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Research paper

Studies on the stability of the chloramphenicol in the microemulsion free of alcohols

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Abstract

Microemulsion composed of Span20 + Tween20 isopropyl myristate (IPM) + H_2O were investigated as potential drug delivery systems for eye drops. The system is important in that all its components are food grade so that the microemulsion is almost free of toxicity and irritation. The phase transition was investigated using the electrical conductivity measurements. The chloramphenicol is used to treat the eye diseases such as trachoma and keratitis. However, this drug in the common eye drops hydrolyzes easily. The main product of the hydrolysis is glycol. Here, the chloramphenicol was trapped into the oil-in-water (o/w) microemulsions free of alcohols. Its stability was investigated by the high performance liquid chromatography (HPLC) assays in the accelerated experiments of 3 months. The location of the chloramphenicol molecules in the microemulsion formulations was determined by means of dynamic light scattering (DLS) and ^{1}H NMR spectroscopy. The results of HPLC revealed that the content of the glycols in the microemulsion formulation was much lower than that in the commercial eye drops at the end of the accelerated experiments. It implied that the stability of the chloramphenicol in the microemulsion formulations was increased remarkably. The results of DLS and NMR confirmed that the chloramphenicol molecules should be trapped into the hydrophilic shells of the microemulsion drops, which were composed of many oxyethylene groups. The benzene rings of the chloramphenicol molecules were near the group of α2-CH_2 and the oxyethylene groups of the surfactant molecules. It was this reason that enabled the chloramphenicol molecules in the microemulsions to be screened from the bulk water and its stability to be increased remarkably.

Keywords: Non-ionic surfactants; Microemulsion; Phase behavior; Chloramphenicol eye drops; HPLC; DLS; ^{1}H NMR; Stability

1. Introduction

Eye drops are the most used dosage form by ocular route and chloramphenicol (see Fig. 1) is one of the main effective drugs in the common used eye drops. However, the drug delivery system of eye drops have several disadvantages, such as a very low bioavailability (1–10%) of the drugs which must be absorbed at this site and must be inserted several times a day [1]. Also, the effective component, that is chloramphenicol, has very low solubility in water and easily hydrolyzes (see Equation 1). The main product of the hydrolysis is glycol. If the content of the glycols becomes higher than the authorized amount, it would cause the content of chloramphenicol to be lower than the standard (According to the pharmacopoeia of P. R. China [2], the amounts of the chloramphenicols in the eye drops should not be less than 0.25%). Then, the eye drops of chloramphenicol turn into unqualified [2].

The microemulsions are optically isotropic and thermodynamically stable systems [3,4]. There are three different basic structural types of microemulsions: oil-in-water (o/w), water-in-oil (w/o) and finally, bicontinuous structures (B.C.). In all these cases, the surfactant forms a film at the internal interface that separates the water and oil domains. The presence of the co-surfactant is often required in order to lower the interfacial tension of this interface because a low interfacial tension is essential for the formation of microemulsions [5].

For several years, microemulsions have been investigated as new drug delivery systems, and their potential uses in ophthalmology have been studied by several research teams [5]. Formulations based on microemulsions have several interesting characteristics such as enhanced drug solubilization, good thermodynamic stability and ease of preparation

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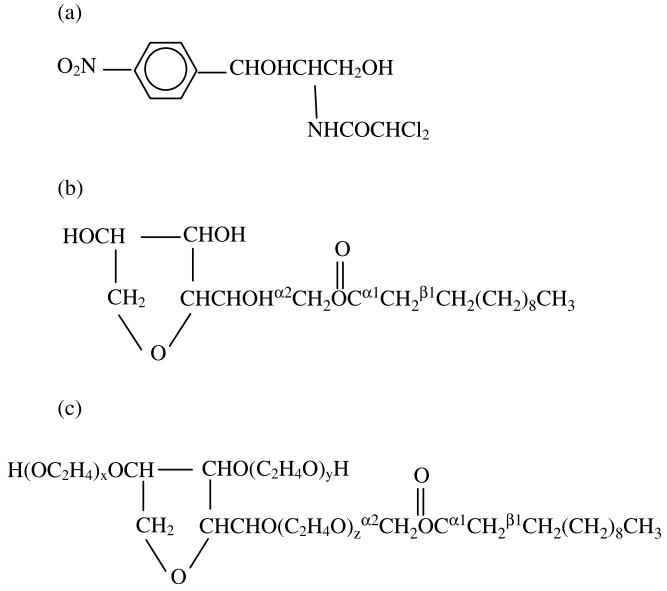
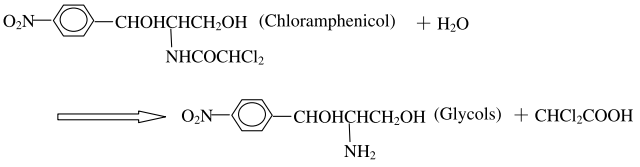


Fig. 1. Chemical structures of the compounds: (a) chloramphenicol; (b) Span20; (c) Tween20.

[6,7]. However, the most critical problem regarding to the microemulsion-based drug carriers is the toxicity of the components. Recent efforts have been focused on how to decrease or eliminate the toxicity or irritation of the microemulsion formulations [5]. Other important questions with the microemulsion-based drug delivery systems are where the drug is solubilized and how the stability of the drug is increased [5]. The methods of ^{1}H NMR spectroscopy, HPLC assay and DLS experiments have been proven to be useful in these fields [8–10].

As far as eye drops of chloramphenicol is concerned, it is desirable that the chloramphenicol could be incorporated into the o/w microemulsions, so the hydrolysis is avoided and its stability could be increased. Furthermore, if chloramphenicol is incorporated into the o/w microemulsions, it may be trapped into the oil core or in the palisade layer of the microemulsion drops and the release may be delayed, thus a delayed effect would be expected [11]. In the present work, the pseudo-ternary phase diagram of a microemulsion formed with non-irritant components was constructed and the phase transition was investigated by the electrical conductivity measurements. The stability of the chloramphenicol was monitored in the accelerated experiments through HPLC assays and the location of the chloramphenicol in the microemulsion is determined by DLS measurements and ^{1}H NMR spectroscopy. Since the present system is composed of food-grade agents and free of alcohols, it is very important for the improvement of the formulation of eye drops in future.



Equation 1. The hydrolysis equation of chloramphenicol.

2. Materials and methods

2.1. Materials

Span20 (sorbitan monolaurate), Tween20 (Polyethylene glycol sorbitan monolaurate) and IPM (isopropyl myristate) were purchased from Sigma Chemical Co., USA. Chloramphenicol was kindly given by FREDA BIOCHEM CO. LTD, China. All other chemicals were AR. Grade and used without further purification. The water was double-distilled.

2.2. Experimental techniques

2.2.1. Phase behavior

The phase behavior was studied following a pseudo-ternary phase diagram for the four component systems. The surfactant/oil ratio was kept constant. The pseudo-ternary phase diagram was investigated by adding the water to the surfactant/oil mixtures. After each addition, the samples were stirred to reach the equilibrium. Isotropic phases were assigned by visual inspection through normal light and crossed polarized light. Temperature was thermostated at 25 ± 0.1 °C.

2.2.2. Electrical conductivity

The electrical conductivity, κ, was measured by means of a DDS-11A conductivity meter (Rex Instruments Factory, Shanghai, China) equipped with a DJS-1 platinum conductance electrode which was coated with platinum black. Temperature was also thermostated at 25 ± 0.1 °C.

2.2.3. Accelerated experiments

The accelerated experiment was carried out at the abnormal conditions (given below) and the stability of the drug could be determined relatively quickly by accelerating the process of physical and chemical changes of the formulations (According to the pharmacopoeia of P. R. China, the changes of the chloramphenicol molecules in the eye drops in the accelerated experiments of three months are about the same as that of the chloramphenicol molecules in the eye drops at normal conditions of a year [2]. Normal conditions mean to the general conditions, such as room temperature and general humidity). The experiments were carried out with the temperature being thermostated at 40 ± 2 °C and the relative humidity being controlled at $75 \pm 5\%$ [2]. The chloramphenicol was solubilized in the selected o/w microemulsions and the compositions of the chloramphenicol-loaded microemulsions (shortened as ME) were as follows (according to the phase diagram, wt/wt): aqueous solution (95.00%); oil phase (IPM) (0.47%); surfactants (Span20 and Tween20) (4.26%) and chloramphenicol (0.27%). The commercial eye drops of chloramphenicol were prepared simultaneously and the contents of chloramphenicol were also 0.27% (wt). Then, the formulations (ME and the commercial eye drops) were divided into three groups in the same commercial package, respectively, and put into the thermostat. The amounts of glycols in the formulations were assayed by HPLC at the end of each month.

2.2.4. HPLC assay

The amounts of glycols in each formulation were measured by high-performance liquid chromatography (HPLC) and each measurement was repeated for three times. The glycols in the formulations were quantified by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (LC10-AD), a vis-UV detector (SPD-10,), a data station (Shimadzu, Kyoto, Japan), and a 25 cm-C18 column (LiChrospher, Merck, Darmstadt, Germany). The mobile phase was a mixture of methanol, 0.21% sodium pentylsulfonate and glacial acetic acid at a ratio of 40:50:10 (v/v/v). The flow rate was fixed at 1.0 ml/min and the UV detector was set at $\lambda = 270$ nm. The injection volume was 20 μl.

2.2.5. Dynamic light scattering (DLS)

The mean hydrodynamic diameters (D_h) of the microemulsion droplets with different concentrations of chloramphenicol (the contents of other components in the microemulsions were remained the same) were characterized at 25 °C by DLS measurements (90 plus, Brookhaven, New York, USA). Results were obtained on filtered (0.45 um) microemulsions at an angle of 90°. Scattering intensity data were analyzed by a digital correlator and fitted by the method of inverse Laplace transformation. The D_h of the microemulsion droplets was calculated through the Stokes–Einstein equation. For each sample, the D_h value was measured three times and the results presented were the mean values.

2.2.6. ^{1}H NMR spectroscopy

^{1}H NMR measurements were performed at 25 °C on a Bruker AMX 400 system. Chemical shifts of all microemulsion components were determined relatively to internal tetramethylsilane. The microemulsion was prepared using the D_2O (Cambridge Isotope Laboratories, Inc. D, 99.9%) and the amount of various components was calculated according to the pseudo-ternary diagram. The contents of surfactants and oil all remained the same and the chloramphenicol was solubilized in these o/w microemulsions with different concentrations. The highest concentration of chloramphenicol was 0.6% (wt) and the solution was still transparent indicating that the system was microemulsion.

3. Results and discussions

3.1. Phase behavior

The compositions of the microemulsions generally include the medium chain length alcohols as co-surfactants. However, most alcohols are harmful to the human body, so the microemulsion-based drug delivery systems may have irritation or toxicity inevitably. However, the most remarkable characteristic of the present system is that it is free of alcohols and all the components are food-grade agents. The non-ionic surfactants (Fig. 1, Span20 and Tween20) and the oil of IPM are almost free of toxicity and irritation at low concentrations. They are included in the pharmacopoeia of P. R. China and are suitable for the use in drug delivery systems [2]. Therefore, the present system is very important for the improvement of the formulations of eye drops in future.

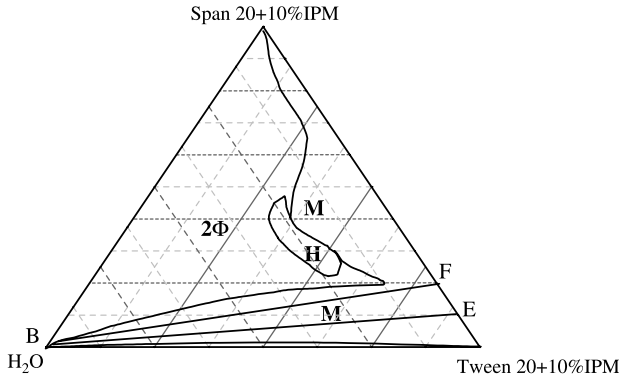


Fig. 2. The pseudo-ternary phase diagram of Span20 + Tween20 + IPM + H_2O (in which the ratio is weight ratio).

At first, the pseudo-ternary phase diagram of Span20 + Tween20 + IPM + water was constructed. The phase diagram was presented in Fig. 2, in which water was one component, another one was Span 20 + 10%IPM (wt) and the third component was Tween20 + 10%IPM (wt). The phase boundary was determined by observing the transition of the sample appearance from turbid to transparent or from transparent to turbid. The contents of each component in the systems were derived from precise mass measurements.

Fig. 2 shows the phase diagram of the present systems. The region marked M is the one-phase microemulsion and the region marked H is hexagonal liquid crystal. The other area (2Φ) is two-phase region. It is clear that the areas of the microemulsion range from w/o to B.C. and to o/w continuously over a wide water content in the phase diagrams.

3.2. Electrical conductivity and phase transition of the microemulsions

The physiological environment in the human body is hydrophilic and the chloramphenicol should be incorporated into the o/w microemulsion in order to favor the applications in future. Therefore, it is important to investigate the phase transition in the microemulsions mentioned above. Electrical conductivity is a structure-sensitive property and there are some studies reported about the systems of non-ionic surfactants [12,13]. In this study, the electrical conductivities of the microemulsion composed of non-ionic surfactants were measured, and the results indicated that this method could be used for this system. Fig. 3 shows a typical experimental result. The electrical conductivity was measured as a function of the composition of the system, which was changed, for example, along the line EB or FB in Fig. 2. It is seen in Fig. 3 (The electrical conductivity values in Fig. 3 are obtained along the line EB.) that the plot of κ versus water content (wt) exhibits the profile characteristic of percolative conductivity [14]. At first, the conductivity remains low up to a certain weight fraction of water, ϕ_c; however, when the water content is raised above ϕ_c, the value of κ increases linearly and steeply up to κ_b

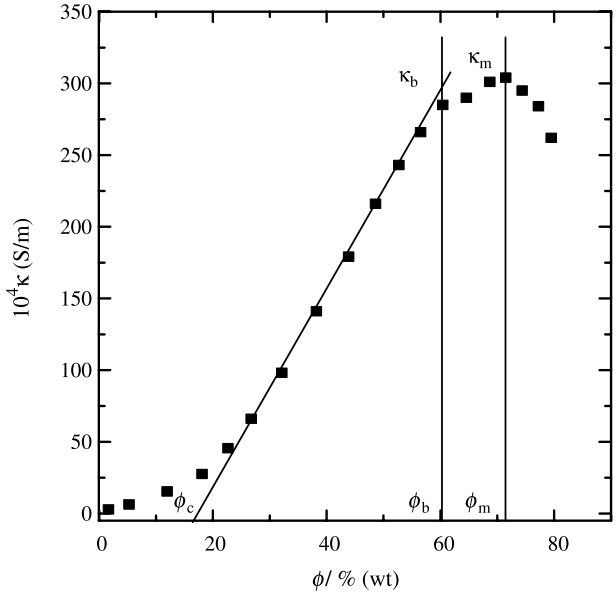


Fig. 3. Variation of the electrical conductivity, κ, as a function of water content in microemulsion region of Fig. 2 (along line EB).

at the concentration of ϕ_b, then it increases continuously but with relatively moderate rate up to κ_m at the concentration of ϕ_m, and after reaching the maximum value, κ decreases with the increase of water content.

Combined the results of electrical conductivity such as EB or FB, different microemulsion regions could be divided as follows: a water-in-oil region in water content up to 60% (wt), an oil-in-water region in water content larger than 72% (wt), and a bicontinuous region in water content between 60 and 72% (wt) [12,14].

3.3. Stability of chloramphenicol in the formulations

The chloramphenicol was solubilized into the selected o/w microemulsion and the formulations in the accelerated experiments included the chloramphenicol-loaded microemulsion (ME) and the commercial eye drops. The stability of the chloramphenicol in the formulations was determined by HPLC assays through the accelerated experiments [8,9]. The results were shown in Fig. 4. At the beginning of the accelerated experiments, the amounts of glycols are similar and very low in both formulations. With the prolongation of the monitored time, the contents of the glycols in the formulations all begin to increase. For example, the glycols in commercial eye drops range from 1.20% (wt) at the start to 11.28% (wt) at the end of the first month, to 20.88% (wt) at the end of the second month and finally, to 27.11% (wt) at the end of the third month, indicating that the hydrolysis of the chloramphenicol in the commercial eye drops becomes more and more remarkable. The same changes could be found in the ME formulations. However, it is worth noting that from the end of the first month, the contents of the glycols in the formulations become different. The contents of glycols in the ME always remain lower than that in the commercial eye drops. At the end of the third month, the contents of glycols are already 27.11% (wt) in

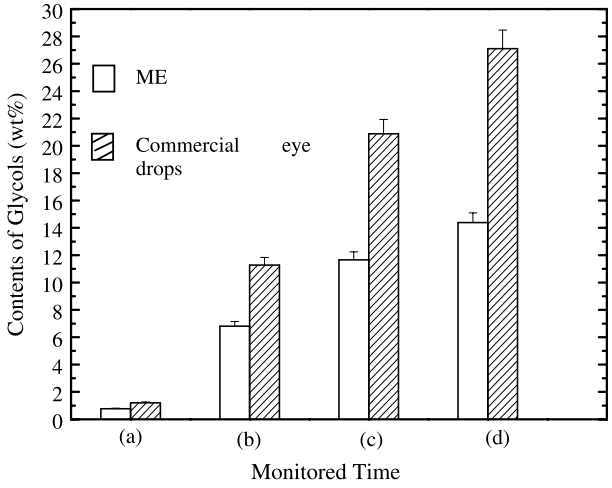


Fig. 4. The contents of glycols in the formulations at the end of each month in the accelerated experiments: (a) 0 month; (b) 1 month; (c) 2 month; (d) 3 month.

the commercial eye drops, but only 14.38% (wt) in ME formulations. The contents of glycols in the microemulsion formulations are much lower than that in the commercial eye drops. This is strong evidence indicating that the microemulsion could improve the stability of the chloramphenicol remarkably.

3.4. The location of the chloramphenicol molecules in the microemulsion formulations

To shed some light on the location of chloramphenicol molecules in the microemulsion formulations, the DLS measurements and the ^{1}H NMR experiments were performed on the microemulsions [8,9].

3.4.1. Dynamic light scattering

The hydrodynamic diameters of D_h could represent the droplet size of the microemulsions and reflect the possible site where the drug is solubilized in the microemulsions [16,17]. The mean D_h values of various microemulsions obtained from the DLS measurements were summarized in Table 1 (the concentrations of the surfactants and oil are fixed at the same and only the amounts of chloramphenicol in the microemulsions are varied). With the addition of chloramphenicol, the diameters of the microemulsion drops all become to increase. For example, the D_h of microemulsion *A* is only 38.5 nm, however, the D_h of the chloramphenicol-loaded microemulsion

Table 1
The mean hydrodynamic diameters of microemulsions with different contents of chloramphenicol

Microemulsions	Contents of chloramphenicol (wt%)	D_h (nm)	Polydispersity index
A	0.0%	38.5	0.426
B	0.2%	53.0	0.472
C	0.4%	56.5	0.489
D	0.6%	59.5	0.481

(microemulsion B, C and D) are all larger than 50 nm. It implies that the chloramphenicol is not dissolved in water but entrapped into the drops of the microemulsions. So the solubilization of chloramphenicol molecules causes the D_h of the microemulsion drops to increase. On the other hand, the increase of the D_h is not directly correlated to the contents of chloramphenicol. This reveals that the distribution of chloramphenicol molecules in the droplets of the microemulsion is not uniform. Some droplets may contain high amount of the chloramphenicol molecules and others may contain relatively low amount.

3.4.2. ^1H NMR spectroscopy

In order to find out the specific location of the chloramphenicol molecules in the microemulsion formulations, the ^1H NMR spectroscopy was performed on the microemulsion systems. The water, surfactants and oil were all fixed at the same content in the microemulsions and only the content of chloramphenicol was varied. The present microemulsion is consisted of Span20 + Tween20 + IPM + D_2O, in which the Span20, Tween20 and IPM all have long hydrocarbon chains and the Tween20 have many oxyethylene groups. So many materials and functional groups would result in a complex ^1H NMR spectra. However, if the experiments are designed finely and studied very carefully, there is still some information obtained. The main results about the ^1H NMR spectra of the microemulsions were summarized in Tables 2 and 3. It can be seen that after the addition of chloramphenicol, the chemical shifts of the functional groups display different changes. For example, the chemical shifts of protons of the methylene near the ether bond (α2-CH_2), are the most sensitive to the addition of chloramphenicol ($0.15\ \text{ppm} \le \Delta\delta \le 0.25\ \text{ppm}$); the second is the oxyethylene groups ($(CH_2CH_2O)_n$)($0.10\ \text{ppm} \le \Delta\delta \le 0.19\ \text{ppm}$). The chemical shifts of other functional groups, such as ω-CH_3, $(CH_2)_n$, β1-CH_2 and α1-CH_2, all change very small ($\Delta\delta \le 0.02$ ppm). Since the changes of the chemical shifts of various groups are closely connected with the addition of chloramphenicol, the location of chloramphenicol molecules in the microemulsions could be confirmed according to these changes. As mentioned above, the chemical shift patterns of ω-CH_3, $(CH_2)_n$, β1-CH_2 and α1-CH_2 are not sensitive to the addition of chloramphenicol, however, the chemical shift changes of α2-CH_2 and $(CH_2CH_2O)_n$ are relatively large. It implies that the chloramphenicol molecules are solubilized neither in the palisade layer nor the oil core of the o/w microemulsions drops, but in the hydrophilic shells of the microemulsion drops, which are composed of many oxyethylene groups (see Fig. 5). However, by inspection carefully, one could find another interesting phenomenon: after the addition of chloramphenicol, the chemical shifts of the α2-CH_2 and $(CH_2CH_2O)_n$ all move to high field and $\Delta\delta > 0$ (Table 3). It reveals the specific location of chloramphenicol molecules in the microemulsions. It is well known that the benzene ring has a strong screening effect and it could enable the chemical shift values of other compounds near it to decrease ($\Delta\delta > 0$) [15]. Therefore, the chloramphenicol is solubilized into the hydrophilic shell of the microemulsion drops. Its benzene ring is near the groups of α2-CH_2 and the $(CH_2CH_2O)_n$ of the surfactant molecules. It is this reason that enables the chloramphenicol in the microemulsions to be screened from the bulk water and its stability to be increased remarkably.

Combined with the results of DLS experiments and ^1H NMR spectroscopy, it is concluded that the chloramphenicol is

Table 2
^1H NMR parameters of surfactant molecules (Span20 and Tween20) in the microemulsions with different contents of chloramphenicol

Functional group	δ (ppm)			
	δ^0	$\delta^{0.2}$	$\delta^{0.4}$	$\delta^{0.6}$
ω-CH_3	0.86650	0.86297	0.85048	0.85163
$(CH_2)_n$	1.15801	1.1554	1.14737	1.16355
β1-CH_2	1.56088	1.55873	1.55062	1.55192
α1-CH_2	2.14686	2.14566	2.13872	2.13920
$(CH_2CH_2O)n$	3.89105	3.79512	3.71379	3.69942
α2-CH_2	4.01523	3.86855	3.80733	3.76267

δ^0 is the chemical shift of the surfactants in the microemulsion which is free of chloramphenicol; $\delta^{0.3}$ is the chemical shift of the surfactants in the microemulsion which contains 0.3% (wt) chloramphenicol; So as $\delta^{0.6}$ and $\delta^{0.9}$.

Table 3
Changes of ^1H NMR chemical shifts of surfactant molecules (Span20 and Tween20) with the addition of chloramphenicol in the microemulsions

Functional group	$\Delta\delta$ (ppm)		
	δ^0–$\delta^{0.2}$	δ^0–$\delta^{0.4}$	δ^0–$\delta^{0.6}$
ω-CH_3	0.0035	0.0160	0.0149
$(CH_2)_n$	−0.0026	0.0106	−0.0055
β1-CH_2	0.0022	0.0103	0.0090
α1-CH_2	0.0012	0.0081	0.0077
$(CH_2CH_2O)_n$	0.0959	0.1773	0.1916
α2-CH_2	0.1467	0.2079	0.2526

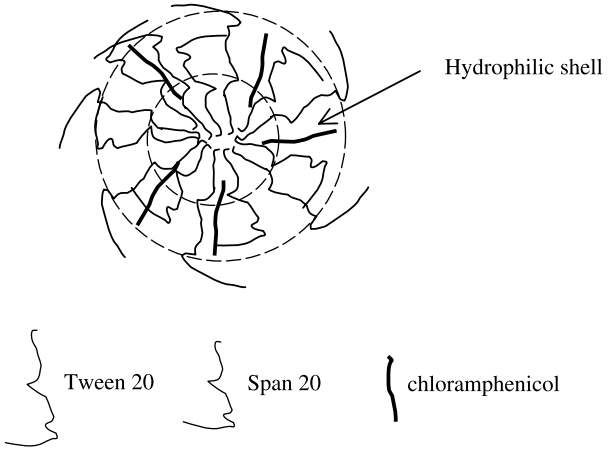


Fig. 5. Sketch of the location of chloramphenicol molecules in the microemulsions. The arc area among the dotted lines represents the hydrophilic shells of the microemulsion drops, which are composed of many oxyethylene groups of the surfactant molecules.

solubilized into the hydrophilic shells of the microemulsion drops (Fig. 5). The benzene rings of the chloramphenicol molecules are near the groups of α2-CH_2 and the $(CH_2CH_2O)_n$ of the surfactant molecules. This location causes the D_h of the microemulsion drops to increase and the chemical shifts of α2-CH_2 and $(CH_2CH_2O)_n$ of the surfactant molecules to be the most sensitive to the addition of chloramphenicol. There is a good agreement between the results of the DSL measurements and NMR spectroscopy.

Of course, due to the chloramphenicol could partly dissolve in water, there are still some glycols in the ME formulations (Fig. 4). However, most chloramphenicol molecules could be encapsulated into the hydrophilic shells of the microemulsion drops as mentioned above, so the stability of chloramphenicol is increased dramatically. As far as the commercial eye drops are concerned, it is mainly a solution of chloramphenicol dissolved in water, thus, it is not surprised to find that the contents of glycols in the commercial eye drops are much higher than that in the ME formulations.

Besides the enhanced stability of chloramphenicol in the microemulsion formulations, other effects are expected, such as a prolonged adherence time and a delayed release [5]. It is reported that the charge of the droplets which constitute the internal phase could influence their absorption in the ocular route. As the corneal area is negatively charged, the positively charged droplets might bind to the sites. The residence time of the microemulsion on the cornea would be increased and thus influence the drug release (The charge is provided by a positively charged surfactant, for example, the stearylamine and the intravenous injections show that it has no side effect on systemic absorption) [18,19]. Other studies show that in the presence of the surfactant molecules, the nanodroplets of the microemulsion would adsorb on the corneal membrane very quickly and only the fraction of the drug dissolved in the aqueous phase is eliminated by drainage. For conventional eye drops, the fraction of the drug eliminated by drainage is more important because only the aqueous phase is present. As for the delayed release of the drug in the microemulsion, Siebenbrodt and Keipert compared the in vitro release of indomethacin and sodium diclofenac in aqueous solutions and in microemulsions. The studies of diffusion kinetics indicated that the diffusion and kinetic release decreased for the drug when it was formulated as microemulsions as opposed to the formulations of aqueous solutions [20,21]. As far as the microemulsion-based chloramphenicol eye drops, further studies of the in vitro and in vivo drug release are still being performed by the collaboration with other institutions, and the results will be reported later.

4. Conclusions

(1) A microemulsion system free of alcohols was investigated as potential drug delivery systems for eye drops. Its components are all food-grade agents and almost free of any irritation and toxicity, so it is very important for the improvement of the eye drops formulations in future. The pseudo-ternary phase diagram is constructed and the phase transition of the microemulsions is studied by the electrical conductivity measurement.

(2) The stability of the chloramphenicol in the microemulsions was monitored by HPLC assays through the accelerated experiments. The results reveal that the amounts of glycols in the microemulsions are much lower than that in the commercial eye drops. It implies that the microemulsion formulations could improve the stability of the chloramphenicol remarkably.

(3) The location of the chloramphenicol molecules in the microemulsion formulations was determined by ^1H NMR spectroscopy and DLS measurements. It is inferred from the results of ^1H NMR and DLS that the chloramphenicol should be solubilized in the hydrophilic shells of the microemulsion drops, which are composed of many oxyethylene groups of the surfactant molecules. The benzene rings of the chloramphenicol molecules are near the α2-CH_2 and the $(CH_2CH_2O)_n$ of the surfactant molecules. It is this reason that enables the chloramphenicol in the microemulsion to be screened from the bulk water and its stability to be increased remarkably.

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