International Journal of Pharmaceutics 398 (2010) 185–189

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

PAMAM dendrimers as solubilizers and hosts for 8-methoxypsoralene enabling transdermal diffusion of the guest

Katarzyna Borowska[a], Barbara Laskowska[a], Agnieszka Magoń[b], Bogdan Mysliwiec[b], Marek Pyda[c], Stanisław Wołowiec[a,*]

[a] Department of Cosmetology, University of Information Technology and Management in Rzeszów, 2 Sucharskiego Str., 35-225 Rzeszów, Poland
[b] Faculty of Chemistry, Rzeszów University of Technology, 6 Powstańców Warszawy Ave., 35-959 Rzeszów, Poland
[c] Department of Pharmacy, Poznań University of Medical Sciences, 61-701 Poznań, Poland

ARTICLE INFO

Article history:
Received 15 March 2010
Received in revised form 8 July 2010
Accepted 13 July 2010
Available online 22 July 2010

Keywords:
Psoralene
PAMAM dendrimers
Host–guest complexes
Transdermal permeation

ABSTRACT

PAMAM dendrimers form host–guest complexes with 8-methoxypsoralene (8-MOP) – the photosensitizer for PUVA therapy. The stoichiometry of complexes was studied by ^{1}H NMR spectroscopy in solution and by differential scanning calorimetry in neat mixtures containing 8-MOP and dendrimers of generations **G2.5**, **G3**, **G3.5**, and **G4**. The dendrimers showed solubilization effect for 8-MOP resulting in increase of 8-MOP concentration in methanol up to 15 molecules of 8-MOP per **G2.5** and **G3** and 30 molecules of 8-MOP per **G3.5**, and **G4**. Isolation of oily host–guest complexes containing 3 or 7 molecules of 8-MOP per **G3** and **G4**, respectively corroborate well with DSC results; glass transition temperature of neat host–guest complexes increases with number of host molecules in comparison with **G3** or **G4**, until the capacity of host is exceeded. The oily host–guest complexes of stoichiometry 3:1 and 7:1 of 8-MOP to **G3** and **G4**, respectively are well soluble in water. The 3:1 host–guest complexes diffused slowly through polyvinyldifluoride and pig ear skin membranes, when released from o/w emulsion. The host–guest complex 8-MOP-**G3** was proposed as convenient formulation for psoralene skin administration in PUVA therapy.

1. Introduction

Dendrimeric polymers are molecules of strictly defined molecular weight, shape and size. The polyamidoamine dendrimers (PAMAM) were synthesized at the beginning of this century (Tomalia et al., 2003; Tomalia, 2005). The PAMAM dendrimers were obtained by alternate addition of methyl acrylate to amine groups and condensation of ester group with diamine to form amide bond, beginning from diamine core. Thus the first generation PAMAM dendrimer; **G1** has 8 surface amine groups, which are able to bind 16 equivalents of methyl acrylate to give dendrimer **G1.5**. The latter can be converted into **G2** by condensation with excess of diamine. When diamine used in synthesis is ethylenediamine, the **G3** and **G4** are globular molecules of *ca.* 7 and 14 kDa molecular weight, 3.5 and 4.6 nm diameter and posses 32 and 64 surface amine groups, respectively, which render them perfectly soluble in water. PAMAM dendrimers were proven to be transdermal and able to pass the cell membrane barrier and therefore they were attempted as vehicles to transport several water-insoluble drugs into cell (Svenson, 2009) like *ibuprofen* (Kolhe et al., 2003), *indomethacin* (Chauhan et al., 2004), *flurbiprofen* (Asthana et al., 2005), *methotrexate* (Kukowska-Latallo et al., 2005), *tamsulosin* (Wang et al., 2003), *niclosamide* (Devarakonda et al., 2005), *doxorubicin* (Papagiannaros et al., 2005), and many others (D'Emanuele and Atwood, 2005). The PAMAM dendrimers are transdermal, biodegradable macromolecules which can be used as drug carriers (Hans and Lowman, 2002).

Therefore we are attempting to use them for deep skin administration of *psoralens*, which are photosensitizers for treatment of hyperproliferative skin diseases like psoriasis, vitiligo, mycosis fungoides, atopic eczema and many others (Saïd et al., 1997). The treatment of skin by UVA irradiation after oral or topical administration of *psoralene* (in PUVA therapy the 8-methoxypsoralene, **1** is used mostly) causes phototoxic and other side-effects such as gastrointestinal, glaucoma and increased risk of carcinogenesis (Stern et al., 1997). Thus the decrease of skin load of *psoralene* is critical for PUVA therapy. Here we present the results on simple host–guest chemistry between PAMAM dendrimers and **1** in solution and in neat dendrimers **G2.5**, **G3**, **G3.5**, and **G4** studied by the ^{1}H NMR and differential scanning calorimetry (DSC) methods in order to establish the proper formulation of the drug (Craig and Reading, 2007). Preliminary in vitro studies of diffusion through artificial model

* Corresponding author. Tel.: +48 17 8551453; fax: +48 17 8661222.
E-mail address: swolowiec@wsiz.rzeszow.pl (S. Wołowiec).

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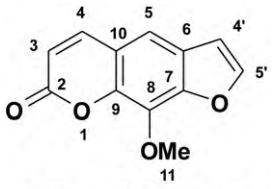
doi:10.1016/j.ijpharm.2010.07.019

membrane (polyvinyldifluoride) and pig ear skin membrane of **1** absorbed in **G3** were also performed.



8-Methoxypsoralen, 1

2. Material and methods

2.1. Reagents

Psoralene (**1**, MW = 216.2 g/mol, m.p. = 142–148 °C, Xanthotoxin, 99% purity from Fluka) was used as received. All solvents and reagents were of reagent grade purity (Aldrich) and used without purification prior to synthesis or measurements.

2.2. Syntheses

2.2.1. PAMAM dendrimers

PAMAM dendrimers of generation 2.5, 3, 3.5, and 4 (**G2.5**, **G3**, **G3.5**, and **G4**, respectively) on ethylenediamine core were synthesized according to the published method by alternate addition of methyl acrylate to **Gn** and condensation of ethylenediamine with **Gn,5** (Tomalia et al., 2003). The dendrimers were characterized by the ^{1}H and ^{13}C NMR spectra in methanol-d_4 using standard 1D and COSY, HMBC, and HSQC 2D methods with 500 MHz Bruker UltraShield spectrometer.

2.3. Solubilization of *1* in methanol and water containing host dendrimers

The solubility of **1** in methanol-d_4 estimated by addition of reference chloroform into the saturated solution of **1** is 0.0198 mol dm^{-3}. The assignment of ^{1}H and ^{13}C resonances has been done based upon 1D and 2D NMR experiments.

^{1}H NMR: 8.00 (1H, H^4, d, $J_{3,4}$ = 9.6 Hz), 7.87 (1H, H$^{5'}$, d, $J_{4',5'}$ = 2.2 Hz), 7.52 (1H, H^5, s), 6.93 (1H, H$^{4'}$, d), 6.36 (1H, H^3, d), 4.23 (3H, H^{11}, s).

^{13}C NMR: 162.9 (C^2), 149.2 (C^7), 148.7 (C$^{5'}$), 146.8 (C^4), 144.3 (C^9), 134.0 (C^8), 128.2 (C^6), 118.2 (C^{10}), 115.2 (C^3), 115.0 (C^5), 108.1 (C$^{4'}$), 61.9 (C^{11}).

The solubilization of **1** in methanol was studied by simple addition of solid **1** into the 700 μL solution of **Gn** or **Gn,5** of variable concentration in methanol-d_4 in the presence of known amount of chloroform as concentration reference or 700 μL solution of Gn in D_2O with *tert*-butanol reference. Solubilization of **1** in water was time consuming and occurred within 4 h only when elevated temperature (80 °C) and vigorous mixing of crystalline **1** and 0.1 molar aqueous solution of **G3** were applied. The examination of the ^{1}H NMR spectrum showed the chemical conversion of **1** and partial decomposition of **G3**.

2.4. Differential scanning calorimetric studies on PAMAM-*1* stoichiometry

Glass transition temperature T_g was examined using standard mode of heat-flux differential scanning calorimeter DSC, Q1000^{TM} from TA Instruments, Inc., equipped with a mechanical refrigerator from temperatures 183.15 K (−90 °C) to 393.15 K (120 °C) (dry nitrogen gas with a flow rate of 50 mL min^{-1} was purged through the DSC cell in the instrument. Cooling was accomplished with a refrigerated cooling system). Samples of host–guest complexes were prepared by dissolving of **Gn** or **Gn,5** in methanol followed by addition of known amount of **1** and extensive vacuum evaporation of the solvent. The oily samples exhibited T_g higher than neat dendrimers until capacity of host was exceeded. At that moment the separation of **1** and dendrimer occurred, accompanied by appearance of crystalline **1** after evaporation of solvent and return of T_g of sample into original value of dendrimer. The values of obtained T_g are collected in Table 1.

Table 1
Glass transition temperatures [K] for **Gn** dendrimers and mixtures of **Gn** and **1**.

1:Gn ratio	**G2.5**	**G3**	**G3.5**	**G4**
0:1	229	253	224	248
1:1	237	270	244	257
2:1	239	272	245	258
3:1	241	274	246	259
4:1	243	253	251	260
5:1	238		243	262
6:1	239		229	265
7:1	238		223	269
8:1	228			250

2.5. In vitro permeation of *G3–1* complexes

Permeation of **G3–1** complexes was studied using Franz diffusion assembly (Thermo Scientific (UK) model DC 600 equipped with 6 cm^3 acceptor compartments). The o/w emulsion was used as donor. The emulsion was prepared using cetyl alcohol (2.0 g) and polysorbate 60 (1.0 g) as emulsifiers and liquid paraphine (1.0 g), vaseline (5.0, g), and water (15.0 g). The emulsion samples containing **G3–1** complex were prepared typically as follows: **G3** (200 mg) was dissolved in 10 ml methanol, then 17.2 mg of **1** dissolved in 10 ml methanol was added, the solvent was removed under reduced pressure, and resulting oil was added to 1.0 g of emulsion, and stirred firmly to homogenize sample. For permeation study *ca.* 250 mg samples were mounted over commercial polyvinyldifluoride (PVDF) membrane or prepared pig ear skin (PES) membrane. The receptor medium was 0.1 M phosphate buffer pH = 7.4: ethanol 7:3 (v/v) as in (Fang et al., 2008). The progress of diffusion was monitored spectrophotometrically at 302 and 246 nm wavelength using the extinction coefficients calculated for the solution of **1** in receptor solution (Fig. 1). The receiving solution was stirred magnetically with 1000 rpm at 32 °C. The 10 ml aliquots of receptor solution were taken at 0.5 h or longer time intervals and the receiver compartment was filled with new 6 ml portion of receptor solution. The experiments were conducted until 25% of initial amount of **1**

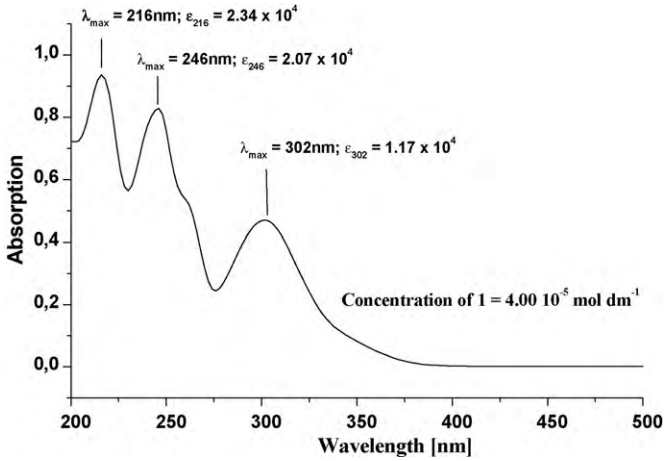


Fig. 1. The UV–vis spectrum of **1** in receptor solution.