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Stability of Tetracycline Hydrochloride in Reverse Micelles

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ABSTRACT – The objective of this study was to investigate the stability of tetracycline HCl on encapsulation into and inside reverse micelles. To do so, tetracycline HCl was first mixed with cetyltrimethylammonium bromide, water and ethyl formate to make reverse micelles. The degradation kinetics of tetracycline HCl inside the reverse micelles was then assessed by scrutinizing its stability data. Under our experimental conditions, the reverse micelles formed spontaneously in absence of any mixing devices. During the preparation of the reverse micelles, however, considerable portions of tetracycline HCl underwent a chemical reaction (e.g., epimerization). For instance, 51.4 ± 0.6% of an initial concentration of tetracycline HCl was transformed into a degradation product. Once dissolved inside the reverse micelles, the degradation of tetracycline HCl followed an exponential decay pattern. The plot of log{the degradation rate of tetracycline HCl} versus log{tetracycline HCl concentration} made it possible to determine the order of degradation reaction and rate constant. It was proven that the degradation of tetracycline HCl inside the reverse micelles followed a first order kinetics with a rate constant of 0.0027 $hour^{-1}$. Meriting further investigation might be formulation studies to stabilize tetracycline HCl on encapsulation into and inside the reverse micelles.

Key words – Reverse micelles, Tetracycline HCl, Stability, Ethyl formate

Since poly-*d,l*-lactide-*co*-glycolide (PLGA) polymers have excellent biocompatibility and biodegradability, they have been increasingly used to deliver drugs over a wide range of periods. Variations in physicochemical properties including molecular weight, molar ratio of lactide to glycolide and crystallinity make it possible to manipulate the rate of PLGA degradation. The polymer-based dosage forms, such as implants and microspheres, are approved for human use in many international health authorities. At present, a number of microencapsulation techniques are available to load bioactive materials into PLGA microspheres. Popular microencapsulation procedures make use of solvent evaporation/extraction, double emulsion, phase separation, and spray drying principles.[1,2]

Cultures of certain streptomyces species furnish a broad spectrum antibiotic tetracycline hydrochloride. Its anti-microbial effects are exerted by the inhibition of protein synthesis. It is indicated for treatment of infections caused by a variety of gram-positive and gram-negative microorganisms. There have been interests in utilizing PLGA microspheres for the controlled release of tetracycline hydrochloride.[3-5] In these studies, the microspheres were prepared using either spray drying or double emulsion-based solvent evaporation techniques.

Reverse micelles are nanoscale aqueous droplets surrounded by a surfactant layer in nonpolar solvents. They can be used not only as simple models of biological membranes but also as hosts for proteins, small molecules, genes, and nucleic acids.[6-10] Recently, we have reported elsewhere a new reverse micellar microencapsulation process to successfully load tetracycline hydrochloride into PLGA microspheres.[11] The encapsulation method is distinguished from a conventional double emulsion one, judged from the perspectives of manufacturing attributes, microsphere characteristics, and drug loading efficiency. Our primary focus was on the preparation of reverse micelles, as well as the effect of manufacturing variables upon the characteristics of PLGA microspheres. A major point that was overlooked at that time was the stability of tetracycline hydrochloride in the reverse micelles. To address such an issue adequately, our present study has aimed to investigate the stability of tetracycline hydrochloride during the preparation of the reverse micelles and inside them. To do so, tetracycline hydrochloride stability study on encapsulation into the reverse micelles has been first carried out. After that, the kinetics and pattern of tetracycline hydrochloride degradation in the reverse micelles have been characterized.

Experimental

• **Materials:** Tetracycline hydrochloride (TC), cetyltrimeth-

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ylammonium bromide (CTAB), and trifluoroacetic acid (TFA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They were used as received without further purification. Aldrich (Milwaukee, WI, USA) was the supplier of analytical grade ethyl formate.

• **Preparation of reverse micelles:** TC (20 mg), CTAB (30 mg), and water (0.15 ml) were added into a vial containing 3 ml of ethyl formate. The mixture was kept still at 37°C overnight inside an oven, until a clear and optically transparent solution was obtained. Any mixing devices such as magnetic stirrer and sonicator were not used to prepare the reverse micelles, since they were formed spontaneously under our experimental conditions.

• **Determination of TC stability on encapsulation into reverse micelles:** During the preparation of the reverse micelles, TC dissolved slowly in ethyl formate via a reverse micellar solubilization process. The amount and nature of TC dissolved in the organic phase were evaluated. To do so, at predetermined time intervals up to 20 hours, the TC/CTAB/water/ethyl formate mixtures were taken out of the oven and centrifuged shortly. Aliquots of the clear organic phase were pipetted and, after dilution, were subject to HPLC analysis.

• **Stability study with reverse micellar systems:** A TC-containing reverse micellar solution was kept inside an oven and its temperature was maintained at 37°C. At time intervals specified, 50 μl of the sample solutions were taken and diluted with 5 ml of water. Aliquots (20 μl) of the diluted solutions were subject to HPLC analysis.

• **HPLC analysis:** The Shimadzu HPLC system was used in this study. The instrument consisted of two pumps (LC-10AT$_{VP}$), an autosampler (Sil-10AD$_{VP}$), a UV detector (SPD-10A$_{VP}$) and a data processing system (Class-V$_p$ version 6.10 integrator). A Luna 5 μm C8(2) column (150 × 4.6 mm; Phenomenex, Torrance, CA, USA) fitted with a Security Guard cartridge was used as a stationary phase. A mobile phase consisting of acetonitrile and 1% TFA-containing water (1:3, by v/v) was used at a flow rate of 1 ml/min. TC and its degradation compound were detected at 254 nm. The concentrations of TC used for constructing a standard calibration curve varied from 125 to 12.5 μg/ml.

• **Data analysis:** The degradation pattern of TC in the reverse micelles was evaluated by differentiating the rate of TC change as a function of storage time. Relevant equations also were derived to calculate its half-life ($t_{1/2}$).

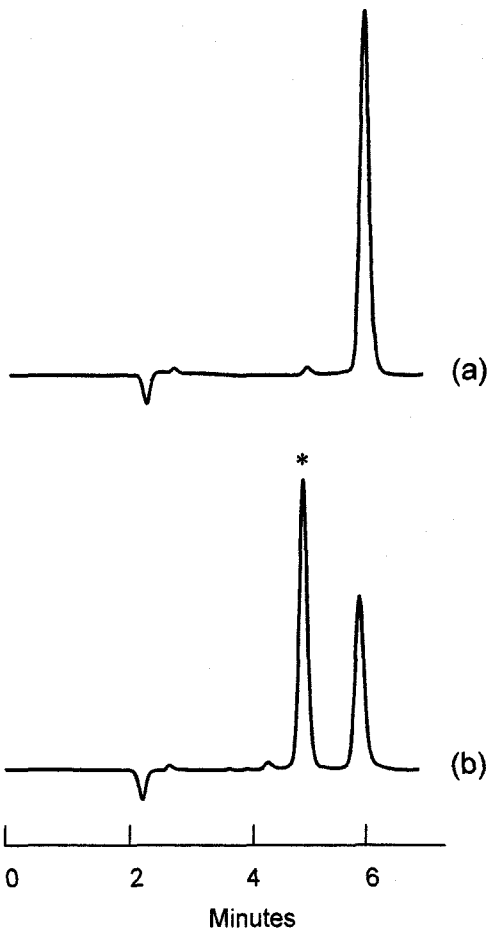

Figure 1–HPLC chromatograms of tetracycline hydrochloride (a) before and (b) after encapsulation into the reverse micelles. A degradation product (*) appears when TC, CTAB, water, and ethyl formate are mixed and incubated at 37°C.

Results and Discussion

Under our experimental conditions, 20 mg of TC was well encapsulated into the CTAB/water/ethyl formate reverse micelles. The resultant reverse micellar solution was clear and transparent. However, it was found that TC was subject to a chemical reaction during encapsulation into the reverse micelles (Figure 1). A degradation product was observed after CTAB, TC, water, and ethyl formate were mixed and incubated at 37°C. As seen in Figure 2, its amount increased with ongoing incubation. Analysis of all HPLC chromatograms led to a conclusion that a rise in the amount of the degradation product followed a sigmoidal pattern (Figure 3). For instance, the degradation product amounted to 9.9 ± 2.1% (mean ± s.d.) after incubation continued for 4 hours. Its amount increased to

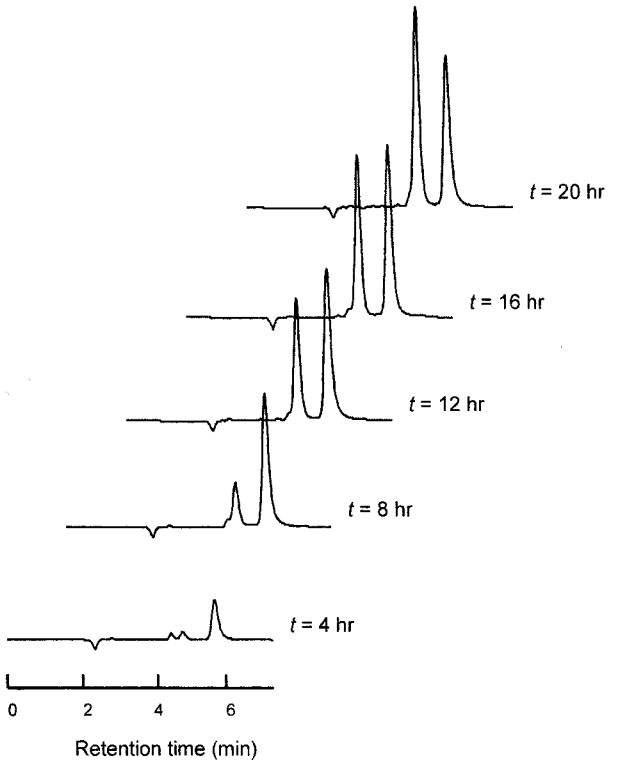

Figure 2–HPLC chromatograms demonstrating a rise in the amount of the degradation product as a function of incubation time. The peak area and height standing for the degradation product rise with ongoing incubation.

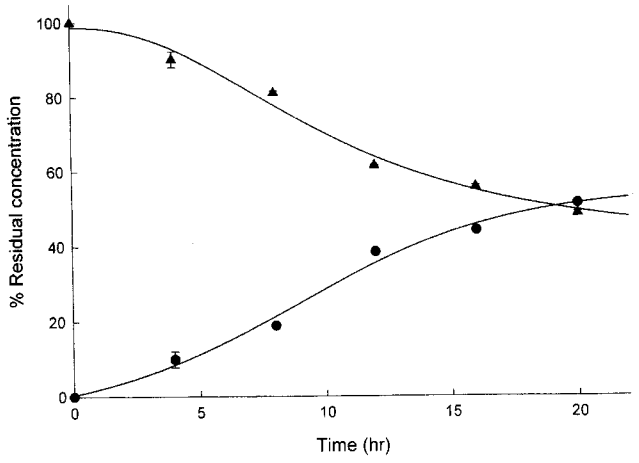

Figure 3–Percentage change in the amount of the degradation product during the preparation of the CTAB/water/ethyl formate reverse micelles. Its amount increases with incubation time (●), whereas TC declines as incubation proceeds (▲).

51.4 ± 0.6% in 20 hours. It was demonstrated before that TC transformed rapidly to 4-epi-tetracycline hydrochloride as a result of epimerization at the C-4 position.[12,13] These reports make it probable to conclude that the degradation product is 4-epi-tetracycline hydrochloride. Further studies such as NMR and LC/MS should be performed to support this supposition. Our HPLC study also indicates that if a UV-Vis spectrophotometer is used to quantify TC in samples, the result is quite likely to contain error: since the degradation product has the similar UV absorbance as the parent compound TC, one may encounter an overestimation of TC.

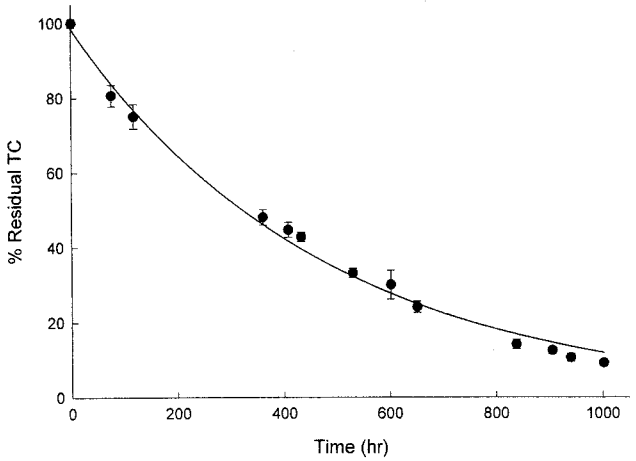

Figure 4–Percentage change of TC inside the reverse micelles observed during stability study. Its amount declines exponentially as a function of storage time.

Figure 4 shows the amount of remaining TC inside the reverse micelles as a function of storage time. Its degradation seemed to follow an exponential decay pattern. The kinetics of TC degradation in the reverse micelles was assessed by the following method. The rate of TC degradation (dC/dt) was described by:

$$-\frac{dC}{dt} = R = k[C]^a \quad (1)$$

where C represents TC concentration at time t; a indicates the order of degradation reaction; and k is its rate constant. Several rates could be attained from our experimental data:

$$-\frac{dC_1}{dt} = R_1 = k[C_1]^a,\ -\frac{dC_2}{dt} = R_2 = k[C_2]^a, \ldots\ldots\ldots\ldots,$$
$$-\frac{dC_n}{dt} = R_n = k[C_n]^a \quad (2)$$

The above equation was rearranged to provide a linear plot as follows:

$$\log\{-\frac{dC_n}{dt}\} = \log\{R_n\} = \log\{k\} + a \cdot \log\{C_n\} \quad (3)$$

The plot of $\log\{R_n\}$ against $\log\{C_n\}$ generates a linear curve with the slope of a, which is the order of degradation reaction. To draw such a plot, a polynomial equation was firstly developed to fit the data of TC stability shown in Figure 4:

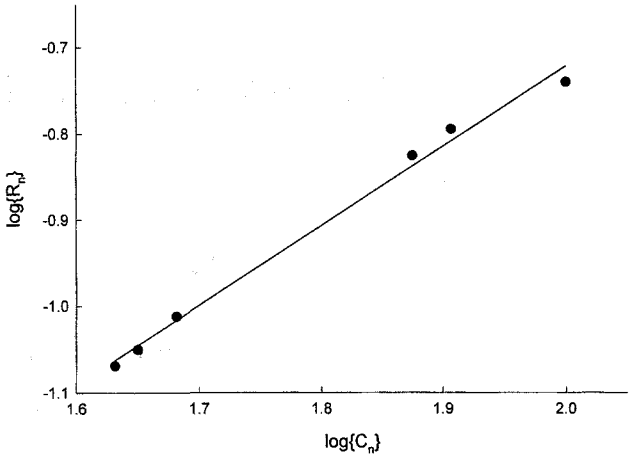

Figure 5–Determination of the degradation reaction order of TC inside the reverse micelles. It can be estimated from a slope of the $\log\{R_n\} - \log\{C_n\}$ plot.

$$C = 96.73 - 0.1816\ t + 1.4559 \times 10^{-4}\ t^2 - 5.2696 \times 10^{-8}\ t^3 \quad (r^2 = 0.99) \qquad (4)$$

Differentiating the above equation provides a rate at each corresponding time t. The relevant rates were calculated using the early 6 time points. Based on these data, a linear regression equation was obtained from the $\log\{R_n\} - \log\{C_n\}$ plot (Figure 5):

$$\log\{R_n\} = 0.92\ \log\{C_n\} - 2.568\ (r^2 = 0.99) \qquad (5)$$

A slope of 0.92 suggests that the degradation of TC in the reverse micelles follows a first order kinetics. Since the calculated reaction rate constant is 0.0027 hr^{-1}, $t_{1/2}$ is estimated to be 256 hours.

Conclusion

Under the experimental conditions reported in this study, it was possible to dissolve TC in the CTAB/water/ethyl formate reverse micelles. However, TC seemed to have a propensity for epimerizing on encapsulation into the reverse micelles. It would be definitely worth investigating such a reaction in detail. Once dissolved into the reverse micelles, the degradation pattern of TC was characterized by a first-order kinetics. Formulation approaches to stabilize TC on encapsulation into and inside the reverse micelles also merit further investigation.

Acknowledgments

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