Understanding drug distribution and release in ophthalmic emulsions through quantitative evaluation of formulation-associated variables

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ABSTRACT

Establishing bioequivalence (BE) of ophthalmic emulsions in the absence of in vivo data is challenging. In these emulsions, drug release is a complex process due to drug distribution among various phases which are difficult to characterize. The objective of this study is to investigate the process of drug distribution and mechanism of drug release in the context of formulation-associated variables. A previously reported kinetic method for determining drug partitioning was used to quantitatively evaluate the drug distribution within a simplified biphasic (emulsion) system employing cyclosporine and difluprednate as model drugs. The impacts of formulation variables, such as the amount of polysorbate 80, glycerin, and carbomer copolymer as well as the area of oil-water interface were investigated. Polysorbate 80 was found to have the greatest influence on the drug distribution. It enhanced both the rate and extent of the drug distribution from oil to aqueous phase. Glycerin was found to slightly reduce the rate and extent of drug distribution of cyclosporine into the aqueous phase, probably by suppressing the solubilization capability of the micelles. Carbomer slowed down the diffusion of drug into the oil phase and shifted the equilibrium drug distribution towards the aqueous phase. Furthermore, increase in the interfacial area significantly increased the rate of drug diffusion across the oil-aqueous interface but had negligible effect on the extent of drug distribution. It is noteworthy that the experimental setup utilized a planar interface rather than an interface with curvature, which may have slightly underestimated the influence of globule size on equilibrium drug distribution. The findings of this study give insight into the drug distribution and diffusion in complex ophthalmic emulsions and assist with formulation design as well as development of in vitro methods to support BE assessment of ophthalmic emulsions.

1. Introduction

Oil-in-water (O/W) emulsions have been successfully used as a delivery vehicle of hydrophobic drugs for ophthalmic delivery. It improves the drug's ocular bioavailability as well as patient compliance. The commercial examples include Restasis® (cyclosporine ophthalmic emulsion) and Durezol® (difluprednate ophthalmic emulsion), which were approved for the treatment of tear production suppression associated with keratoconjunctivitis sicca (or dry-eye) [1] and endogenous anterior uveitis/ocular surgery inflammation and pain [2], respectively. However, for both reference listed drugs (RLDs) no generic has been approved. The unique factors such as lack of reliable systemic absorption and short residence time of drug due to the anatomy and physiology of the eye present challenges in determining the bioequivalence

(BE) of these products [3,4]. Furthermore, the clinical trials for BE assessment can be costly and insensitive, especially for a drug indicated for the chronic treatment (i.e., prolonged and insensitive dose-response) or with large inter-subject variabilities [5–7]. In addition, the in vivo studies for BE assessment can be confounded by multiple in vivo covariates that can give rise to issues in study design and assessment sensitivity [8]. Given these challenges and absence of other viable alternatives, an in vitro option for demonstrating BE that relies on comprehensive physicochemical characterization and in vitro release testing (IVRT) has been considered [9,10]. For example, for generic ophthalmic emulsions having the same qualitative (Q1) and quantitative (Q2) composition as the RLD, parameters like globule size distribution [11,12], viscosity [13], pH, zeta potential, osmolality, surface tension, and drug distribution in different phases within the

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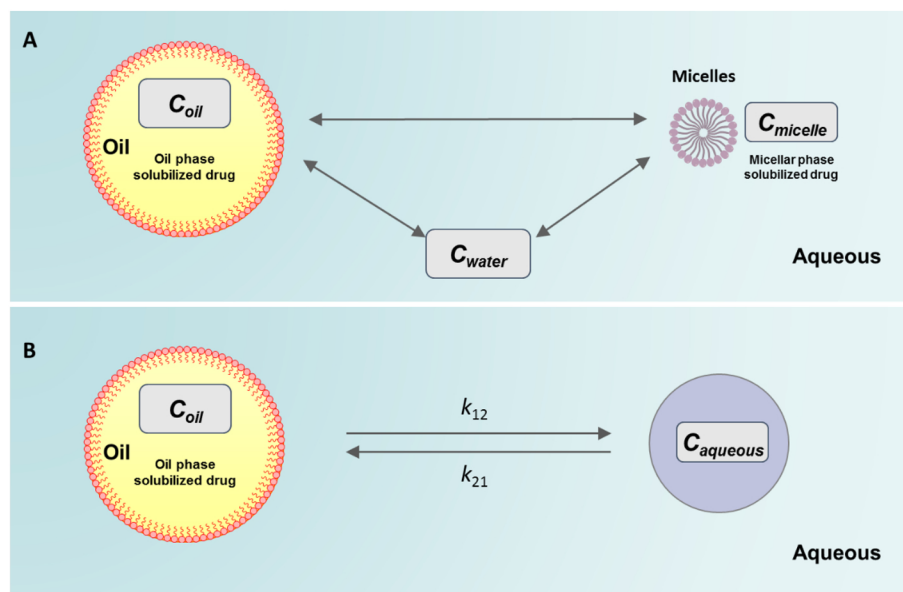**Fig. 1.** A) The phase composition and complex drug diffusion in the microenvironment of an emulsion formulation; B) The simplified scenario of phase composition and drug diffusion in an emulsion formulation.

formulation are identified as critical physicochemical characteristics that are important to support the similarity of different products. However, beyond characterizing and controlling the aforementioned physicochemical parameters, there is an increasing need to understand the function of these parameters particularly for complex biphasic systems like emulsions.

Emulsions are dispersed systems in which an oil phase (e.g., castor oil, mineral oil or vegetable oil) is dispersed as globules in an aqueous phase (Fig. 1A). These oil globules are stabilized by surfactants and viscosity enhancing polymers (e.g., carbomer). In terms of the drug distribution, it is generally understood that hydrophobic drug is predominately solubilized in the oil globules relative to the external aqueous phase. However, it is important to note that excess surfactant in the formulation may also contribute to solubilization of the drug through micellization. The drug distribution in such a complex system is presumed to be constant and in a state of thermodynamic equilibrium during long-term storage, and may be impacted by both the formulation factors (e.g., type and amount of excipients) [14] and the manufacturing process conditions [13]. For instance, higher percentage of drug could be solubilized in the micellar phase if the bulk surfactant concentration increases (i.e., relative to the oil globule surface bound surfactant), which could be impacted by the total globule surface area (related to globule size distribution, see later discussion). Furthermore, the equilibrium distribution may shift with a change in the environment (e.g., temperature fluctuation during storage, dilution upon administration, or dilution in the release medium during an IVRT experiment). The processes to reach and/or shift equilibrium involve complex drug diffusion [15,16] between various phases (Fig. 1A). For example, after topical administration, drug in the water phase is mixed with the lacrimal fluid and thus diluted [17], triggering drug diffusion from micelles and oil globules to the water phase. The transfer of drug between micelle and water phase is considered fast, generally at the time scale of milliseconds to minutes due to rapid micelle relaxation kinetics [18], as opposed to the longer time needed for drug transfer from oil phase to water phase. Therefore, the drug distributed in the water and micellar phases is presumed to be readily available after administration, and the two phases could thus be treated as a single combined aqueous phase (Fig. 1B).

Drug distribution in different phases within the formulation (drug distribution in short is used in the rest of the paper) is considered to be important for the performance of ophthalmic emulsions as it may affect

drug release from the different phases and bioavailability [9,10,13]. However, very few investigators have studied the underlying mechanism and process of drug distribution as a function of formulation variables. To quantitatively evaluate the impacts of formulation variables on drug distribution and transfer among phases of emulsion, the kinetic method developed by Dong et al. [19] for determining the biphasic partitioning of the drug was employed in this study.

In classical thermodynamics, partitioning is considered an equilibrium phenomenon [20]. Unlike other experimental methods that focus on the equilibrium distribution of a compound in two phases [20–23], the newly developed kinetic method enables the determination of partition coefficient via the rate constants (kinetics) of the distribution process across two immiscible phases [19].

In the present study, cyclosporine and difluprednate were selected as hydrophobic model drugs, with predicted log *P* values (octanol/water) of 4.12 [24] and 3.4 [25], respectively. Castor oil was used as the oil phase and water containing various amounts of formulation components were used as the aqueous phase. The formulation variables included polysorbate 80, glycerin, and carbomer copolymer (tonicity adjusting agent and gel-forming stabilizer). The manufacturing process variables potentially affect the area of oil-water interface by altering globule size distribution which in turn may influence the drug distribution. Therefore, the impact of the area of oil-water interface on drug distribution was also investigated.

2. Materials and methods

2.1. Chemical

Cyclosporine USP (> 99%) and difluprednate (> 97%) were purchased from RIA International LLC (East Hanover, NJ). Castor oil was purchased from Fisher (Pittsburgh, PA) and Welch, Holme and Clark (Newark, NJ). Polysorbate 80 was purchased from Acros Organics (Morris Plains, NJ). Glycerol USP was purchased from Thermo Fisher Scientific (Waltham, MA). Carbomer Copolymer Type A (Pemulen™ TR-2 NF) was provided by Lubrizol LifeSciences (Cleveland, OH). Unless otherwise specified, all materials were of analytical grade.

2.2. Kinetic method to investigate the effect of formulation components

The effects of formulation variables on biphasic drug distribution

were investigated using the fiber optic dissolution work station equipped with 25 mL mini vessels (Pion μDiss Profiler™, Billerica, MA) (Fig. 2A [19]). Briefly, 20 mL of aqueous phase was added in each of the 7 vessels where agitation was precisely controlled at 125 ± 1 rpm or 400 ± 1 rpm (for highly viscous carbomer solution only). Additional 20 μL of aqueous drug stock ethanol solution was pipetted in vessel 2 through 4, where the drug diffusion direction was from aqueous to oil phase, to make the initial aqueous concentration reach 7 mg/mL for both drugs. To start the experiment, 2 mL of castor oil that was either blank oil solution (for vessel 1 through 4) or drug-containing oil (for vessel 5 through 7) was placed on top of the aqueous phase. The initial oil concentrations of cyclosporine and difluprednate were 70 mg/mL and 7 mg/mL, respectively. Immediately afterwards, the concentration change in the aqueous phase was monitored using an in situ UV fiber optic probe at a minimal interval of 1 min. Apparent partition coefficient (log P_{app}) values were determined based on the diffusion rate constants, i.e., log $P_{app} = k_{21}/k_{12}$ (kinetic method, refer to [19] for theoretical basis and the procedure to calculate the rate constants). For comparison, a second set of log P_{app} values was calculated using the equilibrium concentrations, log $P_{app} = [C_{oil}]_{eq}/[C_{aq}]_{eq}$. After the equilibrium was reached, aliquots of sample (200 μL) were withdrawn from both oil and aqueous phases for analysis by an HPLC method. To obtain measurable concentrations of the hydrophobic drugs, the oil samples were diluted with 70% (v/v) ethanol and further diluted with 90% (v/v) ethanol prior to HPLC analysis. The initial drug concentrations in either oil or aqueous phase were estimated per the predicted log P values. Castor oil was the oil phase in all experiments. Different aqueous phases contained polysorbate 80, glycerin and carbomer (for cyclosporine only) at various concentrations, and their influence on drug distribution was evaluated (Table S1). Experiments were conducted in paired triplicate (i.e., three aqueous-to-oil diffusions and three oil-to-aqueous diffusions) and with one blank as a control to subtract the

interference from UV active materials, e.g., polysorbate 80. A zero-intercept method (ZIM) based on the second-derivative UV spectrum was used to remove interference from castor oil [19]. As determined by ZIM, the wavelengths utilized for cyclosporine and difluprednate in the kinetic method were 250 nm and 267 nm, respectively, where the second-derivative UV of the drug changed proportionally to its concentration while the UV signal of the interfering component remained zero regardless of its concentration. The experiments were thermostatically controlled at 34 °C.

2.3. Kinetic method to investigate the impact of interfacial area

To accommodate the need to adjust the interfacial area, a different setup was used which consisted of 7 jacketed beakers (1000 mL), each with a custom designed insert to control the interfacial area (Fig. 2B). The agitation was maintained using a magnetic stirrer and the temperature was controlled using a circulating water bath. Unlike the small volume used with mini vessels, 400 mL of aqueous phase containing polysorbate 80 was added in each of the 7 beakers, on top of which was 40 mL of blank oil solution (for beaker 1 through 4) or drug-containing oil solution (for beaker 5 through 7, where drug diffuses from oil to aqueous). The interfacial area was varied from 2.51 cm^2 to 83.0 cm^2 by adjusting the diameter of opening at the bottom of each insert (Fig. 2B). Other experimental conditions and procedure were same as those with mini vessels (Table S1).

2.4. Assay by high-performance liquid chromatography (HPLC)

Equilibrium drug concentrations were analyzed by HPLC method. The HPLC system consisted of an Agilent 1200 Series (Agilent Technologies, Wilmington, DE) equipped with degasser, binary solvent pump, autosampler, thermostatted column compartment, and a diode
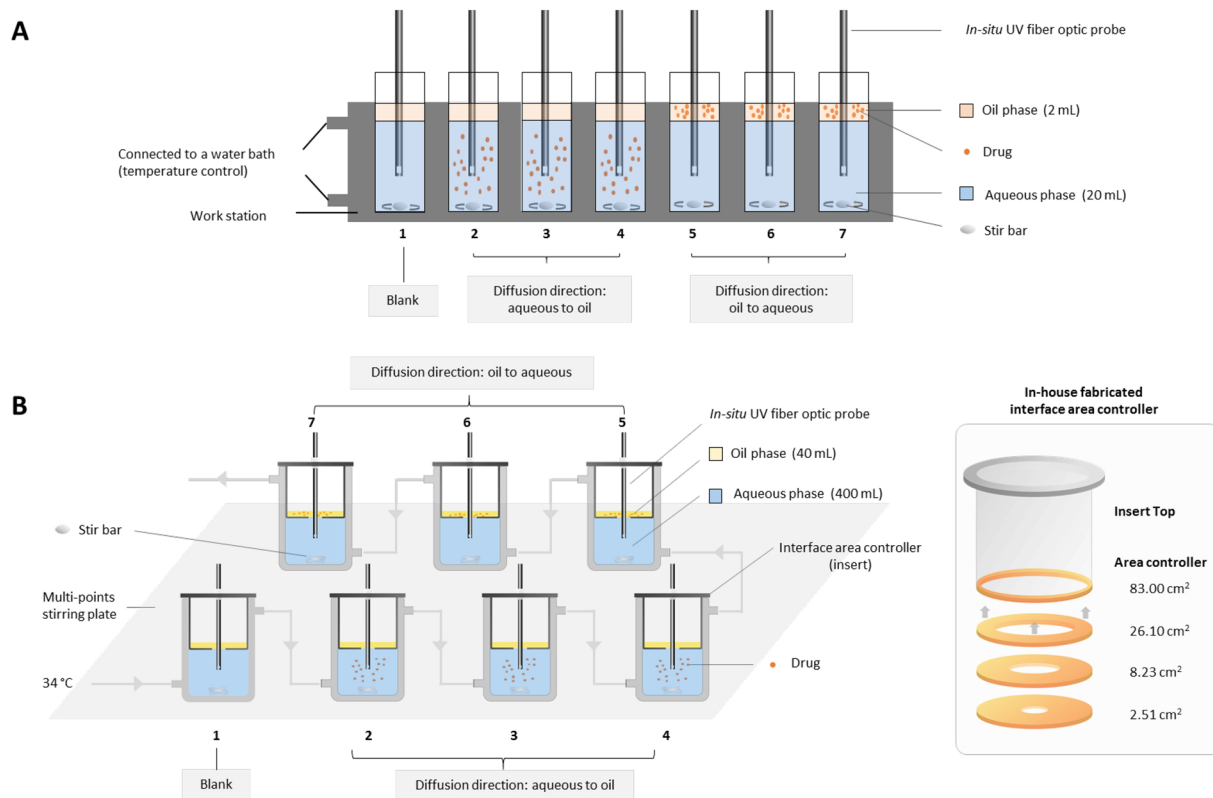
Fig. 2. A) The experimental setup for evaluating the effects of formulation variables, including the fiber optic dissolution work station and 25 mL mini vessels, adapted from [19] with permission from Elsevier. B) The experimental setup for evaluating the effect of interfacial area, consisting of 1000 mL jacketed beakers and in-house fabricated interfacial area controller with switchable bottom inserts.

array detector. Data collection and analysis were performed using OpenLAB chromatographic software. The current method (adapted from [19] employed a reversed phase Luna C8(2), 4.6 mm × 150 mm (3 μm packing) column (Phenomenex, Torrance, CA). For analysis of cyclosporine, the column temperature was maintained at 60 °C. The mobile phase consisting of an isocratic mixture of acetonitrile and 0.1% (v/v) phosphoric acid (70:30, v/v) was pumped at a flow rate of 1 mL/min. A sample volume of 100 μL was injected onto the column and the eluted cyclosporine detected at 210 nm. For analysis of difluprednate, the column temperature was maintained at 40 °C. The mobile phase consisting of an isocratic mixture of acetonitrile and water (50:50, v/v) was pumped at a flow rate of 1 mL/min. A sample volume of 100 μL was injected onto the column and the eluted difluprednate detected at 240 nm.

2.5. Assay of cyclosporine by ultra-performance liquid chromatography (ULPC)

At higher concentrations (e.g. > 0.1%, w/w), polysorbate 80 was found to interfere with the assay of cyclosporine by HPLC; and in those scenarios UPLC method was used. The UPLC system consisted of a Waters Acquity UPLC I-Class (Waters Corporation, Milford, MA) equipped with degasser, binary solvent pump, autosampler, thermostatted column compartment, and a photo diode array detector. CORTECS UPLC C8, 2.1 mm × 150 mm (1.6 μm packing) column (Waters Corporation, Milford, MA) was used along with a CORTECS UPLC C8, 2.1 mm × 5 mm (1.6 μm packing) vanguard precolumn (Waters Corporation, Milford, MA). Gradient elution method (Table 1) was developed by using a flow rate of 0.6 mL/min while maintaining the column temperature at 65 °C. Appropriate dilutions were performed to avoid any matrix effects. A sample volume of 50 μL was injected onto the column by installing a 100 μL extension loop into the system and the eluted cyclosporine was detected at 210 nm. Data collection and analysis were performed using Empower 3 software.

3. Results and discussions

3.1. Effect of polysorbate 80 on the rate and extent of drug distribution in biphasic systems

Polysorbate 80 serves as a stabilizer in the emulsions. It reduces the interfacial tension at the oil (globule)/water interface which lowers the tendency for globules to coalesce and/or phase-separate. Polysorbate 80 is always used in excess, relative to its critical micelle concentration (CMC) and the available surfaces needing stabilization. The excess surfactant spontaneously forms micelles which also provide additional solubilization capacity for the lipophilic compounds like cyclosporine and difluprednate. To determine the impact of solubilization by micelles on the drug diffusion rates as well as the distribution between oil and water phases, a series of concentrations of polysorbate 80 was added into the aqueous phase. With the in situ UV fiber optic probe, the rapid concentration change was captured and the biphasic diffusion rate constants (i.e., k_{12}, oil to aqueous; or k_{21}, aqueous to oil) were determined based on the method reported previously [19].

Table 1
UPLC gradient elution method used for the determination of cyclosporine.

Time (min)	% Acetonitrile	% (0.1% Phosphoric acid in DI Water: Methanol, 68:32 (%, v/v)
0.0	45	55
19.0	45	55
20.5	92	8
23.5	92	8
25.0	45	55
27.0	45	55

The results in Fig. 3 show that the rate constants increased (i.e., faster diffusion) in the direction of oil to aqueous phase (i.e., k_{12}) with increasing concentration of polysorbate 80, which contrasts the decreasing trend of the k_{21} in the opposite direction (i.e., slower diffusion), for both cyclosporine and difluprednate. While the k_{21} values were comparable between the cyclosporine and difluprednate, the k_{12} values of difluprednate were significantly ($p < 0.05$) larger than those of cyclosporine (e.g., 6.58E-08 s^{-1} and 4.19E-09 s^{-1} with 0.1% (w/w) polysorbate 80, respectively), indicating the rate of diffusion from the oil to aqueous phase was inversely related to the drug's hydrophobicity. More importantly, the log of the rate constants ratio (i.e., log P_{app} = log k_{21}/k_{12}) decreased from 4.764 ± 0.109 (w/o polysorbate 80) to 3.299 ± 0.078 (with 1.0% polysorbate 80) for cyclosporine, and from 3.542 ± 0.084 (w/o polysorbate 80) to 2.413 ± 0.062 (with 0.4% polysorbate 80) for difluprednate, suggesting that polysorbate 80 increased not only the rate (i.e., faster transfer from oil to aqueous) but also the extent of drug distribution into aqueous phase (i.e., more drug in the aqueous phase) (Fig. 4).

Apparent partition coefficient values of two model drugs in castor oil and in polysorbate 80 solutions were also calculated using the equilibrium concentrations, i.e., log ($[C_{oil}]_{eq}/[C_{aq}]_{eq}$), and found to be consistent with the values determined using the kinetic method (Table 2). For example, the addition of polysorbate 80 decreased the apparent partition coefficient from 4.533 ± 0.367 (w/o polysorbate 80) to 3.204 ± 0.042 (with 1.0% w/w polysorbate 80) for cyclosporine, and from 3.504 ± 0.066 (w/o polysorbate 80) to1.885 ± 0.076 (with 4.0% w/w polysorbate 80) for difluprednate. Again, the decrease in log P_{app} confirmed that the addition of surfactant leading to micelle formation could significantly shift the distribution equilibrium towards aqueous phase, providing more drug that is readily available for release or absorption.

The relevance of determining the apparent partition coefficient of drug in a biphasic system similar to a proposed formulation is that it provides an estimate of the equilibrium drug distribution as well as the extent of drug re-distribution upon dilution. Taking a difluprednate emulsion for example, let's assume the emulsion contains 1% (v/v) oil and 99% (v/v) aqueous phase, which leads to an oil-to-aqueous volume ratio of 1/99. If we also assume the emulsion contains 0.4% (w/w) polysorbate 80 (according to Table 3, difluprednate has an apparent partition coefficient of 2.413 ± 0.062), the ratio of the equilibrium concentration of oil-to-aqueous is then estimated to be 10^ (2.413 ± 0.06), or between 220.2 and 304.2 (95% confidence interval, CI). By multiplying the volume ratio and the concentration ratio, we can calculate the difluprednate mass distribution ratio of oil-to-aqueous to be 2.2 to 3.0, or in terms of mass percentage of the drug in the aqueous phase to be 24.7% to 31.2% (95% CI). This contrasts the predicted percentage of only 2.3% to 3.4% (95% CI) when no polysorbate 80 was added (similar calculation as shown above). Therefore, by adding excess polysorbate 80, the extent of aqueous phase solubilized difluprednate increases drastically. Another utility of the apparent partition coefficient is to estimate how much drug can be released upon dilution. For example, assuming again a difluprednate emulsion with an oil-to-aqueous ratio of 1/99 and a polysorbate 80 concentration of 0.4% (w/w), we can estimate the amount of difluprednate that can re-distribute (e.g., release) into the medium to be 44% and 77% after 10- and 100-times dilution with water, respectively. It should be noted, however, that the calculation described here focused primarily on the extent of drug release, and to estimate the rate of release one needs to also consider the diffusion rate constants mentioned earlier.

3.2. Effect of glycerin on the rate and extent of drug distribution

Glycerin commonly servers as a viscosity enhancer and or tonicity adjusting agent in emulsion formulations [26]. To determine if viscosity of the aqueous phase has an impact on the rate and extent of drug distribution, increasing percentage of glycerin was added into the
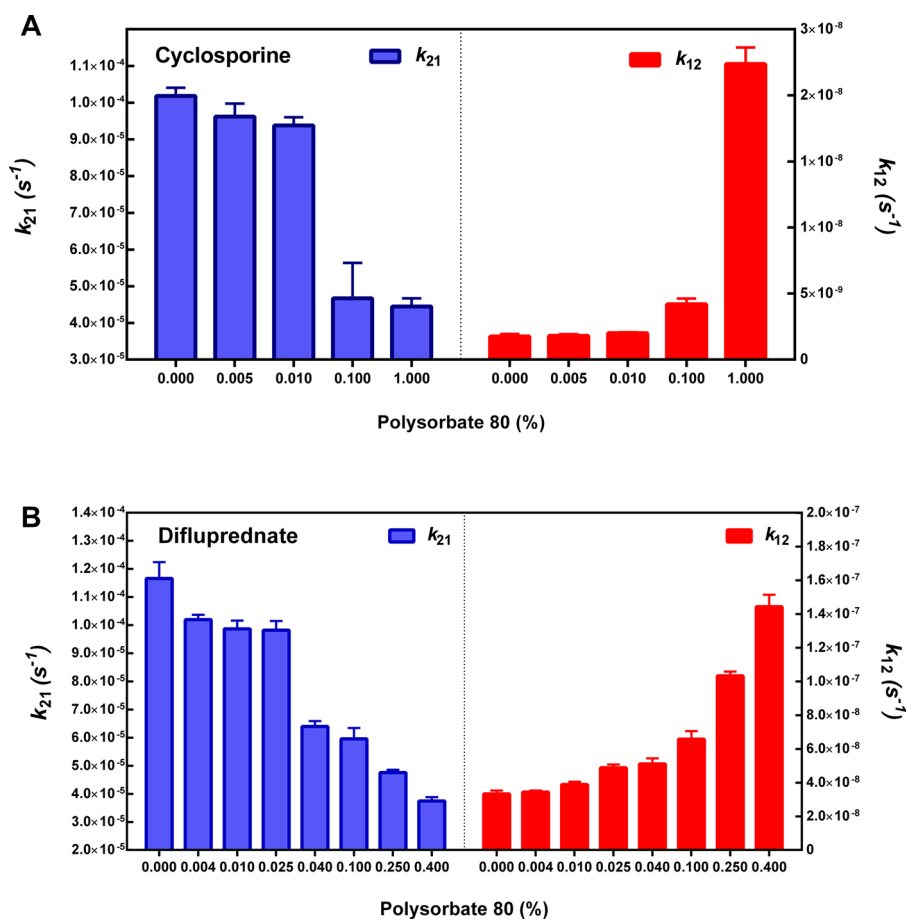
Fig. 3. Biphasic diffusion rate constants determined by the kinetic method with respect to the concentrations of polysorbate 80. A) Cyclosporine; B) Difluprednate. k_{12} and k_{21} are diffusion rate constants in the directions of oil to aqueous and aqueous to oil, respectively n = 3.
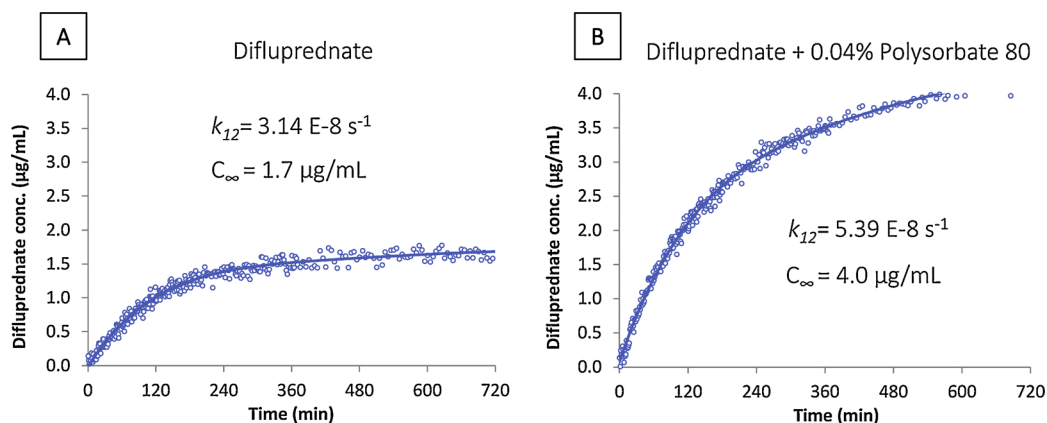

Fig. 4. Effect of surfactants on the diffusion kinetics of difluprednate from castor oil to aqueous phase. A) From castor oil to water; B) From castor oil to water which contained 0.04% (w/w) polysorbate 80.

aqueous phase (from 0% to 2.0%, w/w). The results in Fig. 5 show that the diffusion rates of cyclosporine and difluprednate across the oil and aqueous phase responded differently to the addition of glycerin. In case of cyclosporine, a small amount of glycerin (i.e. 0.2% w/w) caused a slight increase in the rate constants (both k_{12} and k_{21}), but as the concentration of glycerin was increased further (e.g., to 2.0% w/w), the rate constants (k_{12} and k_{21}) decreased significantly ($p < 0.05$). The exact cause for the slight increase in rate constants with small amount of glycerin is unknown but is likely related to cyclosporine's solvent-dependent conformation and hydrogen-bonding capability [27] which might have changed the diffusivity of the drug [28]. While the

decreased diffusion rate from aqueous to oil phase may be explained by the increased viscosity of aqueous solution (e.g., 0.736 cp to 0.783 cp [29], and through the Stokes-Einstein relationship of $D = k_B T/6\pi\eta r$), the drop observed in the diffusion rate from oil to aqueous phase was not expected. It is likely that glycerin inhibited the micellization of surfactants by reducing the cohesive force between polysorbate 80, and thus decreasing the solubilization of the drug through micellization [30]. The decrease in solubilizing capacity of polysorbate 80 led to, not only a reduction in cyclosporine transfer rate from oil to aqueous phase, but also to a lower extent in the aqueous phase (e.g., the log P_{app} increased from 4.669 ± 0.043 without glycerin to 5.006 ± 0.164 with

Table 2
Apparent partition coefficient values of cyclosporine and difluprednate with respect to polysorbate 80 concentration determined by kinetic method and equilibrium concentration method (n = 3).

Drug	Concentration of Polysorbate 80 (%, w/w)	log P_{app}	
		Kinetic method = log (k_{21}/k_{12})	Equilibrium concentration method = log ([C_{oil}]$_{eq}$/[C_{aq}]$_{eq}$)
Cyclosporine	0	4.764 ± 0.109	4.533 ± 0.367
	0.005	4.723 ± 0.083	4.607 ± 0.108
	0.01	4.669 ± 0.043	4.551 ± 0.194
	0.1	4.047 ± 0.231[a]	4.115 ± 0.120
	1.0	3.299 ± 0.078[a]	3.204 ± 0.042
Difluprednate	0	3.542 ± 0.084	3.504 ± 0.066
	0.004	3.471 ± 0.028	3.425 ± 0.008
	0.01	3.405 ± 0.052	3.350 ± 0.070
	0.025	3.304 ± 0.050	n/d
	0.04	3.098 ± 0.071	3.174 ± 0.009
	0.1	2.957 ± 0.096	2.893 ± 0.020
	0.25	2.662 ± 0.030	n/d
	0.4	2.413 ± 0.062	2.476 ± 0.097
	4.0	n/d	1.885 ± 0.076

n/d: Not determined.
[a] Aqueous concentrations were analyzed by HPLC or UPLC after sampling.

Table 3
Apparent partition coefficient values of cyclosporine and difluprednate with respect to changes in several formulation variables as determined by the kinetic method (n = 3).

Drug	Formulation variable	Tested condition	Log P_{app}
Cyclosporine	Glycerin (w/w) in polysorbate 80 (0.1%, w/w)	0%	4.669 ± 0.043
		0.2%	4.691 ± 0.133
		1.0%	4.881 ± 0.269
		2.0%	5.006 ± 0.164
	Carbomer (w/w)	0%	4.764 ± 0.109
		0.005%	4.354 ± 0.111
		0.05%	3.898 ± 0.258
		0.005% in polysorbate 80 (0.1%, w/w)	4.287 ± 0.170
	Interfacial area to aqueous volume ratio (cm²/mL)	0.006	4.414 ± 0.265
		0.020	4.774 ± 0.330
		0.065	4.658 ± 0.207
		0.207	4.764 ± 0.180
Difluprednate	Glycerin (w/w) in polysorbate 80 (0.4%, w/w)	0%	3.205 ± 0.042
		0.2%	3.137 ± 0.072
		1.0%	3.145 ± 0.076
		2.0%	3.236 ± 0.057
	Interfacial area to aqueous volume ratio (cm²/mL)	0.006	2.904 ± 0.392
		0.020	3.246 ± 0.310
		0.065	3.137 ± 0.212
		0.207	3.216 ± 0.131

2.0% w/w glycerin) (Table 3). For difluprednate, rate constants did not show a clear trend with increasing glycerin concentrations, and the apparent partition coefficients also remained constant. These results suggest that glycerin could play a critical role in the drug distribution of cyclosporine which may lead to variations in bioavailability upon administration or the initial release in an IVRT study.

3.3. Effect of carbomer copolymer on the rate and extent of cyclosporine distribution in castor oil and aqueous phase

In addition to polysorbate 80 and glycerin, carbomer can also serve as a stabilizer by forming a swollen micro-gel network over the oil globules [31]. To study the impact of carbomer on cyclosporine distribution, various amounts of carbomer were introduced into the aqueous solutions. The agitation was controlled at 125 rpm except for

0.05% (w/w) carbomer where 400 rpm was used due to the high aqueous phase viscosity. As reported earlier [19], agitation during the diffusion experiment is important to ensure the uniformity of the aqueous phase concentration but the change in agitation speed was not found to impact the equilibrium drug distribution. Accordingly, in this study log P_{app} across various carbomer concentrations were compared directly despite the differences in the agitation speed.

The results in Fig. 6 show that the diffusion rate constant from aqueous to oil (i.e., k_{21}) declined while the rate in the opposite direction (i.e., k_{12}) increased upon addition of carbomer (i.e., 0% to 0.005% w/w), with a net effect of increasing the rate of distribution from oil to aqueous phase. Further increase in carbomer concentration to 0.05% (w/w) resulted in a decrease in the rate constant in both directions, probably due to increase in viscosity of the solution.

The results in Table 3 show that the carbomer significantly ($p < 0.01$) reduced the apparent partition coefficient of cyclosporine from 4.764 ± 0.109 (no carbomer) to 3.898 ± 0.258 (with 0.05% w/w carbomer), with nearly 7 times increase in the concentration of cyclosporine in aqueous phase. One possibility is the presence of an interaction between carbomer and cyclosporine. Carbomer (copolymer type A) has been reported to contain long chain (C10-C30) alkyl acrylates (a lipophilic modification to its backbone) and is crosslinked with allylpentaerythritol [32]. The hydrophobic drug molecules, such as cyclosporine, could interact with the long alkyl chains through hydrophobic interactions which could lead to the shift in the equilibrium drug distribution towards the aqueous phase.

When carbomer was added in lower amount (e.g., 0.005%, w/w) to the aqueous phase containing lower amount of polysorbate 80 (e.g., 0.1% w/w), the log P_{app} of cyclosporine increased slightly, from 4.047 ± 0.231 (Table 2) to 4.287 ± 0.170. This is likely a result of interaction between polysorbate 80 and carbomer, through the hydrogen-bonding between the oxyethylene group of polysorbate 80 and carboxylic group of carbomer [33]. The association resulted in an increase in stability of polysorbate 80 monomers in water [34] which suppressed the solubilization of cyclosporine through micellization. However, when using higher concentration of polysorbate 80 and carbomer, the log P_{app} could be reduced, even lower than using the polysorbate 80 or carbomer alone. The additive effect of solubilization by the surfactant and carbomer could thus result in increase in both the rate and extent of drug transfer from oil to the aqueous phase. Given the complex interactions between carbomer, drug and surfactant, it is important to consider carbomer when estimating the rate and extent of drug distribution in oil and aqueous phases and under various IVRT conditions.

3.4. Effect of interface (surface) area on the rate and extent of drug distribution

At the oil and aqueous interface, the mass exchange of species (e.g., the drug) occurs due to passive diffusion [35] and is generally driven by the concentration gradient. In an emulsion where the globules can be as small as 100 nm, the interface (surface) area to volume ratio of the globules as well as the globule total surface area could be orders of magnitude higher than an emulsion with larger globules (e.g., 1 μm). To determine if the properties of the interface, particularly the interfacial area, influences the rate and extent of drug distribution, the oil and aqueous interface was varied in this study using a special setup (Fig. 2B).

As shown in Fig. 7, increase in the interfacial area resulted in proportional increase in the diffusion rate constants of both cyclosporine and difluprednate in both oil-to-aqueous and aqueous-to-oil directions. This confirms that the larger surface area to volume ratio can result in faster transfer, and in the case of emulsions, a faster process to reach equilibrium distribution. This is particularly important to understand the drug distribution (or re-distribution after a disturbance like dilution in the IVRT medium). For example, it has been determined that the
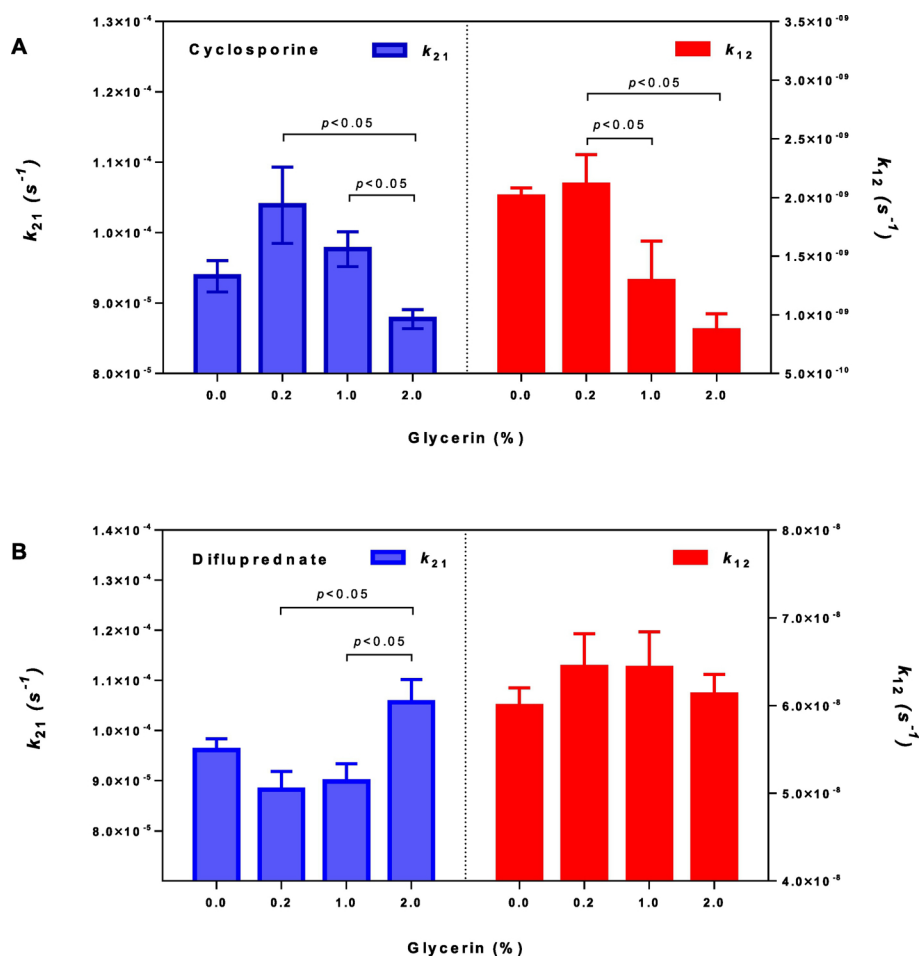
Fig. 5. Biphasic diffusion rate constants as a function of the glycerin concentrations. A) Cyclosporine; B) Difluprednate. k_{12} and k_{21} are diffusion rate constants in the directions of oil to aqueous and aqueous to oil, respectively n = 3.
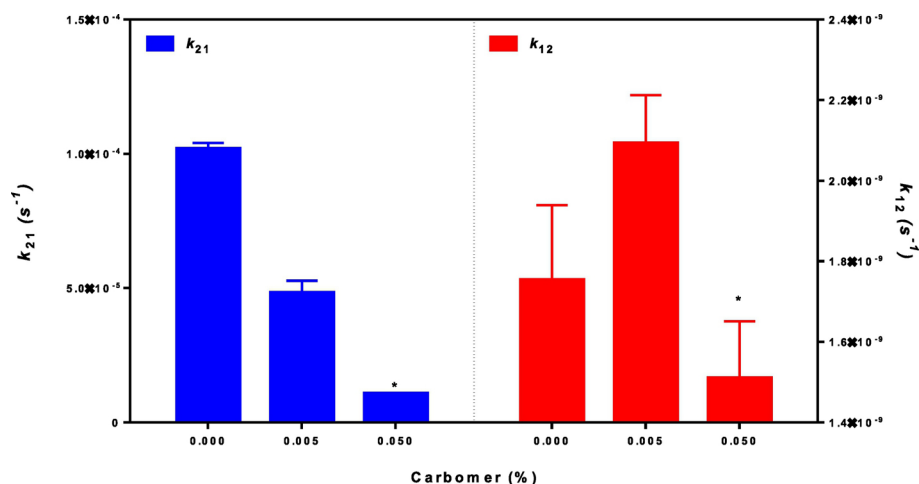

Fig. 6. Biphasic diffusion rate constants of cyclosporine determined by the kinetic method with respect to the concentrations of carbomer (*, The experiment was performed at 400 rpm due to the high viscosity of carbomer solution) n = 3.

commercial cyclosporine ophthalmic emulsion has a globule size span from 30 nm to a few hundred nanometers with majority smaller than 100 nm [12]. Using the actual globule size distribution, we have estimated that the effective (oil-to-aqueous) interfacial area in the commercial cyclosporine ophthalmic emulsion is approximately 15,000 cm²/mL. Therefore, the transfer rate of drug across the oil-water interface in an actual cyclosporine emulsion should be much faster

(e.g., in the range of 10E-3 s^{-1} to 10E-4 s^{-1}) than the values reported here (i.e., 10E-9 s^{-1} to 10E-10 s^{-1}).

With respect to the extent of drug distribution, while the increase in the interfacial area did not change the average values of the apparent partition coefficients of both drugs (Table 3), larger surface area did lead to smaller variations. This suggests that to reduce the estimation error of the apparent partition coefficients, one should choose the setup
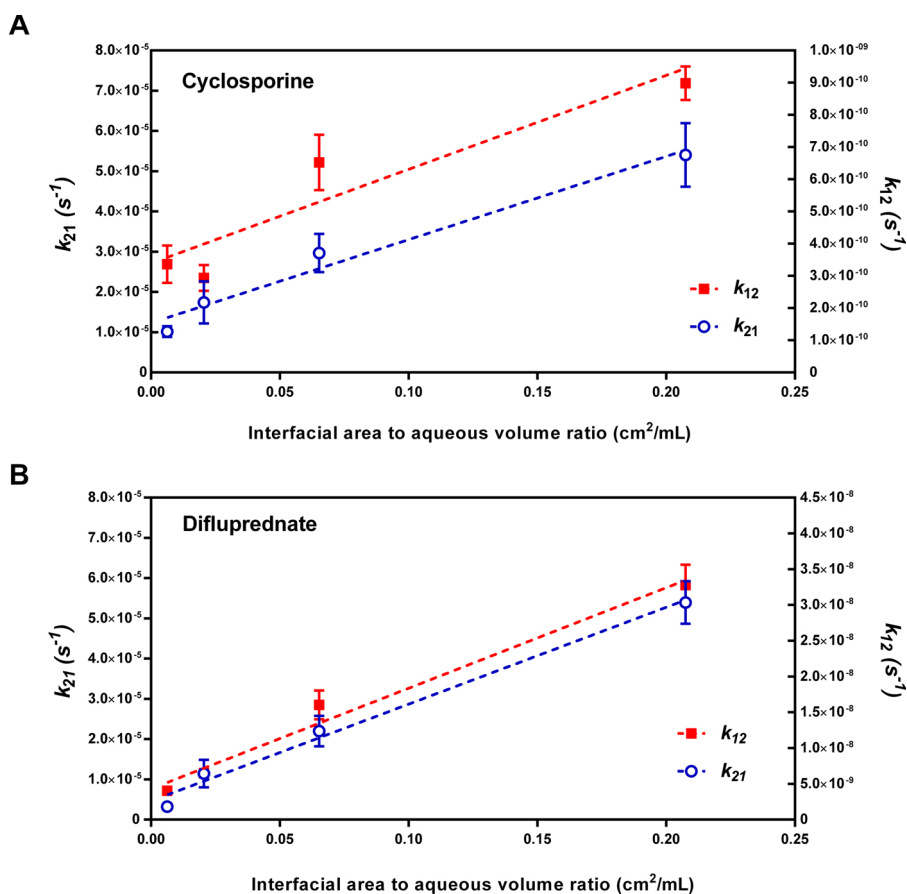
Fig. 7. Biphasic diffusion rate constants determined by the kinetic method with respect to the interfacial area. A) Cyclosporine; B) Difluprednate n = 3.
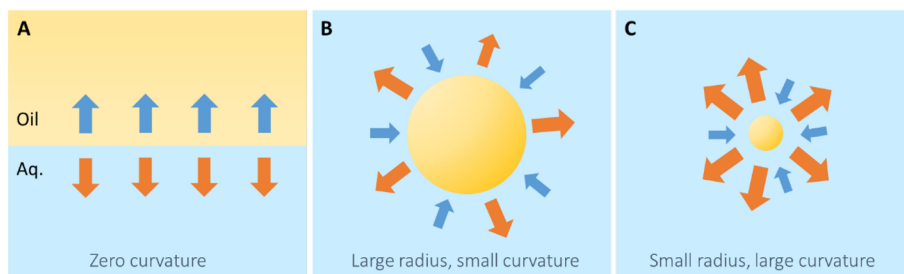

Fig. 8. Schematic showing the effect of interface curvature on the probability of transfer. (A) An interface with zero curvature; (B) A large globule with small interface curvature; and (C) A small globule with large interface curvature. Note: the size of the arrow is not drawn to scale.

with larger surface area to volume (e.g., > 0.1 cm^2/mL). Furthermore, the current investigation on diffusion (distribution) was conducted at a planar interface (between oil and aqueous phase) and the curvature effect was not accounted for. For example, it was assumed that there was no difference in escaping tendency of drug from oil-to-aqueous and aqueous-to-oil phase, which was solely driven by the differences in the chemical potentials. However, in an emulsion where the globule size can be as small as 100 nm, the curvature (i.e., Kelvin effect) is expected to influence the mass transfer across the interface, especially favoring the transfer through a concave interface (i.e., oil-to-aqueous phase). This effect can be explained by the Kelvin equation, i.e., $ln(c/c_0) = 2\gamma V_m/rRT$, where c is the concentration across a curved interface, c_0 is the concentration across a planar interface, γ is the interfacial tension between oil and aqueous phase, V_m is the molar volume, r is the radius of the curved interface (oil globule radius in this case), R is the gas constant and T is the temperature. Therefore, globules of smaller size (i.e., greater curvature) can increase the effective concentration of drug in aqueous phase (over the concaved interface) relative to the oil phase.

A similar effect may also be inferred based on the probability of transfer (Fig. 8). However, the impact of curvature on the equilibrium drug distribution is not yet possible for verification or determination.

3.5. Relating emulsion globule size distribution to the drug distribution

Based on the determined rate constants of drug transfer and apparent partition coefficient values, the underlying relationship among the three key physicochemical parameters (i.e., globule size distribution, drug distribution in different phases, and release characteristics) were explored. As noted above, higher surfactant concentration in aqueous phase leads to lower log P_{app}, suggesting more drug is present in the aqueous phase. In emulsion formulations with Q1/Q2 sameness, it is hypothesized that the size of the globules, which is indirectly related to the available globules surface areas, can influence the relative distribution of the surfactant (i.e., over the globule surface vs. in the bulk aqueous phase). A theoretical derivation of the relationship between various material/physical attributes and the relative distribution
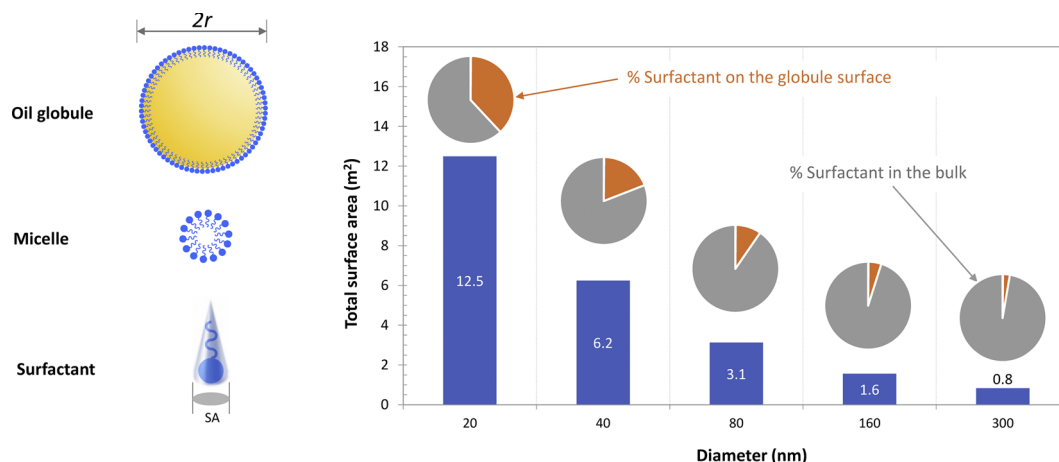
Fig. 9. Schematic showing the structure of oil globule (with surfactant), micelle, and individual surfactant shape; and the estimation of total globule surface area and relative distribution of surfactant with varying globule sizes.

of surfactant is presented below.

It is assumed that the oil globules are spherical in shape and composed of only castor oil (Fig. 9). It is also assumed that the surfactants form a monolayer coverage over the surface of the oil globules [36]. Furthermore, it is assumed that the excess surfactants form micelles and micelle does not contain castor oil.

Accordingly, for an oil globule i with known size, the globule surface area (S_i) and mass (m_i) are given as,

$$S_i = 4 \bullet \pi \bullet r_i^2 \tag{1}$$

$$m_i = \frac{4}{3} \bullet \pi \bullet r_i^3 \bullet \rho_{\text{oil}} \tag{2}$$

where r_i and ρ_{oil} are oil globule radius and oil density, respectively. With known polysorbate 80 polar head surface area (S_A), one can calculate how many surfactant molecules (k_i) are required to stabilize the globule i (with a mono-layer coverage),

$$k_i = \frac{S_i}{S_A} \tag{3}$$

Given the frequency distribution (P_i) for globule i, number of globules (n_i) having the same size as i can be calculated:

$$n_i = \frac{C_{\text{oil}}}{m_i} \bullet P_i \tag{4}$$

where C_{oil} is the castor oil concentration. From the surfactant concentration, the total surfactant number (n_T) can be calculated,

$$n_T = \frac{C_T}{\text{MW}_T} \bullet N \tag{5}$$

where C_T and MW_T are the concentration and molecular weight of polysorbate 80, respectively; and N is the Avogadro constant (6.02×10^{23} mol^{-1}). The fraction of polysorbate 80 occupying the globule surface (F_s) can then be determined,

$$F_s = \frac{\sum_i n_i \bullet k_i}{n_T} = \frac{\sum_i \frac{C_{\text{oil}}}{m_i} \bullet P_i \bullet \frac{S_i}{S_A}}{n_T} = \frac{3 \bullet C_{\text{oil}} \bullet \text{MW}_T}{S_A \bullet C_T \bullet \rho_{\text{oil}} \bullet N} \sum_i \frac{P_i}{r_i} \times 100\%$$
$$= K \sum_i \frac{P_i}{r_i} \times 100\% \tag{6}$$

where, the constant K is,

$$K = \frac{3 \bullet C_{\text{oil}} \bullet \text{MW}_T}{S_A \bullet C_T \bullet \rho_{\text{oil}} \bullet N} \tag{7}$$

Fraction available to form micelles (F_m) can be determined based on the mass balance,

$$F_m = 1 - F_{\text{cmc}} - F_s \tag{8}$$

where F_{cmc} and F_s are the fractions of polysorbate 80 CMC and oil globule surface coverage, respectively. As shown above, the fraction of surfactant needed for globule surface is proportional to the oil concentration and molecular weight of the surfactant, but inversely proportional to the surfactant polar surface area, concentration of the surfactant, density of the oil, and size of the oil globule.

An estimation is given in Fig. 9, assuming that for an emulsion formulation with 4% (w/w) castor oil and 3% (w/w) polysorbate 80, the following are known: castor oil density (ρ_{oil}) is 0.961 g/cm^3, polysorbate 80 molecular weight (MW$_T$) is 1310 g/mol [37], polysorbate 80 CMC (F_{cmc}) is 0.0014% w/w, and the polysorbate 80 polar head surface area (S_A) is 224 A^2 [38]. It is determined that the fraction of surfactant in the aqueous phase can vary from 59.52% to 97.26% of the total amount of polysorbate 80 with globule size ranging from 20 nm to 300 nm, which is expected to lead to a difference in drug distribution based on the above experimental data. Therefore, accurately determining globule size distribution of complex emulsion formulations with high polydispersity is critical for predicting drug distribution. Qu et al. [12] developed a high-resolution method using asymmetric flow field flow fractionation to accurately characterize the globule size distribution of cyclosporine ophthalmic emulsion, which also offers the potential for more accurate estimation of drug distribution.

Drug distribution within the formulation also plays a role in drug

Factor change	Rate (to aq.)	Extent (to aq.)
Surfactant (e.g., polysorbate 80) conc. ↑	⬆⬆⬆	⬆⬆⬆
Carbomer conc. ↑	Cyclosporine ⬆	Cyclosporine ⬆
Glycerin conc. (viscosity) ↑	Cyclosporine ⬇ Difluprednate ▬	Cyclosporine ⬇ Difluprednate ▬
Total surface area (i.e. globule size) ↑	⬆⬆	▬ (underestimating due to curvature effect)

Fig. 10. Summary of effect of formulation-related variables on the rate and extent of drug distribution into the aqueous phase.

release from emulsion (Fig. 10), particularly the release at initial time point. For example, to initiate an IVRT process, emulsion formulations are diluted with the release media at an elevated temperature (typically 34 °C for ophthalmic products) and the surrounding microenvironment changes accordingly. Drug that is originally distributed in the aqueous phase (including micelles and water) is diluted immediately, which lowers the drug concentration outside of the oil globule, disrupts the original distribution equilibrium and triggers the drug diffusion from oil globules towards aqueous phase (release media). Thus, initial release is associated with drug distributed in the aqueous phase where no rate limiting diffusion process is involved as compared to drug diffusion from oil to aqueous phase.

4. Conclusion

This study has shown that formulation composition and area of oil-water interface effects the drug distribution and diffusion in biphasic emulsion systems. It has also demonstrated that polysorbate 80 and the area of the oil-water interface accelerate the drug distribution in such systems. Also, higher concentrations of glycerin were found to interact physically with polysorbate 80 and led to the suppression of its solubilizing capacity for drugs. Carbomer decreased the rate and extent of cyclosporine transfer from the aqueous to the oil phase and shifted the drug distribution slightly in favor of aqueous phase. However, in formulations with Q1/Q2 sameness, glycerin and carbomer are expected to exert equivalent effects on drug distribution within the formulations and consequently not affect bioequivalence. A direct relationship was estimated between the emulsion globule size distribution and the relative distribution of surfactant could be linked to the rate and extent of drug distribution in complex emulsions. These findings may be helpful in formulation design and development of in vitro methods to assess BE of ophthalmic emulsions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jconrel.2019.09.010.

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