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Phase behavior of the microemulsions and the stability of the chloramphenicol in the microemulsion-based ocular drug delivery system

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Abstract

Microemulsion systems composed of Span20/80 + Tween20/80 + *n*-butanol + H_2O + isopropyl palmitate (IPP)/isopropyl myristate (IPM) were investigated as model systems of drug carriers for eye drops. Effects of chloramphenicol, normal saline, sodium hyaluronate and various oils on the phase behavior were studied. The phase transition was investigated by the electrical conductivity measurements. The electrical conductivity of the microemulsion was affected by the encapsulation of the drug into the system, and the addition of normal saline and sodium hyaluronate. The chloramphenicol is used to treat the diseases such as trachoma and keratitis. However, this drug in the common eye drops hydrolyzes easily. The main product of the hydrolysis is glycols. Here, the chloramphenicol was trapped into the oil-in-water (o/w) microemulsions and its stability was investigated by the high performance liquid chromatography (HPLC) assays in the accelerated experiments of 3 months. Its location in the microemulsion formulations was determined by means of ^{1}H NMR spectroscopy. The results of HPLC revealed that the contents of the glycols in the microemulsion formulations were 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 NMR experiments confirmed that the chloramphenicol molecules should be trapped into the hydrophilic shells of the microemulsion drops, which was composed of many oxyethylene groups. The nitro-groups of the chloramphenicol molecules were near the α2-CH_2 of the surfactant molecules and the benzene rings of the chloramphenicol molecules were near 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: Microemulsion; Chloramphenicol eye drops; Phase behavior; Electrical conductivity; Stability

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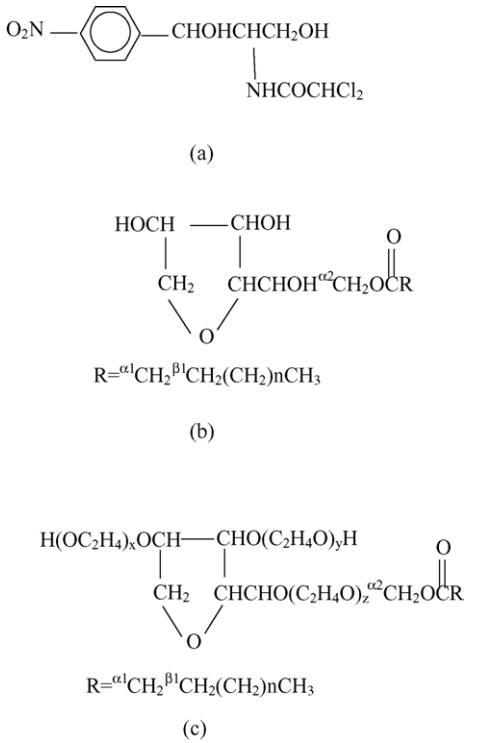


Fig. 1. Chemical structures of the compounds: (a) chloramphenicol; (b) Span: Span20 with R = $C_{11}H_{23}$; Span80 with R = $C_{17}H_{33}$; (c)Tween ($x + y + z = 20$): Tween20 with R = $C_{11}H_{23}$; Tween80 with R = $C_{17}H_{33}$.

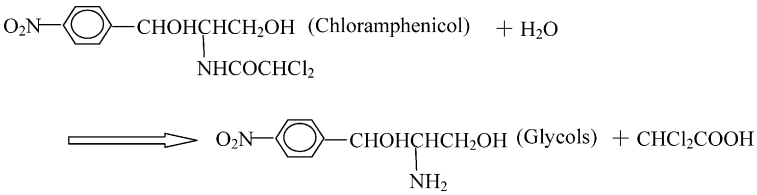
1. Introduction

The microemulsions are transparent, thermodynamically stable multi-component fluids (Eicke et al., 1994; Gradzielski and Hoffmann, 1994), normally composed of an aqueous component, an oily component, an amphiphile as emulsifying agent and frequently a co-surfactant (usually an alkanol of intermediate chain length). Basically, there are three different types of microemulsions: oil-in-water (o/w), water-in-oil (w/o) and finally, bicontinuous structures (B.C.).

Eye drops are the most used dosage form by ocular route and chloramphenicol (see Fig. 1) is the main effective drug in the common used eye drops. However, the 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 (Jarvinen et al., 1995). Also, the effective component, that is chloramphenicol, has very low solubility in water and easily hydrolyzes (see Scheme 1). The main product of the hydrolysis is glycols. 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 (the content of chloramphenicol in the eye drops should not be less than 0.25%). Then, the chloramphenicol eye drops turn into unqualified (The Pharmacopoeia Committee of State, 2000).

For several years, microemulsions have been investigated as new drug delivery systems and their potential applications in ophthalmology have been studied by several research teams (Vandamme, 2002). Formulations based on microemulsions have several interesting characteristics such as the enhancement of the drug solubility, good thermodynamic stability and ease of preparation (Peira et al., 2001; Trotta et al., 2003). However, the points, which are needed to study, of the drug-loaded microemulsions are where the drug molecules are located and how the stability of the drug molecules are increased. The methods of ^{1}H NMR spectroscopy and HPLC assay have been proven to be particularly useful in this field (Kreilgaard et al., 2000; Soderman and Nyden, 1999).

As far as chloramphenicol eye drops are concerned, it is desirable that the chloramphenicol molecules could be incorporated into the oil core or palisade layer of the o/w microemulsion drops, so the hydrolysis is



Scheme 1. The hydrolysis equation of chloramphenicol molecule.

avoided and its stability could be increased. Furthermore, the release of the drug molecules from the drops of the microemulsion may be delayed, thus a delayed effect would be expected (Sarciaux et al., 1995). In the present work, the pseudo-ternary phase diagrams of various microemulsion systems were constructed and the phase transitions were investigated by the electrical conductivity measurements. Effects of chloramphenicol, normal saline, sodium hyaluronate and various oils on the phase behavior were studied. The stability of the chloramphenicol molecules was monitored in the accelerated experiments through HPLC assay and the locations of the chloramphenicol molecules in the microemulsions were determined by ^{1}H NMR spectroscopy.

2. Materials and methods

2.1. Materials

Span20 (sorbitan monolaurate), Span80 (sorbitan monooleate), Tween20 (polyethylene glycol sorbitan monolaurate), Tween80 (polyethylene glycol sorbitan monooleate), isopropyl palmitate (IPP) and isopropyl myristate (IPM) were purchased from Sigma Chemical Co., USA. Chloramphenicol and sodium hyaluronate were kindly provided 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. 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 thermostated at $25 \pm 0.1\,^{\circ}$C.

2.2.2. 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 PR China, the changes of the chloramphenicol molecules in the eye drops in the accelerated experiments of 3 months are about the same as that of the chloramphenicol molecules in the eye drops at normal conditions of a year (The Pharmacopoeia Committee of State, 2000)). The experiments were carried out with the temperature being thermostated at $40 \pm 2\,^{\circ}$C and the relative humidity being controlled at $75 \pm 5\%$ (The Pharmacopoeia Committee of State, 2000). The chloramphenicol was solubilized in two selected o/w microemulsion formulations (ME-1 and ME-2). The compositions of the two chloramphenicol-loaded microemulsions are shown in Table 1. Then, all the formulations (ME-1, ME-2 and the commercial eye drops) were divided into three groups in the same commercial package, respectively, and put all the samples into the thermostat. The amounts of glycols in the formulations were assayed by HPLC at the end of each month.

2.2.3. 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 HPLC system consisted of a pump (LC10-AD), a UV–vis detector (SPD-10), a data station (Shimadzu, Kyoto, Japan), and a 25-cm C18 column (LiChrospher, Merck, Darmstadt, Germany).

Table 1
Compositions of the tested formulations in the accelerated experiments

	Formulations	Surfactants (wt%)	Oil phase (wt%)	Aqueous phase (wt%)	Chloramphenicol (wt%)
ME-1	S_{80} + T_{80}(1:1) + *n*-butanol + IPP + H_2O	10.65	9.35	80.00	0.27
ME-2	S_{80} + T_{80}(1:2) + *n*-butanol + IPP + H_2O	10.65	9.35	80.00	0.27
	Commercial eye drops				0.27

The surfactants refer to the S_{80} (Span80) and T_{80} (Tween80); the oil phase refer to the *n*-butanol and IPP.

The mobile phase was a mixture of methanol, 0.21% sodium pentyl-sulfonate 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.

2.2.4. ^1H 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 relative 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 chloramphenicol was solubilized in the o/w microemulsions with different concentrations. The highest concentration of chloramphenicol was 0.9 wt% and the solution still remained transparent indicating that the system was microemulsion.

3. Results and discussions

3.1. Phase behavior

At first, the pseudo-ternary phase diagrams of the systems of Span20 + Tween20 + *n*-butanol + IPP + water and Span80 + Tween80 + *n*-butanol + IPP + water were constructed. The phase diagrams are presented in Fig. 2, in which water was one component, another one was *n*-butanol (co-surfactant) + 10 wt% IPP or 10 wt% IPM, and the third component was surfactant + 10 wt% IPP or 10 wt% IPM. In all phase diagrams, the surfactants were a mixture of Span20 + Tween20 or Span80 + Tween80, in which the molar ratio of Span/Tween was fixed at 1:1. The pseudo-ternary phase diagrams were constructed adopting a simple titration technique. The mixtures of the surfactants and the oil (IPP or IPM) were prepared by mixing the desired amounts, and then an appropriate amount of *n*-butanol was introduced into the system. Water was added drop by drop and during the titration, samples were magnetically stirred in order to reach the equilibrium quickly. The phase boundary was determined by observing the changes of the sample appearance from turbid to transparent or from transparent to turbid. The content of each component in solutions was derived from precise mass measurements.

Fig. 2 shows the phase diagrams of some selected systems. The regions mark 1Φ (ABCD) are the one-phase microemulsion. The other areas (2Φ) are two-phase region. At very low water content, for example, <8 wt%, liquid crystal phase was often generated, which was not shown in the figures. It is clear that the areas of microemulsion range from w/o to bicontinuous and to o/w continuously over a wide water content in all the phase diagrams.

As it is shown in Fig. 1(c) (The Pharmacopoeia Committee of State, 2000), the hydrophilic group of the chloramphenicol, such as the $–NO_2$ and –OH are at the end of the molecule, while the hydrophobic group of phenyl ring in the middle is rigid, so it could not be dissolved in water easily. Furthermore, when it is solubilized, the two groups of –Cl at the end of the molecule would hydrolyze easily (see Scheme 1). In the present work, the chloramphenicol was solubilized in *n*-butanol with the concentration of 5 wt% and its effect on the phase behavior of the chosen systems was investigated. It is seen that the added drug slightly extends the area of the microemulsion, although the effect is not so big (the phase diagrams with chloramphenicol were almost the same as Fig. 2(a) and (b), so they were not presented in Fig. 2). It is possible since the chloramphenicol molecules are amphiphilic (the hydroxyl groups and the nitro-groups are all hydrophilic, while the part of phenyl is hydrophobic). And it may act as cosurfactant and could insert into the palisade layer of the microemulsion drops. So, it is reasonable that a small amount of chloramphenicol could extend the region of microemulsion slightly.

Other factors that affect the phase behavior of the present systems were also studied. First is the chain length of various oils. In the phase diagrams of Fig. 2, it is seen that the region of the microemulsion in Fig. 2(a) is a little larger than that in Fig. 2(b). It can be seen clearly that the area of microemulsion in Fig. 2(c) is much larger than that in Fig. 2(a). These results may be understood in terms of the law of chain length compatibility. Chain length compatibility of surfactant and oil is a very important factor regarding the formation of microemulsions (Hou and Shah, 1987; Garti et al., 1995). Bansal et al. showed that the maximum water solubilization by surfactants occurred when $l_a + l_o = l_s$ (l_a, l_o, and l_s are the lengths of hydrocarbon chains in alcohol, oil and surfactant, respectively). The equation

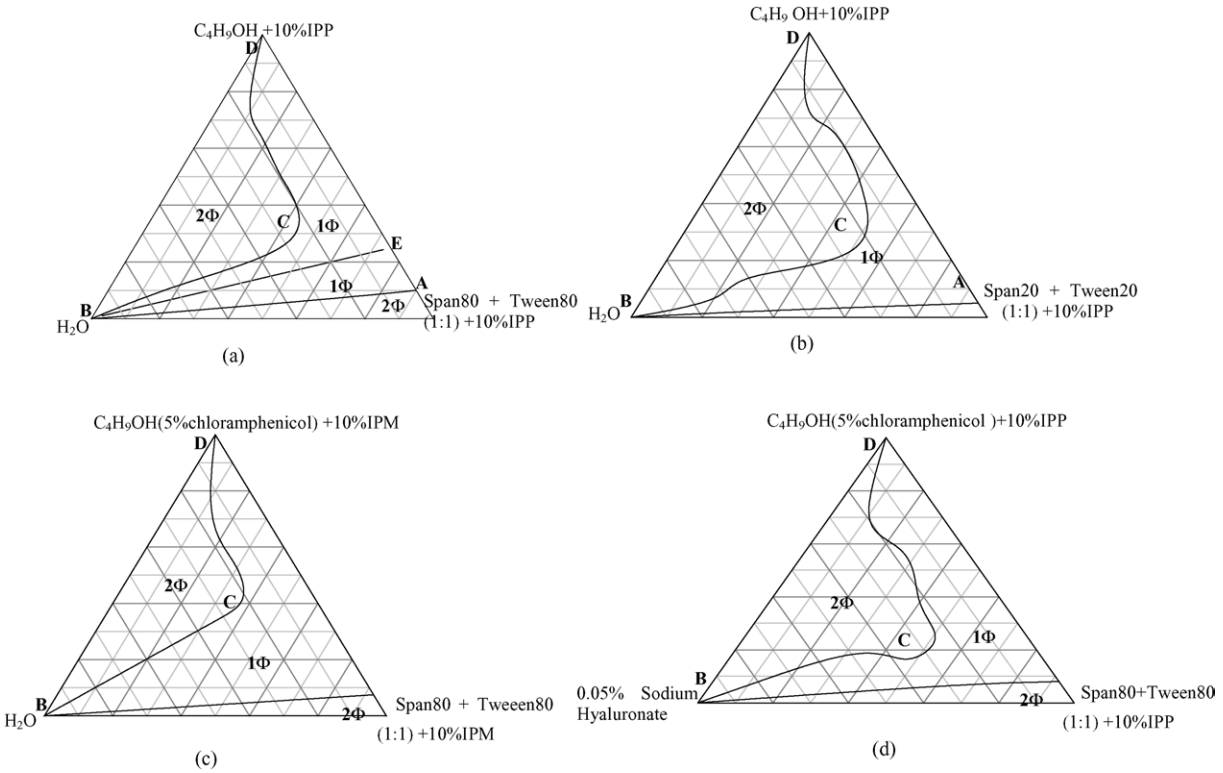


Fig. 2. The pseudo-ternary phase diagrams of various systems at 25 °C: (a) Span80 + Tween80 (1:1) + *n*-butanol + IPP + H_2O; (b) Span20 + Tween20 (1:1) + *n*-butanol + IPP + H_2O; (c) Span80 + Tween80 (1:1) + *n*-butanol (5% chloramphenicol) + IPM + H_2O; (d) Span80 + Tween80 (1:1) + *n*-butanol (5% chloramphenicol) + IPP + 0.05% sodium hyaluronate. All the ratios mentioned above are weight ratios except the ratios of Span/Tween are molar ratios.

of $l_a + l_o = l_s$ is also called BSO equation and reflects the requirements of chain length compatibility (Garti et al., 1995; Eastoe et al., 1996; Shiao et al., 1998). As far as the present systems are concerned, according to the BSO equation, when $l_s = 18$ for the mixed surfactants (see Fig. 2a and b, the fixed molar ratio of Tween80/Span80 = 1:1) and $l_a = 4$ for *n*-butanol, the maximum solubilization of water should occur when l_o is 14. So it is not surprised to find that the system containing IPM (Fig. 2c) has a larger area of microemulsion than that of the system containing IPP (Fig. 2a). The same reason could explain the difference between Fig. 2(a) and (b). Due to $l_s = 12$ for these surfactants (the fixed molar ratio of Tween20/Span20 = 1:1), the 20 systems have a shorter hydrocarbon chain than 80 systems, thus the hydrocarbon chain length of IPP is relatively large for them, so the region of microemulsion in Fig. 2(b) is smaller than that in Fig. 2(a). It is thought that the chain length compatibility is the main factor to determine the microemulsion area in the present work. However, the chain length compatibility is an experimental rule and not necessarily fit to all systems. Considering the complexity of the phase behavior, the theory should be testified through the experiments.

It is well known that the sodium hyaluronate can improve the percolation of the blood vessel and lubricate the human joint. It is also called natural moisturizing factor, so adding a little amount of sodium hyaluronate into the formulations can increase the physiological compatibility of the eye drops and improve the eye drops' performance greatly (Acosta et al., 1996; Ling and Zhang, 2000). The solution of sodium hyaluronate with the concentration of 0.05 wt%

was used to replace the pure water and its effect on the phase behavior of the microemulsion was studied. In order to mimic the physiological condition and adjust the osmotic pressure of the formulations, the solution of 0.9 wt% NaCl was used to replace the pure water and its influence on the phase behavior was also investigated. Interestingly, the two solutions exhibit quite different effects on the phase behavior. For example, the solution of 0.9% NaCl has little effect on the phase diagram (Fig. 2a), but the solution of 0.05% sodium hyaluronate induces a large decrease of the microemulsion area (Fig. 2d). Since Span and Tween are nonionic surfactants, they must be relatively insensitive to the salts, and the NaCl concentration of 0.9% is too low to cause any phase transition (Peira et al., 2001). But the situation for sodium hyaluronate is different. It is a kind of polymer, which is partial ionization and partial hydrolysis in aqueous solutions, its hydrocarbon chains may have certain interactions with the surfactant molecules. So the solution of 0.05 wt% sodium hyaluronate has large effect on the phase behavior of the systems.

3.2. Electrical conductivity and phase transition of the microemulsions

Electrical conductivity is a structure-sensitive property and there are some studies reported about the systems of nonionic surfactants (Mehta and Bala, 2000; Zheng et al., 2003). In this study, the electrical conductivities of several systems of microemulsions composed of nonionic surfactants were measured, and the results indicated that this method could be used for such systems. The electrical conductivity was measured as a function of the composition of the system, which was changed, for example, along the line EB in Fig. 2(a). Fig. 3 shows a typical experimental result. It is seen that the plot of κ versus water content (wt) exhibits the profile characteristic of percolative conductivity (Lagues et al., 1980; Gennes and Taupin, 1982; Grest et al., 1986). 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 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.

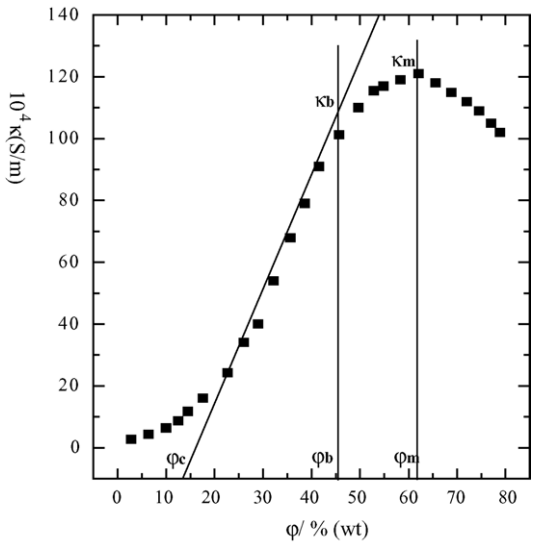


Fig. 3. Variation of the electrical conductivity (κ) as a function of water content (along line EB in the microemulsion region of Fig. 2a).

The electrical conductivity curve in Fig. 3 illustrates excellently three microemulsion regions: a water-in-oil region in water content up to 45 wt%, an oil-in-water region in water content larger than 62 wt%, and a bicontinuous region in water content between 45 and 62 wt%.

The electrical conductivity curves of the system containing the 0.9% NaCl and the 0.05% sodium hyaluronate are shown in Fig. 4, respectively. It is interesting to compare them with each other. At first, it is obvious that the behaviors of the curves in Fig. 4 are different. The curve in Fig. 4(a) demonstrates a classical picture of the conductivity behavior of microemulsions as interpreted above. However, the experimental curve in Fig. 4(b) is abnormal, i.e., the electrical conductivity is increased continuously even in the region of o/w microemulsion, although the rise in the end is somewhat mild. It is possibly because that sodium hyaluronate is a kind of polymer and the concentration of 0.05 wt% is too low, so it contributes very little to the system's conductivity (Fig. 4a). Thus, the solution of 0.05% sodium hyaluronate does not influence the shape of the conductivity curve of the microemulsion. However, NaCl is an inorganic electrolyte. The concentration of 0.9 wt% is not so high as to induce phase transition, but enough to influence the conductivity of the system (Fig. 4b).

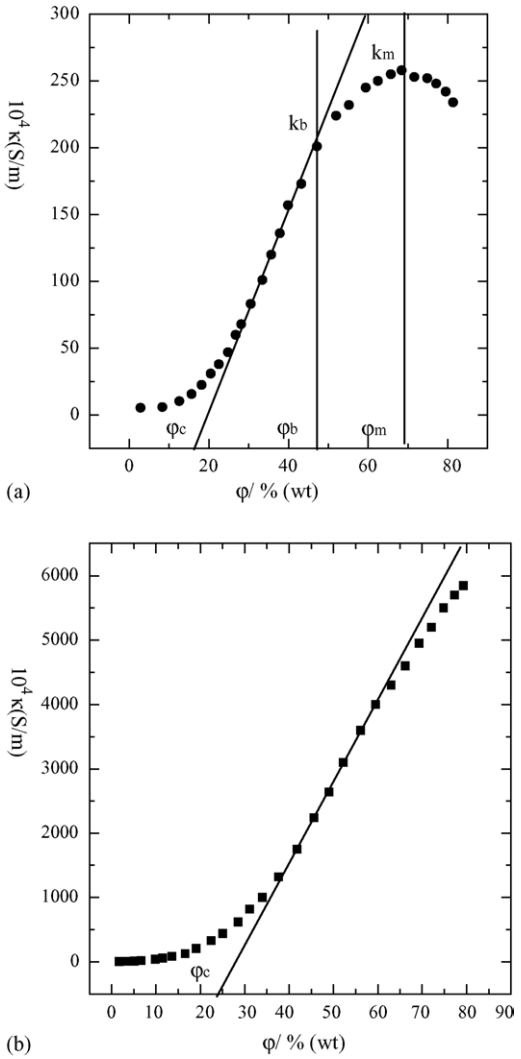


Fig. 4. The effects of sodium hyaluronate and sodium chloride on the electrical conductivity of the microemulsion: (a) Span20 + Tween20(1:1) + *n*-butanol(5% chloramphenicol) + IPP + 0.05% sodium hyaluronate; (b)Span20 + Tween20 (1:1) + *n*-butanol(5% chloramphenicol) + IPP + 0.9% NaCl. All the ratios are weight ratios except the ratio of Span20/Tween20 is molar ratio.

The phase transition could be identified clearly for Fig. 4(a): a water-in-oil region with water content up to 42 wt%, an oil-in-water region with water content larger than 70 wt%, and a bicontinuous region in water content between 42 and 70 wt%.

The effect of chloramphenicol on the electrical conductivity was also investigated (Fig. 5). The results

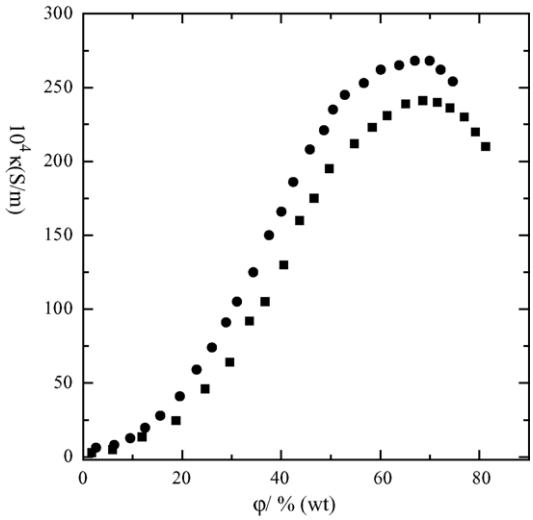


Fig. 5. Variation of the electrical conductivity of the microemulsion, κ, as a function of water content with and without chloramphenicol: (●) Span20 + Tween20(1:1, molar ratio) + *n*-butanol (5 wt% chloramphenicol) + IPP + H_2O; (■) Span20 + Tween20 (1:1, molar ratio) + *n*-butanol + IPP + H_2O.

show that the addition of chloramphenicol increases the electrical conductivity of the system. It is reasonable since chloramphenicol is a polar molecule.

3.3. Stability of chloramphenicol in the formulations

The stability of chloramphenicol in the formulations was determined by HPLC assays through the accelerated experiments (Soderman and Nyden, 1999; Kreilgaard et al., 2000). The results are shown in Fig. 6. At the beginning of the accelerated experiments, the amounts of glycols are similar and very low in all formulations. With the prolongation of the monitored time, the contents of the glycols in the formulations all begin to increase. For example, the amounts of glycols in commercial eye drops range from 1.20 wt% at the start to 11.28 wt% at the end of the 1st month, to 20.88 wt% at the end of the 2nd month and finally, to 27.11 wt% at the end of the 3rd month. It indicates that the hydrolysis of the chloramphenicol in the commercial eye drops becomes more and more remarkable. The same changes of glycols' contents could be found in the formulations of ME-1 and ME-2. However, it is worth noting that from the end of

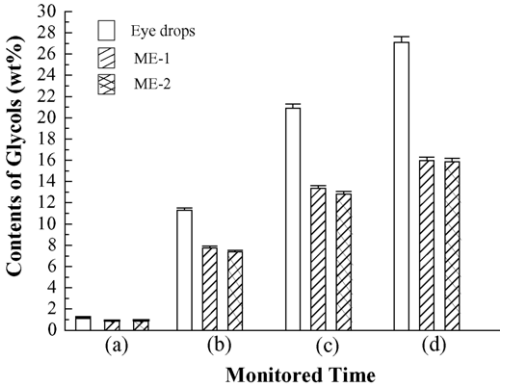


Fig. 6. The contents of glycols in the formulations at the end of each month in the accelerated experiments: (a) 0 month; (b) 1 month; (c) 2 month; (d) 3 month. The temperatures are thermostated at $40 \pm 2\,^{\circ}C$ and the relative humidity is controlled at $75 \pm 5\%$.

the 1st month, the contents of the glycols in the formulations become different. The contents of glycols in the microemulsions always remain lower than that in the commercial eye drops. At the end of the 3rd month, the contents of glycols are already 27.11 wt% in the commercial eye drops, but only 15.99 wt% in ME-1 and 15.87 wt% in ME-2, respectively. The contents of glycols in the microemulsions are much lower than that in the commercial eye drops. This reveals that the microemulsion formulations could improve the stability of the chloramphenicol remarkably. Besides, the HPLC assays (Fig. 6) also show that the contents of glycols in ME-1 and ME-2 are near the same throughout the accelerated experiments. It could be attributed to the similarities of the compositions of the microemulsion formulations. ME-1 is composed of Span80 + Tween80 (1:1) + *n*-butanol + IPP + H_2O and the ME-2 is composed of Span80 + Tween80 (1:2) + *n*-butanol + IPP + H_2O. Due to the similar compositions of the two microemulsion formulations, it is assumed that the chloramphenicol molecules are solubilized in the microemulsions in the similar position. This results in the similar stability of chloramphenicol molecules in ME-1 and ME-2.

3.4. The location of chloramphenicol molecules in the microemulsion formulations

To shed some light on the location of the chloramphenicol molecules in the microemulsion formulations, the ^1H NMR experiments are performed on the microemulsions (Soderman and Nyden, 1999; Kreilgaard et al., 2000). Due to the compositions of ME-1 is similar to ME-2, the ^1H NMR experiment was only performed on the ME-1 in detail. The amounts of water, surfactants, alcohol and oil were fixed at the same in all the microemulsion systems and only the content of chloramphenicol was varied. The present microemulsion is consisted of Span80 + Tween80 (1:1) + *n*-butanol + IPP + D_2O, in which the Span80 and *n*-butanol both have hydroxyl groups and the Tween80 have many oxyethylene groups. So many materials and functional groups would result in a complexing ^1H NMR spectra of the microemulsions. 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 ME-1 are summarized in Table 2. From Table 2, it can be seen that after the addition of chloramphenicol, the chemical shifts of the functional groups display different changes. The chemical shifts of protons of the oxyethylene groups, $(CH_2CH_2O)_n$, are the most sensitive to the addition of chloramphenicol ($\Delta\delta \geq 0.15$ ppm); secondly is the methylene near the ether bond, α2-CH_2 ($|\Delta\delta| \geq 0.04$ ppm), and 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 ($\Delta\delta \leq 0.005$ 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. In the

Table 2
^1H NMR chemical shifts of Span80 and Tween80 in the microemulsions with various chloramphenicol concentrations

Functional group	δ (ppm)			
	δ^0	$\delta^{0.3}$	$\delta^{0.6}$	$\delta^{0.9}$
ω-CH_3	0.9549	0.9340	0.9553	0.9525
$(CH_2)_n$	1.3882	1.3911	1.3880	1.3906
β1-CH_2	1.6390	1.6357	1.6434	1.6355
α1-CH_2	2.2415	2.2410	2.2464	2.246
$(CH_2CH_2O)_n$	3.9506	3.7921	3.7791	3.7889
α2-CH_2	4.1978	4.2429	4.2494	4.2407

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 ^{1}H NMR chemical shifts of Span80 and Tween80 in the chloramphenicol-loaded microemulsions

Functional group	$\Delta\delta$ (ppm)		
	$\delta^0 - \delta^{0.3}$	$\delta^0 - \delta^{0.6}$	$\delta^0 - \delta^{0.9}$
ω-CH_3	0.0024	−3E−4	0.0025
$(CH_2)_n$	−0.0029	3E−4	−0.0024
β1-CH_2	0.0033	−0.0044	0.0035
α1-CH_2	5E−4	−0.0049	−0.0048
$(CH_2CH_2O)_n$	**0.1585**	**0.1715**	**0.1618**
α2-CH_2	**−0.0451**	**−0.0517**	**−0.0430**

chloramphenicol-loaded microemulsions, 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 $(CH_2CH_2O)_n$ and α2-CH_2 are relatively large (Table 3). It implies that the chloramphenicol is not solubilized in the palisade layer or the oil kernel of the drops of the o/w microemulsions, but in the hydrophilic shell of the microemulsions drops, which is composed of many oxyethylene groups (see Fig. 7). However, there is another interesting phenomenon. With the addition of chloramphenicol, the chemical shifts of oxyethylene groups all move to high field and $\Delta\delta > 0$; on the contrast, the chemical shifts of α2-CH_2 all move to low field and $\Delta\delta < 0$. The different changes of $(CH_2CH_2O)_n$ and α2-CH_2 reveal the specific location of chloramphenicol molecules in the microemulsions formulations. It is well known that the benzene ring has a strong screening effect and it could enable the chemical shift values of other groups near it to decrease ($\Delta\delta > 0$) (Xing and Xu, 1993). On the other hand, the nitro-group, –NO_2, is a famous group of electron withdrawing. The chemical shift of functional groups of other compounds near the nitro-group would move to low field ($\Delta\delta < 0$) (Xing and Xu, 1993). Therefore, the chloramphenicol is solubilized into the hydrophilic shell composed of many oxyethylene groups of the surfactants. Its nitro-group is near the group of α2-CH_2 of the surfactant molecules and its benzene ring near the group of $(CH_2CH_2O)_n$ of the surfactants. It might be this reason that enables the chloramphenicol molecules in the microemulsion formulations to be screened from the bulk water and its stability to be increased remarkably.

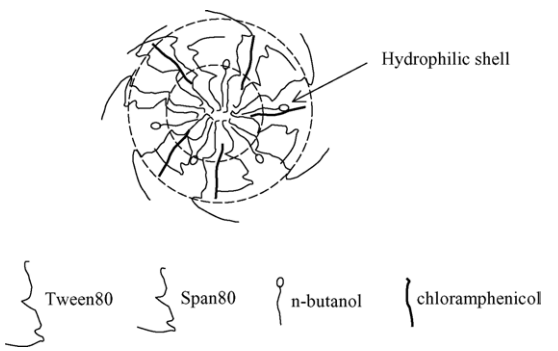


Fig. 7. Sketch of the locations of chloramphenicol molecules in the microemulsions. (The arc area among the dotted lines represents the hydrophilic shell of the microemulsion drops, which are composed of many oxyethylene groups.)

Of course, due to the chloramphenicols could partly dissolve in water and there are still some glycols in the microemulsion formulations (Fig. 6). However, most chloramphenicol molecules could be solubilized into the hydrophilic shell of the microemulsion drops as mentioned above, so the stability of chloramphenicol is increased dramatically. As far as the commercial eye drops is concerned, it is mainly a solution of choramphenicols 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-1 and ME-2.

4. Conclusions

A series of presudo-ternary phase diagrams were constructed and the differences of the microemulsion area were interpreted using the BSO equation. The solution of 0.9 wt% NaCl has little effect on the phase behavior of the systems, but the solution of 0.05 wt% sodium hyaluronate induces a sharp decrease of the microemulsion area of the systems.

The phase transition of the chloramphenicol-trapped microemulsions was studied by the electrical conductivity measurement. The chloramphenicol and the solution of 0.05% sodium hyaluronate have little effects on the electrical conductivity of the system. However, the solution of 0.9% NaCl could affect the electrical conductivity of the microemulsions strongly.

The chloramphenicol-loaded microemulsions are investigated as model drug delivery systems for eye drops. The stability of the chloramphenicol molecules

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

The locations of the chloramphenicol molecules in the microemulsions are determined by ^{1}H NMR spectroscopy. It is inferred from the results of ^{1}H NMR that the chloramphenicol molecules might be solubilized in the hydrophilic shell of the microemulsion drops, which are composed of many oxyethylene groups of the surfactants. The chloramphenicol molecule's nitro-group is near the α2-CH_2 of the surfactant molecules and its benzene ring is near 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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