Effect of Polyamidoamine (PAMAM) Dendrimers on the In Vitro Release of Water-Insoluble Nifedipine From Aqueous Gels

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ABSTRACT

The objective of this study was to determine the effect of ethylenediamine core PAMAM dendrimers on the release of nifedipine suspended in aqueous gels and to correlate release to the increase in solubility afforded by the dendrimers. Drug release from aqueous 5% HPMC gels containing nifedipine (2% wt/vol) through 0.2-µm membranes was measured using Enhancer cells and 50% ethanolic solution as the receptor medium. The release from gels containing PAMAM G-3 and G-5 (0.25%-1% wt/vol) was compared with gels containing the cosolvent isopropyl alcohol (10%-80% vol/vol). PAMAM dendrimers significantly increased the solubility of nifedipine. This caused a significant increase in the release rate of nifedipine from the gel suspensions. The increase in drug release depended on the concentration and generation size of the dendrimers added. For higher generations (G-5) lower concentrations were needed to obtain equivalent increases in release. Although the increase in solubility and release was not as high as from gels containing high concentrations of the cosolvent isopropyl alcohol, the dendrimers prevented the recrystallization of the drug that was observed when the gels containing isopropyl alcohol were left open.

KEYWORDS: PAMAM dendrimers, Nifedipine, Release, Gel.

INTRODUCTION

Nifedipine (4-(2-nitrophenyl)-2, 6-dimethyl-3, 5-dicarbomethoxy-1, 4-dihydropyridine) (Figure 1) is a calcium channel blocking agent used in the treatment of various cardiovascular diseases.[1] Nifedipine is also used topically for the treatment of severe vascular occlusive wounds, pathologies of the anal canal, acute and chronic fissures, rhagades, spasm, hemorrhoids, and tenesmus, as well as ischemic colotis, bladder tenesmus, and spasm alone or in combination with anti-inflammatory agents and local anesthetics.[2-6] Nifedipine also causes a dose-dependent fall of intraocular pressure, which lasts 4 hours or more after topical administration.[7] The permeability of this lipophilic drug across biological membranes depends on its water solubility and on its lipid-protein partition coefficient in relation to the stratum corneum.[8]

Kondo et al[9] studied the effect of N,N-diethyl-m-toluamide (DEET) and 1-dodecylazacycloheptan-2-one (Azone), on the skin permeability of nifedipine, taking into account their effects on the thermodynamic activity of the drug. Azone exerted a genuine effect on the skin and produced marked improvement in the penetration of the drug but the effect of DEET was interesting because it exhibits excellent solubilizing properties and penetrates the skin easily. Accordingly, it was concluded that DEET functions simply as a cosolvent to produce saturated or supersaturated solutions of the drug. This increases the disappearance of the drug from the vehicle, and thereby maximizes the thermodynamic activity of the drug.

Recently a new class of macromolecules, the polyamidoamine (PAMAM) dendrimers, has received much attention for their ability to solubilize water-insoluble drugs and their ability to promote the transport of drugs across biomembranes.[10-13] In one study an efficient transdermal drug delivery system (TDDS) consisting of a polyhydroxyalkanoate (PHA)-based system with a polyamidoamine dendrimer was examined for the transdermal delivery of tamsulosin.[14] The dendrimer was found to act as the weak enhancer. By adding the dendrimer, the dendrimer-containing PHA matrix achieved the clinically required amount of tamsulosin permeating through the skin model.

In another study, the transdermal delivery of aqueous formulations of indomethacin, a model drug, with different concentrations of 3 types of dendrimer showed a linear increase in flux with increasing concentration of each of the dendrimers.[10] This result was in contrast to phase solubility, where Higuchi's A_N profiles were observed.[10] The steady-state flux of the drug increased significantly and was highest with the G4-NH_2 dendrimer at 0.2% wt/vol, which

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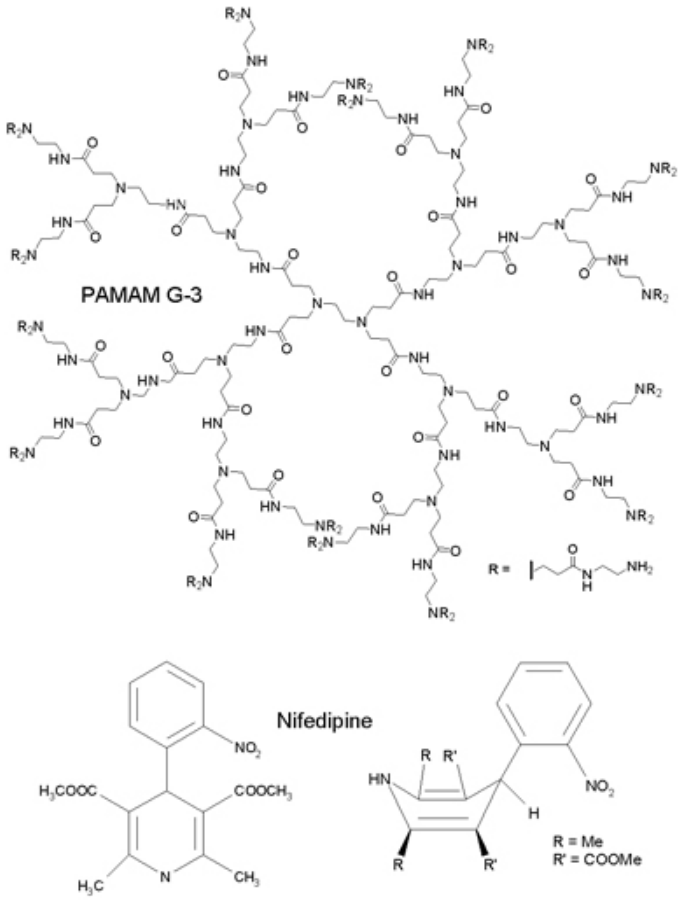

Figure 1. Chemical structures of PAMAM G-3, nifedipine, and a 3-dimensional representation of the nifedipine molecule.

showed an enhancement factor of 4.5 compared with the pure drug suspension. In vivo, a steady-state flux was achieved in 5 hours, and the C_{max} values were significantly higher with PAMAM G4-NH_2 and PAMAM G4-OH dendrimer formulations. Purohit et al[15] reported the interaction between cationic dendrons and albumin and their enhanced diffusion through cellulose membranes. PAMAM dendrimers have also been used as ophthalmic vehicles with prolonged drug residence time for ocular delivery of pilocarpine and tropicamide.[16]

A major problem associated with the successful formulation of effective dosage forms containing nifedipine is its poor aqueous solubility, 5 to 6 µg/mL over a pH range of 4 to 13, which may account for its low and irregular bioavailability in humans.[17,18] Recently we reported the increase in the solubility of nifedipine when combined with PAMAM dendrimers.[19] Based on the results of this study, the objective of the present study was to determine the effect of ethylenediamine core PAMAM dendrimers on the release of nifedipine suspended in aqueous gels and to correlate release to the increase in solubility afforded by the dendrimers.

Materials and Methods

Materials

Nifedipine and G-5 PAMAM dendrimer were purchased from Sigma Chemical Co (St Louis, MO). Ethylenediamine, methylacrylate, methanol (high-performance liquid chromatography [HPLC] grade), ethanol, sodium dihydrogen phosphate, citric acid, tromethamine, sodium borate, potassium chloride, and standard pH buffers were obtained from Spectrum Chemical Co (Gardena, CA). Amine terminated full generation dendrimers (G-3) were synthesized as described earlier (Devarakonda et al[19]). For solubility studies, HPLC analysis, and release measurements distilled deionized water was used.

HPLC analysis of nifedipine

Nifedipine in solubility samples was analyzed by HPLC (Spectrum System, AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of detection λ_{max} = 254 nm. Chromatographic separation was performed using a C_{18} column (Econosil, 5-µm particles, 250 × 4.6 mm, Alltech, Deerfield, IL). The mobile phase was methanol:water (2:1 vol/vol); flow rate 0.7 mL/min; injection volume 20 µL. The retention time for nifedipine was 11 minutes and the limit of detection was 1.0 ng/mL. Results are the mean of 3 analyses. Throughout the study, solutions were protected from light. Standard curves were constructed from plots of the peak areas of solutions containing known amounts of nifedipine vs concentration ($y = 118873x + 76849$, $R^2 = 0.9998$).

Solubility measurements

Solubility studies were performed using the Higuchi rotating bottle method.[20] The solubility of nifedipine in aqueous isopropyl alcohol solutions (1% to 80% vol/vol) was determined by adding an excess of nifedipine to 3-mL solutions in 5-mL screw-capped amber colored vials containing increasing amounts of the dendrimers. The vials were rotated at 60 rpm while being kept at 30 ± 1.0ºC. Preliminary experiments indicated that 6 hours provided sufficient time to reach equilibrium. After 6 hours, samples were filtered through 0.45-µm cellulose acetate filters (Osmonics Inc, Minnetonka, MN), diluted appropriately with the mobile phase and analyzed by HPLC. Measurement of pH at the end of the solubility studies showed no significant alterations in the pH of the medium. Phase solubility diagrams were constructed by plotting the molar concentrations of nifedipine (solubility) vs molar concentration of dendrimers.

Preparation of nifedipine gels

The 2% nifedipine aqueous gels were prepared as follows: 200 mg of nifedipine was suspended in water, the amount of appropriate enhancer (different concentrations of G-3 and G-5 dendrimers and isopropanol) and gelling agent added, and the volume adjusted to 10 mL. These suspensions were gelled with 5% wt/vol hydroxy propyl methyl cellulose (HPMC). The resulting gels were sonicated for 30 minutes and stored at room temperature for 24 hours before use. To determine the amount of nifedipine in the gels, 10-mg samples of the gels were placed in 50-mL volumetric flasks, diluted to volume with methanol, and stirred for 30 minutes. The samples were then filtered using 0.45-µm cellulose acetate filters (Osmonics Inc, Minnetonka, MN) and analyzed.

In vitro release studies

Nifedipine release from the different gels was measured through 0.2-µm cellulose nitrate membranes (Osmonics Inc, Minnetonka, MN) using an Enhancer cell (Van Kel Industries, Cary, NC). The enhancer cell used in this study consisted of a Teflon load ring, a cap, a membrane, and a drug reservoir. About 0.5 g of gel was placed in the drug reservoir (2-cm diameter) on top of the membrane making certain that no entrapped air was present at the interface of the gel and the membrane. A USP Apparatus 2, Dissolution Tester (Vanderkamp 600, Van Kel Industries) with standard 900-mL vessels and paddles was used to measure the release of nifedipine from the enhancer cell assembly. The receptor compartment was filled with 500 mL of 50% ethanol in water maintained at 37°C and stirred at 100 rpm. At predetermined time intervals, 5-mL aliquots were removed and replaced with the same volume of 50% ethanolic solution. The amount of nifedipine released was determined spectrophometrically at λ_{max} 240 nm ($y = 0.0535x + 0.076$, $R^2 = 0.9991$). The steady-state flux (J_{ss}) was obtained from the linear portion of the cumulative drug permeated vs time graphs. The permeability coefficient was calculated from the equation $K_p = J_{ss}/C_s$, where C_s is the saturation solubility of the drug in the vehicle. An enhancement factor was calculated from the equation: Enhanced flux/Un-enhanced flux.

Particle size analysis

Samples from the gels were studied under an inverted light optical microscope (model PIM-R, World Precision Instruments, Sarasota, FL). The gels were spread out on a glass microscope slide, covered with a coverslip, and photographs taken using an Olympus C-3040 digital camera (Olympus, Melville, NY). A Galai-Cis-1 particle size analyzer (Ashkelon, Israel) was used to measure the particle size distributions in the gels. This instrument uses a unique time-size mapping called the Time-of-Transition theory to directly measure particle size. Particle sizing on this instrument is done by a dual discipline analysis, integrating laser diffraction and image analysis. Samples from the gels before and after sonication and after release suspended in water were placed in small cuvettes and fitted into the analyzer. A small magnetic stirrer inside the cuvette prevented sedimentation of the particles during the measurement. The acquired data were used to construct particle size distribution graphs and to compute the mean volume particle size $D[4,3] = \Sigma d^4/\Sigma d^3$ where d is the diameter of individual particles.

Statistical analysis

The mean values of the release rates of nifedipine from the gels were compared for significant differences using 1-way or 2-way analysis of variance (ANOVA) for single-factor and 2-factor comparisons (SPSS 10.0 for Windows, SPSS, Chicago, IL). The differences between the 2 sample means were observed by pair-wise comparisons using a least significant difference (LSD) test performed.

Results and Discussion

Nifedipine solubility

Nifedipine is a weak acid with a pK_a of 13 (Figure 1); its solubility in Tris-buffers over a range of 4 to 10 is $1.28 (\pm 0.20) \times 10^{-5}$ M (mean ± SD, $N = 21$) (Devarakonda et al[19]). In the presence of full-generation PAMAM and

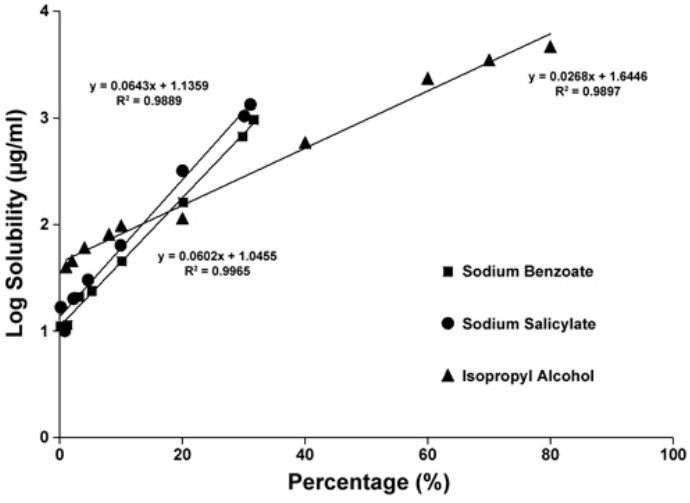

Figure 2. Equilibrium solubility of nifedipine in solutions containing increasing concentrations of the hydrotropic agents sodium benzoate and sodium salicylate[21] and isopropyl alcohol determined experimentally.

Table 1. In Vitro Permeability Parameters of Nifedipine Across Cellulose Nitrate Membranes From Nifedipine Gels Prepared With Different Concentration of Dendrimers and Isopropanol (Iso)

	Flux, J_{ss} (μg/cm^2 × min)	Calculated K_p (× 10^{-5}, cm/min)	Enhancement factor, EF	Measured C_s (μg/mL)
Drug suspension	0.021 ± 0.001	284.8	1.0	7.4 ± 0.6
G3%-0.5%	0.038 ± 0.002	370.5	1.8	10.3 ± 1.3
G3%-1%	0.058 ± 0.008	52.2	2.7	111.8 ± 7.8
G5%-0.25%	0.029 ± 0.003	299.3	1.4	9.8 ± 1.2
G5%-0.5%	0.046 ± 0.008	292.9	2.2	17.5 ± 2.2
Iso-10%	0.044 ± 0.003	72.2	2.1	60.6 ± 5.9
Iso-20%	0.090 ± 0.010	79.2	4.3	114.0 ± 6.2
Iso-40%	0.117 ± 0.016	20.1	5.5	585.1 ± 11.1
Iso-60%	0.160 ± 0.034	6.8	7.6	2341.1 ± 14.4
Iso-70%	0.245 ± 0.012	7.0	11.6	3504.5 ± 19.9
Iso-80%	0.336 ± 0.041	7.2	15.9	4668.2 ± 17.7

half-generation PAMAM dendrimers G-1 to G-3 at pH 7, the solubility of nifedipine increased linearly with an increase in dendrimer concentration. The solubility profiles observed can be classified as A_L-type diagrams (Higuchi and Connors[20]) indicating soluble complexes between nifedipine and the dendrimers with 1:1 stoichiometries.

Solubility studies in solutions containing increasing concentrations of a cosolvent isopropyl alcohol and the hydrotropic agents sodium salicylate and sodium benzoate (Figure 2) showed that these solubilizing agents increased the solubility of nifedipine in a log-linear manner, typical of cosolvent and hydrotropic solubilization.[21] This increase in solubility did not follow the same pattern as that obtained with the dendrimers.[19] Dendrimer solubilization correlated better with that achieved with complexation agents such as cyclodextrins and substituted phenolic ligands, suggesting that dendrimers increased the solubility of nifedipine by complexation.

Table 2. Change in Mean Volume Particle Size of Nifedipine in HPMC Gels After Sonication and After Release From the Gels for 6 Hours*

Product	Mean volume particle size (μm)	
	After sonication	After release
Nifedipine	24.86 ± 2.38	15.97 ± 2.15
10% Isopropanol	23.44 ± 2.86	29.32 ± 3.11
20% Isopropanol	28.26 ± 3.01	
40% Isopropanol	36.23 ± 4.28	
60% Isopropanol	40.82 ± 3.42	
80% Isopropanol	44.26 ± 3.86	
0.25% G-5	19.86 ± 3.51	15.32 ± 2.47
0.50% G-5	20.11 ± 3.22	12.86 ± 2.12
0.50% G-3	20.21 ± 2.45	10.62 ± 1.23
1.0% G-3	17.19 ± 1.88	8.62 ± 1.32

*The initial particle size of the nifedipine was 46.01 ± 3.25 μm.
− indicates not measurable.

Before the release studies were conducted, the solubility of nifedipine in the gel formulations was determined. Table 1 shows the saturated solubility (C_s) of the drug in the gels in the presence of the dendrimers and isopropyl alcohol. Quantitative analysis of the gels for nifedipine content showed that the drug content of all the formulations was within the acceptable range (100% ± 5% wt/wt).

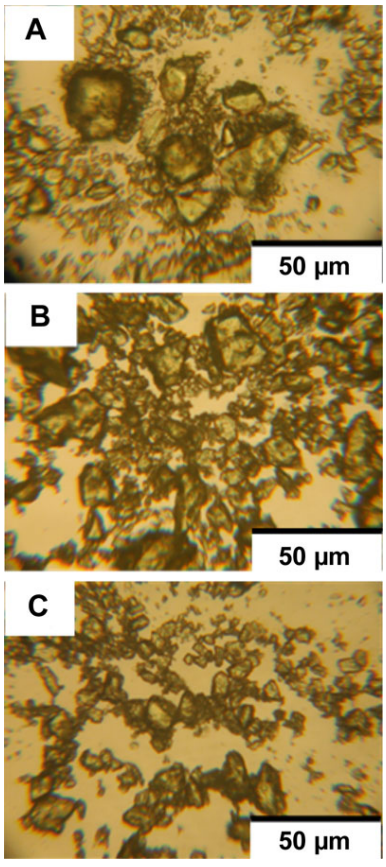

Figure 3. Photomicrographs of HPMC gels showing nifedipine crystals after (a) preparation of the gel, (b) after sonication, and (c) after release.

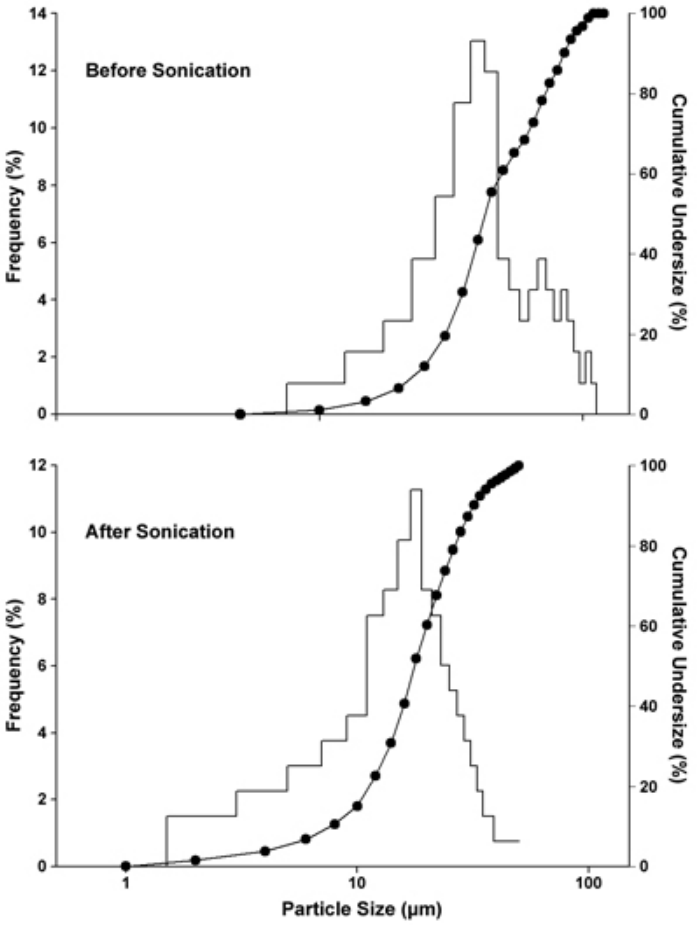

Figure 4. Mean volume particle size distributions of nifedipine in HPMC gel before and after sonication.

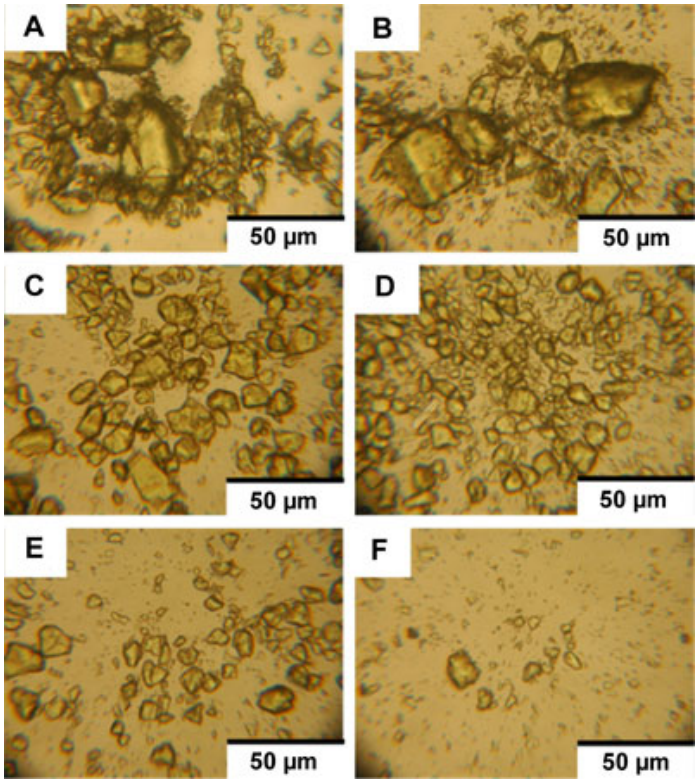

Figure 5. Photomicrographs of HPMC gels containing G-5 at 0.25% (left) and 0.5% (right) showing nifedipine crystals after preparation of the gels (A and B), after sonication (C and D), and after release (E and F).

Nifedipine particle size changes in gels

Since nifedipine is practically insoluble, less than 1 μg/mL, in aqueous media the drug formed suspensions in the 5% HPMC gels. The particle sizes of the crystals in the gels are listed in Table 2, and in Figure 3 photomicrographs of the crystals in an aqueous gel are shown. Sonication reduced the particle size and after 6 hours release was there was a further slight decrease in size of the remaining nifedipine crystals (Figure 4).

In contrast, the addition of the dendrimers significantly decreased the size of the suspended crystals after sonication (as shown for PAMAM G-5 in Figure 5) and after the release studies were completed most of the smaller crystals have dissolved. The addition of 1% PAMAM G-3 produced a suspension from which the fewest number of crystals remained in the gels after release. Solubility and particle size decreased, from the largest to the smallest, in the order of nifedipine > G-5 (0.25%) > G-3 (0.5%) > G-5 (0.5%) > G-3 (1%). The order in which the particle size decreased (Figure 6) correlated with the increase in the solubility of the drug in the gels (Table 1).

As shown in Figure 7, the particle size in the gels prepared with increasing concentrations of the cosolvent isopropyl alcohol decreased even more after sonication. Except for the gel containing 10% isopropyl alcohol, there were so few crystals left in the gel that it was not possible to measure the particle size (Table 2). For these

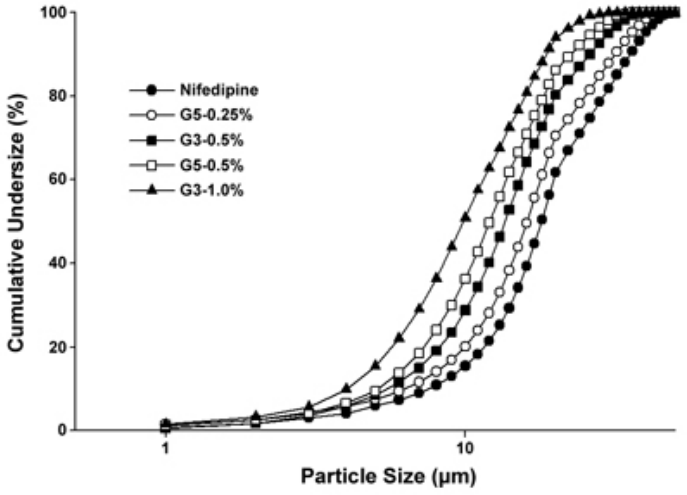

Figure 6. Mean volume particle size distributions of nifedipine in HPMC gels containing dendrimers measured after release study.

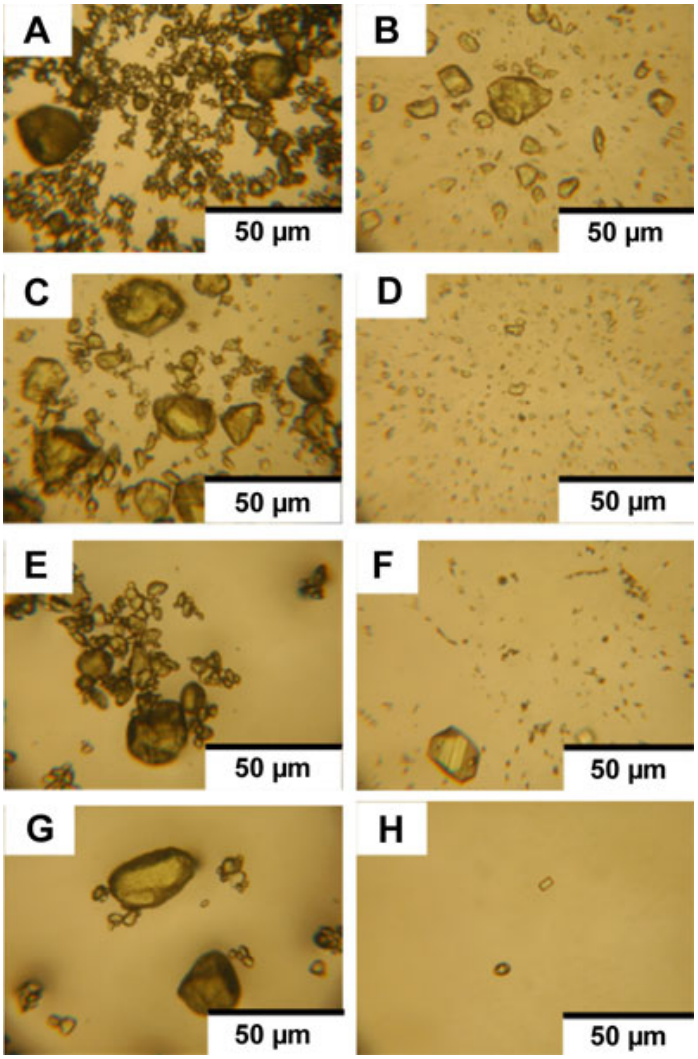

Figure 7. Photomicrographs of nifedipine crystals in HPMC gels containing from top to bottom 10% (A and B), 20% (C and D), 40% (E and F), and 80% (G and H) isopropanol. The pictures on the left were taken after sonication and on the right after 6-hour release.

gels there was a poor correlation between the change in particle size and the solubility. The solubility of the drug increased in a log-linear (exponential) manner with an increase in isopropyl alcohol concentration (Figure 2). However, the particle size increased because when most of the smaller particles dissolved in the isopropyl alcohol, only larger particles were left in the gels. Therefore, the number of particles per volume of gel decreased but the mean particle size increased as shown in Figure 8.

In vitro release of nifedipine from gels

The amount of nifedipine permeated with time from the different gels is shown in Figure 9. From these plots, the corresponding steady-sate flux (J_{ss}), permeability coefficient (K_p), enhancement factor (EF), and amount of drug permeated at the end of 6 hours (Q) for all the formulations were calculated (Table 1). These results suggest that the presence of dendrimers significantly enhanced the amount of nifedipine released from the gel preparations. The steady-state flux of nifedipine observed at 0.5% and 1% wt/vol of G-3 was 0.038 and 0.058 µg/cm^2/min, respectively (Table 1). At 0.25% and 0.5% wt/vol of G-5, the steady-state fluxes were 0.029 and 0.046 µg/cm^2/min, respectively. The solubility of nifedipine was increased by 1.4- and 15-fold with 0.5% and 1% wt/vol of G-3 and 1.5- and 2.4-fold with 0.25% and 0.5% wt/vol of G-5 dendrimer.

In the gels the dendrimers may function as solubility enhancers by virtue of electrostatic interactions in addition to hydrogen bonding and molecular encapsulation in the crevices of the dendrimeric network.[10,19] The resultant solubility enhancement allows the presentation of the drug to the receptor medium in a more diffusible form, such as mechanisms reported for cyclodextrin that have been widely investigated as penetration enhancers for topical use.[22,23] The enhanced steady-state flux of nifedipine in the presence of dendrimers, therefore, could be due to the greater solubility of the drug in the presence of dendrimers.

The amount of nifedipine released increased with increasing concentrations of dendrimers (Table 1). This could be due to increased solubility of the drug with increasing concentrations of dendrimers. At similar concentrations of dendrimers (0.5%), significantly higher flux was observed with G-5 dendrimer than that for G-3 dendrimer. The G-3

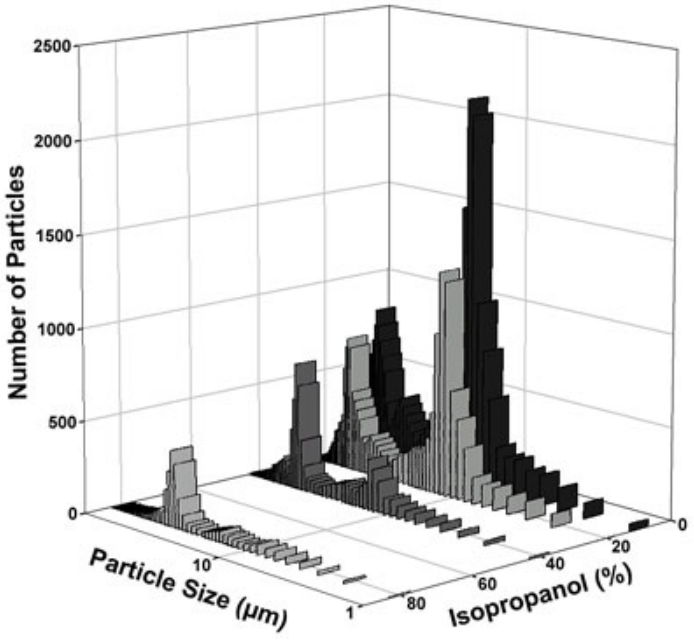

Figure 8. Mean volume particle size distributions of nifedipine in HPMC gels containing increasing concentrations of isopropanol after the gels were sonicated for 30 minutes.

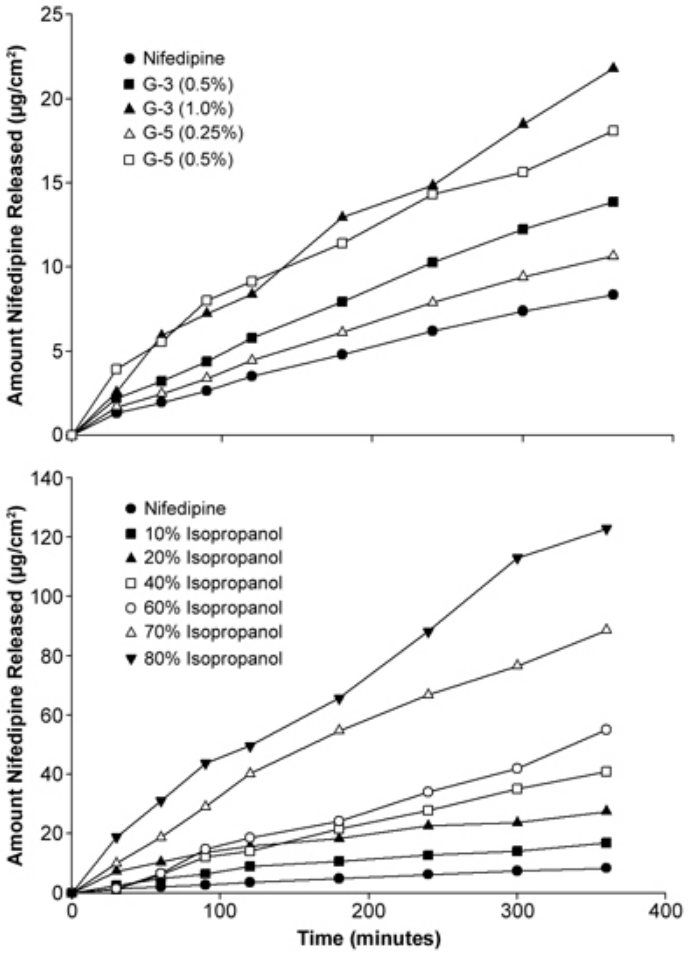

Figure 9. Amount of nifedipine per unit area released from the gels prepared with enhancers: dendrimers (top) and isopropanol (bottom).

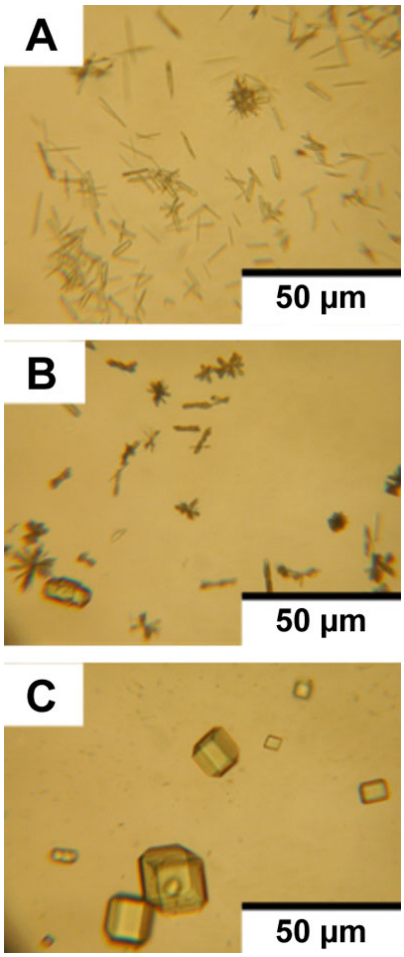

Figure 11. Recrystallization of nifedipine from HPMC gels containing 70% isopropyl alcohol when left open to the atmosphere: Top: a few minutes after exposure; Middle: approximately 15 minutes after exposure; and Bottom: approximately 30 minutes after exposure.

dendrimer has a diameter of 3.6 nm with 32 surface primary amines, whereas the G-5 dendrimer has a diameter of 5.7 nm with 128 surface primary amines.[24] The increased size and number of primary and tertiary amines available for interaction with increased generation size are responsible for the increased solubility and subsequent enhanced flux. However, except at 1% wt/vol of G-3, the permeability coefficients of nifedipine in the presence of dendrimers was not significantly lower than that for the free drug suspension. This indicates the possible permeation of both free drug as well as complexed drug through the membrane since the porosity of the membrane was 200 nm.[10]

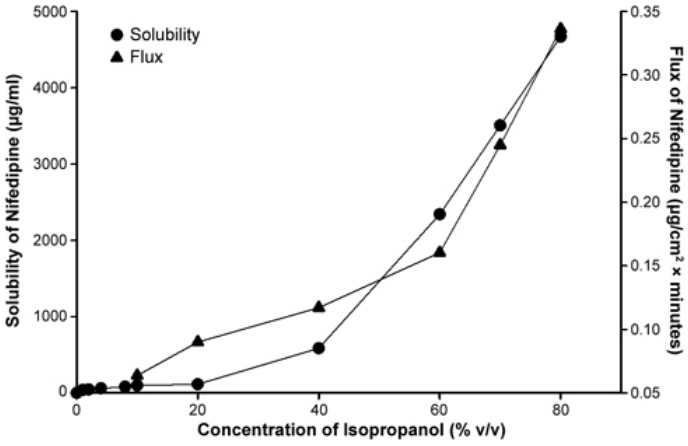

Figure 10. Solubility and steady-state flux of nifedipine as a function of concentration of isopropanol.

The gel formulations prepared with various concentrations of isopropanol also showed enhanced steady-state flux of nifedipine. Vehicles similar to isopropanol such as ethanol and polyethylene glycol are known to enhance the skin permeability of drugs and are recommended for use in various topical formulations.[25,26] In the present study, the observed increase in the nifedipine permeability with isopropanol, therefore, could be due to enhanced thermodynamic activity of the drug by isopropanol. Both the solubility and steady-state flux of nifedipine increased

exponentially as a function of concentration of isopropanol in the gel formulations (Figure 10).

Compared with the gel formulations prepared with isopropanol, the steady-state flux of nifedipine prepared with dendrimers as permeation enhancers was significantly lower (Table 1). From extrapolation of the linear portion of steady-state flux of nifedipine vs isopropanol concentration ($y = 0.0018x + 0.0521$, $R^2 = 0.995$) it was found that only the flux observed with G-3 dendrimer at 1% wt/vol concentration showed an isopropanol equivalent concentration of 3.5% vol/vol isopropyl alcohol. However, the gels containing high concentrations of isopropyl alcohol were thermodynamically unstable since it contained saturated or super-saturated solutions of the drug. When these gels were left open for a short time, less than 10 minutes, isopropyl alcohol started to evaporate and this caused crystallization of nifedipine. Crystallized needles quickly appeared in the gels as shown in Figure 11. These needle crystals quickly transformed into large rhombic crystals, nifedipine Form I.[27] Crystallization was not observed in the dendrimer gels when left open for up to 7 days.

CONCLUSION

PAMAM dendrimers significantly increased the release rate of nifedipine from suspensions in aqueous gels. The increase in permeability depended on the concentration and generation size of the dendrimers added. For higher generations (G-5) lower concentrations were needed to obtain equivalent increases in release. The main reason for the increased release was the increase in nifedipine solubility in the presence of the dendrimers. Although the increase in solubility and release was not as high as what was observed for gels containing high concentrations of the cosolvent isopropyl alcohol, the dendrimers prevented the recrystallization of the drug that was observed when gels containing this cosolvent were left open.

ACKNOWLEDGMENTS

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