temperatures of 25, 35, 45, 60°C and in the refrigerator at 8°C for a period of 2 months. Following this period, gels were evaluated for physical stability (uniformity, color and crystallization).

Chemical stability

For chemical stability, determination of the degree of diazepam degradation was investigated by the non-isothermal method (11). The extent (degree) of reaction was determined by using the graphic methods cited in Martin Physical Pharmacy (12). To accomplish this objective, 2.5 mL samples were placed in incubator at different temperatures of 40, 50 and 60°C . At time intervals of 0.5 h, 1.5, 3, 6, 8 and 24 h, three samples from each formulation were taken

and subjected to quantitative analysis. The data obtained was used to draw standard curve for determination of the degree of reaction. Then, gel samples were packaged into 2.5 mL disposable syringes and placed in a water bath equipped with an adjustable timer that displays temperature rise over a specified time period. Water bath was so adjusted as to show 5 degree increment over a 30 min period between 10°C and 60°C . At appropriate time intervals, 3 samples containing 2.5 mL were removed and analyzed quantitatively as follows:

Each sample was transferred to a 25 mL Erlenmeyer flask, and adjusted to volume with 0.1 N HCl. Then, 1 mL of this solution was placed in a 50 mL volumetric flask and brought to volume with the addition of 0.1 N hydrochloric acid. Absorbance of the solution was read against the control.

Determination of shelf-life

Stability studies are designed to give an insight into the drug degradation mechanism and expiry dating (shelf-life) estimation. Shelf-life is defined as the time required for a drug to decompose to 90% of its initial concentration at a specific temperature (T). The establishment of the prospective expiry date is of prime importance to drug product stability. Accelerated stability testing using the Arrhenius relationship is often employed for stability parameter identification. The well-known classical approach consists of sequential steps, including the determination of the kinetic constant K_T for the correct order of the degradation reaction. This is done through the functional relationship between drug content C and time t at several temperatures T (13). For a first-order reaction, this relation is based on Eq. 1:

$$\ln C = \ln C_0 - K_T t \quad (1)$$

Based on the measured times t and the corresponding concentrations C, it is easy to compute the least-squares estimates of $\ln C$

and K_T . This identification is repeated for several experiments at different temperatures T . Then, the intercept on the vertical axis is $\log A$, from which E_a and A may be obtained and used in the classical Arrhenius Eq. 2:

$$\ln K_T = \ln A - (E_a/RT) \quad (2)$$

Finally, the rate constant K_8 (at refrigerator temperature T_8) (281 K) is deduced from Equation 2 and the predicted shelf-life $t_{90\%}$ is determined from Eq. 3:

$$t_{90\%} = 0.105/K_8 \quad (3)$$

Results and discussion

Identification tests

IR spectra, melting point and λ -max determination confirmed the identification of diazepam (14).

Purity determination

The UV absorbance of different concentrations of diazepam (0.002-0.1 mg/ml) in HCl 0.1 N at 240 nm were determined. The best fit equation for the Beer's law plot of the UV absorbance versus drug concentrations, is given by the equation " $Y = 97.265C - 0.0058$ ". In this equation, Y is the absorbance at 240 nm and C is the concentration of diazepam (mg/mL). This equation was used for determination of diazepam purity and content uniformity in the samples.

Effect of HPMC concentration on viscosity of gels

Different formulations of gel base without drug, containing 3-7% w/w of HPMC were made. These gels were examined from point of view of transparency, syringability and consistency. Gel containing 6% w/w was chosen as the best formulation, because gels containing more than 6% hardly passed through syringe and gels containing less than 6% had low viscosity that was not desirable.

Drug Content in rectal hydrogels

The amounts of diazepam in samples were determined. The results are shown in the table 2. It is seen that diazepam content in different formulations are in the range of 96 to 103% and less than 5% error was observed, indicating there is no interaction between ingredients which were used in the formulations. The difference in drug content from different formulations may be due to their different pH and electrolyte in their formulations.

pH determination

Since pH is an important parameter for stability of a product, pH of different formulations during 60 days after preparation was investigated. It was observed that, time did not have any significant effect on pH of the formulations kept at room temperature from day one to day 60 after preparation.

Table 2: Amounts of diazepam present in the samples of different formulations

Formulation	Percent content \pm SD*
1 (contained buffer benzoate)	101.61 \pm 1.02
2 (contained buffer phosphate)	103.24 \pm 0.85
3 (without buffer)	96.49 \pm 0.61

*Results are the means \pm SD of 3 determinations.

Viscosity determination

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. (15). Table 3 shows the viscosity of 3 formulations after 3, 15, 30 and 60 days of preparation at different rotational speeds. Two studies were carried out on the data obtained.

A: The viscosity of 3 formulations was compared at the time of preparation to find the effect of used materials on the products.

B: Each formulation was investigated separately, after 72 h, 15, 30 and 60 days, in order to find the effect of aging on the viscosity of formulation.

The results obtained from part A showed that formulation No. 3 (without electrolyte) had a significant higher viscosity ($P < 0.001$), compared with the two other formulations that had almost similar viscosities. This finding is due to the effect of electrolyte on cloud point of HPMC gels (16) that causes significant reduction in viscosity (15).

The results obtained from part B of the study showed that the viscosity of all formulations increased up to 72 h and then a small reduction in viscosity was observed during 60 days that was not significant. Mitchel et al., (17) reported

that viscosity of HPMC gels would increase up to 72 h and after that a gradual decrease in viscosity would occur. The highest viscosity which was observed in all formulations up to 72 h was in agreement with finding of Mitchel et al. (17).

Table 4 shows the static comparison between viscosity of 3 formulations after 3, 15, 30 and 60 days of preparation. SPSS software programme was used and the results one-way analysis of variance (ANOVA) followed by Tukey test. P value of 0.05 or less was taken as significant. The data obtained confirmed the significant difference in viscosity of gels prepared without electrolyte or buffer.

Effect of antibacterial preservative

Following incubation, plates containing samples contaminated with prescribed microbial doses, were examined for growth. No growth of microorganisms was observed on plates incubated with fungi. Samples and plates contaminated by bacteria, showed a 4 log decrease after 28 days. Not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days count at 28 days is acceptable as a criteria for antimicrobial effectiveness (10).

Table 3: The viscosity of gels at different rotational speed at days after 3, 15, 30 and 60

Speed	Formulation No. 1 (days)				Formulation No. 2 (days)				Formulation No.3 (days)			
	3	15	30	60	3	15	30	60	3	15	30	60
10	*	*	*	*	*	*	*	*	18	2.9	1.8	1.1
20	15.8	6.9	6.8	5.7	15.2	6.7	6.0	5.6	33.4	25.2	22.1	21.8
30	21.5	14.5	13.2	12.6	18.4	14.1	12.8	12.1	38.4	28.4	28.3	27.8
50	25.3	18.5	18.2	17.8	24.6	17.9	17.7	17.4	42.3	31.5	32.9	32.2
60	26.9	20.1	19.9	19.8	25.9	19.4	18.8	18.6	44.1	33.3	34.1	33.6
100	29.0	25.2	23.6	23.0	28.1	24.6	23.0	21.8	*	42.7	37.0	36.3

*The viscometer did no show any data.

Stability tests

Figures 1, 2 and 3 show the plot of Log C (Log of residue of diazepam) against time (hour) for formulation 1, 2 and 3, respectively. A linear relationship between data was obtained, indicating first order kinetics with r^2 value of >0.994 .

Shelf-life determination

The data obtained from accelerated stability tests were used to determine the shelf life of diazepam rectal gels. The values of K (specific reaction rate of

diazepam) at different temperature of 40, 50 and 60°C (as $1/T$) were calculated. For example, in Fig.4, the slope of the plot ($\text{Ln } C = \text{Ln } C_0 - Kt$) for $K_{60^\circ\text{C}}$ was obtained and thus, it was calculated as $3 \times 10^{-3} \text{ days}^{-1}$. Similar method was used to determine the value of K_{50} and K_{40} which are presented in the table 5. Then, the data of K_{60} , K_{50} and K_{40} were used in Arrhenius equation ($K=Ae^{-E_a/RT}$) or $\text{Ln } K_T = \text{Ln } A - (E_a/RT)$.

Table 4: Static comparison of viscosity between 3 formulations after 72h, 15, 30 and 60 days

No. of Formulation		P value			
		72 h	15 days	30 days	60 days
1	2	0.922	0.993	0.983	0.983
	3	0.002*	0.010*	0.009*	0.009*
2	1	0.922	0.993	0.983	0.983
	3	0.001*	0.008*	0.006*	0.006*
3	1	0.002	0.010*	0.009*	0.009*
	2	0.001*	0.008*	0.006*	0.006*

*Significant

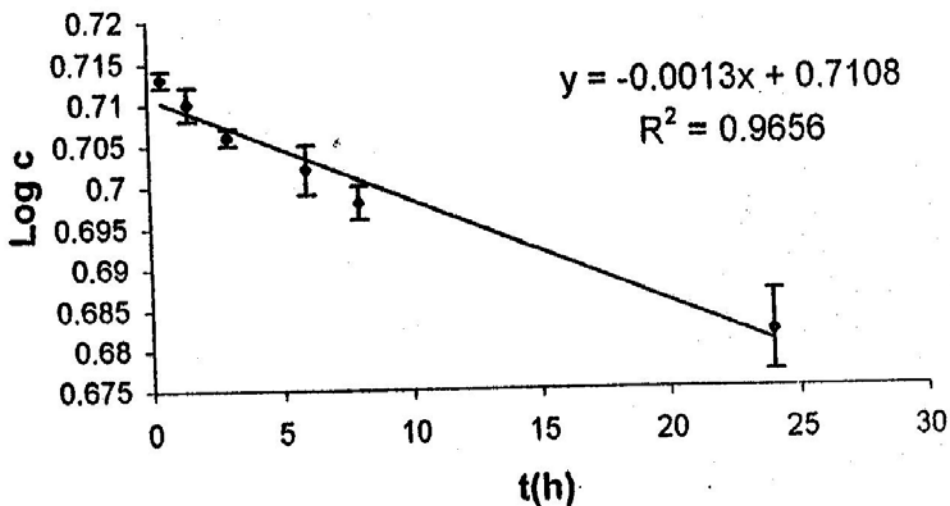


Fig. 1: Plot of log C versus time (h) for drug potency to fall to 90% of its original value for rectal gel formulation no. 1 containing benzoate buffer.

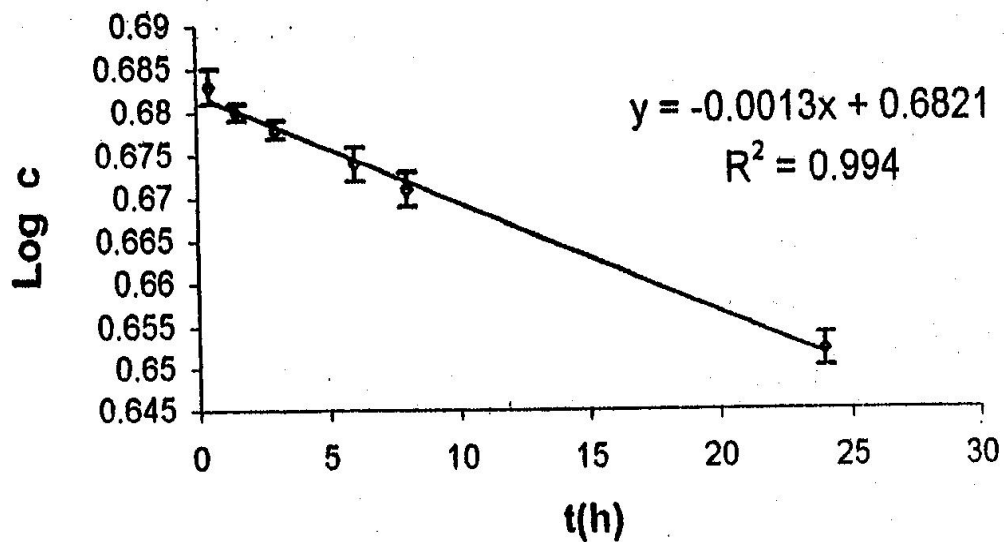


Fig. 2: Plot of log C versus time (h) for drug potency to fall to 90% of its original value for rectal gel formulation no. 2, containing phosphate buffer.

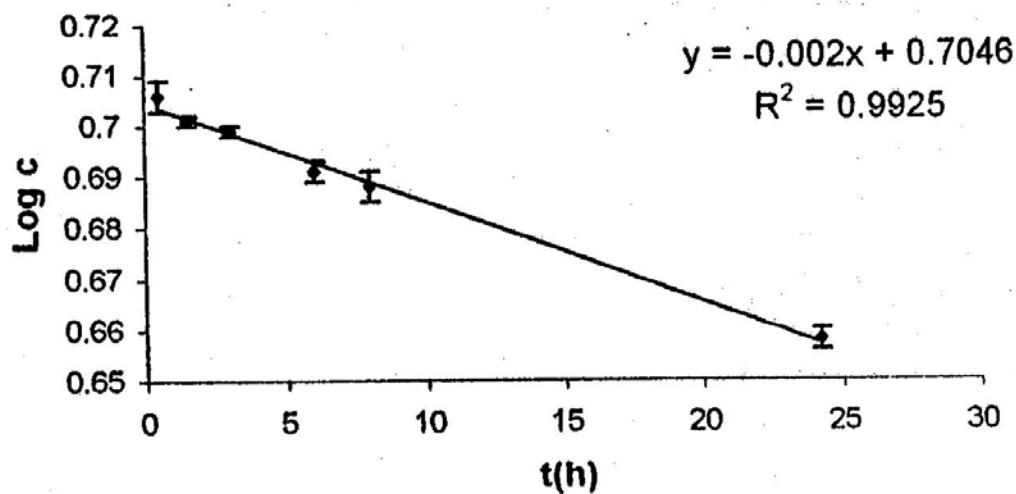


Fig. 3: Plot of log C versus time (h) for drug potency to fall to 90% of its original value for rectal gel formulation no. 3 without buffer.

Fig. 4 shows the plot of log K against reciprocal absolute temperature (1/T) for formulation No. 3 (without buffer). From this plot the slope of $Y = -4.819x + 8.674$ ($R^2 = 0.998$) was obtained. By using this equation, one can calculate the value of K_8 . Therefore, using the above mentioned procedure, it was observed that formulation 3 (without buffer) was the best formulation, because its shelf-life was 458 days at refrigerator condition and its pH was 6.5 which was in the acceptable range (Diazepam is stable between pH of 6.2 to 6.9) (7). In the second rank, was formulation 1 (containing benzoate buffer) with a shelf-life of 336 days and a pH similar to rectal pH. Formulation No. 2, containing buffer

phosphate had the lowest shelf-life of 254 days at refrigerator condition.

Dodow et al (9), prepared diazepam rectal gel containing 5 mg diazepam in 2.5 mL of HPMC gels.

They determined physico-chemical parameters of prepared gels after the 1st, 2nd, 3rd and 4th month after hydrogels preparation and reported that the preparations were maintained over a period of 4 months at a temperature of $26 \pm 0.5^\circ\text{C}$ (9). Dawson et al (18) reported that the rate of chemical reactions doubles for every 10°C rise in temperature. Therefore, for more stability of diazepam rectal gel, it is recommended to keep the preparation in refrigerator at $8-10^\circ\text{C}$.

Table 5: The values of K at different temperatures

Temperature $^\circ\text{C}$	Absoute temperature (T)	1/T $\times 10^3$	K	Ln K
40	313	3.195	0.00119	- 6.733802
50	323	3.096	0.00198	- 6.224466
60	333	3.003	0.003	- 5.809143

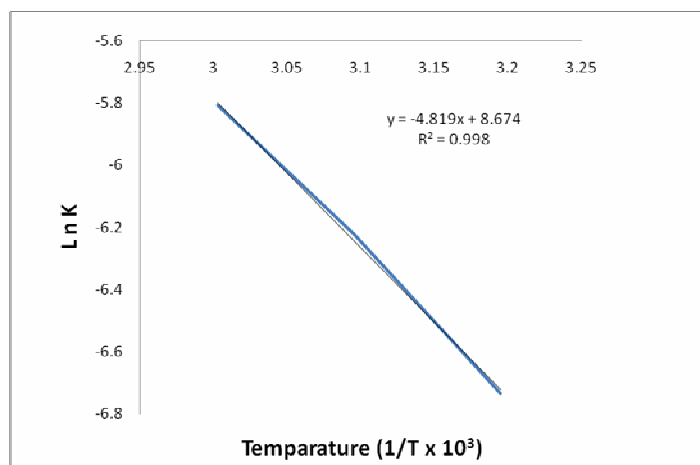


Fig. 4: A plot of Ln K against 1/T for the thermal decomposition of diazepam.

Conclusions

The HPMC hydrogels containing 5 mg of diazepam in 2.5 mL prefilled syringe intended for rectal administration have been prepared and evaluated. Three types of formulation containing benzoate buffer, phosphate buffer and without buffer were prepared and evaluated. The gels manifested good quality in respect to physico-chemical parameters (pH value, drug content and viscosity). Diazepam content in different formulations was in the range of 96 to 103 % which had less than 5% error. pH determination from all formulations did not change significantly during 60 days of preparation. Prepared gels showed good antimicrobial efficiency. Viscosity determination showed that viscosity of the formulation No. 3 (without buffer) was the highest among all the formulations. A linear relationship between decomposition of diazepam versus time was obtained, indicating first order kinetic. It can be summarized that formulation No.3 was the best formulation and at the refrigerator condition, it was stable for a period of 458 days.

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