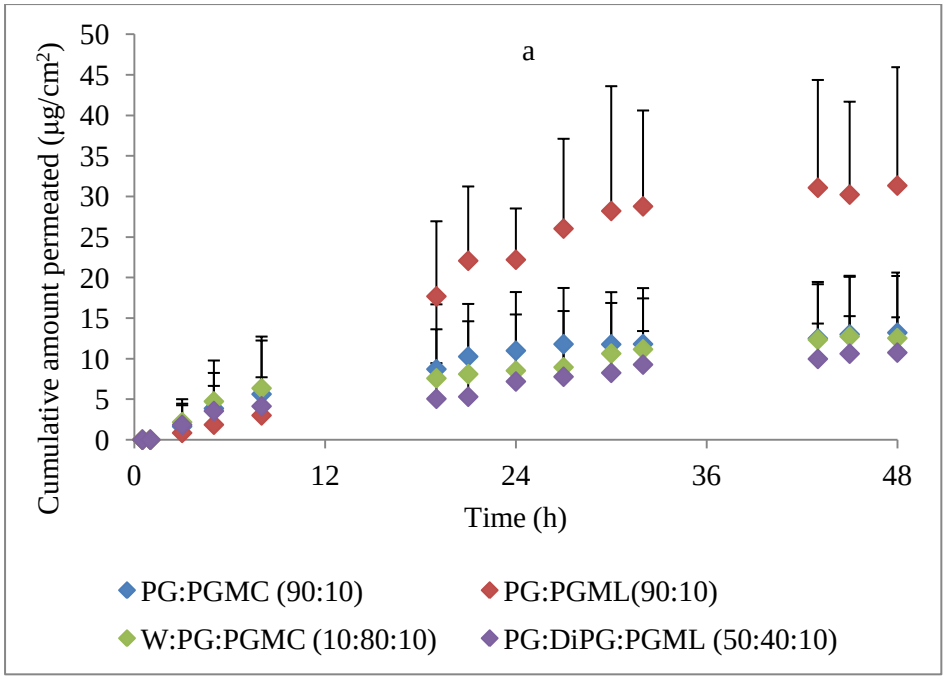
ABSTRACT

Anthramycin (ANT) is a member of the pyrolobenzodiazepine family and is a potent cytotoxic agent. Previously, we reported the topical delivery of ANT from a range of solvents that may also act as skin penetration enhancers (SPEs). The skin penetration and uptake was monitored for simple solutions of ANT in propylene glycol (PG), dipropylene glycol (DiPG), Transcutol P (TC), isopropyl myristate (IPM), propylene glycol monocaprylate (PGMC) and propylene glycol monolaurate (PGML). The amounts of PG, DiPG and TC that were taken up by, and that penetrated the skin were also measured, with a clear dependence of ANT penetration on the rate and extent of PG and TC permeation. The present work investigates ANT skin delivery from a range of binary and ternary systems to determine any potential improvement in skin uptake compared with earlier results for the neat solvents. Following miscibility and stability studies a total of eight formulations were taken forward for evaluation in human skin *in vitro*. Binary systems of PG and water did not result in any skin permeation of ANT. Combining PG with either PGMC or PGML did promote skin penetration of ANT but no significant improvement was evident compared with PG alone. More complex ternary systems based on PG, DiPG, PGMC, PGML and water also did not show significant improvements on ANT permeation, compared with single solvents. Total skin penetration and retention of ANT ranged from 1 – 6 % across all formulations studied. Where ANT was delivered to the receptor phase there were also high amounts of PG permeation with >50% and ~35% PG present for the binary systems and ternary vehicles respectively. These findings along with our previous paper confirm PG as a suitable solvent / SPE for ANT either alone or in combination with PGML or PGMC. The results also underline the necessity for empirical testing to determine whether or not a vehicle is acting as a SPE for a specific active in a topical formulation.

Key words:

Anthramycin, pyrrolobenzodiazepine, solvents, skin, permeation, mass balance

***Graphical Abstract (for review)**



Topical delivery of anthramycin II. Influence of binary and ternary solvent systems

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ABSTRACT

Anthramycin (ANT) is a member of the pyrolobenzodiazepine family and is a potent cytotoxic agent. Previously, we reported the topical delivery of ANT from a range of solvents that may also act as skin penetration enhancers (SPEs). The skin penetration and uptake was monitored for simple solutions of ANT in propylene glycol (PG), dipropylene glycol (DiPG), Transcutol P (TC), isopropyl myristate (IPM), propylene glycol monocaprylate (PGMC) and propylene glycol monolaurate (PGML). The amounts of PG, DiPG and TC that were taken up by, and that penetrated the skin were also measured, with a clear dependence of ANT penetration on the rate and extent of PG and TC permeation. The present work investigates ANT skin delivery from a range of binary and ternary systems to determine any potential improvement in skin uptake compared with earlier results for the neat solvents. Following miscibility and stability studies a total of eight formulations were taken forward for evaluation in human skin *in vitro*. Binary systems of PG and water did not result in any skin permeation of ANT. Combining PG with either PGMC or PGML did promote skin penetration of ANT but no significant improvement was evident compared with PG alone. More complex ternary systems based on PG, DiPG, PGMC, PGML and water also did not show significant improvements on ANT permeation, compared with single solvents. Total skin penetration and retention of ANT ranged from 1 – 6 % across all formulations studied. Where ANT was delivered to the receptor phase there were also high amounts of PG permeation with >50% and ~35% PG present for the binary systems and ternary vehicles respectively. These findings along with our previous paper confirm PG as a suitable solvent / SPE for ANT either alone or in combination with PGML or PGMC. The results also underline the necessity for empirical testing to determine whether or not a vehicle is acting as a SPE for a specific active in a topical formulation.

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1. Introduction

Pyrrolobenzodiazepines (PBDs) are sequence-selective minor groove DNA-interacting agents that restrict cellular processes thus leading to apoptosis. The term PBD derives from the three ring system of benzene, diazepine and pyrrole in the chemical structure of these compounds (Hartley, 2011). The first PBD molecule to be isolated was anthramycin (ANT) and the compound (Figure 1) was subsequently shown to be a potent cytotoxic agent both *in vitro* (Tendler and Korman, 1963) and *in vivo* (Grunberg et al., 1966). Because of dose dependent cardiotoxicity when ANT was administered intraperitoneally the molecule was not investigated further as a drug (Cargill et al., 1974). However, the possibility of reduced cardiotoxicity of ANT following topical application has never been investigated.

Because of the potent antitumour activity of ANT, PBDs have been of considerable interest to several research groups and sibiromycin, tomaymycin, and neothramycin were later isolated from natural sources. Currently, the PBD group comprises of these naturally occurring PBDs, synthetic PBD monomers, PBD conjugates and PBD dimers (Hartley, 2011). About eighty PBD monomers were synthesised by C2-aryl substitution and a number showed promising results when tested in the National Cancer Institute (NCI) 60 cancer cell line screening model. In order to increase DNA binding affinity and sequence selectivity, PBD conjugates were subsequently synthesised (Hartley, 2011). A number of C8-linked PBD-conjugates showed enhanced DNA binding affinity and cytotoxic potencies compared to the monomers. To date, the PBD dimer, SJG-136 (or SG 2000) (Gregson et al., 2001) is the most widely evaluated PBD and shows superior DNA binding affinity compared with other PBDs as well as minor groove inter-and intrastrand cross-linking and mono-alkylation ability (Rahman et al., 2009). This PBD dimer also showed significant *in vitro* cytotoxic potency and *in vivo* antitumour activity (Alley et al., 2004; Hartley et al., 2004), reached Phase II clinical trials in leukaemia and ovarian cancer (Mantaj et al., 2017) and is still being evaluated in a canine lymphoma clinical trial (Mellinas-Gomez et al., 2009). More recently, PBD-type molecules have been attached to antibodies *via* chemical linkers to form Antibody-Drug Conjugates (ADCs), and several of these are at various stages of clinical trials, with two in Phase III (Mantaj et al., 2017).

As promising results have been reported for SJG-136 in systemic chemotherapy trials (Janjigian et al., 2010) we hypothesised that PBDs might be

useful in the management of actinic keratoses and non-melanoma skin cancers (Haque et al., 2017). There is a clear need for non-invasive and tissue salvaging approaches for management of these conditions yet few topical therapies are currently available (Madal et al., 2010; Haque et al., 2015). To this end we investigated whether ANT, as a model PBD compound, could penetrate the skin and we also identified a number of solvents capable of promoting uptake of ANT into the skin (Haque et al., 2017). Finite dose permeation studies were performed using Franz cells with excised human skin. A range of vehicles was studied including 1,3-butanediol (BD), dipropylene glycol (DiPG), propylene glycol (PG), Transcutol P® (TC), isopropyl myristate (IPM), propylene glycol monolaurate (PGML) and propylene glycol monocaprylate (PGMC). As well as monitoring penetration of ANT the extent to which PG, TC, BD and DiPG permeated the skin was measured. ANT was delivered through the skin for PG, TC and PGML with the active "tracking" the skin penetration of both PG and TC. For PGMC, PGML and TC, skin uptake of ANT also correlated with the solvent uptake by the tissue. A number of previous publications from our group has also shown that combinations of solvents and/or skin penetration enhancers may have a synergistic effect on skin permeation of actives (Oliveira et al., 2012; Mohammed et al., 2014; Parisi et al., 2016). The aims of the present work were therefore (i) to identify potential binary and ternary solvent systems for topical delivery of ANT and (ii) to probe any relationship between skin uptake of active and vehicle components.

2. Materials and methods

2.1 Materials

ANT, sodium azide, Sudan III, PG, IPM, ethanol (EtOH) and acetone were supplied by Sigma Aldrich, UK. PGMC (Type II) and PGML (Type II) were kind donations from Gattefossé, St. Priest, France. DiPG and methylene blue were obtained from Acros Organics (UK). BD was purchased from Wako Pure Chemical Industries Ltd., Japan. Phosphate buffered saline (PBS) tablets were purchased from (Oxoid Limited, UK).

2.2 Methods

2.2.1 Miscibility, solubility parameter calculation, solubility and stability studies.

Miscibility studies of binary solvents were conducted at solvent ratios of 10:90; 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10. For ternary miscibility determinations, the three solvents were added at different percentages, varying by 10%. Samples were mixed and where necessary, Sudan III and methylene blue were used to confirm miscibility. Tubes were left to stand for at least 24 h to test for phase separation. For ternary systems, phase diagrams were constructed using OriginPro® 8.0 software (OriginLab Cooperation, USA). Stability and solubility studies were conducted as reported previously at 32±0.5°C; stability was monitored up to 96 h and solubility was determined over 48 h (Haque et al., 2017). The concentration of ANT used in the stability studies ranged from 0.03 to 0.43 mg/mL depending on the binary or ternary system evaluated. Samples and solvents were analysed using the HPLC and GC methods described earlier (Haque et al., 2017). All analytical methods were validated according to ICH guidelines also as reported in this publication.

Molecular Modelling Pro® (ChemSW Inc., USA) software was used to calculate the van Krevelen Hoftyzer three dimensional solubility parameter (δ) values. For the binary and ternary solvents that were miscible, the solubility parameter (δ_m) was calculated using the following equations:

$$\delta_{m\ (binary)} = \frac{\delta_1 \Phi_1 + \delta_2 \Phi_2}{\Phi_1 + \Phi_2} \quad \text{.....Equation 1}$$

$$\delta_{m\ (ternary)} = \frac{\delta_1 \Phi_1 + \delta_2 \Phi_2 + \delta_3 \Phi_3}{\Phi_1 + \Phi_2 + \Phi_3} \quad \text{.....Equation 2}$$

Where, Φ_1, Φ_2 and Φ_3 are the respective mole fractions for solvents 1, 2 and 3; and δ_1, δ_2 and δ_3 are their respective solubility parameters y (Breon et al., 1980).

2.2.2 Finite dose permeation and mass balance studies of ANT and solvents from the mixed solvent systems

Vertical glass Franz type diffusion cells were used for the ANT permeation studies in human skin as reported previously (Oliveira et al., 2012). Skin was obtained from the abdomen of donors who had undergone surgery following informed consent and ethical approval (Research Ethics Committee reference 07/H1306/98). The skin

was prepared by the heat separation method with samples comprising of stratum corneum and part of the viable epidermis (Kligman and Christophers, 1963). All skin samples were of the same thickness. The receptor phase was prepared by dissolving PBS tablets in 1 L of deionised water (pH 7.3 ±0.1) with the addition of 0.002% (w/v) sodium azide. Finite doses (~10 μL/cm^2) of saturated solutions of ANT in the various vehicles were applied in the donor chamber under non-occluded conditions and permeation of ANT and vehicles was monitored up to 48 h, as described in detail for the previous study (Haque et al., 2017). After the 48 h permeation study, the receptor medium was removed completely. The mass balance study was conducted and validated. The skin surface was washed twice with 1 ml of methanol and three times with 1 ml of methanol-water (50:50) mixture. The skin was removed from the Franz cell and placed in an Eppendorf® tube with 1 ml of methanol. All the tubes were placed in a rotor (rotating at 40 rpm) in a temperature-controlled oven (32 ± 0.5 °C) for 3 h. After extraction was completed, the tube was centrifuged at 13,000 rpm at 32 °C for 15 min. Aliquots were taken from the supernatant portion and analysed by HPLC and GC in order to quantify the amounts of ANT and respective solvents on the surface of the skin and inside the skin. The washing and extraction methods were validated as reported previously (Haque et al., 2017).

2.2.3 Statistical analysis

The data were analysed in MS Excel® 2007 and the results presented as mean±SD. IBM SPSS Statistics software (version 22.0) was used for statistical evaluation. The Shapiro-Wilk test was used to check the normality of the results. If the p value calculated from the test was greater than 0.05, the data were assumed to be normally distributed (parametric) and one way analysis of variance (ANOVA) was conducted. Multiple comparisons between each individual group were performed by a post hoc Tukey test. For non-parametric data, a Kruskal-Wallis one way ANOVA followed by multiple comparisons between groups was performed. Equal variance was assumed in all cases. $p<0.05$ was considered as a statistical significant difference.

3. Results and discussion

3.1 Miscibility, stability, solubility parameter values and solubility

All hydrophilic solvents were miscible at all ratios in binary systems. PGMC and PGML were miscible with all candidate hydrophilic and hydrophobic solvents with the exception of water. IPM was miscible with TC, PGMC and PGML in all ratios and immiscible in PG, BD and water (W). IPM was miscible in DiPG only at ratios of 10:90 or 90:10. Subsequently, ternary systems comprising PG-TC-IPM, W-TC-PGMC, W-TC-PGML, W-DiPG-PGMC and W-PG-PGMC systems were prepared based on miscible regions of their respective ternary phase diagrams (data not shown).

Recovery values of ANT for binary systems are shown in Figure 2. ANT is not stable in TC (Haque et al., 2017) and rapid degradation occurred in PG:TC (50:50) with ~6.5% of the initial concentration present after 96 h. The recoveries of ANT at 96 h in EtOH:W (70:30), W:TC (50:50), W:DiPG (50:50) and PG:TC (80:20) were ~51.5, 54, 64.5 and 38%, respectively. The lower recoveries of ANT in these vehicles were accompanied by colour changes. The other solutions remained transparent throughout the experiment. With the exception of W:PG (20:80), where ~88% of ANT was recovered, greater than 90% recovery of ANT was found for all other binary mixtures at 96 h. Eleven ternary solvent systems were evaluated for the stability of ANT (Figure 3). Good recovery of ANT (>90%) was found for W:PG:PGMC (10:80:10), PG:DiPG:PGMC (50:40:10) and PG:DiPG:PGML (50:40:10). ANT recovery was ~81.5% of the initial concentration for W:DiPG:PGMC (40:50:10). In PG:TC:IPM (40:50:10) the lowest recovery of ANT (3.69%) was evident. In the other ternary solvent systems less than 35% of ANT remained at 96 h. As for the binary systems, colour changes were evident for those ternary systems that demonstrated marked instability. Where the recovery of ANT was <80% at 96 h, binary and ternary systems were not evaluated further. ANT contains an amide group and it can also have an imine bond between the N10-C11 positions in anhydroanthramycin, which is interconvertible with the carbinolamine form. In addition, oxidation is possible at the C9 position to generate an ortho quinone imine, a degradant that is cardiotoxic.

The solubility parameters of the final candidate vehicles and corresponding ANT solubility values are shown in Table 1. Previously, the solubility parameter of ANT was calculated as 15.35 $(cal/cm^3)^{1/2}$ and high solubility of the molecule had been observed for solvents with comparable solubility parameters (Haque et al., 2017).

However, highest solubility for the binary mixtures, was observed with PG:PGMC and PG:PGML. These vehicles have comparatively lower solubility parameter values than the W:PG vehicle, that has the closest value to ANT. For the ternary systems, highest solubility is evident for W:PG:PGMC compared with PG:DiPG:PGMC and PG:DiPG:PGML. The solubility parameter value for W:PG:PGMC, 14.54 $(cal/cm^3)^{1/2}$, is also the closest of all the values of the ternary systems to that for ANT.

3.2 Finite dose permeation and mass balance studies for ANT

Figure 4a shows the permeation profiles of ANT for binary and ternary solvent systems for finite dose applications under non-occluded conditions. For the eight vehicles, ANT permeation was only evident for PG:PGMC, PG:PGML, W:PG:PGMC and PG:DiPG:PGML. Cumulative amounts that permeated at 48 h were as follows: PG:PGML - 31.34 μg/cm^2 , PG:PGMC - 13.20 μg/cm^2, W:PG:PGMC – 12.53 μg/cm and PG:DiPG:PG - 10.74 μg/cm^2. The amounts of ANT that permeated from these mixtures were not significantly different ($p>0.05$) and are comparable to ANT permeation observed for single solvents (Haque et al.., 2017). The percentage permeation profiles (Figure 4b) confirm the highest value for PG:PGML (2.63%) and the corresponding values for PG:PGMC, PG:DiPG:PGML and W:PG:PGMC were 1.4, 1.2 and 0.9% respectively. For the neat solvents the highest percentage permeation was observed for PGML (13.16%) with ANT cumulative permeation of 8.5 μg/cm^2 (Haque et al., 2017). The cumulative amounts of ANT permeated between PGML and PG:PGML (90:10) were found to be equal to the significance threshold ($p=0.05$). There was no significant increase in amount of ANT that permeated from PG:PGML (90:10) compared with neat PG ($p>0.05$).

The mass balance results for ANT binary formulations are summarised in Table 2 and confirm that most of the applied dose was recovered from the surface of the skin. The percentages of ANT remaining in the skin for PG:PGML, W:PG (20:80), PG:PGMC and W:PG (50:50) were ~3.4, 1.3, 1.0 and 0.8% respectively. Table 2 shows less than 80% of total recovery values of ANT from the surface, inside the human skin and from the receptor fluid for W:PG (50:50), W:PG (20:80) and PG:PGML (90:10). However, the stability results of ANT in these three systems were almost 88% and above. When the stability testing was conducted, ANT was kept only

with the respective solvent system. However, during the skin experiment studies, the drug solution was kept in contact with the viable epidermis for 48 hours. The extent of skin metabolism of ANT is not known. Therefore, skin metabolism of ANT may have contributed in the lower recovery values of ANT. As for the binary systems most of the applied amount of ANT was recovered from the skin surface for the ternary systems (Table 2). ANT skin retention was comparable for PG:DiPG:PGML (3.01%) and W:PG:PGMC (1.93%). PG:DiPG:PGML deposited more ANT in skin compared with W:DiPG:PGMC and PG:DiPG:PGMC ($p<0.05$). For the PG:DiPG:PGML and W:PG:PGMC systems, the skin retention of ANT was also similar to that previously observed for neat PG (Haque et al., 2017). More than 80% of ANT was recovered in total for all ternary formulations.

3.3 Finite dose permeation and mass balance studies of solvents for binary and ternary systems

The permeation of PG was quantified for solutions of ANT in W:PG (50:50) and W:PG (20:80), PG:PGMC and PG:PGML (Table 3). Extraction and washing of the skin surface was also conducted to determine any residual PG in or on the skin. For W:PG (50:50) and (20:80), cumulative amounts of PG permeation were not significantly different at 48 h (17.12 and 24.55%, respectively, $p>0.05$). The cumulative percentage permeation of PG at 48 h when applied as a neat vehicle was reported as ~21.87% (Haque et al., 2017) and is not significantly different ($p>0.05$) from the data reported here for the PG binary systems. Overall recovery of PG from the W:PG vehicles did not exceed ~ 30%, also comparable to recovery of PG for the solution of ANT in neat PG (Table 3). Interestingly no ANT permeation was observed for these systems (Section 3.2) and this may reflect the rapid depletion and evaporation of PG following application of these binary systems to the skin. No PG could be quantified from the surface and inside the skin for W:PG (50:50). For W:PG (20:80), 4.35±2.86 % and 2.20±0.88% of PG were recovered from the surface and extracted from the skin respectively. These values are comparable to the amounts recovered for the solution of ANT in neat PG (Table 3).

For the PG:PGMC and PG:PGML systems, significantly higher ($p<0.05$) permeation of PG was observed compared with the W:PG systems, with values of

52.11 and 71.92%, respectively at 48 h. The addition of 10% of either ester also results in significantly increased ($p<0.05$) permeation of PG compared with neat PG (Table 3). However, PG could not be quantified on the surface and inside the skin for either system. Approximately 30% of the applied dose of PGML was retained inside the skin but PGMC could not be quantified in the skin, with most of the compound recovered from the skin surface. Total recovery for both PGMC and PGML was ~100% (Table 3). The high permeation of PG from the PG/ester systems mirrors the superior ANT permeation from these mixtures, compared with the failure of other binary systems to delivery ANT where comparatively lower skin penetration of PG is evident (Section 3.2, Table 3).

Mass balance results for PG and DiPG at 48 h for ANT solutions in the neat solvents and ternary systems across human skin are shown in Table 3. The amounts of PG that permeated from W:PG:PGMC, PG:DiPG:PGML and PG:DiPG:PGMC were 39.6%, 35.0% and 26.5%. As for the binary systems, the two ternary systems demonstrating comparatively higher PG permeation also achieved effective skin penetration of ANT. However, there were no significant differences in the amounts of PG that permeated for the three ternary systems and no differences compared with neat PG ($p>0.05$). PG could not be quantified in the skin for any of the ternary systems. No PG was detected on the surface of the skin for W:PG:PGMC; for PG:DiPG:PGMC and PG:DiPG:PGML, ~9 and 5%, of PG respectively were recovered from the skin surface. The total recoveries of PG in all ternary systems were poor and this may reflect evaporation of PG from the formulation

DiPG permeation was evaluated for W:DiPG:PGMC, PG:DiPG:PGMC and PG:DiPG:PGML. For PG:DiPG:PGML, a significantly higher percentage of DiPG permeation was evident (44.95%), compared with W:DiPG:PGMC and PG:DiPG:PGMC where the corresponding amounts that permeated were 17.13 and 17.90%, respectively ($p<0.05$). The PG:DiPG:PGML system also enhanced permeation of DiPG to a significant extent ($p<0.05$) compared with neat DiPG. As for PG, high DiPG permeation from the PG:DiPG:PGML vehicle is consistent with ANT skin permeation, in contrast to the other two DPG mixtures where no ANT was detected in the receptor phase (Section 3.2). DiPG skin retention was highest (8.32%) for PG:DiPG:PGML. For W:DiPG:PGMC and PG:DiPG:PGMC similar retention values (3.93 and 3.50%, respectively) and permeation (17.13 and 17.90%) of DiPG

were evident. Thus, the total absorption values of DiPG in PG:DiPG:PGML (50:40:10) were higher (53.27%) than the other two ternary systems. The total recovery of DiPG was >85%. No PGMC was measured in the skin nor in the receptor phase for any of the PGMC ternary systems. The total recovery of PGMC in the three systems ranged from 68 to 81%. For PG:DiPG:PGML, 19.05% of PGML was retained in the skin and 40.67% was recovered from the skin surface (Table 3).

4. Conclusions

Previous work had confirmed the dependence of ANT skin permeation on the corresponding penetration of specific solvents/skin penetration enhancers. Although more complex systems were evaluated in the present work, it is evident that significant skin uptake and the presence of vehicle components in the receptor phase is critical for effective percutaneous delivery of the active. Binary W:PG systems did not result in any ANT skin penetration. In contrast, combinations of PG with PG-fatty acid esters did promote delivery of ANT, although PG is present in high amounts (90%). The ANT permeation from these vehicles is also associated with significant amounts of PG in the receptor phase (>50%). In addition, only those ternary systems containing PG resulted in skin penetration of ANT, consistent with results from the earlier work with neat PG. When PGMC is selected as the ester in the ternary system, high amounts of PG are required (80%) for penetration of the active, compared with the corresponding systems with PGML where the PG content is 50%. The overall delivery observed for the binary and ternary systems is still comparable to that for neat solvents, suggesting that the best formulation strategy should be based on a single solvent or skin penetration enhancer and specifically, PG.

Acknowledgement

The authors would like to thank the Commonwealth Scholarship Commission for funding the project.

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Figure(s)

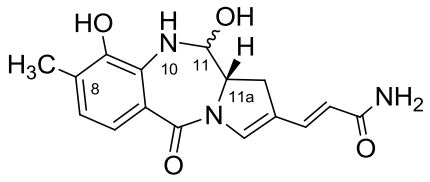

Figure 1. Anthramcyin

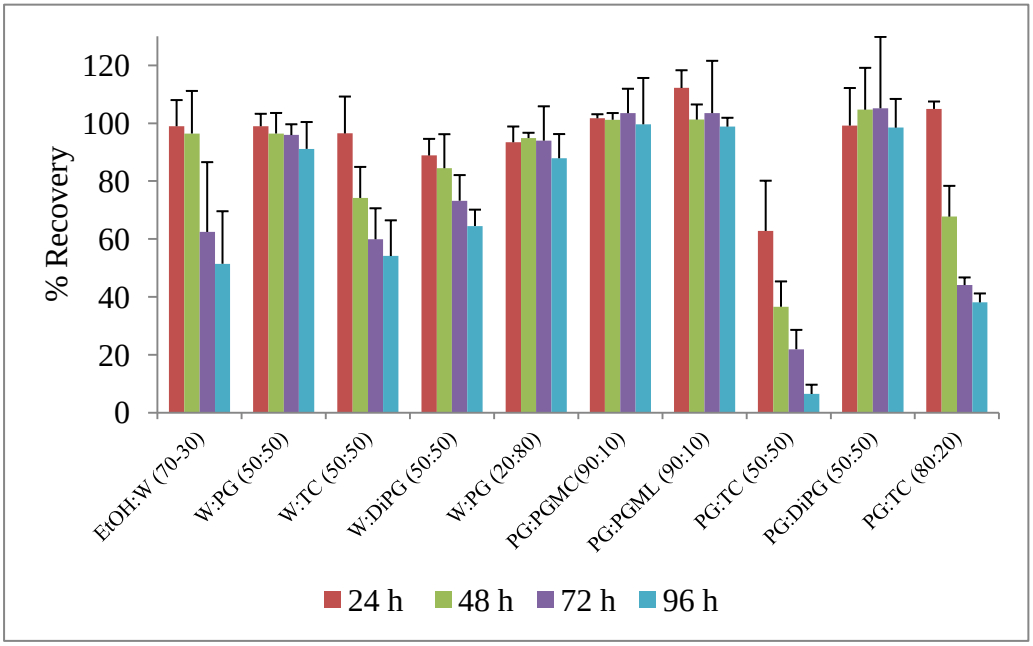

Figure 2. Percentages of the applied ANT recovered in binary mixtures at 24, 48, 72 and 96 h at 32±0.5°C (n=3, mean±SD).

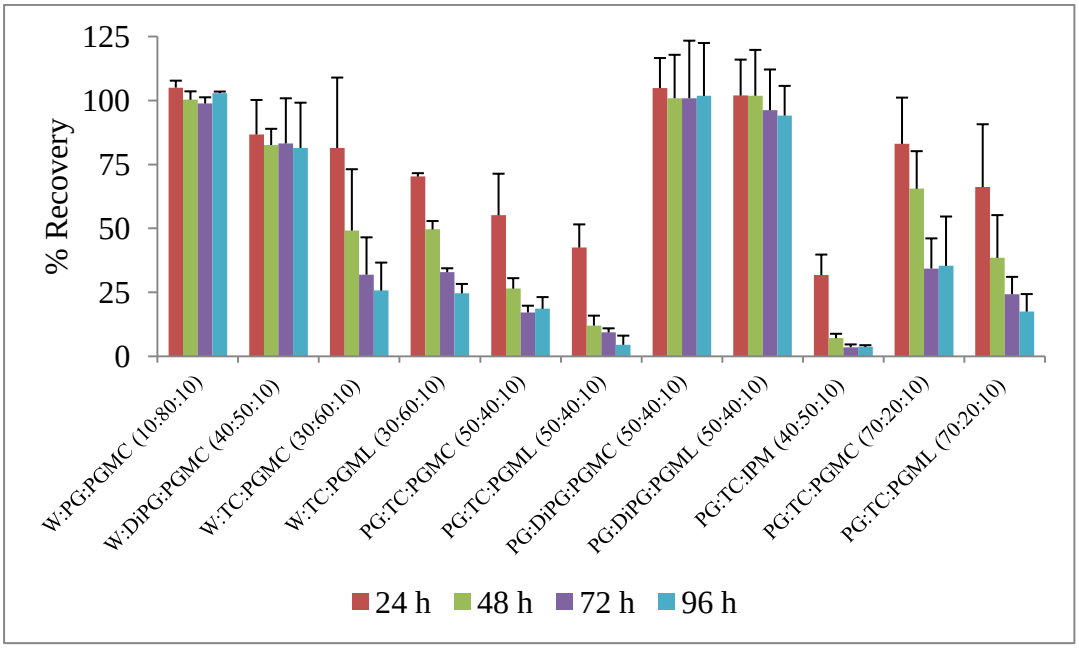

Figure 3. Percentages of the applied ANT recovered in ternary mixtures at 24, 48, 72 and 96 h at 32±0.5°C (n≥3, mean±SD).

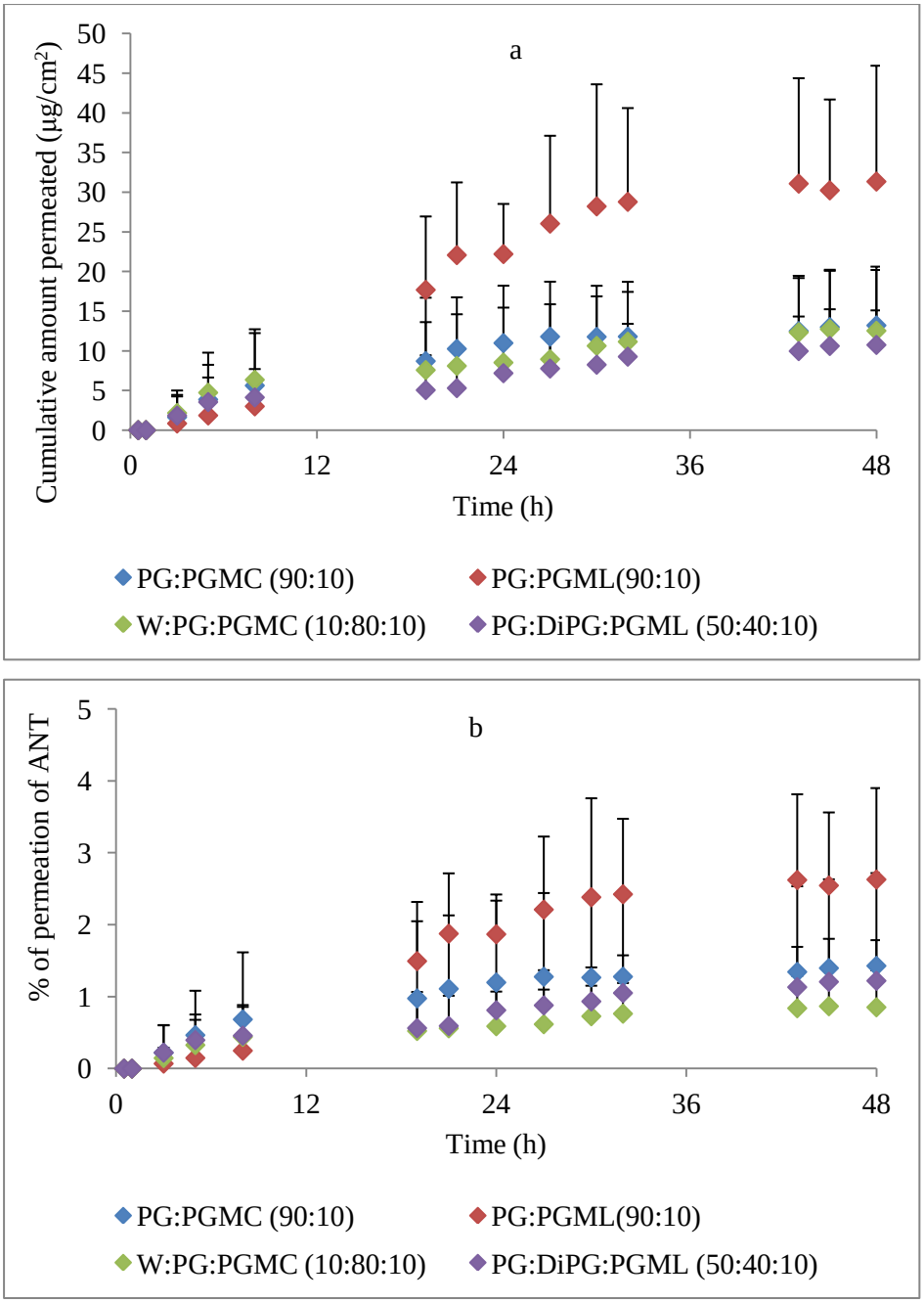

Figure 4. Plot of (a) cumulative amount of ANT and (b) percentage of applied dose of ANT permeated as a function of time through human skin in binary and ternary solvent systems (n≥3, mean±SD).

List of Tables

Table 1. Solubility parameters for binary and ternary systems and corresponding solubility values of ANT* at 32±0.5°C (n=3, mean±SD).

Solvent mixtures	Solubility Parameter [$(cal/cm^3)^{1/2}$]	Solubility (mg/ml)
W:PG (50:50)	18.52	47.09±4.06
W:PG (20:80)	15.85	117.15±3.96
PG:PGMC (90:10)	13.65	128.29±19.86
PG:PGML (90:10)	13.61	123.08±16.20
W:PG:PGMC (10:80:10)	14.54	145.07±9.22
W:DiPG:PGMC (40:50:10)	16.27	80.59±2.10
PG:DiPG:PGMC (50:40:10)	12.90	115.80±21.06
PG:DiPG:PGML (50:40:10)	12.86	82.40±18.07

*Solubility parameter for ANT - 15.35 $(cal/cm^3)^{1/2}$

Table 2. Percent recovery of the applied dose of ANT from binary and ternary systems after 48 h permeation studies (n≥3, mean±SD).

Solvent(s)	% of the applied dose recovered			
	Skn surface	In skin	Receptor phase	Total
PG	80.12±3.15	1.31±0.74	0.64±0.07	82.06±3.76
DiPG	98.49±15.34	0.21±0.01	NQ	98.71±15.34
PGMC	73.79±16.79	17.50±8.16	NQ	91.29±21.22
PGML	75.10±21.35	4.01±0.99	13.14±0.41	92.25±22.15
W:PG (50:50)	68.02±1.91	0.76±0.14	NQ	68.78±1.83
W:PG (20:80)	71.92±6.95	1.32±1.06	NQ	73.24±7.06
PG:PGMC (90:10)	79.00±22.85	1.04±0.85	1.43±1.29	81.47±22.09
PG:PGML (90:10)	68.15±7.87	3.42 ±1.31	2.63±1.27	74.20±6.99
W:PG:PGMC (10:80:10)	98.77±7.34	1.93±0.71	0.85±0.51	101.55±7.80
W:DiPG:PGMC (40:50:10)	82.83±8.62	0.39±0.27	NQ	83.22±8.53
PG:DiPG:PGMC (50:40:10)	85.09±3.11	0.20±0.09	NQ	85.28±3.10
PG:DiPG:PGML (50:40:10)	76.86±1.89	3.01±1.12	1.22±0.56	81.09±3.13

* NQ= not quantified.

Table 3. Percent recovery of the applied dose of solvent(s) from binary and ternary systems after 48 h permeation studies (n≥3, mean±SD).

Solvent(s)	Vehicle	Dose applied (mg)	% of the applied dose recovered			
			Skin surface	In skin	Receptor phase	Total
PG		10.36	6.86±2.01	2.14±1.44	21.87±10.27	30.87±13.68
DiPG		10.20	98.86±4.08	2.11±0.48	7.98±2.27	108.95±3.03
PGMC		9.50	96.63±8.86	7.45±0.40	ND	104.08±8.86
PGML		9.31	95.88±6.12	4.17±1.26	ND	100.06±6.19
W:PG (50:50)	PG	5.18	NQ	NQ	17.12±1.02	17.12±1.02
W:PG (20:80)	PG	8.29	4.35±2.86	2.20±0.88	24.55±7.93	31.10±7.10
PG:PGMC (90:10)	PG	9.32	NQ	NQ	52.11±13.93	52.11±13.93
	PGMC	0.95	99.59±2.81	NQ	ND	99.59±2.81
PG:PGML (90:10)	PG	9.32	NQ	NQ	71.92±8.40	71.92±8.40
	PGML	0.93	70.60±7.57	29.04±1.82	ND	99.65±7.03
W:PG:PGMC (10:80:10)	PG	8.29	NQ	NQ	39.61±6.03	39.61±6.03
	PGMC	0.95	67.89±3.84	NQ	ND	67.89±3.84
W:DiPG:PGMC (40:50:10)	DiPG	5.1	81.04±2.02	3.93±1.10	17.13±4.29	102.11±5.12
	PGMC	0.95	81.35±1.33	ND	ND	81.35±1.33
PG:DiPG:PGMC (50:40:10)	PG	5.18	9.12±6.92	NQ	26.54±4.52	35.66±7.95
	DiPG	4.08	67.32±7.09	3.50±0.90	17.90±3.36	88.73±6.60
	PGMC	0.95	81.35±3.03	NQ	ND	81.35±3.03
PG:DiPG:PGML (50:40:10)	PG	5.18	5.07±2.41	NQ	35.01±5.81	40.08±4.92
	DiPG	4.08	51.44±23.52	8.32±2.27	44.95±20.41	104.71±8.37
	PGML	0.93	40.67±1.47	19.05±0.17	ND	59.72±1.61

*ND= Not detected and NQ= not quantified.