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Full Length Research Paper

PAMAM dendrimers affect the *in vitro* release of clotrimazole from hydrogels irrespective of its molecular state

Katarzyna Sosnowska* and Katarzyna Winnicka

Department of Pharmaceutical Technology, Faculty of Pharmacy, Medical University of Bialystok, Kilinskiego 1, 15-089 Bialystok, Poland.

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Insufficient aqueous solubility of clotrimazole (CLO) is the main problem in designing pharmaceutical formulations and limits its therapeutic efficiency. Polyamidoamine (PAMAM) dendrimers give the opportunity to decrease this limitation because of their solubilising and permeation enhancement properties. The aim of this study was to examine the influence of PAMAM dendrimers on the release of CLO from hydrogels and water suspensions. PAMAM dendrimers improved permeation of CLO from all analyzed formulations in the following order: PAMAM-NH_2 G3 > PAMAM-NH_2 G2 > PAMAM-OH G3 > PAMAM-OH G2. The effect evoked by PAMAM dendrimers was the most potent from hydrogels containing dissolved drug.

Key words: Polyamidoamine (PAMAM) dendrimers, clotrimazole, hydrogels.

INTRODUCTION

Clotrimazole (CLO) is a local imidazole-derivative antifungal agent that is used for the treatment of skin and vaginal infections caused by various species of pathogenic dermatophytes and yeasts (Holt, 1974; Henry et al., 2000). An optimal therapeutic effect of dermal drug depends on the appropriate dosage system, physicochemical properties of the substance, type of vehicle (lipophilic, hydrophilic) and the presence of skin absorption enhancers, which influence diffusion of a medical agent from vehicle to skin.

One of the main problems in transdermal delivery is restricted permeation of the drug through *stratum corneum*. In order to improve the cutaneous penetration in dermal preparations, chemical enhancers are used (Pathan and Setty, 2009), which increase active substance's solubility in the vehicle, improve the partition coefficient or modify barrier properties of *stratum corneum*. Recently, dendrimers, a new class of polymers were found to improve solubility of water-insoluble drugs and to expedite the transportation of drugs across biomembranes. Dendrimers are monodispersed, nanometer size range and hyperbranched macromolecules, which make them suitable as carriers binding guest molecules in the interior of dendrimers or with the surface groups (Nanjwade et al., 2009). One of the most examined families of dendrimers is polyamidoamine (PAMAM) dendrimers, which are being investigated as permeation enhancer and carriers for several routes of delivery: transdermal, oral, ocular or even intravenous (Cheng et al., 2007a; Borowska et al., 2012).

The aim of this work was to examine the influence of concentration and type of surface groups of PAMAM dendrimers generation 2 and 3 (G2, G3) on *in vitro* release of CLO. Additionally, the mechanism of permeation increase, the diffusion of CLO from hydrogels containing the drug

*Corresponding author. E-mail: katarzyna.sosnowska@umb.edu.pl. Tel/Fax: 0048 85 748 56 16.

in two molecular states and from water suspensions were also studied.

MATERIALS AND METHODS

Clotrimazole was provided by Aflofarm (Pabianice, Poland). PAMAM dendrimers G2 and G3 with -NH_2 or -OH surface groups and Tween 80 were provided by Sigma Aldrich (St. Louis, MO, USA), together with other chemicals and buffers used. Carbopol® 980 was donated by S&D Polska (Warsaw, Poland). Clotrimazolum GSK® cream is a product of GlaxoSmithKline Pharmaceuticals SA (Poznan, Poland). Cuprophan®, natural cellulose membrane (MWCO 10,000 Da) was purchased from Medicell (London, UK).

Preparation of hydrogels with CLO in different molecular states (dissolved or dispersed)

All hydrogels were prepared using mechanical stirrer model DT 200 (Witko, Lodz, Poland). Carbopol 980 was gradually added to the water and stirred for 45 min until homogenous mixture appeared, then mixture was neutralized by dropwise addition of 20% solution of sodium hydroxide to allow gel formation. Methyl p-hydroxybenzoate (0.1%) and propyl p-hydroxybenzoate (0.1%) dissolved in ethanol, Tween 80, and propylene glycol were added to the hydrogel bases. To obtain hydrogels with dissolved drug, CLO in aqueous solutions of PAMAM dendrimers G2 or G3 with -NH_2 or -OH surface groups was mixed up with hydrogels bases. Hydrogels with dispersed drug were prepared by mixing CLO with hydrogel bases, and then aqueous solutions of PAMAM-NH_2 G3 dendrimers were added. Final concentration of PAMAM dendrimers in hydrogels was 0.3, 3.0 and 30.0 mg/g. As CLO is stable in alkaline medium and in acidic environment hydrolyzes to (*o*-chlorophenyl)diphenyl methanol and imidazole (Hoogerheide and Wyka, 1982); pH of all preparations was adjusted to 6.9. Composition of prepared hydrogels is shown in Table 1.

Preparation of water suspensions of CLO

CLO was suspended in water or in mixture of water with aqueous solutions of PAMAM G2 or G3 with -NH_2 or -OH surface groups at concentration of 0.3, 3.0 and 30.0 mg/g. Composition of prepared suspensions is shown in Table 1.

The *in vitro* release of CLO from hydrogels

The *in vitro* release of CLO from prepared hydrogels and comercially available product was performed in enhancer cell (Agilent Technologies, Cary, NC, USA), using natural cellulose membrane (Cuprophan®, Medicell, London, UK), previously moistened with water. The diameter of the cell was 2.2 cm, providing 3.80 cm^2 effective constant area. The enhancer cell consisted of Teflon chamber with adjustable capacity (0.5 to 5.0 g) and a screw cap to hold the membrane. About 3 g of each formulation ("infinite dose") was placed in the drug reservoir on the top of the membrane (10 µm thick) without entrapped air at the interface of the gel and membrane. A United States Pharmacopeia (USP) Apparatus 2, Dissolution Tester (Agilent 708-DS, Agilent Technologies, Cary, NC, USA) with mini vessels (250 ml) and mini paddles with a rotating speed of 75 rpm were used to measure the release of CLO from the enhancer cell assembly. The receptor compartment was filled with 150 ml of acetate buffer pH 5.5 with sodium dodecyl sulfate (SDS; 1%) to provide the sink conditions and maintained at 32 ± 0.5°C. Aliquots (1 ml) of the acceptor phase were collected at the predetermined time intervals (0.5, 1, 2, 3 and 4 h) and replaced with an equal volume of fresh buffer solution (Thakker et al., 2003). The drug content in the examined samples was determined by the high performance liquid chromatography (HPLC) method. The results are expressed as means (%) ± standard deviation (SD) for 6 independent experiments.

The *in vitro* release of CLO from water suspensions

The experiments were performed using cellulose membrane (Cuprophan®, Medicell, London, UK) and acetate buffer pH 5.5 with SDS (1%). The dialysis bags containing 3 ml of investigated suspensions were placed in beakers with 150 ml of acceptor fluid preheated to 32 ± 0.5°C. The total area for diffusion was approximately 12 cm^2. The dialysis bags in beakers were shaken (75 rpm) in a water bath. Aliquots (1 ml) of the acceptor phase were collected at predetermined time intervals (0.5, 1, 2, 3 and 4 h) and replaced with an equal volume of fresh buffer solution. The drug content in examined samples was determined by the HPLC method. The results are expressed as means (%) ± SD for 6 independent experiments.

HPLC analysis of CLO

The amount of released CLO was determined by Agilent Technologies 1200 HPLC system equipped with a G1312A binary pump, a G1316A thermostat, a G1379B degasser and a G1315B diode array detector (Agilent, Waldbronn, Germany). Data collection and analysis were performed using ChemStation 6.0 software. Isocratic separation was obtained on a Zorbax Eclipse XDB–C18, 4.6 × 150 mm, 5 µm column (Agilent, Waldbronn, Germany). Mobile phase was methanol and phosphate buffer pH 7.4 (80:20 v/v); the flow rate was 1.0 ml/min and ultraviolet (UV) detection was performed at a wavelength of 210 nm (Hájková et al., 2007). The retention time for CLO was 5.5 min. The column's temperature was maintained at 25°C. For injection into the HPLC system, 20 µl of sample was used. Standard calibration curve was linear over the range of 1 to 100 µg/ml ($y = 154.4x + 6.0672$, $R^2 = 0.9995$). All reagents used for analysis were HPLC grade. The amount of CLO released (µg/cm^2) was plotted against square root of time (√h) and then linear regression analysis of the plot was accomplished (Higuchi's equation). The validity of applying Higuchi's equation was indicated by correlation coefficient ($R^2 \geq 0.998$).

Data analysis

The results were analysed by means of analysis of variance (ANOVA) and multiple comparisons were made to check statistical significance. The statistical significance between means was verified by Sheffe's comparison test accepting $p < 0.05$ as significant.

RESULTS AND DISCUSSION

The membrane permeability of drug depends on its solubility and *stratum corneum*/vehicle partition coefficient (Michaels et al., 1975). CLO belongs to lipophilic substances, therefore is able to penetrate the skin relatively

Table 1. Composition of prepared formulations.

Ingredient (g)	Formulation code for hydrogels												
	H-1	**H-2**	**H-3**	**H-4**	**H-5**	**H-6**	**H-7**	**H-8**	**H-9**	**H-10**	**H-11**	**H-12**	**H-13**
CLO	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Carbopol 980	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Sodium hydroxide (20%)	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Ethanol (760 g/l)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Propylene glycol	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methy p-hydroxybenzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl p-hydroxybenzoate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tween 80	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
PAMAM-NH_2 G2		0.03	0.3	3.0									
PAMAM-NH_2 G3					0.03	0.3	3.0						
PAMAM-OH G2								0.03	0.3	3.0			
PAMAM-OH G3											0.03	0.3	3.0
Purified water (up to)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Formulation code for suspensions												
	S-1	**S-2**	**S-3**	**S-4**	**S-5**	**S-6**	**S-7**	**S-8**	**S-9**	**S-10**	**S-11**	**S-12**	**S-13**
CLO	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PAMAM-NH_2 G2		0.03	0.3	3.0									
PAMAM-NH_2 G3					0.03	0.3	3.0						
PAMAM-OH G2								0.03	0.3	3.0			
PAMAM-OH G3											0.03	0.3	3.0
Purified water (up to)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

easier than hydrophilic ones and it should diffuse faster from hydrophilic vehicle than from lipophilic bases. However, poor aqueous solubility of CLO (0.49 µg/ml) (Pedersen et al., 1998) is the main problem in technology of effective dosage forms, because only dissolved fraction of drug is able to cross the barrier. Various methods, like forming microcapsules, liposomes (Ning et al., 2005), cyclodextrin complexes (Bilensoy et al., 2006) or solid dispersions (Balata et al., 2011) were used to improve the solubility of CLO and its permeation through the membranes.

PAMAM dendrimers are an interesting and relatively new class of compounds, which act not only as solubility enhancers (Filipowicz et al., 2011), but also possess the ability to improve anti-bacterial or antifungal activity of drugs (Strydom et al., 2013; Cheng et al., 2007b; Winnicka et al., 2011). Since properties of dendrimers depend on their size and surface charge (Malik et al. 2000), lower generation of PAMAM are more preferred for examination in dermal formulations, because of their stronger ability to enhance the permeation and lower toxicity (Heiden et al., 2007; Winnicka et al., 2009). PAMAM-NH_2 in contrast to neutral and anionic dendrimers can be accumulated in the skin upper layers (Yang et al., 2012), which makes them proper carriers for topical drug de-livery. The effect of PAMAM dendrimers G2 and G3 with various surface groups on the release of

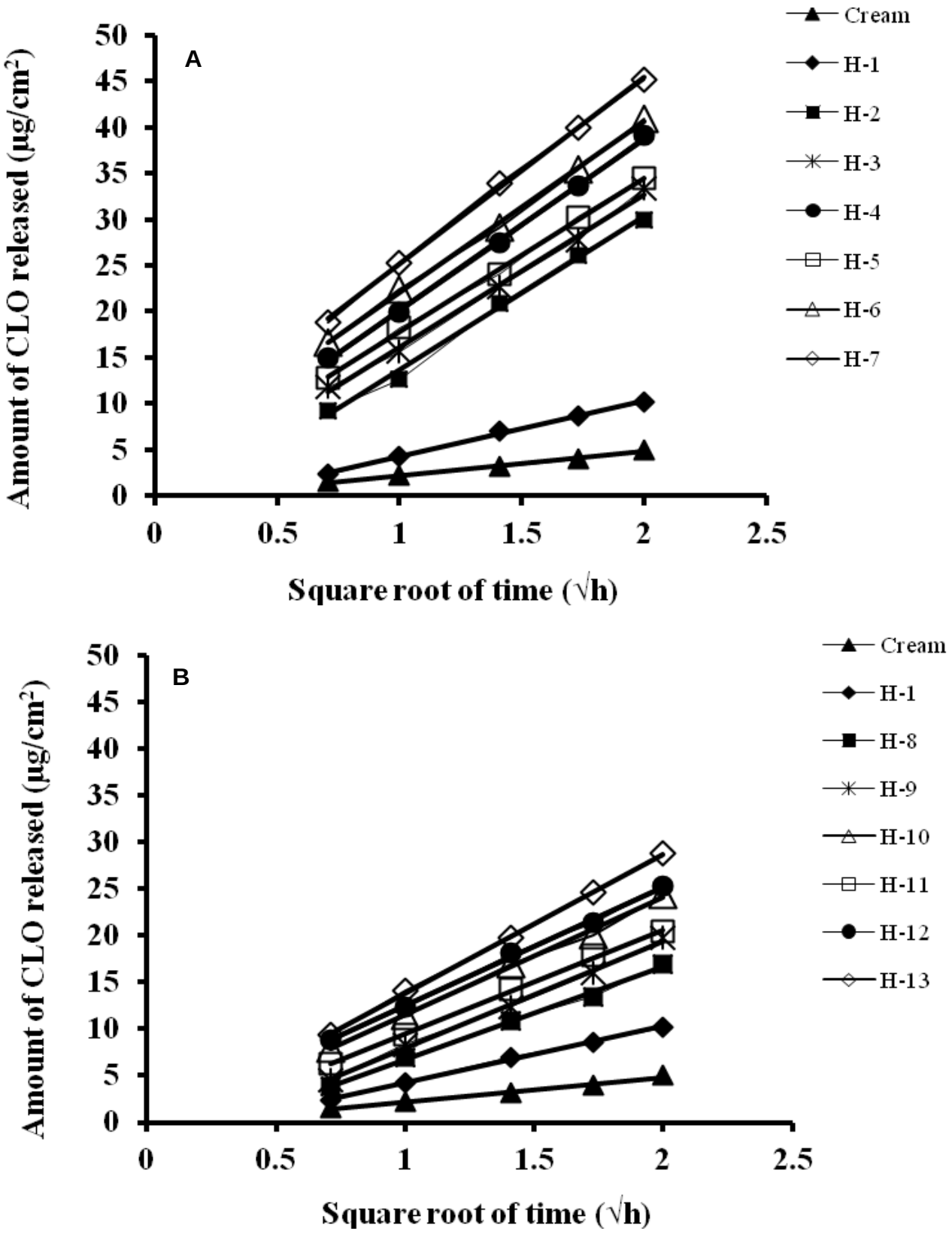

Figure 1. Cumulative amount of CLO released from hydrogels with PAMAM-NH_2 (A) or PAMAM-OH (B) containing dissolved CLO as a function of square root of time.

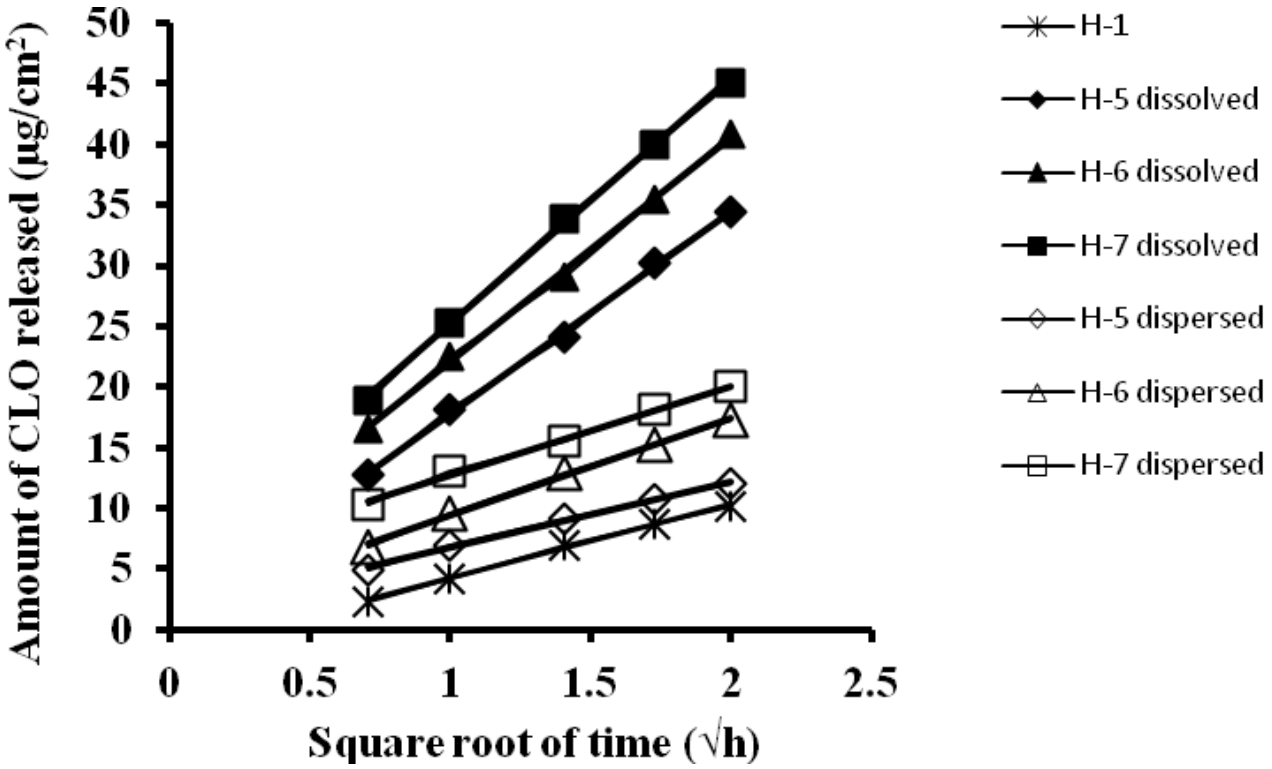

Figure 2. Comparison of cumulative amount of CLO released from hydrogels with PAMAM-NH_2 G3 containing dissolved or dispersed CLO as a function of square root of time.

CLO from hydrogels with CLO in two different molecular states and from water suspensions was studied, because the size and surface charge of dendrimers can influence the permeation of the drug. As shown in Figure 1, PAMAM-NH_2 dendrimers were more potent enhancers of CLO release than PAMAM-OH. After 4 h, the cumulative amount of CLO released from hydrogel with PAMAM-NH_2 G2 (H-4), hydrogel with PAMAM-OH G2 (H-10), hydrogel without PAMAM (H-1) and from the commercially available product was 39.11 ± 0.25, 24.34 ± 0.24, 10.16 ± 0.08 and 5.0 ± 0.05 µg/cm^2, respectively. Moreover, enhancing effect of PAMAM dendrimers on the release of CLO from hydrogels containing dissolved drug was generation and concentration dependent (Figure 1). For example, PAMAM-OH G3 induced 3-fold and PAMAM- from hydrogel with dispersed form of drug) (Figure 2). As it is shown in Figures 1 and 2, the straight plots with correlation coefficient ($R^2 \geq 0.998$) indicate that the data fit Higuchi's equation and the mechanism of release process is simply diffusion of the drug through membrane. Diffusion is a common mechanism controlling release and according to Higuchi's theory, the increase in thermodynamic activity of drug improves diffusion of the drug from topical formulation (Kobayashi et al., 1999).

To examine if dendrimers also affect the diffusion of CLO in dispersed form, the *in vitr o* release from water OH G2 2- fold increase in diffusion of CLO in comparison with hydrogel without PAMAM. The increase in generation number affects growth in size and number of primary and tertiary amines groups available for interaction with the drug and can be responsible for its dissolution enhancement in a vehicle, which in consequence can improve the *in vitro* release of the drug (Devarakonda et al., 2004). Additionally, to examine if diffusion of CLO from hydrogels depends on its molecular state, CLO was dissolved or dispersed in hydrogels with the most potent PAMAM-NH_2 G3 dendrimers. The increase in release rate of CLO was higher from hydrogels with dissolved CLO (PAMAM-NH_2 G3 dendrimers 3.4 to 4.4-fold improved the *in vitro* release of CLO from hydrogel with dissolved and 1.19 to 2-fold suspensions of CLO with PAMAM, without additional excipients was studied. The results revealed that the enhancement in amount of CLO release evoked by PAMAM was size and concentration dependent (Figure 3). However, compared with hydrogels containing a dissolved form of the drug, the improvement in CLO release from water suspensions was definitely lower (PAMAM-NH_2 G3 caused 3.4 to 4.4-fold and 1.5 to 1.8-fold increase in diffusion of CLO from hydrogels with dissolved CLO and from suspensions, respectively).

The most potent enhancers of the *in vitro* release of

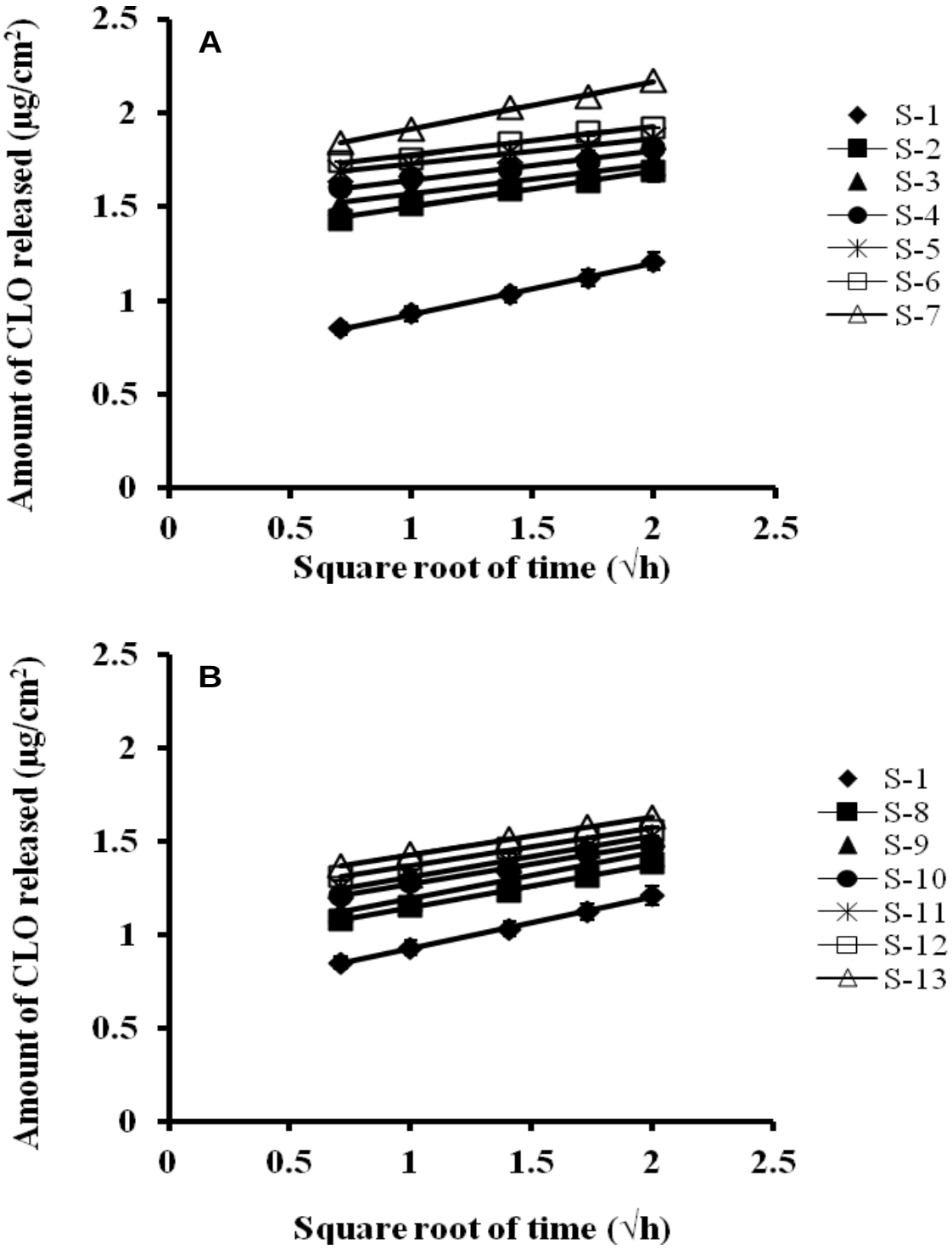

Figure 3. Cumulative amount of CLO released from water suspensions with PAMAM-NH_2 (A) or PAMAM-OH (B) as a function of square root of time.

CLO from hydrogels containing drug in two molecular states and suspensions were PAMAM-NH_2 G3. PAMAM dendrimers as co-solvent can generate saturated solution of the drug in the vehicle, maximize the thermodynamic activity of the drug, and as a result, improve the *in vitro* release of CLO from prepared formulations.

Conclusion

This study shows that PAMAM dendrimers with -NH_2 and -OH surface groups increased the release of CLO from both hydrogels, irrespective of its molecular state and suspensions.

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REFERENCES

Balata G, Mahdi M, Bakera RA (2011). Improvement of solubility and dissolution properties of clotrimazole by solid dispersions and inclusion complexes. Indian J. Pharm. Sci. 73:517-526.

Bilensoy E, Abdur Rouf M, Vural I, Sen M, Atila Hincal A (2006). Mucoadhesive, thermosensitive, prolonged-release vaginal gel for clotrimazole: β-cyclodextrin complex. AAPS PharmaSciTech. 7(2):E54-E60.

Borowska K, Wołowiec S, Rubaj A, Głowniak K, Sieniawska E, Radej S (2012). Effect of polyamidoamine dendrimer G3 an12d G4 on skin permeation of 8-methoxypsoralene – *In vivo* study. Int. J. Pharm. 426:280-283.

Cheng Y, Man N, Xu T, Fu R, Wang X, Wang X, Wen L (2007a). Transdermal delivery of nonsteroidal anti-inflammatory drugs mediated by polyamidoamine (PAMAM) dendrimers. J. Pharm. Sci. 96:595-602.

Cheng Y, Qu H, Ma M, Xu Z, Xu P, Fang Y, Xu T (2007b). Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: an *in vitro* study. Eur. J. Med. Chem. 42:1032-1038.

Devarakonda B, Hill RA, de Villiers MM (2004). The effect of PAMAM dendrimer generation size and surface functional group on the aqueous solubility of nifedipine. Int. J. Pharm. 284:133-140.

Filipowicz A, Wołowiec S (2011). Solubility and *in vitro* transdermal diffusion of riboflavin assisted by PAMAM dendrimers. Int. J. Pharm. 408:152-156.

Hájková R, Sklenářová H, Matysová L, Šrecová P, Solich P (2007). Development and validation of HPLC method for determination of clotrimazole and its two degradation products in spray formulation. Talanta 73:483-489.

Heiden TCK, Dengler E, Kao WJ, Heideman W, Peterson RE (2007). Developmental toxicity of low generation PAMAM dendrimers in zebrafish. Toxicol. Appl. Pharm. 225:70-79.

Henry KW, Nickels JT, Edlind TD (2000). Upregulation of ERG genes in Candida species by azoles and other sterol biosynthesis inhibitors. Antimicrob. Agents Chemother. 44:2693-2700.

Holt RJ (1974). Laboratory and clinical studies with clotrimazole. Postgrad. Med. J. 50(Suppl. 1):24-27.

Hoogerheide JG, Wyka BE (1982). Clotrimazole. In: Florey K (ed.), Analytical Profiles of Drug Substances, Vol. 11. Academic Press, Salt Lake City. pp. 225-255.

Kobayashi N, Saitoh I (1999). Factors influencing the *in vitro* drug release from a liquid droplet dispersion system ointment. Chem. Pharm. Bull. 47:647-651.

Malik N, Wiwattanapatapee R, Klopsch R, Lorenz K, Frey H, Weener JW, Meijer EW, Paulus W, Duncan R (2000). Dendrimers: relationship between structure and biocompatibility *in vitro* and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. J. Control. Release 65:133-148.

Michaels AS, Chandrasekaran SK, Shaw JE (1975). Drug permeation through human skin: Theory and *in vitro* experimental measurement. J. AIChE 21:985-996.

Nanjwade BK, Bechra HM, Derkar GK, Manvi FV, Nanjwade VK (2009). Dendrimers: Emerging polymers for drug-delivery systems. Eur. J. Pharm. Sci. 38:185-196.

Ning M, Gu Z, Pan H, Yu H, Xiao K (2005). Preparation and *in vitro* evaluation of liposomal/niosomal delivery systems for antifungal drug clotrimazole. Indian J. Exp. Biol. 43:150-157.

Pathan IB, Setty CM (2009). Chemical penetration enhancers for transdermal delivery systems. Trop. J. Pharm. Res. 8:173-179.

Pedersen M, Bjerregaard M, Jacobson J, Sorensen AM (1998). A genuine clotrimazole γ-cyclodextrin inclusion complex-isolation, antimycotic activity, toxicity and an unusual dissolution rate. Int. J. Pharm. 176:121-131.

Strydom SJ, Rose WE, Otto DP, Liebenberg W, de Villiers MM (2013). Poly(amidoamine) dendrimer-mediated synthesis and stabilization of silver sulfonamide nanoparticles with increased antibacterial activity. Nanomedicine 9:85-93.

Thakker KD, Chern WH (2003). Development and validation of *in vitro* release tests for semisolid dosage forms–Case study. Dissolut. Technol. 10:10-15.

Winnicka K, Bielawski K, Rusak M, Bielawska A (2009). The effect on generation 2 and 3 poly(amidoamide) dendrimer on viability of human breast cancer cells. J. Health Sci. 55:169-177.

Winnicka K, Sosnowska K, Wieczorek P, Sacha P, Tryniszewska E (2011). Poly(amidoamine) dendrimers increase antifungal activity of clotrimazole. Biol. Pharm. Bull. 34:1129-1133.

Yang Y, Sunoqrot S, Stowell Ch, Ji J, Lee CW, Kim JW, Khan SA, Hong S (2012). Effect of size, surface charge, and hydrophobicity of poly(amidoamine) dendrimers on the skin penetration. Biomacromolecules 13:2154-2162.