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Original Article

Invitro Release Study of Sodium Salicylate from Lecithin Based Phospholipid Microemulsions

Reza Aboofazeli*, Seyed Alireza Mortazavi, Pooneh Khoshnevis

Department of Pharmaceutics, School of Pharmacy, Shaheed Beheshti University of Medical Sciences and Health Services, Tehran, Iran.

Abstract

Sodium salicylate containing microemulsions have been formulated, based on the previous phase diagram studies, using a pharmaceutically acceptable surfactant and oil. The effects of formulation variables on the release profile of the drug from microemulsion through intact rat skin were also determined experimentally. In this investigation, two commercially available lecithins (namely Epikuron 200 and Epikuron 170), three short chain alcohols (*n*-butanol, *iso*propanol and *n*-propanol) and isopropyl myristate (IPM) were used as surfactant, cosurfactant and oil, respectively. The water phase was composed of sodium salicylate solution (2% w/v). To investigate the release profile, samples with 25% wt% total surfactant content were prepared with different surfactant/cosurfactant weight ratios (K_m of 1:1 and 1.5:1) and various amounts of drug solution (from 7 to 35 wt%), depending on the nature of alcohol. Two compartment Franz diffusion cells, equipped with rat skin as the absorption membrane, were employed for release studies. All experiments were performed at room temperature and sampling was taken over 12 hours with one-hour intervals. The amount of drug released was determined spectrophotometrically and the permeation parameters were then calculated. Results showed that systems formulated with 7 and 9% drug solution, were not capable of releasing the drug with a lower rate, compared to the corresponding drug solutions, while in other systems, a lower release rate was observed in comparison to the control samples. In general, among the systems investigated, those prepared with *n*-propanol at K_m of 1.5:1 and 11-20 wt% and 29% dispersed phase showed a relatively lower absorption rate, comparing to the corresponding control samples , regardless of the nature of surfactant and cosurfactant. Under passive conditions, the flux from microemulsions followed the zero order behavior with respect to the donor concentration.

Keywords: Phospholipid; Microemulsion; Sodium salicylate; Release study.

Introduction

Microemulsions are bicontinuous systems that are essentially composed of bulk phases of water and oil separated by a surfactant/cosurfactant rich interfacial region. These systems have advantages over conventional emulsions in that they are thermodynamically stable liquid systems and are spontaneously formed (1).

Microemulsions are currently the subject of many investigations because of their wide range of potential and actual utilizations. The high capacity of microemulsions for drugs makes them attractive formulations for pharmaceuticals. These systems also offer several benefits for oral administration, including increased absorption, improved clinical potency and decreased toxicity (2). In this regard, a great deal of dosage form development activities have been focused on the use of microemulsions as oral and intramuscular delivery systems (3-7). Furthermore, microemulsions have shown themselves to be an effective way of delivering active ingredients transdermally for

* Corresponding author:
E-mail: raboofazeli@itmrc.org

pharmaceutical and cosmetic applications (8). Some research works have been focused on the use of microemulsions in several other fields such as chemistry and physicochemistry. In biopharmaceutics, microemulsions were used to stabilize drugs and to improve systemic and topical availability (9-14).

Most activities in the dosage form development field have been also focused on the formulation of lecithin based microemulsions and some recent studies have shown that microemulsions containing pharmaceutically acceptable oils as vehicles for sparingly soluble substances can be formulated using lecithin and a cosurfactant (15-19). Lecithin is a naturally occurring biocompatible material and is capable of producing isotropic solutions of water and oil in the presence of suitable cosurfactants.

In our previous study , the phase behavior of systems containing isopropyl myristate (as oil), lecithin (as surfactant), short chain alcohols (as cosurfactant) and sodium salicylate solution was reported. Using the appropriate surfactant/cosurfactant weight ratios, microemulsion systems in the oil rich part of the phase diagrams were obtained (20). The major objective of this research was to evaluate the potential of lecithin based microemulsions for an effective delivery of a hydrophilic solute, and the absorption rate of a model water soluble drug (sodium salicylate) from w/o phospholipid microemulsions was determined. Based on the predetermined phase diagrams, microemulsions with constant surfactant/cosurfactant weight percent were prepared and the influence of formulation variables on the release rate and release kinetics of the drug was then investigated.

Experimental

Materials

Two commercially available soybean lecithins, Epikuron 200 (E200) and Epikuron 170 (E170) were obtained as a donation from Faratin Company (Lucas Meyer Representative in Iran) and used without further purification. Isopropyl myristate (IPM) and sodium salicylate were purchased from Sigma-Aldrich Chemical Company (Dorset, UK). *n*-Butanol, *n*-propanol and *iso*-propanol were obtained from Aldrich-Sigma Chemical Company (Dorset, UK). Triple distilled water was used throughout the study.

The permeation data (flux and permeability coefficient) for these systems (25 wt %) surfactant/cosurfactant content) were determined through intact hairless rat skin. In order to determine the flux, the amount of the drug penetrated through the skin membrane per unit area was plotted against time and the slope which represents the steady state flux was calculated by linear regression of all points. The slope (J) was then substituted into the following equation for the determination of permeability coefficient (K_p):

$$J = K_p . C_v$$

where, C_v is the concentration of the drug in microemulsion vehicle.

Methods

Preparation of microemulsions

For the preparation of microemulsions, a base mixture without sodium salicylate solution was made by adding the components (wt %). Lecithin and alcohol were dissolved in IPM and mixed with other components under agitation. Sodium salicylate solution (2% w/w) was then added to the base mixture and stirred until a transparent multicomponent system was formed. The total surfactant/cosurfactant content (with 1:1 and 1.5:1 weight ratios) in all samples was kept constant at 25% (w/w).

Partition studies

Partitioning of sodium salicylate between water and IPM, in the presence or absence of alcohol, was determined. Sodium salicylate was dissolved in a water phase (40 mg/ml) saturated with IPM. This solution and an oil phase saturated with water were filled into a suitable separating funnel and shaken for 12 hours at room temperature until distribution equilibrium was reached. After equilibrium, the volumes of the aqueous and oil phases were measured and the sodium salicylate concentration was determined in the aqueous phase spectrophotometrically. The apparent partition coefficient was then calculated. Each partition measurement was performed in triplicate. The same procedure was performed when alcohols were added to both saturated phases, based on their partitionings between water and IPM.

Construction of calibration curve

Standard stock solutions of sodium salicylate were prepared with the concentration range of 5-35 mg/Liter. Each solution was analyzed by spectrophotometric method at 296 nm in triplicate and a calibration curve was constructed using a least square regression.

Transdermal delivery experiments

The release studies were carried out using a Franz diffusion cell. Freshly excised hairless rat skin was clamped between the donor and receptor chambers of diffusion cells (4.19 cm^2). The sodium salicylate containing microemulsion (2 mL) was placed into the donor compartment while the receptor compartment (45 ml) was filled with phosphate buffer (pH 6.5). The temperature of cell assembly was maintained at 37 ± 2°C and contents of the receptor compartment were stirred using a magnetic stirrer. Hourly samples were collected up to 12 h and the cells were refilled with equal volumes of fresh media. The amount of drug released was determined spectrophotometrically at 296 nm.

Results and Discussion

One of the main strategies considered in the development of pharmaceutically acceptable microemulsions, is the substitution of hydrocarbon oils by biocompatible oils such as IPM. A second important strategy is to minimize the likelihood of toxicity problems associated with some surfactants (21).

Lecithin was found to be most appropriate for our purpose, for being pharmaceutically acceptable and having moderately high water and drug solubilization capacity. However, since lecithin is not capable of producing isotropic solutions, in order to prepare microemulsions, it is necessary to use a short chain alcohol as cosurfactant. Table 1 represents the compositions of drug loaded microemulsions investigated in this study. Figures 1 shows the plot of the amount of sodium salicylate transported through the skin from the microemulsions. Table 2 summarizes the permeation parameters calculated for all microemulsion systems studied. It should be noted that the in vitro release behavior of sodium salicylate from the microemulsions was studied in comparison with aqueous solutions.

In general, the following results were observed:

a) Formulations enriched with 7 and 9% drug solution showed a release rate faster than those of the corresponding control samples.

b) The flux from formulation 1E200nB11 was found to be higher than that from the aqueous sample.

c) The fluxes in systems prepared with *iso*-propanol and E170 and 11% sodium salicylate solution, did not show any significant difference with those observed in control samples, regardless of the surfactant/cosurfactant weight ratio.

d) The fluxes from other formulations containing 11% sodium salicylate solution were found to be lower than those observed from the corresponding aqueous solutions.

e) The release rates between the microemulsion systems prepared with 14-35% of sodium salicylate solution, under the test condition, were significantly lower than those observed from the control samples.

f) It was observed that the difference in the flux between microemulsions and control samples became more significant as the percentage of the drug solution increased. For example, drug cumulative delivery of microemulsion vehicles with 32% sodium salicylate solution was 7 fold lower than that of the aqueous solution, while the difference decreased to 0.5 fold for systems formulated with 14% drug solution.

g) Sodium salicylate flux from the different microemulsions was indistinguishable between formulations at early time points. However, by the end of the experiments, it was possible to differentiate two groups of microemulsions.

h) The release kinetics for all systems followed pseudo zero order behavior.

As mentioned earlier, microemulsions are capable of solubilizing drug molecules in their internal phase. The reservoir effect of the internal phase for lyophobic drugs, compared to the external phase, could lead to a sustained release of drug from these systems. Despite of low tendency, drug molecules would continuously diffuse into and saturate the external phase, until the internal phase is depleted from the drug (22). When the effect of microemulsions on the release rate of loaded drug is discussed, the flux, permeability constant (K_P) and the partition coefficient (P) of

Table 1. Composition of lecithin based microemulsions that were loaded with sodium salicylate and investigated for release rate.

Sample *	Sodium Salicylate Solution (%)	Surfactant/ Cosurfactant Weight Ratio	Cosurfactant	Surfactant
1E200nB9	9			
1E200nB11	11	1:1	*n*-Butanol	E200
1E200nB14	14			
1.5E200nB11	11			
1.5E200nB14	14	1.5:1	*n*-Butanol	E200
1.5E200nB17	17			
1.5E200nB20	20			
1E200nP11	11			
1E200nP14	14	1:1	*n*-Propanol	E200
1E200nP17	17			
1E200nP20	20			
1.5E200nP14	14			
1.5E200nP17	17			
1.5E200nP20	20			
1.5E200nP23	23	1.5:1	*n*-Propanol	E200
1.5E200nP26	26			
1.5E200nP29	29			
1.5E200nP23	32			
1E200isoP20	20	1:1	*iso*-Pronaol	E200
1E200isoP29	29			
1.5E200isoP20	20	1.5:1	*iso*-Pronaol	E200
1.5E200isoP29	29			
1 E170nB9	9	1:1	*n*-Butanol	E170
1E170nB11	11			
1.5 E170nB11	11			
1.5E170nB14	14	1.5:1	*n*-Butanol	E170
1.5E170nB17	17			
1E170nP7	7			
1E170nP9	9	1:1	*n*-Propanol	E170
1E170nP11	11			
1.5E170nP11	11			
1.5E170nP14	14	1.5:1	*n*-Propanol	E170
1.5E170nP17	17			
1.5E170nP20	20			
1E170isoP11	11	1:1	*iso*-Pronaol	E170
1.5E170isoP11	11	1.5:1	*iso*-Pronaol	E170
1.5E170isoP17	17			

Table 2. Steady state flux and permeability constant of sodium salicylate from microemulsions through rat skin.

Sample	Flux ($\mu g/cm^2/hr$)	Permeability Constant $(cm/hr) \times 10^3$	Correlation Coefficient
1E200nB9	7.542	4.77	0.993
1E200nB11	9.423	4.67	0.991
1E200nB14	5.464	2.22	0.993
1.5E200nB11	4.129	2.07	0.991
1.5E200nB14	4.886	1.96	0.991
1.5E200nB17	6.431	2.06	0.997
1.5E200nB20	7.963	2.18	0.996
1E200nP11	4.941	2.52	0.999
1E200nP14	4.851	1.89	0.998
1E200nP17	5.330	1.73	1.000
1E200nP20	6.330	1.74	0.999
1.5E200nP14	4.697	1.84	0.999
1.5E200nP17	5.137	1.65	1.000
1.5E200nP20	4.672	1.25	0.999
1.5E200nP23	4.782	1.13	0.997
1.5E200nP26	5.672	1.20	0.999
1.5E200nP29	3.262	0.595	0.989
1.5E200nP32	6.579	1.00	0.998
1E200isoP20	4.268	1.16	0.994
1E200isoP29	4.679	0.848	0.999
1.5E200isoP20	3.675	0.98	0.990
1.5E200isoP29	4.609	0.801	0.997
1 E170nB9	3.816	2.37	0.992
1E170nB11	5.447	2.78	0.999
1.5 E170nB11	5.134	2.61	0.990
1.5E170nB14	5.094	1.99	0.992
1.5E170nB17	5.921	2.02	0.995
1E170nP7	3.756	2.98	0.995
1E170nP9	3.790	2.28	0.999
1E170nP11	3.609	1.81	0.998
1.5E170nP11	3.985	1.98	0.998
1.5E170nP14	3.510	1.37	0.997
1.5E170nP17	3.543	1.19	0.997
1.5E170nP20	5.449	1.54	0.998
1E170isoP11	6.565	3.35	0.996
1.5E170isoP11	5.142	2.52	0.972
1.5E170isoP17	4.139	1.32	0.994
Control 7%	2.733	2.10	0.997
Control 9%	3.373	2.04	0.990
Control 11%	6.073	2.90	0.995
Control 14%	8.464	3.34	0.997
Control 17%	10.348	3.39	0.994
Control 20%	14.083	4.05	0.998
Control 23%	18.913	4.4	0.997
Control 26%	21.959	4.56	1.000
Control 29%	32.425	6.17	0.994
Control 32%	41.922	6.42	0.995

drug between the internal and external phases are considered. Trotta and his coworkers have studies the release of five steroids with different lipophilicity and observed various release rates from microemulsions formulated with IPM, Aerosol OT and *n*-butanol (23). Their results also showed a linear but reverse relation between K_P and P such that a decrease in P (i.e. high tendency for the external aqueous phase) resulted in a linear increase in K_P. Gasco and his collaborators have determined the P value of prednisolone between the oil and water phases and justified the decrease in the release rate of the drug from o/w microemulsions, compared to aqueous solutions (22). They suggested that due to the presence of *n*-butanol, the partitioning of the drug into the lipophilic phase could increase, leading to a decrease in K_P and release rate. It should be noticed that various factors, including oil/water phase ratio, droplet size, concentration, type of cosurfactant and the viscosity of the external phase, could influence the partition coefficient of drugs.

Our results on systems prepared with 7 and 9% of drug solution showed that release rate was higher from microemulsions compared to the corresponding control samples. In our previous work on the same drug-free microemulsions, we found that droplets were formed when more than 9% water was incorporated as the internal and we therefore suggested that those isotropic transparent

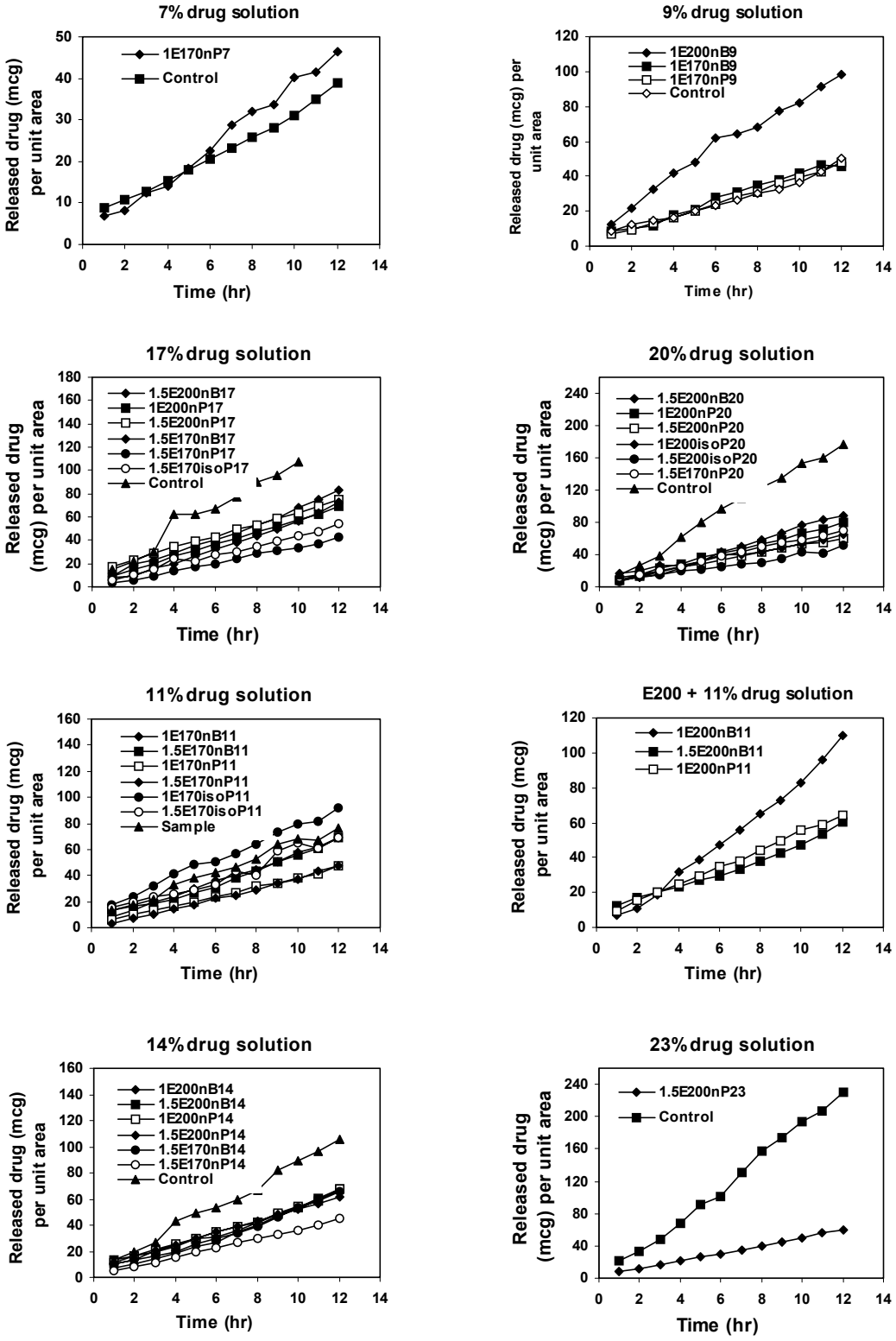
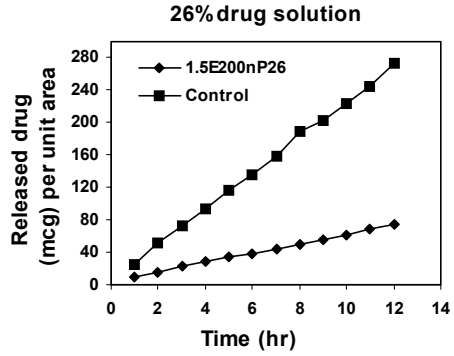
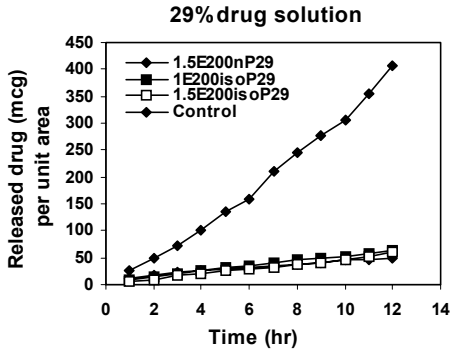
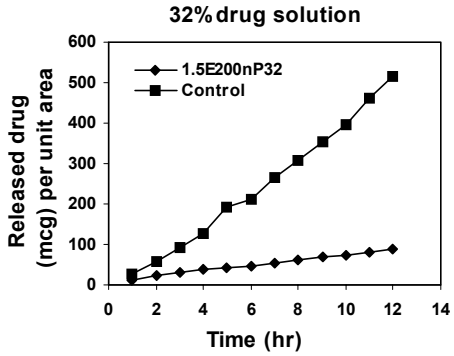


Figure 1. Drug release profile from lecithin based microemulsions loaded with sodium salicylate and from aqueous solutions containing the same amount of drug as control samples.

Figure 1. Continued.







mixtures composed of less than 9% water could be possibly cosolvent systems (24). Higher fluxes observed in microemulsions prepared with less than 9% of sodium salicylate solution could be attributed to the insufficient surfactant content in order microdroplet formation to be induced. Lecithin and IPM are known as penetration enhancers and hence may influence the partitioning of sodium salicylate between the skin and the vehicle. Therefore, it is believed that the penetration enhancement effect of these compounds in systems formulated with 7 and 9% of drug solution (which could exist as cosolvent systems) would increase the absorption rate of the drug through the skin. As mentioned earlier, the flux of drug from salicylate – containing microemulsions and salicylate solutions was monitored under passive conditions. The release profiles showed that there is no significant difference in the observed fluxes when comparing systems 1E170nB9, 1E170nP9, 1E170isoP11 and 1.5E170isoP11 and the corresponding aqueous solutions, which is possibly due to the role of the skin barrier (playing the rate-determining step in the permeation process). However, the fluxes from salicylate solutions are higher than those obtained with microemulsions, loaded with more than 11% of drug solution. This most likely reflects the presence of microemulsion droplets, inducing the reservoir effect. Low partition coefficient of sodium salicylate between IPM and water, even in the presence of alcohols (Table 3), would probably be lower than concentration of the drug in the external phase, located in the vicinity of the membrane. This effect could justify a decrease in the flux, as more drug solution is incorporated.

From the view point of surfactant purity, a significant difference is generally observed between the fluxes obtained, when the type of surfactant changed. Based upon the phase behavior studies and high solubilizing capacity of systems composed of E200, it could be suggested that the interfacial film constructed by E200 is possibly more flexible than that formed by E170. On the other hand, due to the presence of more hydrophilic components in E170, the hydrophilicity of the microemulsion core may increase which in turn leads to the remaining of drug inside the core.

With the exception of systems containing 20% of drug solution, in general, the flux from microemulsions stabilized with *n*-propanol was lower than that obtained when *n*-butanol was replaced. This would suggest that the partitioning of drug is relatively affected by the substitution of alcohols. Water soluble, hydrophilic cosurfactants would be expected to be distributed primarily between the aqueous phase and in the polar part of the interfacial layer, while oil soluble hydrophobic cosurfactants would be expected to be distributed mainly between the oil phase and in the hydrocarbon part of the interface. *n*-Propanol and *iso*-propanol are partitioned

Table 3. Partition coefficient of sodium salicylate between IPM and water in the presence of alcohols.

Alcohol	L og *P*
n-butanol	-1.268
n-propanol	-1.602
iso-propanol	-1.721

mainly in the aqueous phase, thus increasing the tendency of the drug to the internal phase. However, *n*-butanol is less soluble; therefore it increases the tendency of the drug to be partitioned mainly in the external phase. It seems difficult to establish a general trend for the effect of surfactant/cosurfactant weight ratio and the amount of drug solution incorporated, on the release pattern of sodium salicylate from microemulsions. It should be noticed that an increase in the drug content had no effect on the release rate, although the difference between the flux from a microemulsion system and its corresponding drug solution increased as the drug content increased.

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