

Effect of polymeric micelles on the characteristics and stability of diazepam-loaded hydrophilic and lipophilic suppositories

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Abstract

The polymeric micelles can overcome the poor solubility of drug in physiological fluid. However, the incorporation of polymeric micelles in suppository may affect the characteristics and stability of suppository. The aim of this study was to investigate the effect of diazepam-loaded polymeric micelles (DZ-PM) on the characteristics and stability of hydrophilic and lipophilic rectal suppositories in comparison with DZ powder (DZ-PD). The suppositories containing DZ-PD or DZ-PM equivalent to 5 and 10 mg of DZ were prepared by fusion method using PEG4000:PEG400 and Suppocire[®] AM:BM at 1:1 weight ratio as hydrophilic and lipophilic suppository bases, respectively. The results revealed that the incorporation of DZ-PM had no effect on the uniformity of mass and melting range of all formulations as compared to those containing DZ-PD. In addition, the DZ-PM did not affect the dissolution behaviors of hydrophilic suppository (>80% within 45 min). Meanwhile, the micelles dramatically enhanced the dissolution efficiency of DZ from the lipophilic suppository by at least 5.5 times and reduced the mean dissolution time by 2.0 times as compared to DZ-PD. After stored at 2-8°C for 180 days, the appearance, uniformity of mass, %drug content and dissolution behaviors of all formulations remained unchanged with respect to the initial time. All formulations remained the %weight deviation less than 5% and the %drug remaining was in the range of 93.66-103.06%. It can be concluded that the DZ-PM significantly enhanced the dissolution of drug from lipophilic suppository but slightly affected the properties of hydrophilic suppository.

Keyword: Diazepam, Hydrophilic, Lipophilic, Polymeric micelles, Suppository

1. INTRODUCTION

Diazepam (DZ) is one of the benzodiazepine anticonvulsants generally used for the treatment of seizure^{1,2}. It is considerably effective in the treatment of acute attack of febrile seizure in pediatrics. To prevent further brain damage, seizure must be ceased as immediately as an attack is noticed. Currently, the commercially available DZ products in Thailand are in the forms of tablet and solution for injection. The tablet is inconvenient and impractical to administer to the patients during the attack. Moreover, the

administration of solution for injection requires well-trained and high skill. Although the use of such solution by rectal administration is presently preferred in hospital, the drug leakage after administration may often occur. Therefore, the alternative formulation for the rectal administration of DZ is the suppository.

The formulation development of DZ is problematic due to its practical insolubility in water. The development of rectal formulation for poorly water-soluble drugs is typically limited by a very small volume of rectal fluid.

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One approach to overcome such problem is the use of polymeric micelles. Normally, the polymeric micelles, made from amphiphilic polymers or surfactants, consist of hydrophobic core surrounded by hydrophilic corona³. The hydrophobic drug can be incorporated or entrapped in the core of polymeric micelles and thus its water solubility can be improved. As previously reported, we have successfully developed the DZ-loaded polymeric micelles (DZ-PM) by using d- α -tocopheryl polyethylene glycol 1000 succinate (TPGS)⁴. Since the polymeric micelles were prepared using amphiphilic polymers, the incorporation of polymeric micelles in the suppository base may alter the characteristics of suppository and thus affect the dissolution and stability of dosage form.

The suppository is categorized into 3 types according to physical properties, namely oleaginous or lipophilic base, water-soluble or hydrophilic base and water-dispersible base. From our preliminary study, the water-dispersible base provided the unsatisfied characters⁵. Therefore, the hydrophilic and lipophilic suppositories were chosen to incorporate the DZ-PM in comparison with the diazepam powder (DZ-PD). The hydrophilic suppository was prepared by the mixture of two different polyethylene glycol polymers with molecular weights of 4000 and 400 (PEG4000:PEG400) at the weight ratio of 1:1. Meanwhile the lipophilic suppository base was made up of the mixture of Suppocire[®] AM and BM (AM:BM) at the ratio of 1:1 by weight.

The aim of this study was to investigate whether the incorporation of DZ-PM does affect the characteristics and stability of hydrophilic and lipophilic rectal suppositories in comparison with DZ-PD. The suppositories contained the amount of drug equivalent to 5 or 10 mg. The prepared suppositories were evaluated in terms of appearance, melting range, uniformity of mass, %drug content and dissolution behaviors. According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline, the stability of rectal suppositories was investigated at 2-8°C for 180 days.

2. MATERIALS AND METHODS

2.1. Materials

Diazepam was kindly supplied by Defense Pharmaceutical Factory, Bangkok, Thailand. TPGS, and poloxamer 407 (P407) were kindly gifted from BASF, Ludwigshafen, Germany. The lyophilized DZ-PM was prepared according to the previous report⁴. Suppocire[®] AM and BM (saturated C8-C18 triglyceride fatty acids) pastilles were kindly provided by Gattefosse, Nanterre, France. Polyethylene glycol 400 (PEG400, PETRONAS Chemicals, Kuala Lumpur, Malaysia) and 4000 (PEG4000, INEOS Oxide, Zwijndrecht, Belgium) were used as received. Methanol (high performance liquid chromatography grade, HPLC) and absolute ethanol were purchased from Honeywell Burdick&Jackson, Michigan, USA. Sterile water for injection was bought from General Hospital Products Public Co., Ltd., Pathum Thani, Thailand.

2.2. Determination of displacement value of DZ-PM

The displacement value of DZ-PM was determined by calculating the weight difference of DZ-PM-loaded suppository with respect to the corresponding suppository base. Briefly, each component of suppository base was melted in a casserole and casted by a suppository mold. After that the weight of solidified suppository base was recorded. The suppository base was melted again and stirred until almost congealing. The known amount of DZ-PM was incorporated in the congealed suppository base which was then casted in the mold. After the suppository had solidified, the accurate weight of DZ-PM-loaded suppository was recorded. The displacement value was calculated according to equation 1. In case of DZ-PD, the displacement value of DZ-PD was not determined since the weight of DZ-PD was very small as compared to that of suppository.

$$\text{Displacement value} = \frac{W_{\text{DZ-PM}}}{W_{\text{B}} - W_{\text{B,DZ-PM}} - W_{\text{DZ-PM}}} \quad (1)$$

where $W_{\text{DZ-PM}}$ is the actual weight of DZ-PM added in the formulation. W_{B} and

$W_{B,DZ-PM}$ are the recorded weights of suppository base and DZ-PM-loaded suppository, respectively.

2.3. Preparation of suppository

The DZ-PD- and DZ-PM-loaded suppositories were prepared by fusion method. Briefly, the accurate weight of each component was melted in the casserole. After completely melted, the mixture was stirred until almost congealing and then DZ-PD or DZ-PM was added. The suppository was casted in the calibrated suppository mold which was allowed to solidify at room temperature. Finally, the drug-loaded suppository was collected for further analysis.

2.4. Physical appearance observation

The physical appearance of suppository was visually observed in terms of color, smoothness of surface, cracking and bubble.

2.5. Melting point determination

The melting range of the suppository was determined by capillary tube method.⁶ Shortly, the capillary tube was filled with suppository for 1 cm in height by pressing on the suppository. The tube was tied up with a thermometer and placed in the middle of test tube. Then the test tube was immersed in a water bath. The temperature was recorded when the suppository in the test tube started to be melted (T_1) and was completely melted (T_2).

2.6. Determination of uniformity of mass

The weight of suppository was individually recorded and the %deviation of suppository weight was calculated according to equation 2. The measurement was performed in triplicate.

$$\% \text{ Deviation} = \frac{(x_i - \bar{x}) \times 100}{\bar{x}} \quad (2)$$

where x_i and \bar{x} are the individual weight and average weight of suppository, respectively.

2.7. Drug content analysis

The DZ was extracted from the suppository as follows. The suppository was dissolved

in 20 mL of solvent with aid of heating on a water bath. The solution was allowed to cool down and transferred to 50-mL volumetric flask. The volume was adjusted to 50 mL with solvent. After sonicated for 15 min, the solution was centrifuged at 4,500 rpm for 30 min. One milliliter of supernatant was taken and mixed with 4 mL of HPLC mobile phase. The sample was then centrifuged at 4,500 rpm for 15 min. The supernatant was filtered through 0.45 μm nylon syringe filter and the drug concentration was analyzed by HPLC. Methanol and ethanol were used as solvents for PEG4000:PEG4000 and AM:BM formulations, respectively. The %drug content was calculated according to equation 3.

$$\% \text{ Drug content} = \frac{\text{Analyzed amount of DZ}}{\text{Initially added amount of DZ} \times 100} \quad (3)$$

2.8. Dissolution testing

The dissolution of DZ from the suppository was conducted by dissolution USP apparatus I (ERWEKA DT80 dissolution tester, ERWEKA GmbH, Heusenstamm, Germany) in 500 mL of 0.05 M phosphate buffer pH 7.4 at $37 \pm 0.5^\circ\text{C}$ ⁷⁻⁹. The rotation speed was set at 50 ± 0.1 rpm. Five milliliters of dissolution medium were withdrawn at 5, 15, 30, 45, 60, 120, 240 and 360 min and replaced with an equal volume of fresh medium immediately after sampling. The taken sample was filtered through 0.45 μm cellulose acetate membrane syringe filter prior to HPLC analysis.

2.9. HPLC analysis

The amount of DZ was quantified by HPLC method according to the previously published method⁴ using Shimadzu HPLC machine (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with SPD-20A UV/VIS detector. The drug was eluted through Luna C18 reverse phase column (5 μm , 150 \times 4.6 mm, Phenomenex Inc., Macclesfield, UK) plus a C18 guard column at a flow rate of 1 mL/min and detected at a wavelength of 230 nm. The mixture of methanol and water at 65:35 v/v was used as a mobile phase. The amount of DZ was calculated from the standard curve

over the concentration range of 0.5-50 µg/mL with r^2 of at least 0.9995. The interday and intraday precisions were less than 2%.

2.10. Stability study of DZ-loaded suppository

The stability study of DZ-loaded suppository was performed at 2-8°C for 180 days according to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline¹⁰. At day 180, the appearance, uniformity of mass, %drug content and dissolution profile of the samples were analyzed as previously described and compared with those at initial time.

2.11. Dissolution parameter calculation

The dissolution parameters were determined and reported in terms of relative dissolution rate at 30 min (RD_{30min}), mean dissolution time (MDT) and %dissolution efficiency (%DE).¹¹ The dissolution profiles were statistically compared using the difference (f_1) and similarity factors (f_2)^{12,13}. These parameters were calculated according to the equations shown below. The suppository containing DZ-PD was used as a reference for comparison. In case of stability testing, the dissolution data of suppository after preparation was used as a reference.

Relative dissolution rate at 30 min (RD_{30min})

$$RD_{30min} = \frac{\%Cumulative\ of\ drug\ dissolved_{Test}}{\%Cumulative\ of\ drug\ dissolved_{Reference}} \quad (4)$$

Mean dissolution time (MDT)

$$MDT = \frac{\sum_{j=1}^n \hat{t}_j \Delta M_j}{\sum_{j=1}^n \Delta M_j} \quad (5)$$

where j is the sample number, N is a number of dissolution sample times, \hat{t}_j is the time at the midpoint between t_j and t_{j-1} and M_j is the additional amount of drug dissolved between t_j and t_{j-1} .

%Dissolution efficiency (%DE)

$$\%DE = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100\% \quad (6)$$

where y is the percent drug dissolved at time t .

Difference factor (f_1)

$$f_1 = \frac{\sum_{j=1}^n |R_j - T_j|}{\sum_{j=1}^n R_j} \times 100 \quad (7)$$

where n is the sampling number and R_j and T_j are the percent drug dissolved of reference and test products at each time point j .

Similarity factor (f_2)

$$f_2 = 50 \times \log \left\{ \left[1 + \left(\frac{1}{n} \sum_{j=1}^n W_j |R_j - T_j|^2 \right)^{0.5} \times 100 \right] \right\} \quad (8)$$

where W_j is an option weight factor and R_j and T_j are the percent drug dissolved of reference and test products at each time point j .

2.12. Statistical analysis

The results are expressed as mean \pm SD from at least three measurements. The data was statistically analyzed using student's t-test or one-way ANOVA with the Scheffe test applied post hoc to compare the data of two or multiple groups, respectively. The results are considered to be significant when p -value is less than 0.05 at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1. Characteristics of suppositories after preparation

The displacement value of DZ-PM in PEG4000:PEG400 and AM:BM bases was found to be 0.518 and 0.805, respectively, suggesting the lower density of DZ-PM as compared to suppository bases. The physical appearance of the freshly prepared suppositories is illustrated in Figure 1. The PEG4000:PEG400 formulations had white and smooth surface and no cracking. The DZ-PM-contained hydrophilic suppository had a few bubbles inside the suppository. Meanwhile the appearance of AM:BM formulations was white to off-white and smooth without cracking or bubble. The melting range of suppositories is demonstrated in Figure 2. The melting range of suppositories is determined by the temperature at which the suppository

started to be melted and was completely melted as recorded as T_1 and T_2 , respectively. The melting range of PEG4000:PEG400 and AM:BM suppositories containing DZ-PD was in the range of 42.4-50.9°C and 33.0-36.3°C, respectively. The PEG4000:PEG400 suppositories had significantly higher melting range than the AM:BM formulations due to higher melting point of PEG4000:PEG400. The incorporation of DZ-PM did not affect the T_1 value while it tended to increase the T_2 value of both suppositories. The increase of amount of DZ-PM increased

the T_2 value especially for the formulations containing 10 mg of DZ.

As summarized in Table 1, the average weights of PEG4000:PEG400 and AM:BM formulations were in the range of 2.246-2.467 g and 1.821-1.873 g, respectively. The %deviation of all suppositories was less than 5% conforming the acceptance criteria stated in the uniformity of mass in the British Pharmacopoeia 2014.¹⁴ The %drug content of PEG4000:PEG400 and AM:BM suppositories were in the range of 90.94-104.19% and 94.81-100.30%, respectively.

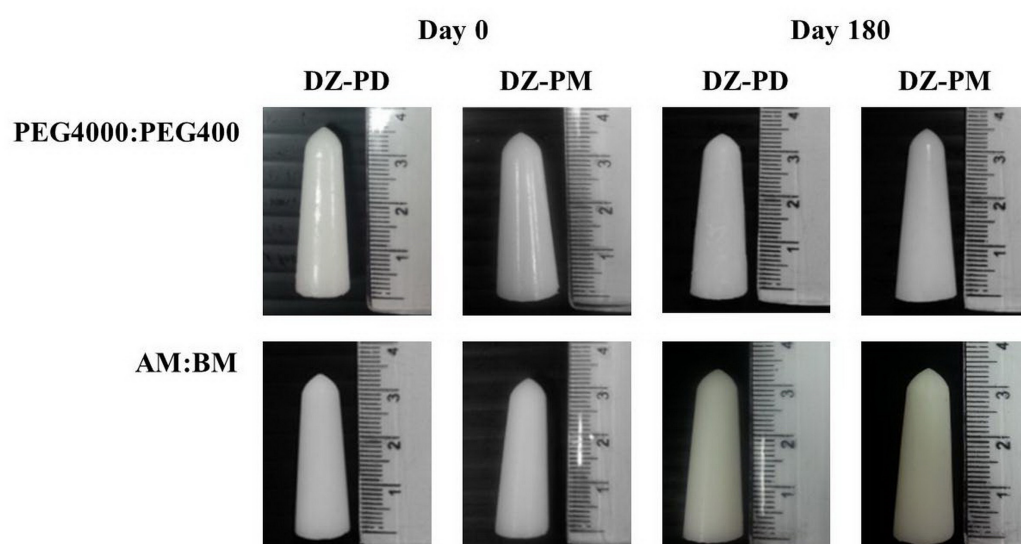


Figure 1. Exemplified appearance of PEG4000:PEG400 and AM:BM suppositories containing the equivalent amount of DZ at 10 mg.

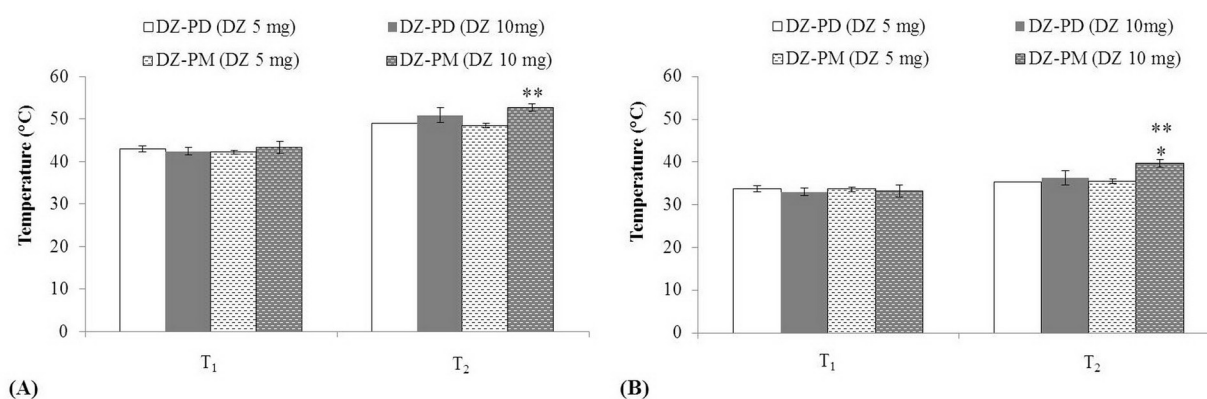


Figure 2. Melting range of PEG4000:PEG400 (A) and AM:BM (B) suppositories (T_1 : Temperature at which the suppository started to be melted, T_2 : Temperature at which the suppository was completely melted). *Significantly different comparing between the formulations containing DZ-PM and DZ-PD at the same equivalent amount of DZ. **Significantly different comparing the different equivalent amount of DZ using the same type of DZ in the formulation. (n = 5)

Table 1. Weight, %deviation of weight and %drug content of suppositories containing DZ-PD or DZ-PM equivalent to 5 mg or 10 mg of DZ (n ≥ 3)

Formulation	Time	Equivalent amount of DZ	Weight (Mean±SD) (g)		%Deviation		%Drug content or %Drug remaining ^a (Mean±SD)	
			DZ-PD	DZ-PM	DZ-PD	DZ-PM	DZ-PD	DZ-PM
			PEG4000: PEG400	Day 0	5 mg	2.332 ± 0.009	2.246 ± 0.023	(-0.46)-0.78
		10 mg	2.454 ± 0.014	2.467 ± 0.018	(-1.13)-1.18	(-1.09)-1.02	102.92 ± 4.26	90.94 ± 1.65
	Day 180	5 mg	2.329 ± 0.010	2.251 ± 0.015	(-0.59)-0.91	(-1.14)-1.26	95.24 ± 3.90 ^a	96.58 ± 2.69 ^a
		10 mg	2.426 ± 0.018	2.476 ± 0.051	(-1.64)-0.92	(-2.42)-3.80	97.01 ± 4.59 ^a	96.35 ± 2.32 ^a
AM:BM	Day 0	5 mg	1.859 ± 0.011	1.873 ± 0.005	(-0.76)-1.17	(-0.48)-0.33	100.30 ± 7.85	96.18 ± 6.34
		10 mg	1.821 ± 0.007	1.866 ± 0.016	(-0.45)-0.78	(-1.03)-1.46	96.02 ± 3.84	94.81 ± 1.20
	Day 180	5 mg	1.865 ± 0.008	1.866 ± 0.008	(-0.82)-0.68	(-0.65)-0.88	101.54 ± 4.29 ^a	103.06 ± 2.93 ^a
		10 mg	1.816 ± 0.014	1.852 ± 0.012	(-0.97)-1.57	(-0.92)-1.02	93.66 ± 4.80 ^a	97.30 ± 2.28 ^a

^aCalculated with respect to 100% of drug content at the initial time

Table 2. Dissolution parameters of PEG4000:PEG400 and AM:BM suppositories at day 0 and 180

Formulation	Type of DZ	Equivalent amount of DZ	Dissolution parameters							
			Day 0				Day 180			
			RD _{30 min} ^a	MDT _{80%} ^b	MDT _{6h} ^c	%DE _{6h} ^d	RD _{30 min} ^a	MDT _{80%} ^b	MDT _{6h} ^c	%DE _{6h} ^d
			(min)	(min)	(%)		(min)	(min)	(%)	
PEG4000: PEG400	DZ-PD	5 mg	-	11.56	-	84.11	1.06 ^f	9.88	-	87.37
		10 mg	-	11.60	-	83.17	1.03 ^f	10.41	-	84.76
	DZ-PM	5 mg	92.84 ^e	13.19	-	82.72	1.04 ^f	11.93	-	84.18
		10 mg	19.72 ^e	15.61	-	79.96	1.10 ^f	13.46	-	83.10
AM:BM	DZ-PD	5 mg	-	-	173.75	4.33	2.71 ^f	-	173.86	5.21
		10 mg	-	-	170.25	3.97	2.36 ^f	-	181.87	8.47
	DZ-PM	5 mg	0.97 ^e	-	80.12	23.80	1.07 ^f	-	94.75	17.84
		10 mg	0.88 ^e	-	87.23	28.28	1.04 ^f	-	98.07	20.07

^aRD_{30 min} = Relative dissolution rate at 30 min

^bMDT_{80%} = mean dissolution time at 80% drug dissolved

^cMDT_{6h} = mean dissolution time at 6 h

^d%DE_{6h} = %dissolution efficiency at 6 h

^eComparing between DZ-PM and DZ-PD

^fComparing with the initial time

3.2. Dissolution testing

The dissolution of suppositories was conducted in phosphate buffer pH 7.4 at 37°C for 6 h, using dissolution tester USP apparatus type I. The dissolution profiles of PEG4000:PEG400 and AM:BM suppositories are illustrated in Figure 3. The results found that the %drug dissolved of DZ-PD- and DZ-PM-loaded PEG 4000:PEG400 suppositories was higher than

80% within 45 min. The dissolution profiles of DZ-PD- and DZ-PM-loaded PEG4000:PEG400 suppositories were comparable ($f_1 < 15$, $50 < f_2 < 100$). Regarding the dissolution parameters (Table 2), the $MDT_{80\%}$ and % DE_{6h} of all PEG4000:PEG400 suppositories were around 11.56-15.61 min and 79.96-84.11%, respectively. The incorporation of DZ-PM and increasing amount of DZ slightly increased $MDT_{80\%}$ and decreased % DE_{6h} .

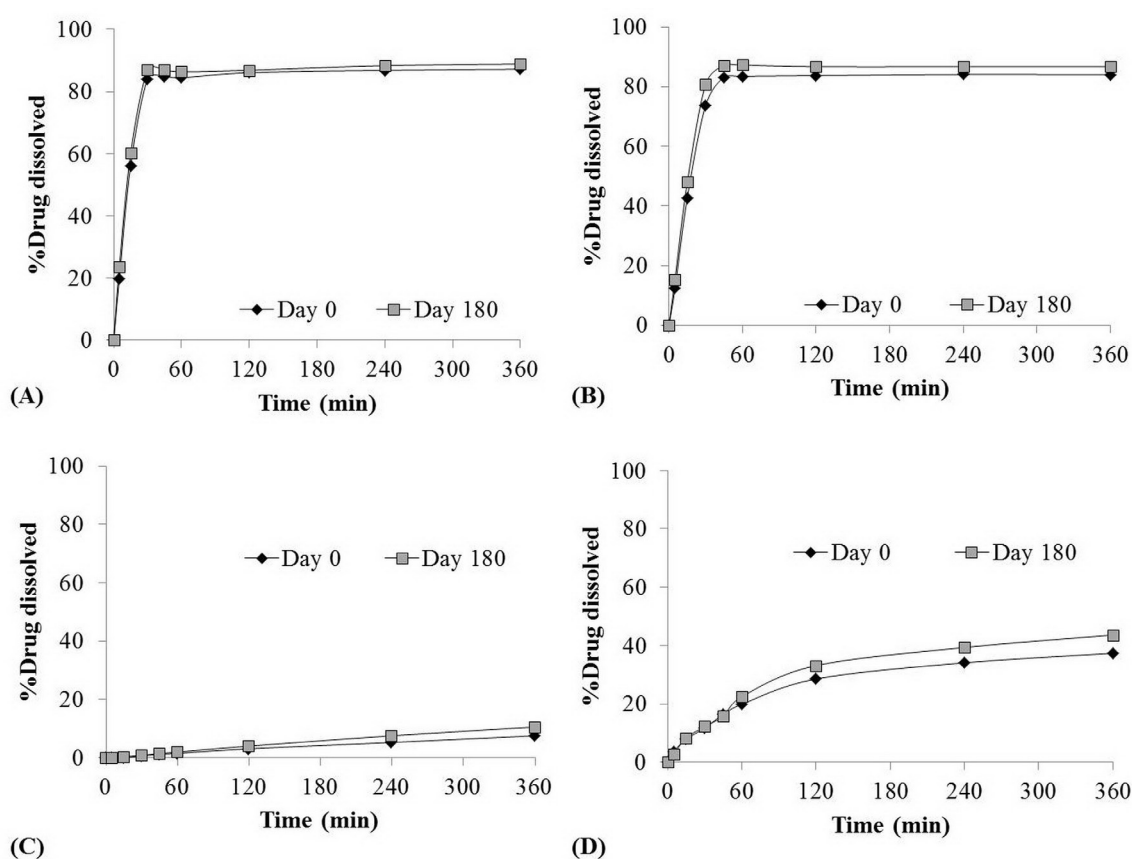


Figure 3. Examples of dissolution profiles of PEG4000:PEG400 and AM:BM suppositories after preparation (black diamond) and after stored for 180 days (gray square). A: PEG4000:PEG400 formulation containing DZ-PD, B: PEG4000:PEG400 formulation containing DZ-PM, C: AM:BM formulation containing DZ-PD and D: AM:BM formulation containing DZ-PM. An error bar indicates the standard deviation from three measurements.

In the meantime, the incorporation of DZ-PM significantly altered the dissolution profile of DZ from AM:BM suppositories as compared to DZ-PD ($f_1 > 15$, $f_2 < 50$). The %drug dissolved of DZ-PD-loaded AM:BM suppository was considerably low (less than 10%) over 6 h. However DZ-PM dramatically enhanced the

%drug dissolved by 31-37% which was around 3.7-5.0 times higher than DZ-PD. The increasing amount of DZ of all formulations from 5 to 10 mg did not affect the dissolution profiles. The $MDT_{80\%}$ of AM:BM formulations could not be determined since the %drug dissolved of these formulations was less than 50% over 6 h.

However, these formulations had the MDT at 6 h of 170-173 min and %DE as small as 3.97-4.33% when incorporated with DZ-PD. Interestingly, DZ-PM reduced the MDT_{6h} by 2.0 times and increased %DE by at least 5.5 times as compared to DZ-PD. The enhanced dissolution of DZ was obviously due to the solubilizing effect of polymeric micelles.

These results suggested that the polymeric micelles significantly enhanced the dissolution of DZ from lipophilic AM:BM suppositories but did not significantly alter that of hydrophilic PEG4000:PEG400 suppositories.

3.3. Stability study

After storage at 2-8°C for 180 days, no change in physical appearance of all formulations was observed (Figure 1). The average weight of PEG4000:PEG400 and AM:BM suppositories was in the range of 2.251-2.476 and 1.816-1.873 g, respectively. The %deviation of all formulations remained less than 5%. The %drug contents of PEG4000:PEG400 and AM:BM formulations were higher than 90% comparing with the initial time conforming the British Pharmacopoeia 2014. The dissolution profiles of all formulations remained unchanged as compared to the initial time ($f_1 < 15$, $50 < f_2 < 100$). The $MDT_{80\%}$ of PEG4000:PEG400 formulations slightly decreased while the %DE_{6h} increased. On the contrary, the MDT_{6h} of AM:BM suppositories minimally increased and their %DE_{6h} slightly declined. The RD_{30min} of DZ-PD-loaded AM:BM suppository was about 2. The change in dissolution parameters of AM:BM suppositories may be related to the polymorphic change of lipid component in the formulation which is being under investigation.

4. CONCLUSION

The incorporation of DZ-PM did not significantly affect the characteristics and stability of PEG4000:PEG400 suppositories while it obviously enhanced the dissolution behaviors of AM:BM suppositories as compared to DZ-PD. The increment of DZ from 5 to 10 mg did not affect the characters of all formulations. All formulations were physically and chemically stable after stored at 2-8°C for 180 days.

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