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In vitro studies on the application of colloidal emulsion aphrons to drug overdose treatment

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Abstract

Colloidal emulsion aphrons (CEAs) are considered as the micron-sized water-in-oil (W/O) emulsion-cores encapsulated by a "soapy shell" consisting of multi-layer surfactant molecules. In this dispersion, the emulsion-core sizes are mainly in 10–100 μm and that of the inner phase droplets are in 1–5 μm. CEAs not only behave analogously to emulsion liquid membrane (ELM) in extraction with advantages of high concentration ratio, counter-concentration extraction and combination of extraction with backwash together, but also have the large interface areas, easy scatteration and quick extraction which colloidal liquid aphrons (CLAs) possess. CEA extraction overcomes the restriction of partition equilibrium between the water and the oil phase that CLAs have. They have greater extraction capacity than CLAs. In this study, the application of CEAs to drug overdose treatment was studied using salicylic acid as the model drug, paraffin oil as the membrane phase, PEG-30 dipolyhydroxystearate (P135) as the hydrophobic surfactant, nonylphenol ethoxylate-10 (NP10) as the hydrophilic surfactant and NaOH solution as the receptor phase. Also some factors affecting the stability of this dispersion and extraction ratio were investigated. In order to prepare CEAs successfully, the concentrations of NP10 and P135 should be in 1.5–3.0% (w/v) and 0.25–1.0% (w/v), respectively, together with the ratio of the volume of oil phase to the volume of inner aqueous phase of CEAs, $R_{oi} \geq 1{:}1$. For the extraction of salicylic acid, the pH value of the feed phase was supposed to be lower than 2.0 and the suitable NaOH concentration of the receptor phase was higher than 0.02 mol/L. Under this condition, more than 98.7% of salicylic acid was transported into receptor phase in half a minute.

Keywords: Colloidal emulsion aphron; Detoxification; Drug overdose; Extraction

1. Introduction

There are many cases of human poisoning involving drug ingestion each year (Chiu, 1996; Hanley et al., 1998; Byard et al., 2000; Ostamo and Lonnqvist, 2001; Romain et al., 2003). Traditionally, acute poisoning treatment involves attempts to reduce drug absorption from the gastrointestinal tract. Emetics, peritoneal dialysis are often used for the therapies (Morris et al., 1974; Stein et al., 2001; Jeremy et al., 2001). Orally uptake activated charcoal has been applied for this purpose (Raffa et al., 2000; Deshpande et al., 1999; Sato et al., 2003) and also the application of emulsion liquid membranes (ELMs) has been investigated (Frankenfeld et al., 1976; Chiang et al., 1978), although these methods have some limitations.

Colloidal emulsion aphrons (CEAs) are composed of micron-sized water-in-oil (W/O) emulsion-cores encapsulated by a "soapy shell" consisting of multi-layer surfactant molecules (seen in Fig. 1) (Deng et al., 2005). The emulsion-core sizes are mainly in 10–100 μm and that of the inner phase droplets are in 1–5 μm. The structure of this dispersion can be considered as the combo of ELM (Li, 1968; Patnaik, 1995) and colloidal liquid aphron (CLA) dispersions (Sebba, 1987; Lye and Stuckey, 1998). The extraction with CEAs probably has the advantages of high concentration ratio, counter-concentration extraction and combination of extraction and backwash together which ELM extraction has, and also has the large interface areas, easy scatteration and quick extraction which colloidal liquid aphrons possess. On the other hand, CEA extraction overcomes

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Nomenclature

c_{HSa}	total concentration of salicylic acid (mol/L)
c_{NaOH}	concentration of sodium hydroxide (mol/L).
E	extraction ratio of salicylic acid
HSa	salicylic acid molecule
K_a	ionization constant of salicylic acid
n_{HSa}/n_{NaOH}	molar ratio of salicylic acid to sodium hydroxide
PVR	ratio of the volume of water-in-oil emulsion-cores to the volume of hydrophilic surfactant aqueous solution which encapsulating emulsion-cores of CEAs or the ratio of the volume of oil phase to the volume of continuous hydrophilic surfactant aqueous solution of CLAs.
R_{dc}	treat ratio, which is the ratio of the volume of feed phase to the volume of CEAs or CLAs.
R_{oi}	ratio of the volume of oil phase to the volume of inner aqueous phase of water-in-oil emulsion.
Sa^-	salicylic acid anion

Greek symbols

α	ratio of the equilibrium concentration of non-dissociated form to that of the total salicylic acid in the aqueous solution
υ	extraction velocity

the restriction of partition equilibrium between the water and the oil phase that CLAs have. They have greater extraction capacity than CLAs. Compared with ELMs, CEAs can disperse in the aqueous phase more easily. The extraction with CEAs may meet the demand of quick extraction or the extraction without sufficient stirring.

To understand the properties of CEAs in drug overdose treatment, in vitro studies were conducted under different conditions with salicylic acid as a model drug.

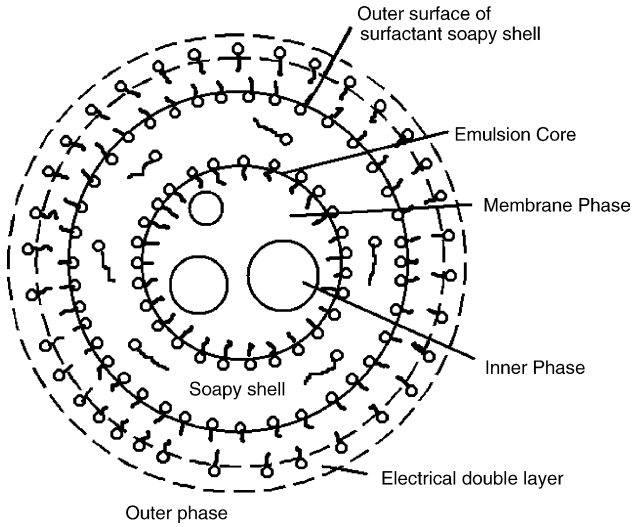


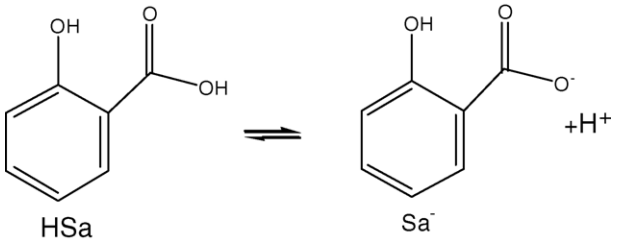
Fig. 1. Proposed structure of the CEA aphron (Deng et al., 2005).

2. Principles

The inner aqueous phase of CEAs can be formulated to form a high capacity sink for the drug in the feed phase by (a) pH control, (b) plasma proteins to bind the drug, (c) activated charcoal, or (d) specific drug antibodies, etc.

Some drugs are organic weak acids (Romain et al., 2003; Jeremy et al., 2001; Deshpande et al., 1999). They can be extracted with CEAs by pH control. In this study, the application of CEAs to drug overdose treatment was studied using paraffin oil as the membrane phase, PEG-30 dipolyhydroxystearate (P135) as the hydrophobic surfactant, nonylphenol ethoxylate-10 (NP10) as the hydrophilic surfactant and NaOH solution as the inner phase.

Salicylic acid (substituted for HSa) is a weak acid. In the aqueous solution, there exists ionization equilibrium in acidic, neutral or basic conditions.



(1)

The dissociation constant K_a (Degim et al., 2001) is

$$K_a = \frac{[H^+][Sa^-]}{[HSa]} = 6.98 \times 10^{-4} \quad (2)$$

where $[H^+]$ is the equilibrium concentration of H^+, $[Sa^-]$ the equilibrium concentration of dissociated form and [HSa] is the equilibrium concentration of non-dissociated form of salicylic acid in aqueous solution.

The total content of salicylic acid in the outer solution c_{HSa}, may be expressed as

$$c_{HSa} = [HSa] + [Sa^-] = \left(1 + \frac{K_a}{[H^+]}\right)[HSa] \quad (3)$$

Then the ratio of the equilibrium concentration of non-dissociated form to that of the total salicylic acid in the aqueous solution is α,

$$\alpha\,(\%) = \frac{[HSa]}{c_{HSa}} \times 100 = \frac{[H^+]}{[H^+] + K_a} \times 100 \quad (4)$$

It is can be seen that the pH influences the existing form of salicylic acid remarkably. The effect of pH on α according to Eq. (4) is shown in Fig. 2. Under pH > 5.2, 99% of the salicylic acid exists in the dissociated form of salicylic anion (Sa^-); however it mainly exists as the non-dissociated form (>99%) when pH < 1.2 in aqueous solution.

Fig. 3 illustrates the principle of extraction by CEAs. The non-dissociated salicylic acid, which is soluble in the membrane oil phase, transports across the soapy shell and the membrane phase into the receptor phase (inner phase). The driving force is simply the concentration gradient of the non-dissociated form of salicylic acid. Because the gastric juice is acidic (pH 1.2)

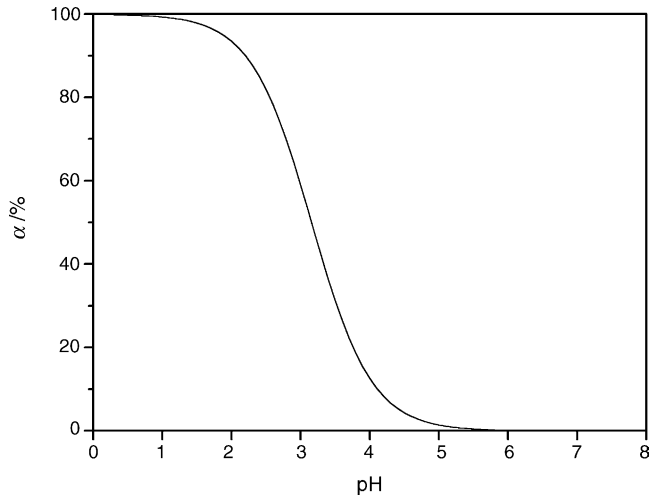


Fig. 2. Effect of pH on HSa and Sa^- shift.

(Xu, 2000), the salicylic acid is mainly in the non-dissociated form under this condition. In order to sustain a large concentration gradient of non-dissociated salicylic acid between the outer feed phase and the inner receptor phase, the strong base solution NaOH was introduced into the inner phase to exhaust the non-dissociated salicylic acid by the following reaction:

$$HSa + OH^- \rightleftharpoons Sa^- + H_2O \quad (5)$$

Due to the basic condition of the inner phase, the salicylic acid dissociates spontaneously and irreversibly into Sa^- (Eq. (5)), which is insoluble in the membrane phase and cannot penetrate the membrane backwards. Thus the salicylic acid can be collected and reserved in the inner phase.

According to the analysis above, the pH of the outer feed solution of salicylic acid must be adjusted to lower than 1.2 and the pH of the receptor phase should be higher than 5.2 in order to keep the maximum concentration gradient between the feed phase and the inner phase to facilitate the extraction of 99% salicylic acid from the feed phase.

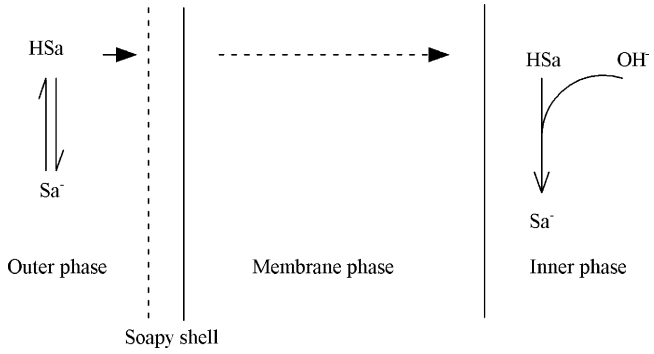


Fig. 3. Mechanism for CEA extraction.

3. Materials and methods

3.1. Regents

The membrane phase used in this work was paraffin oil (A.R. the First Chemical Regent Plant of Tianjin, China). P135, a PEG-30 dipolyhydroxystearate, block copolymer with a molecular weight of 5000 (ICI Surfactants, Wilmington, DE of U.S.A.), was used as hydrophobic surfactant and NP10 (C.P., Wuhan Shengshi Fine Chemical Ltd., China) was employed as hydrophilic surfactant. Salicylic acid (A.R. Beijing Chemical Plant of China) was used as the model drug. The water was deionized water, and all the other regents are analytical pure.

3.2. Equipments

A PHS-3C pH meter, made by Shanghai Leici Instrumental Factory, was employed to measure the pH value of salicylic acid solution. A two-blade paddle impeller of 26 mm diameter with 10 mm width was used for stirring. An X80 ultrasonic generator manufactured by L&R Manufacturing Company of U.S.A. and a D40-2F electric stirrer made by Hangzhou Electric Instrument Factory of China were employed for CEA preparation and extraction. A SZGB-11 tachometer (made by Shanghai Tachometer Factory) was employed to measure the stirring speeds. A 722s spectrophotometer produced by the Main Workshop of Shanghai Analytical Instruments of China and 0.2 μ cellulose acetate filter membranes (Advantec, Japan) were used for measuring the concentration of salicylic acid to determine the extraction ratio of the extraction.

3.3. Preparation of CEAs

There are two essential steps for the preparation of CEAs: (1) the preparation of water-in-oil emulsion and (2) the formation of CEAs.

3.3.1. Preparation of water-in-oil emulsion

Some quantity of paraffin oil containing P135 and some sodium hydroxide aqueous solution were taken into a 125 mL conical flask and then the flask was covered with rubber plug to prevent losses by splashing or evaporation. The oil–water mixture was violently stirred at 2500 rpm for 10 min under ultrasonic condition. The mixture formed a water-in-oil emulsion with the inner aqueous droplets of 1-5 μm in diameter.

3.3.2. Formation of CEAs

To make CEAs, some volume of NP10 aqueous solution was taken into a 125 mL conical flask and shaken until good gas foam was formed. The water-in-oil emulsion previously prepared was gradually dropped into the foam. The foam would soon disappear and some aphrons shaped. The emulsion was being added continuously under low-speed stirring (100 rpm, too violent the stirring may destroy the emulsion and let the inner phase out) at an average rate of 1.0 mL/min until all the required volume emulsion was added and then the CEA dispersion formed. When the PVR (ratio of the volume of hydrophilic surfactant aqueous

solution to the volume of water-in-oil emulsion) reached 5, the dispersion showed viscous and no distinct signs of phase separation for more than 48 h, but too high the PVR would (roughly more than 10) make the CEA dispersion ruined. So all the CEAs used in extraction in this article was with PVR = 5.

3.4. Extraction of salicylic acid

In a 150 mL beaker with 100 mL of 8.0×10^{-4} mol/L salicylic acid, 10 mL of CEAs was added under stirring, the treat ratio, R_{dc} which is the ratio of the volume of feed phase to the volume of CEAs, was 10). The CEAs were quickly dispersed in the salicylic acid solution and 5 mL of samples was taken at intervals and then filtered using membrane filter. The salicylic acid concentration in the filtrate could be determined using spectrophotometric method (Geeta and Baggi, 1988). The extraction ratio of salicylic acid (E) can be calculated by the equation:

$$E\,(\%) = \left(1 - \frac{c_t}{c_0 \times \frac{V_0}{V_d + V_0 \times \frac{1}{PVR+1}}}\right) \times 100 \qquad (6)$$

c_0, c_t are the concentration of salicylic acid in the feed phase at the starting time and t moment, respectively. V_d is the volume of the feed phase. V_0 is the volume of CEAs. $V_0 \times \frac{1}{PVR+1}$ is the volume of the hydrophilic surfactant solution which encapsulating the water-in-oil emulsion-cores in CEAs. The hydrophilic surfactant solution can mix with the outer feed phase to form a homogeneous outer aqueous phase during the extraction. Compared with V_d, the volume of hydrophilic surfactant solution is very small. In order to obtain reproducible results, a comparatively low-speed mixing rate of 250 rpm was adopted. In this study, CEAs were made with $R_{oi} = 1$, 0.08 mol/L NaOH as receptor phase and 0.5% P135 (w/v) of paraffin oil as membrane phase, 2.0% (w/v) NP 10 as the hydrophilic surfactant solution and the extraction of salicylic acid were done with $c_0 = 8.0 \times 10^{-4}$ mol/L, R_{dc} of 10, mixing rate of 250 rpm unless specific declaration.

4. Results and discussion

4.1. Stability of the water-in-oil emulsion and CEAs

The stable water-in-oil emulsion is the precondition for preparing a stable CEA dispersion. In order to get a stable water-in-oil emulsion, some hydrophobic surfactant must be added in the oil. In our experiments, P135, which is an excellent water-in-oil type surfactant, was dissolved into paraffin oil to stabilize the water-in-oil emulsion. If the P135 concentration is lower than 0.25% (w/v), the water-in-oil emulsion cannot be stabilized satisfactorily. Moreover the solubility of P135 in paraffin oil is roughly as much as 1.0% (w/v). The suitable P135 concentration for preparing stable water-in-oil emulsion is in the range of 0.25–1.0% (w/v) and the emulsion prepared can exist stably within 7 days.

There is also a suitable scale of hydrophilic surfactant for the preparation of CEAs. Too low the concentration of NP10 will lead to the hydrophilic surfactant aqueous solution to be encapsulated into the water-in-oil emulsion and the water-in-oil-in-water (W/O/W) type CEAs cannot be obtained. However, too high the NP10 concentration makes the globules of emulsion-core so small that the inner receptor solution leaks out. The suitable NP10 concentration for preparing stable CEAs was found to be in 1.5–3.0% (w/v).

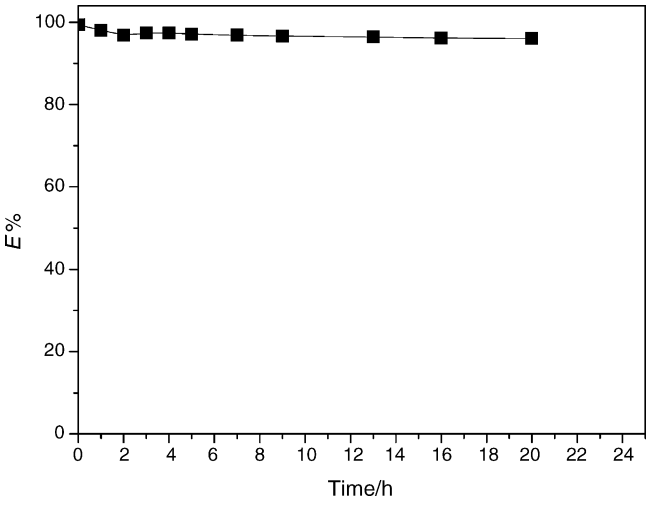


Fig. 4. Changes of HSa extraction ratio with time (pH 2.0, $c_{HSa} = 8.0 \times 10^{-4}$ mol/L, $R_{dc} = 10$).

In order to approach the in vivo situation and to lower the effect of osmotic pressure on the stability of CEAs, 0.5% (w/v) NaCl was added in the feed phase. At the beginning of the extraction, the transfer of salicylic acid reaches equilibrium rapidly within 1 min. After then E slowly decreases because of the breakage of CEA aphrons, therefore the decrease of the salicylic acid extraction ratio E is an important parameter for the determination of CEAs' stability in the extraction process. Fig. 4 is the changes of HSa extraction ratio with time after CEAs were dispersed into the feed phase and then deposit at static state. It is can be seen that CEAs can keep stable in the feed phase for a comparatively long term with minor leakage. The extraction ratio of salicylic acid only decrease from the maximum 98.7% to 96.1% in 20 h.

4.2. Extraction velocity of CEAs

The extraction velocity of the dispersion can be determined by the reduction of the concentration of salicylic acid in the feed phase with time

$$\upsilon = -\frac{dc_{HSa}}{dt}$$

But because the drastic decrease of c_{HSa} within the first half-minute, it is difficult to measure the extraction velocity by the sampling method. The comparison of the extraction of CEAs with that of emulsion liquid membranes under the same mixing rate, concentrations of inner NaOH solution and salicylic acid in the feed phase can be seen from Fig. 5.

The extraction with CEAs was far more rapid than that with emulsion liquid membranes. For the process with emulsion liq-

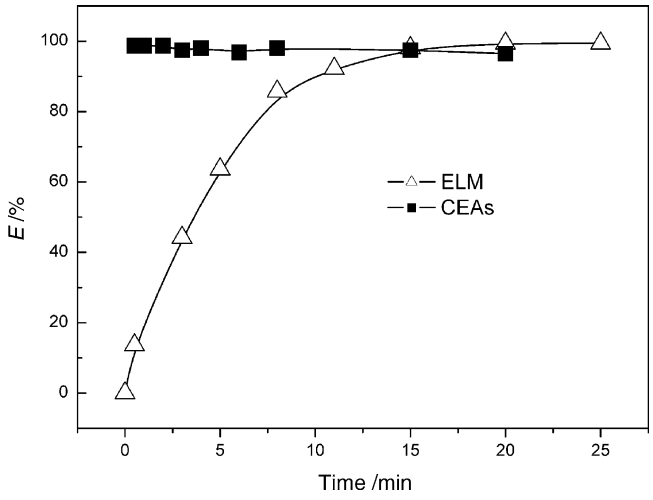


Fig. 5. Comparison of the ELM extraction with CEA extraction (pH 2.0, $c_{HSa} = 8.0 \times 10^{-4}$ mol/L, $R_{dc} = 10$, mixing speed of 250 rpm. The emulsion and CEAs are all made with $R_{oi} = 1$, 0.08 mol/L NaOH as receptor phase and 0.5% P135 of paraffin oil as membrane phase).

uid membranes, the extraction needed 20 min to come to equilibrium, but for that with CEAs, it needed only half a minute. This rapid extraction is very important for the treatment of drug overdose. The quick extraction of CEAs is mainly due to large mass transfer surface areas resulting from the small globule diameters of emulsion-cores.

4.3. Effect of the feed phase pH on extraction ratio

Before each batch of extraction, the pH of the feed phase was adjusted with 1.0 mol/L HCl. The extraction ratio of salicylic acid in different pH value of feed phase was shown in Fig. 6. Compared with Fig. 2, the practical extraction curve with pH was steeper than the theoretical analysis. The reason for this phenomenon is unknown now. The favorable extraction ratio (>98.7%) can be obtained when the pH value of feed phase is lower than 2.0, whereas at pH 3.5, salicylic acid nearly cannot

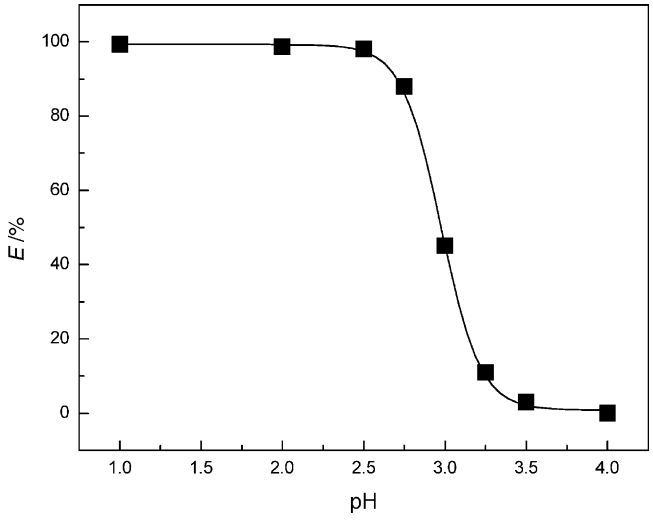


Fig. 6. pH effect of feed phase on salicylic acid extraction ratio ($c_{HSa} = 8.0 \times 10^{-4}$ mol/L, $R_{dc} = 10$, mixing speed of 250 rpm).

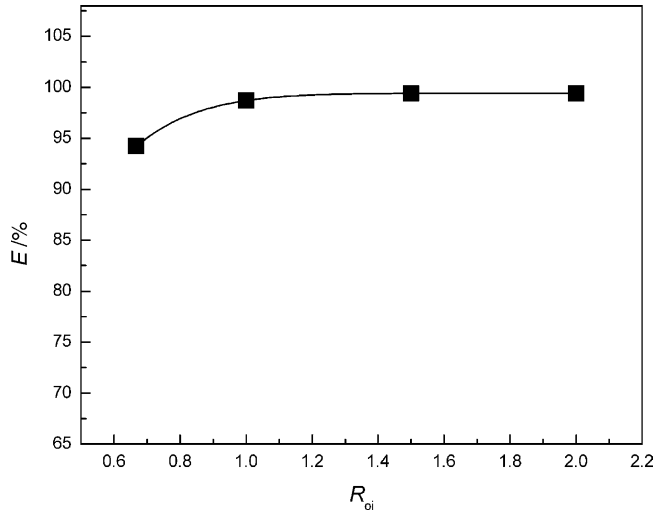


Fig. 7. Effect of R_{oi} on salicylic acid extraction ratio (pH 2.0, $c_{HSa} = 8.0 \times 10^{-4}$ mol/L. $R_{dc} = 10$, mixing speed of 250 rpm. CEAs made with a PVR = 5, 0.08 mol/L NaOH as receptor phase, 0.5% P135 of paraffin oil as membrane phase and 2.0% NP10 as hydrophilic surfactant aqueous solution).

be transported into inner phase. So all the following experiments were conducted at the feed phase pH of 2.0.

4.4. Effect of R_{oi} on extraction ratio

The thickness of the oil phase is important for the stability of liquid membrane, which is mainly determined by R_{oi} (ratio of the volume of oil phase to the volume of inner aqueous phase of CEAs). Extremely low R_{oi} will make the membrane too thin and leads to drastic leakage of the inner phase, therefore leads to low extraction ratio. The effect of R_{oi} on extraction ratio is depicted in Fig. 7. If $R_{oi} < 2/3$, the water-in-oil emulsion is easily broken and CEAs cannot be obtained. When R_{oi} is 1:1 or higher, more than 98.7% salicylic acid can be transported into the inner phase.

4.5. Effect of c_{NaOH} on extraction ratio

NaOH concentration in the inner phase also influences the transfer of salicylic acid. According to reaction (5), higher c_{NaOH} will make the equilibrium shift to right and offers higher driving force for the transport of salicylic acid, thus higher extraction ratio can be achieved. The effect of c_{NaOH} on extraction ratio of salicylic acid is illustrated in Fig. 8. It can be seen when c_{NaOH} is lower than 0.02 mol/L the transport decreases acutely. This is because n_{HSa}/n_{NaOH} was greater than 1.0 and the reaction (5) was nearly completed. So in order to get better extraction ratio, c_{NaOH} should be higher than 0.02 mol/L. On the other hand, too high the c_{NaOH} will make the dispersion unstable, so the selected c_{NaOH} in other experiments was 0.08 mol/L.

4.6. Comparison of the CLA extraction with CEA extraction

The comparison experiment of CLA extraction was done with the same oil phase, concentration of hydrophobic and hydrophilic surfactant as CEAs under the same PVR and R_{dc}.

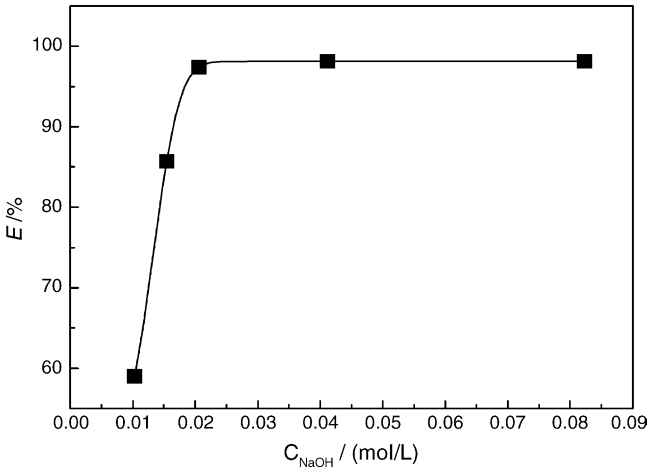


Fig. 8. Effect of c_{NaOH} on salicylic acid extraction ratio (pH 2.0, $c_{HSa} = 8.0 \times 10^{-4}$ mol/L. $R_{dc} = 10$, mixing speed of 250 rpm).

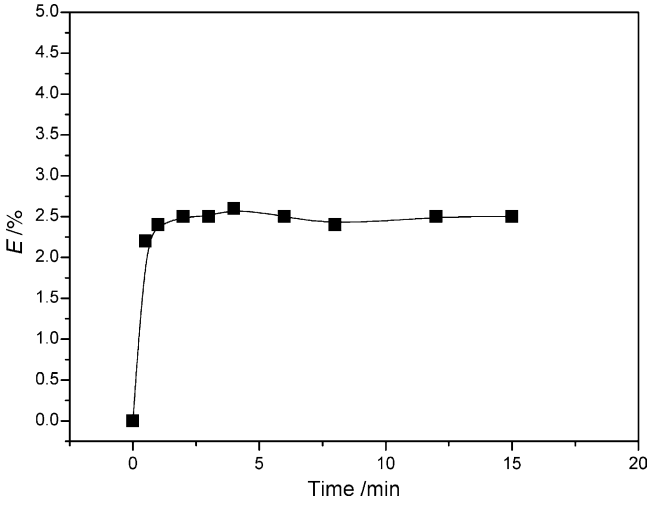


Fig. 9. Comparison of the CLA extraction with CEA extraction (pH 2.0, $c_{HSa} = 8.0 \times 10^{-4}$ mol/L, $R_{dc} = 10$, mixing speed of 250 rpm).

Fig. 9 is the extraction curve with CLAs, because of the low solubility of salicylic acid in paraffin oil and the restriction of the CLA extraction capacity, only 2.5% of salicylic acid was extracted. This phenomenon showed the comparatively high extraction capacity of CEAs.

5. Conclusion

As a new technique, the extraction with CEA dispersion has the fast extraction velocity, easy disperse in feed phase and high extraction ratio. This technique can be carried through by adding CEAs into the feed phase and dissipated by vibration or low-speed stirring. It is suitable for the quick extraction or the extraction without sufficient stirring. It offered a new method for the treatment of drug overdose. Hydrophilic surfactant concentration, hydrophobic surfactant concentration and R_{oi} are important factors for successful preparation of CEAs. In order to get a favorable extraction performance, the pH values of the receptor phase and the feed phase, receptor concentration, R_{oi} should be in suitable ranges. For the extraction of salicylic acid with this technique, the pH value of the feed phase was supposed to be lower than 2.0. The necessary NaOH concentration of the receptor phase is higher than 0.02 mol/L, R_{oi} is needed to be as much as 1:1 or more. More than 98.7% of salicylic acid was transferred into receptor phase in half a minute.

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