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Study and development of microemulsion formulations to increase the permeability of acyclovir

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ABSTRACT

The purpose of this study was to improve the permeability of acyclovir (ACV) by developing a water-in-oil microemulsion, since it is an important factor that influences the oral absorption and, consequently, the oral bioavailability of drugs. Pseudoternary phase diagrams (PTPD) were constructed to identify the regions for obtaining microemulsions (ME). From the region of fluid transparent systems, five ME were chosen to evaluate the effect on ACV permeability by using two-compartment horizontal Franz diffusion cells, using a bio-mimetic artificial membrane, which simulates the typical lipophilic characteristics of the biological membranes. The characterization of ME was carried out by PTPD, conductivity determination and Nuclear Magnetic Resonance studies. The formulation that showed the higher incorporation of ACV (81.57 mg/ml) consisted of 13.3% Polysorbate 80, 13.3% ethanol, 6.6% ginger oil and 66% water. An increase in ACV permeability was observed, reaching an apparent permeability coefficient of $1.99.10^{-6}$ cm/s, corresponding to a fraction of human absorbed dose of 99.97%, according to the correlation previously established by our research group. These results indicated the ability of ME to improve the permeability of ACV, representing a system with the potential to improve the efficacy of ACV in oral administrated pharmaceutical formulation.

1. Introduction

Acyclovir (ACV) is a first-line antiviral drug for the treatment of orofacial, cutaneous and genital herpes, due to its inhibitory activity against herpes simplex virus type 1 and 2, varicella-zoster and the Epstein Barr virus [1,2]. In general, patients infected with herpes are more susceptible to HIV infections, with a rapid progression of the disease [3]. Because of its high selectivity and low toxicity, ACV has revolutionized antiviral therapy [2]. On the other hand, the parenteral route is limited due to the non-compliance of the therapy by the patient. As an alternative, the oral route has been widely investigated. However, ACV presents poor oral bioavailability (15–30%), due to its variable and incomplete absorption, since the administration of high doses is usually required at frequencies of three or four times a day, leading to potential systemic adverse effects, renal failure or neurotoxicity. In addition, it causes noncompliance on the part of the patient, causing an increase in resistance to the treatment [2,3]. It was previously determined that this drug presents an oral dose fraction absorbed in humans (Fa%) of 30% [4]. The Biopharmaceutical Classification System (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. According to the BCS, drug substances are classified as follows:

Class I: High Solubility-High Permeability
Class II: Low Solubility-High Permeability
Class III: High Solubility-Low Permeability
Class IV: Low Solubility-Low Permeability

Abbreviations: ACV, acyclovir; PTPD, pseudoternary phase diagrams; ME, microemulsions; Fa%, oral dose fraction absorbed in humans; BCS, Biopharmaceutical Classification System; API, active pharmaceutical ingredient; W, aqueous phase; O, oil phase; S, surfactants; O/W, oil in water; P_{app}, apparent permeability; P80, polysorbate 80; GO, ginger oil; DMSOd, dimethyl sulfoxide-D6 with a 99.8% deuteration degree; HLB, hydrophilic-lipophilic balance; O/S, oil/surfactant; PDI, polydispersity index; $\Delta\delta$, chemical shifts; PBS, phosphate buffer solution; dQ/dt, increase of the permeated cumulative drug amount versus time (mg/min); V, volume of the receiver compartment; A, surface area of the membrane; C_0, initial drug concentration; F, formulations; S_{app}, apparent solubilities; S_{app}/S_{ACV}, apparent solubility increments; σ, electrical conductivity.

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A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1–6.8 at 37 ± 1 °C. The volume estimate of 250 ml is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with an 8 fluid ounce glass of water. The permeability class boundary establishes that a drug substance is considered to be highly permeable when the systemic bioavailability or the extent of absorption in humans is determined to be 85% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose.

For this reason, ACV is categorized within Class III in the Biopharmaceutical Classification System (BCS) [5], due to their high solubility and low permeability, considering a dose of 400 mg for ACV [6]. However, this active pharmaceutical ingredient (API) is also available in higher doses, 800 mg, for which it has also been classified as Class IV [2,3,6]. For these reasons, it is of great interest to obtain new pharmaceutical systems that make it possible to improve the unfavorable physical and chemical properties of these antiviral agents and, hence, oral treatment.

Microemulsions (ME) are isotropic, optically transparent, nano-structured and thermodynamically stable dispersions, composed of two immiscible liquids an aqueous phase (W) and an oil phase (O), stabilized by an interfacial layer of surfactants (S) generally associated with a cosurfactant [7–11]. One of the main advantages of ME is that they can be obtained using biocompatible components to be used as a vehicle for pharmacologically active ingredients [12–16]. In the last decades, a large number of research on microemulsyfied systems for pharmaceutical application was published [10,11,17–22]. The study of microemulsions for the delivery of drugs is well described in the literature, at the same time as it shows a favorable influence on the modification of the bioavailability of numerous API. For example, the use of oil in water (O/W) ME to modulate the antimicrobial activity of glycerol monolaurate O/W was reported [11] to increase the solubility of drugs such as azithromycin [23], chloramphenicol [10] and various water-soluble peptides of different molecular structures, size and charge [24]. However, the use of microemulsions to enhance the permeability of low permeable drugs has not been extensively described so far. Recent studies performed in our laboratory demonstrated the advantages of using ME [25] and combining the formation of complexes of API with cyclodextrins and ME [26], observing a synergistic increase in the solubility of the hydrophobic drugs sulfamerazine and indomethacin. Furthermore, the permeability of these systems was evaluated using an *in vitro* method developed and validated in our laboratory, which was proved to be fast, efficient, reproducible, and low cost to appropriately predict the permeability of structurally diverse drugs that are absorbed by a passive diffusion mechanism. The apparent permeability (P_{app}) value of allopurinol that presents a Fa% of 90% was set as the high permeability limit for the current method, and it was used to classify the model drugs according to the BCS [27]. It is important to highlight that these ME had a positive effect on the solubility of the studied drugs, but the permeability remained unchanged since SMR and INM presented high permeability, and were classified as type II according to the BCS. Acyclovir was used as one of the 20 drugs used for the validation of the *in vitro* permeability method, and presented a Fa% of 30%.

In this context, the purpose of this project contemplates the development of microemulsions that allow increasing the permeability of ACV, since this property is widely associated with peroral absorption of drugs. We aim to evaluate the apparent permeability of ACV when it is incorporated in the developed microemulsion, using the *in vitro* method previously developed by our research group.

2. Materials and methods

2.1. Materials

Microemulsion excipients in the current study were chosen with respect to biocompatibility, biodegradability and availability. Polysorbate 80 (Tween® 80) (P80) (CAS number 9005-65-6), ginger oil (GO) and acyclovir were provided by Todo Droga®, Argentina. Deuterium oxide (D_2O) was obtained from Sigma-Aldrich, Argentina, and dimethyl sulfoxide-D6 with a 99.8% deuteration degree (DMSOd) was supplied by Merk®, Switzerland. All the other materials and solvents were of analytical reagent grade. A Millipore Milli-Q Water Purification System generated the water used in these studies.

2.2. Pseudo-ternary phase diagram (PTPD)

For the construction of the pseudo-ternary phase diagrams, Hydrophilic-lipophilic balance (HLB) values of surfactant mixtures were calculated. The HLB value describes the simultaneous attraction of the surfactant mixture for the oil and aqueous phase, and when it is similar to the required HLB of the oil phase of the ME systems, it provides the minimum energy condition for ME formation [28]. From these, to obtain the clearest system, a 1:1 Polysorbate 80:Ethanol mixture that presented an HLB value of 15 was selected as S and maintained constant. Ginger oil constituted the oil phase. A titrimetric method was employed by increasingly adding water (W) to the Oil/Surfactant (O/S) semisolid mixtures (1.0 g) in weight ratios ranging from 1:9 to 9:1 (w/w) with constant stirring using a vortex stirrer. The whole study was carried out at 37 °C to mimic the blood temperature so the behavior of the system after administration can be figured out. Also, above this temperature Tween 80 was melted, improving the interaction with the other components. The transitions from semisolid mixture to fluid transparent or semi-transparent systems or separate phase (SP) were sharp and reproducible with 0.1 ml of W.

2.3. Droplet size and polydispersity measurement

The mean droplet size and polydispersity index (PDI) of the ME were determined at room temperature using a dynamic light scattering in a Zetasizer Nano ZS90 instrument (Malvern Instruments, UK). Five formulations (Table 1) selected from the fluid transparent region of the PTPD (Fig. 1, lines A and B) containing different O/S ratios were evaluated, in absence and in presence of acyclovir. The samples were appropriately diluted with water before analysis.

2.4. Study of the effect of O/S ratio on the incorporation of the drugs in the ME

The same five selected formulations described in Section 2.2 were prepared to evaluate the influence of the system composition on the incorporated amount of ACV (Table 1). The samples were prepared by slowly adding the corresponding volume of W with gentle stirring to the polysorbate80/ethanol/ginger oil semisolid mixture after the addition of the oil phase amount to enable the dissolution of the surfactant. The dispersion was then stirred using a vortex agitator for 1 min. Excess amounts of ACV were dissolved directly in the dispersions, which were then stirred for an additional minute. The suspensions were filtered through a 0.45 μm Seringa Cel Reg Sartorius® filter, appropriately diluted with water and analyzed utilizing an Agilent Technologies ® Cary 60 UV visible spectrometer with 1 cm path length cuvettes. The amounts of drug incorporated were plotted against the O/S used.

Table 1
Percentage composition (w/w %); droplet size and polydispersity index (PDI) of unloaded and ACV-loaded formulations (F); apparent solubilities achieved with the systems (S_{app}) in mg/ml and apparent solubility increment achieved using them (S_{app}/S_{Acv}). (See Fig. 1 for phase diagram illustration). *Water solubility of ACV (S_{ACV}):* 1.41 mg/ml at 25 °C [29].

	Percentage composition (w/w %)					Unload ME		ACV-load ME		Solubility studies	
F	P80	ET	GO	W	O/S	Droplet size (nm)	PDI	Droplet size (nm)	PDI	S_{app} (mg/ml)	S_{app}/S_{ACV}
F_1	15.45	15.45	2.49	66.61	0.08	462.4	0.532	344.3	0.406	42 ± 3	29.7
F_2	14.50	14.50	4.17	66.83	0.14	327.0	0.319	108.8	0.214	51 ± 4	36.2
F_3	14.15	14.15	5.00	66.70	0.18	285.2	0.460	113.4	0.152	57.9 ± 0.7	41.1
F_4	13.80	13.80	5.80	66.60	0.22	242.8	0.461	219.0	0.269	78.3 ± 0.2	55.5
F_5	13.30	13.30	6.60	66.80	0.25	324.5	0.466	324.0	0.463	82 ± 3	58.2

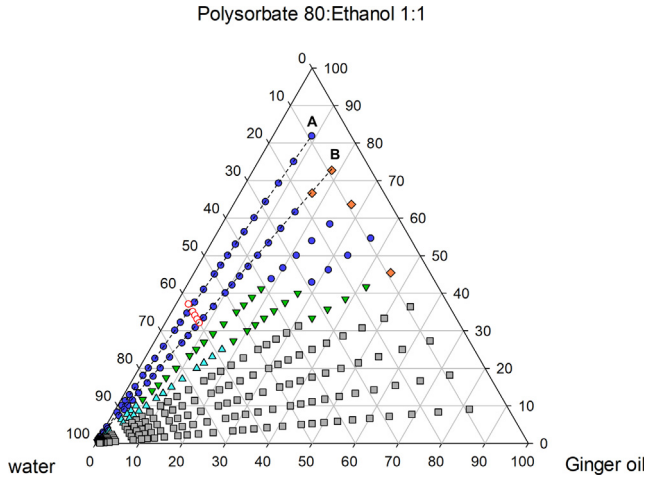


Fig. 1. Pseudo-ternary phase diagram of 1:1 polysorbate 80:ethanol (S) microemulsions containing ginger oil (O) and water (W). ● fluid transparent system; ◆ semi-fluid transparent system; ▼fluid semi-transparent system; ▲fluid hazy system; ■ separation of phases; O selected formulations for droplet size analysis, incorporation studies, conductivity measurements and *in vitro* permeability assay. **Lines A and B**: Formulations obtained from the titration of 1:9 or 2:8O:S semisolid mixture, respectively.

2.5. Conductivity (σ) measurements

The electrical conductivity (σ) of unload and drug-load formulations was measured as a function of the percentage of water (%), the oil/surfactant ration (O/S) and drug percent using a Metler Toledo DG 115-SC conductivity meter. The conductivity-meter was calibrated using a standard solution of 1413 μS/cm before testing. The compositions selected for all studies are presented in Table 1. Measurements were carried out in duplicate at 25 ± 1 °C.

2.6. Nuclear Magnetic Resonance (NMR) studies

^{1}H NMR were performed at 298 K in a Bruker® Advance II High Resolution Spectrometer equipped with a Broad Band Inverse probe and a Variable Temperature Unit using 5 mm sample tubes. Spectra of the unload and ACV-load systems were obtained by incorporating a 0.1 ml of a 5.8/27.6/66.8O/S/W (w/w/w) unload and drug-load ME to 0.5 ml of D_2O. The spectra of the pure components were obtained by diluting appropriate amounts of polysorbate 80 and ethanol in D_2O; acyclovir was dissolved in a DMSOd: D_2O mixture and ginger oil in DMSOd. The reason for this was that acyclovir spectra showed no signals when obtained in D_2O due to its low solubility, and ginger oil is immiscible with D_2O. All the studies were carried out at 400.16 MHz and the data were processed with Bruker® TOPSPIN 2.0 software. The residual solvent signal (4.80 ppm) was used as the internal reference. The induced changes in the ^{1}H NMR chemical shifts ($\Delta\delta$) for the ME components originated due to their interaction were calculated according to the following equations:

$$\Delta\delta_1 = \delta_{unload-ME} - - \delta_{component}$$

and

$$\Delta\delta_2 = \delta_{ACV-load\ ME} - - \delta_{unload\ ME}$$

2.7. In vitro permeability studies

Permeation experiments were carried out using side-by-side two-chamber diffusion cell system (active diffusion area of 1.44 cm^2). In order to mimic the lipophilic properties of biological membranes, a 0.45 μm cellulose ester membrane (Gamafil® S.A., Argentina) was used as support and impregnated by immersion for 30 min, with an 80% of Lipoid ® S100 and 20% of Cholesterol (Sigma Aldrich ®) dispersed in 2 ml of n-octanol in a 10% w/w solution (Table 1). The membranes were then mounted between the donor and receptor compartments. Both phases were thermostated at 37.0 ± 0.5 °C and circulated continuously on two sides of the diffusion cell. A 0.01 M, pH 7.4 phosphate buffer solution (PBS) was used as the diffusion medium in the donor and receptor cells. The test formulation samples in a higher oral dose of 400 mg of acyclovir [6] in 250 ml equivalent quantity (1.6 mg/ml) were suspended in PBS 7.4 and then loaded into the donor compartment. Solutions inside the cell compartments were mechanically stirred at 14,000 rpm (BioTraza® MS-HS10). Samples (2.0 ml) were withdrawn from the receiver compartments at fixed time intervals and replaced with an equal volume of prewarmed PBS. The drug concentration was measured spectrophotometrically at 254 nm using a spectrometer UV vis Agilent Technologies ® Cary 60. All experiments were run in triplicate. To ensure that the experiment was maintained under sink conditions, the relation between drug concentrations in the receptor and donor compartment was monitored at all time points, considering a ratio of 0.1 or lower as appropriate. The apparent permeability coefficients (P_{app}) were calculated from the slope of the linear region of the permeation profile according to the following equation:

$$P_{app} = dQ/dt\ x\ V/(A.C_0.60)$$

where dQ/dt is the increase of the permeated cumulative drug amount versus time (mg/min), V is the volume of the receiver compartment, A is the surface area of the membrane, C_0 is the initial drug concentration in the donor compartment and 60 is the conversion factor from minute into second.

The permeation behavior of ACV from PBS and from the ME was analyzed according to the correlation between the P_{app} values obtained experimentally and the Fa % previously developed in our laboratory [27]. The following equation was used:

$$Fa\% = \left(1 - e^{A.\ Papp}\right) x100$$

where A is the correlation coefficient between the Fa% and the P_{app} obtained by the permeability assay. In addition, considering the permeability limit stablished in the mentioned method, the perme-

ability behavior of the drug from the different ME was classified as low or high.

3. Results

3.1. Pseudo-ternary phase diagram (PTPD)

The PTPD for the systems containing ginger oil (O), 1:1 Polysorbate 80:Ethanol and water is presented in Fig. 1. Microemulsions often need high surfactant concentrations in order to attained very low interfacial tension. However, high surfactant amounts are often not appropriate because of performance, bioincompatibility or economic reasons. The addition of another amphiphile, as cosurfactant, permits the improvement of the surfactant efficiency and concentration required to form the system [29]. For this reason, a small amount of ethanol was added to the formulation, is a short length alcohol and helps to reduce interfacial tension, acting as a co-surfactant/co-solvent. In this study, fluid transparent systems were considered the first indicator of microemulsions, which were later confirmed by droplet size determination. Transparent and fluid systems were obtained when the 1:9O:S semisolid mixture was titred with water (line A), and remained transparent and fluid in the whole range of titration with water. A similar behavior was observed when the 2:8O:S semisolid mixture was titred with water (line B), with the only difference that semi-fluid systems were obtained when the water amount was lower than 20%, and turned fluid with water percentages higher than 20%. Transparent and semi-fluid formulations were obtained when the water amounts were lower than 15%, were added to the 3:7O:S semisolid mixture, and became fluid with water percentages higher than 15%. However, the systems turned semi-transparent when the water percentage reached 40%. A similar behavior was observed when the 4:6O:S semisolid mixture was titred, which was fluid and transparent until reaching 30% of water percentage, where it became semi-transparent and turned hazy at 55% of the water amount. When 4:6O:S semisolid mixture was titred, fluid semi-transparent systems were obtained from 15% to 35% of water, and then separation of phases was observed. Phase separation was obtained when 5:5, 6:4, 7:3, 8:2 and 9:1 semisolid mixtures were prepared and titred. From these results, five stable systems were selected from the fluid transparent region along lines A and B, and are described in Table 1. These formulations were used for subsequent studies.

3.2. Droplet size analysis and polydispersity measurement

The mean droplet size and PDI of the five selected formulations were analyzed, with values obtained in the range 242.8–462.4 nm and 0.319–0.532, for unloaded systems (Table 1). As it was observed for formulations (F) 1–4, the average diameter of the droplets decreased because of the increasing oil content in the internal phase, up to 5.8% of ginger oil. On the other hand, the mean droplet size and PDI of the ACV-load systems presented values in the range 108.8–344.3 nm and 0.152–0.463. The formulations that presented lower droplet size values were F_2 and F_3, with 4.17% and 5.00% of ginger oil, which also presented the lowest PDI. It was interesting to observe that the systems containing ACV presented reduced droplet size in comparison with the unload formulations.

3.3. Study of the effect of O/S ratio on the incorporation of ACV in the ME

In order to evaluate the effect of the O/S ratio on the solubilization of ACV in the ME, incorporation studies were performed using the five formulations selected from the phase diagram (Table 1). The results obtained are presented in Fig. 2 c. In order to analyze the obtained values, it is important to highlight that ACV presents high water solubility (1.41 mg/ml) [29]. The apparent solubilities (S_{app}) for ACV obtained from its incorporation in $ME_{1\text{-}5}$ were 42; 51; 57.9; 78.3 and 82 mg/ml, respectively. In order to analyze the effect of each microemulsion on the solubility of ACV, the apparent solubility increments (S_{app}/S_{ACV}) were calculated, observing that the solubility of ACV was 58 times greater in relation to the water solubility of the drug.

3.4. Conductivity (σ) measurements

The electrical conductivity (σ) was determined as a function of the composition of the microemulsions, since it was previously

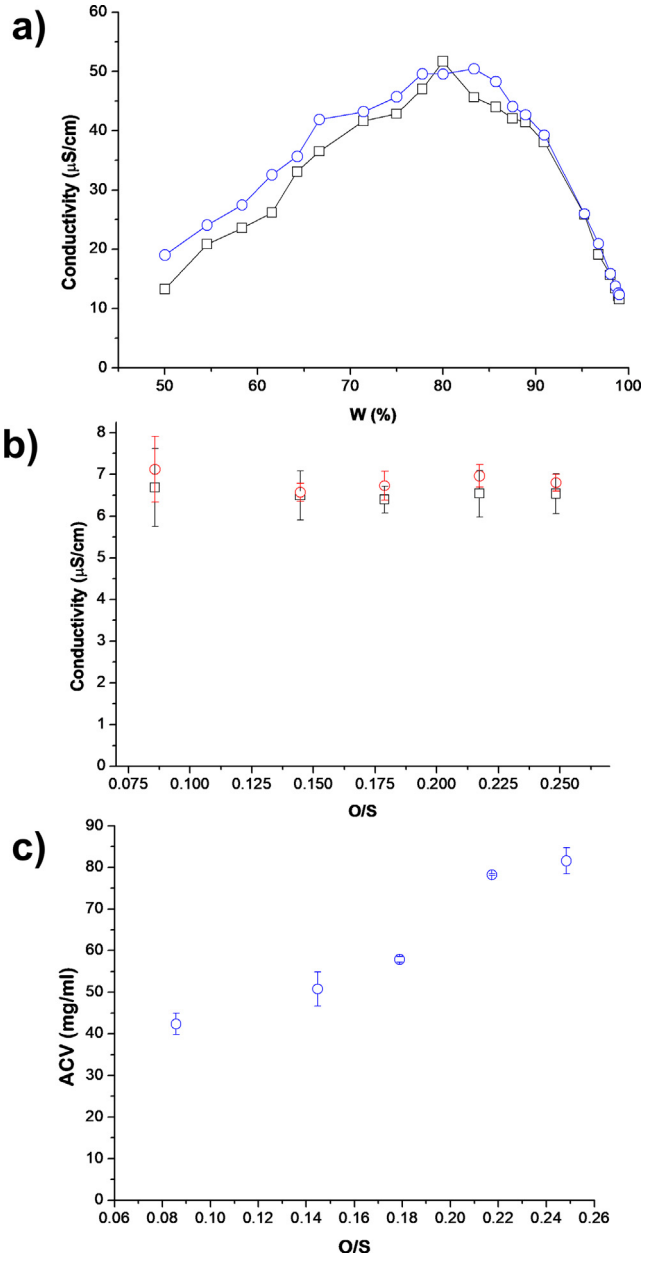


Fig. 2. Variation in the electric conductivity (σ) as a function of: a) water percentage (%) across lines A (□) and B (○) of the PTPD; b) the O/S ratio, for unload (□) and ACV-load (○) microemulsions; and c) Incorporation curve of ACV in W/O 1:1 Polysorbate 80:Ethanol microemulsions containing ginger oil as the oil phase, at 37 °C (See Table 1).

shown that there is a strong correlation between the structure of ME and its electro-conductive behavior [17,30,31]. The conductivity of the different formulations was determined as a function of percentages of water, along lines A and B in Fig. 1, which shows systems that belong to the region where fluid transparent systems were obtained in the PTPD, and according to the particle size, they were classified as microemulsions. Besides, the conductivity was evaluated as a function of O/S ratio for unload and ACV-load systems. In Fig. 2a, when 1:9 and 2:8 oil:surfactant semisolid mixtures were titred with water, it was observed that the conductivity increases with the increase in the water percentage, up to certain amounts of 80 and 83%, for 1:9 and 2:8, respectively. After these values, the conductivity decreases with increasing water content. In Fig. 2b, it was observed that the conductivity remains constant as the O/S ratio increases. In addition, the effect of the presence of ACV was evaluated, where the same behavior was observed in comparison with the unload ME.

3.5. Nuclear Magnetic Resonance (NMR) studies

In order to determine the type of interaction between the components in the microemulsion, ^{1}H NMR studies were developed. Based on the chemical shifts of the pure components, the signals of the unload and ACV-load ME were assigned. The structure of each component and chemical shifts of pure components, unload and ACV-load ME in the corresponding spectra (Fig. 3), are presented in Table 2. Most of the signals in ME were assigned to ginger oil protons and, unfortunately, it was not possible to compare the chemical shift in the pure component with those in the ME, since ginger oil had to be run in DMSO-d because it is insoluble in water. Besides, it was not possible to distinguish proton signals corresponding to acyclovir due to superposition. However, the protons assigned to ethanol in the unload-ME spectra (2-E; 7-F) presented downfield displacement in the unload ME compared to the pure component, with a higher intensity in proton F, which is vicinal to an alcohol substituent. In addition, protons corresponding to the alcohol moiety in the terminal position of lateral chains in the polysorbate molecule (8-N) presented an intense up field displacement, as it can be evaluated by using $\Delta\delta_1$. On the other hand, the proton signals in the ACV-load ME presented an intense downfield shift in the vicinal proton to the alcohol moiety in the ethanol molecule (7-F). Mild upfield displacements were recorded in the protons corresponding to the alcohol moiety in the terminal position of lateral chains in the polysorbate molecule (8-N) and in the protons involved in the double bond of the shogaol molecule, which is an important polyphenolic component of ginger oil.

3.6. Effect of the microemulsions on the apparent permeability of acyclovir

The permeability of ACV dispersed in PBS and contained in the selected ME (Table 1) was evaluated using Franz diffusion cells, and an appropriate bio-mimetic artificial membrane. The ME evaluated in this study showed a tendency of linear permeation, with a progressive increase in the amount of ACV over time (Fig. 4). As it can be seen, the percentage of permeated ACV increased with the increase of O/S ratio, from ME_1 to ME_5.

In the correlation of the experimentally obtained P_{app} values and Fa% data of 20 drugs, previously reported by our research group, it was established that a sample that shows a P_{app} value < $0.64.10^{-6}$ cm s^{-1} presents low permeability and P_{app} value > $0.64.10^{-6}$ cm s^{-1}, which indicates that the drug presents high permeability [27]. According to the P_{app} values obtained experimentally for the selected samples, as shown in Fig. 5 a, ACV presented low permeability when it was dispersed in PBS or ME_1, since the values obtained from P_{app} were 0.0554 and $0.4520.10^{-6}$ cm s^{-1}, respectively. From the rest of the ME, ACV showed high permeability, since P_{app} values greater than $0.64.10^{-6}$ cm s^{-1} were obtained. ACV contained in $ME_{2\text{-}5}$ showed a greater apparent permeability compared to ACV dispersed in PBS. In addition, as it can be seen in Fig. 5b, the %Fa for ACV in PBS was 20.32%, increasing considerably when it was incorporated into the ME, being the highest value obtained with ME_5 (99.97%).

4. Discussion

In the PTPD, the transition from fluid transparent systems to fluid semi-transparent systems was clearly observed, and the latter to hazy systems or separated phases. It was possible to observe a wide range of combinations among the formulation components to obtain fluid transparent systems, in which large volumes of water and ginger oil can be added, maintaining the thermodynamic stability of the system. It could be seen that up to 10% of water is needed to obtain fluid transparent systems, which prevailed in a region below 15% of ginger oil. In order to obtain fluid transparent systems that incorporate higher amounts of oil, greater percentages of surfactants were needed. From the region where fluid transparent system were obtained, five formulations were selected for further studies. Their particle sizes and PDI were determined. The systems with particle sizes lower than 250 nm were considered microemulsions. Unload formulations 3 and 4 accomplished this criteria and, interestingly, in the case of ACV-load systems, formulation 2 also presented a particle size lower than 250 nm. Besides, the particle sizes of all ACV-load formulations were lower than the corresponding ones in absence of the drug. This may be explained by the hypothesis that ACV is located in the interfacial surface of the microemulsion droplets, allowing to reduce the interfacial tension. This will be further discussed based on the NMR results.

The incorporation results show an increase in solubility as a function of the O/S ratio, suggesting that the increasing hydrophobicity of the system is an important factor for the incorporation of the drug. The highest concentration of acyclovir incorporated was 82 mg/ml with ME_5. Considering that the therapeutical dose of this drug goes from 200 to 800 mg, only 2.4 ml would be necessary to reach the minimum dose of 200 mg, and the administration of a 3.25 ml microemulsion three times a day would be enough to reach the maximum dose. However, with the increase in permeability, the needed effect may be achieved with a lower dose.

On the other hand, conductivity measurements were carry out in order to evaluate the microstructure of the microemulsions in relation to their composition. The linear increase was due to the formation of aqueous microdomains ascribed from the partial fusion clustered inverse microdroplets, as it was previously reported by Guiling Li et al. [22]. The percolation theory establishes that with increasing water percentage, the electrical conductivity is altered following three phases: A linear increment due to the formation of aqueous microdomains attributed from the partial combination of clustered inverse microdroplets. The phenomenon indicates that a w/o microemulsion are formed in lower water content region, attributed to the occurrence of a percolation transition. In the primary step, these w/o droplets immersed in nonconducting oil phase, are isolated from each other below percolation threshold, and hence contributed very little to the conductance. As the weight portion of water is above the percolation threshold, some of these conductive droplets began to contact and colloid each other and formed clusters. The number of domains increased suddenly above the percolation threshold, leading to the observed increase of electrical conductivity. The next nonlinear curve increase indicated that the medium underwent further structural transitions and a bicontinuous microstructure was formed, owing

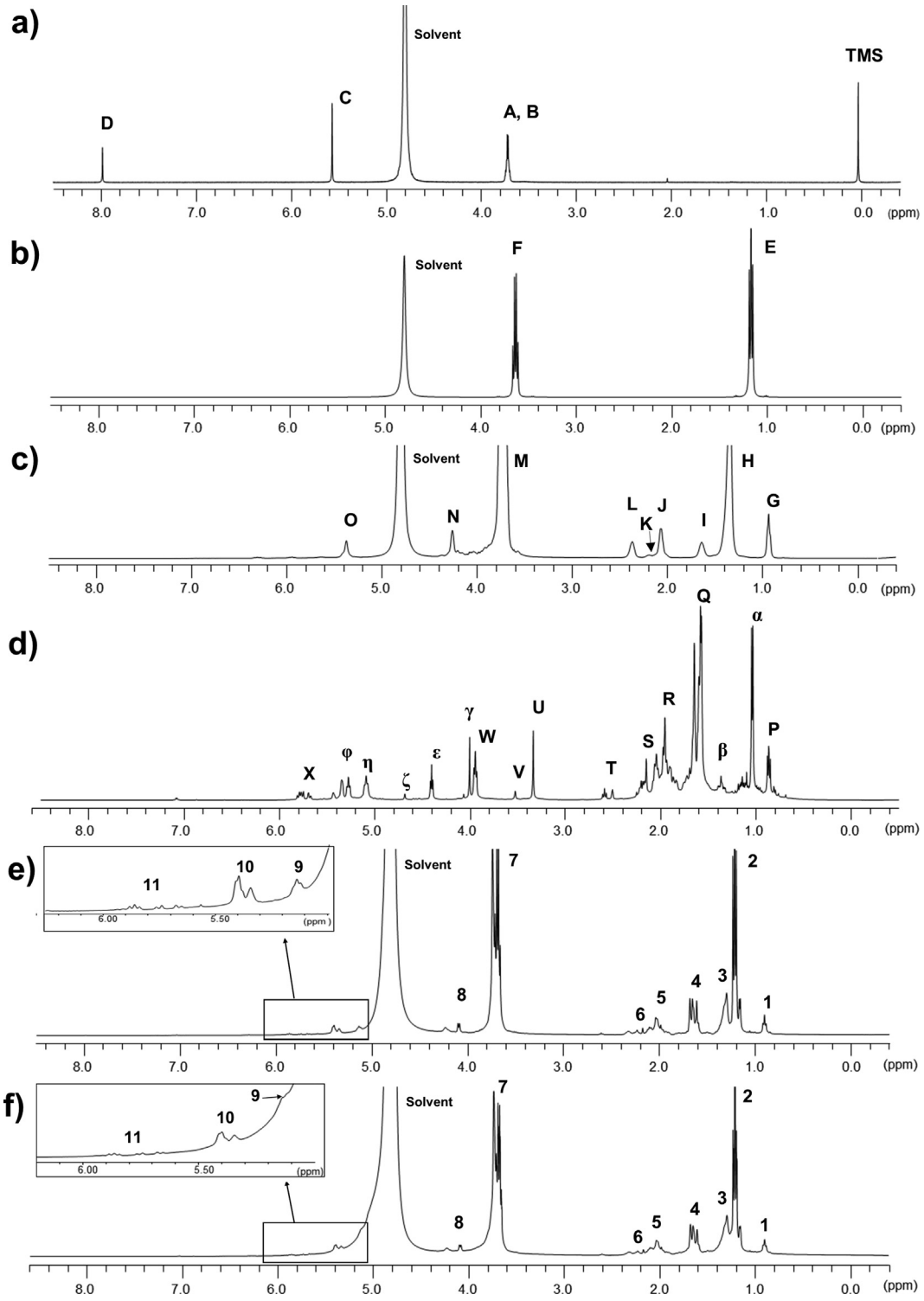
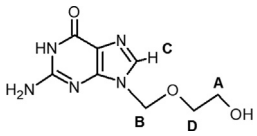
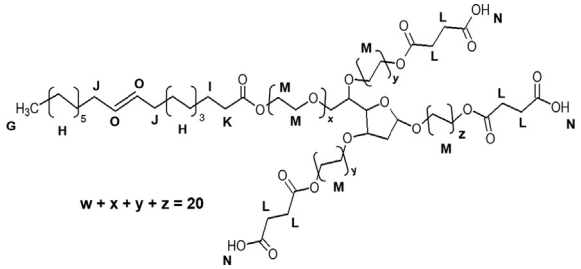
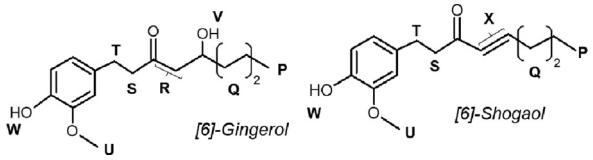


Fig. 3. NMR spectra of: a) pure acyclovir; b) pure ethanol; c) pure polysorbate 80; d) pure ginger oil; e) unload ME and f) ACV-load ME.

to the progressive growth and interconnection of the aqueous microdomains. The final decrease of the slope with increase of water percentage corresponded to water-continuous emulsion type system, indicating the presence of an o/w microemulsion formed at high water percentage. The profile indicated that W/O microemulsions were formed when the water percentage was lower than 80 or 82%, when 1:9 or 2:8O:S semisolid mixtures were titred, respectively. On the other hand, when the water percentage is greater than the critical volume of water, the conductivity values decrease with increasing water percentages, which suggests the occurrence of a percolation transition, in which oil droplets exist in contact with each other, forming domains. Their formation increases rapidly upon reaching percolation, observing large changes in conductivity and a typical profile of O/W ME [22,31]. This behavior suggests that the water content used in the five selected formulations was below the critical fraction values of 80%, that is, it is suitable for obtaining ME with water droplets immersed in a continuous oil phase (W/O). No significant

Table 2
Structures of the main constituent and chemical shifts in the corresponding ^{1}H NMR spectra (a-d in Fig. 2) of each component; and chemical shifts of unload and ACV-load ME in the corresponding spectra (e-f in Fig. 2). $\Delta\delta_1$: δ unload ME - δ component; $\Delta\delta_2$: δ ACV-load ME - δ unload ME.

Component	Structure	Spectrum	Signal	δ (ppm)
acyclovir		**a)**	**A**	0.036
			B	3.7215
			C	5.567
			D	7.981
ethanol	CH_3-CH_2-OH E F	**b)**	**E**	1.171
			F	3.527
polysorbate 80		**c)**	**G**	0.935
			H	1.334
			I	1.64
			J	2.07
			K	2.18
			L	2.369
			M	3.749
			N	4.26
			O	5.375
ginger oil *Polyphenols*		**d)**	**P**	0.862
			Q	1.6085
			R	1.955
			S	2.15
			T	2.544
			U	3.335
			V	3.525
			W	3.943
			X	5.746
Other components	threonine		**α**	1.031
	alanine		**β**	1.365
	fructose		**γ**	4.002
	maleic acid		**ε**	4.401
	β-glucose		**ζ**	4.682
	α-glucose		**η**	5.088
	sucrose		**φ**	5.31

	e)	f)			
ME signal	δ unload ME	δ ACV-load ME	Assigned to	$\Delta\delta_1$	$\Delta\delta_2$
1	0.906	0.911	P (ginger oil)	*	0.005
2	1.218	1.223	E (ethanol)	0.047	0.005
3	1.301	1.305	H (ginger oil)	*	0.004
4	1.657	1.66	Q (ginger oil)	*	0.003
5	2.039	2.043	R (ginger oil)	*	0.004
6	2.105	2.109	L (ginger oil)	*	0.004
7	3.697	3.728	F (ethanol)	0.170	0.031
8	4.094	4.090	N (polysorbate)	−0.166	−0.004
9	5.135	5.136	η (ginger oil)	*	0.001
10	5.367	5.372	φ (ginger oil)	*	0.005
11	5.792	5.789	X (ginger oil)	*	−0.003

changes were observed in the conductivity values when ACV was added to the ME, which suggests that the internal microstructure continued in the W/O ME configuration, even in the presence of the drug.

By NMR studies, it was observed that polar groups play an important role in the microstructure of ME, evidenced by the shift in protons near alcohol moieties in ethanol and in polysorbate 80 in the unload ME in comparison with the pure components. The low upfield shifts in most ACV-load ME protons may indicate the presence of low Van Der Waals interactions between the aromatic ring and lateral chain of ACV with the hydrophobic carbonated chains present in all the ME components. These observations may explain the increment in the incorporation of ACV with increasing O/S ratio. Besides, other important interactions were observed, in which the oxygen in alcohol moiety in polysorbate 80 and ethanol are involved, suggesting that dipole-dipole or hydrogen bond interactions occur, and may interact with the polar groups in the ACV molecule.

In the *in vitro* permeability studies, an increment in the percentage of permeated ACV with increasing O/S ratio was observed, from ME_1 to ME_5. This behavior is probably due to the fact that the oil component of the ME, act as permeation promoters, as previously reported by Bergonzi et al. [32]. Besides, the internal microstructure of the ME is represented by water phase droplets dispersed in the oil continuous phase forming domains, which allow this hydrophilic drug to be incorporated into a much more hydrophobic carrier. ACV is a low permeable drug according to the BCS, since it presents a Fa% of 30% [4]. The Fa% values of ACV incorporated in the microemulsions show the efficacy of these new drug release systems to improve ACV permeation, with great potential application to enhance the oral absorption of this drug. Considering that ACV in $ME_{2\text{-}5}$ presented Fa% higher than 90%, and this drug presents high water solubility, acyclovir in these ME could be classified as Class I systems according to the BCS, which may cause an important positive effect on the therapeutic performance of this antiviral drug.

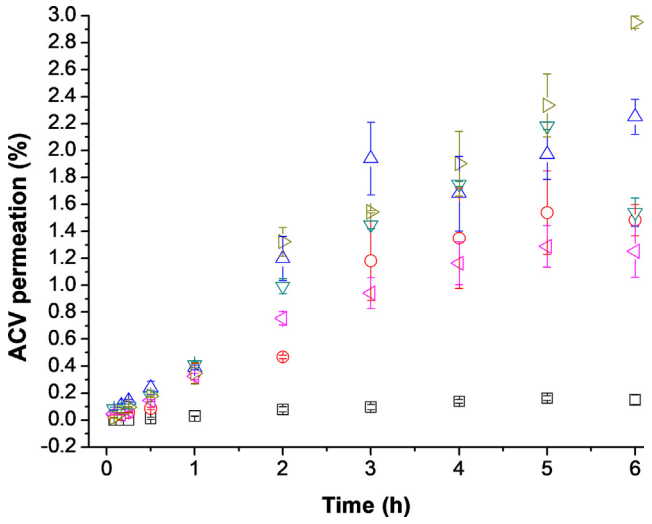


Fig. 4. Permeation profiles of ACV from PBS pH 7.4 (□); ME_1 (○); ME_2 (△); ME_3 (▽); ME_4 (◁) or ME_5 (▷) at 37 ^{0}C (each value represents the average ± S.D. of n ≥ 3).

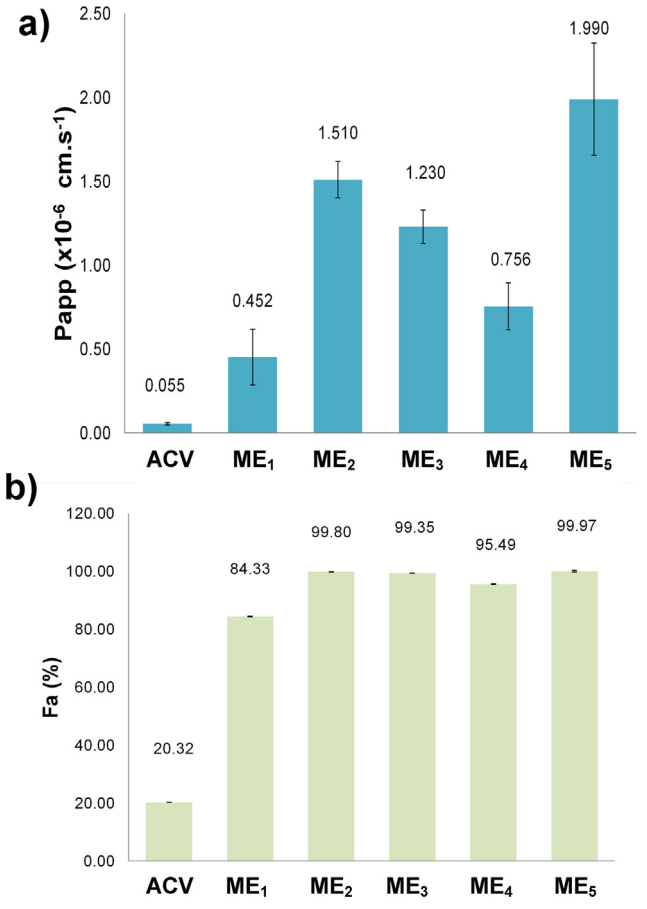


Fig. 5. Permeation parameters of ACV from PBS pH 7.4 (ACV); ME_1; ME_2; ME_3; ME_4 or ME_5 at 37 °C. a) Apparent Permeability Constants (P_{app}) and b) Fraction Absorbed (Fa %).

5. Conclusion

ACV was formulated in microemulsions to improve its permeability. The physical nature of these systems, as well as the physicochemical interactions of the constituents, determined the solubilization and permeation capacity of ACV in the ME. From the obtained results, it could be demonstrated that microemulsions can be obtained by a simple procedure and from biocompatible components, and it was possible to incorporate up to 15% of ginger oil. The conductivity determinations indicated that it is possible to obtain stable ME W/O below the critical fraction values of water of 80 % and suggested that the internal microstructure is represented by water droplets dispersed in the oil continuous phase forming domains, which remain unchanged due to the incorporation of ACV. Incorporation studies demonstrated the ability of microemulsions to incorporate the drug, with an incorporated maximum amount of ACV of 82 mg/ml. The permeability tests showed a considerable increment in the percentage of permeation through the artificial membrane, which represents Fa% values 70% higher with respect to the aqueous ACV solution, which is a low permeable drug according to the BCS, since it presents a Fa% of 30%. In conclusion, the developed drug delivery systems increased the solubility and permeability of ACV, since they were able to incorporate appreciable concentrations of the drug with higher Fa%, which can have a favorable impact on the oral absorption of ACV, thus achieving the main objective of the present work. The results presented have significant relevance and potential for application in the pharmaceutical field.

CRediT authorship contribution statement

Micaela Ponce Ponte: Methodology, Resources, Writing – review & editing. **Martina Bianco:** Methodology, Resources, Writing – original draft. **Marcela Longhi:** Supervision, Writing – review & editing, Funding acquisition, Project administration. **Carolina Aloisio:** Conceptualization, Investigation, Methodology, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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