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pH-Mediated Release in a Model Drug Delivery System

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Abstract **-** Targeted drug delivery systems protect healthy tissue in the patient's body while carrying the therapeutic agent to the diseased tissue. For cancer therapy, the enhanced permeability and retention (EPR) effect takes advantage of porosity of the tumor blood vessels and negligible lymphatic drainage. Nanoparticle drug carriers will accumulate in tumors without external intervention. The general problem is how best to release the drug once it has reached the targeted area. Here we show a model micellar drug delivery system that is stable and secure at a normal blood pH of 7.4, but disperses and releases its substrate as the pH drops below 7.0. The micelle is formed from the ionic surfactant, 10-undecenoate, with the hydrophobic tetrabutylammonium counterion. The pH-dependent structure and function differ markedly from other micellar solutions and suggests a key role of the counterion in both stability and solubilization power of the micelle. Our results indicate that tetrabutylammonium counterions can bring about the pH-mediated release of therapeutic agents from micellar drug delivery systems.

Keywords**:** micelle, solubilization, CMC, pH, model system, drug delivery, hydrophobic interactions

1. Introduction

Targeted drug delivery systems deliver therapeutic agents to specific parts of a patient's body. The carriers protect the drug while it circulates in the bloodstream and then release at the site of the diseased tissue. [1], [2] The enhanced permeability and retention (EPR) effect is one of the simplest approaches to targeted drug delivery for cancer treatment. Newly formed tumor vessels are leaky and lack lymphatic drainage. Nano-carriers circulating in the bloodstream will build up in the tumors without intervention. [3], [4], [5] Many tumors also have a markedly lower pH than healthy tissue due to the production of lactic acid by the anaerobic metabolism of cancer cells. This pH differential can trigger the release of the drug from a suitably designed carrier. [6], [7], [8]

Tetrabutylammonium 10-undecenoate (TBAU, Figure 1) micelles (Figure 2) with oil blue N (OBN, Figure 3) substrate have been shown to be an effective, simple model for isolating the molecular properties responsible for some drug delivery system functions. The hydrophobic tetrabutylammonium cation is particularly effective at stabilizing the micelle (low critical micelle concentration, CMC) and increasing its loading capacity (high solubilization power). [9] The 10-undecenoate ion is polymerizable [10] and oligomerization of the ion leads to enhanced stability of the micelle/substrate complex. [11]

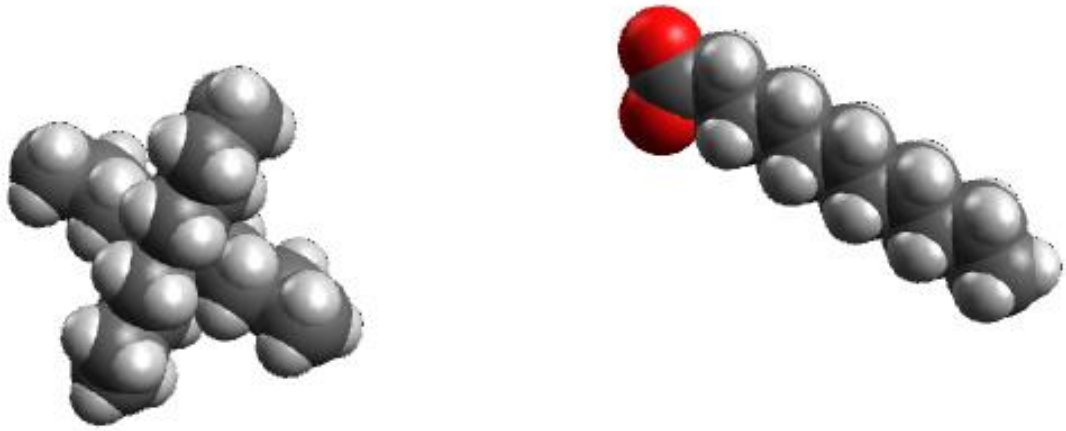

Fig. 1: Tetrabutylammonium 10-undecenoate space filling model.

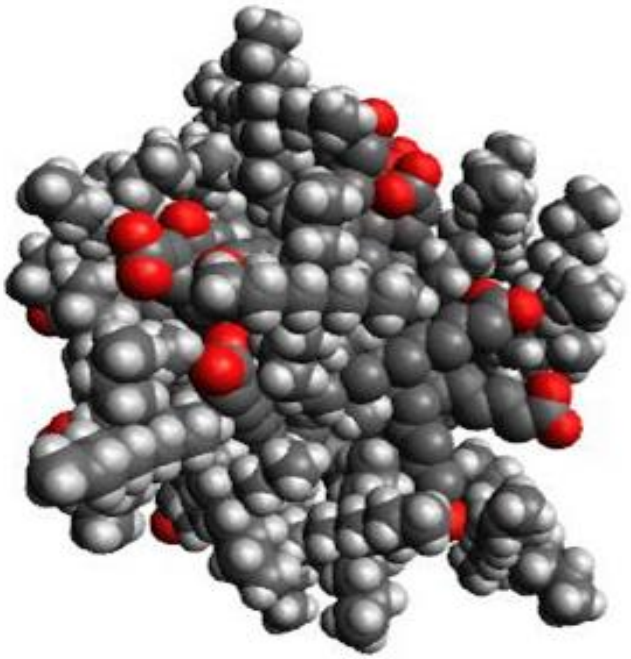

Fig. 2: Tetrabutylammonium 10-undecenoate micelle space filling model comprised of 45 10-undecenoate ions and 30 tetrabutylammonium counterions.

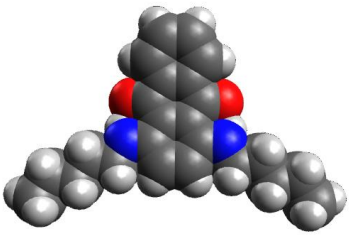

Fig. 3: Oil Blue N space filling model.

The long-term objective of this research is to determine how molecular structure influences the hydrophobic interactions that play a role in the rational design of drug delivery assemblies. The specific goal of this project was to describe the effect of pH on the stability and release of TBAU/OBN micelle/substrate complex.

2. Materials and Methods

All solutions were made up with ACS Reagent Grade, ASTM Type II water (Ricca Chemical Company). Stock solutions of tetrabutylammonium 10-undecenoate were prepared by neutralizing 10-undecenoic acid (Acros, 99%) with tetrabutylammonium hydroxide (Acros, 40% in water) after standardizing with potassium hydrogen phthalate (Fisher, ACS). A stock solution of hydrochloric acid was prepared by dilution (Fisher, ACS, 12M) and standardized with aqueous sodium hydroxide (Ricca Chemical, ACS Reagent grade), that had been standardized with potassium hydrogen phthalate.

Absorbance measurements were made at 600 nm with an Ocean Optics USB4000 UV/Vis spectrometer. The pH measurements were made with a Vernier PH-BTA sensor calibrated with pH 4.00, 7.00, and 10.00 buffer solutions (Fisher, Certified). All measurements were made at room temperature, 295 ± 2 K.

2.1. Critical Micelle Concentrations

Solutions were prepared by adding excess oil blue N (MP Biomedicals) to the surfactant solutions, sonicating for 30 minutes, allowing to settle at least overnight, and filtering (Millipore PVDF 0.45 μm) into glass cuvettes. OBN is a water

insoluble dye and is solubilized only in the presence of micelle solution. [9] Absorbance data for OBN in aqueous TBAU solutions were fit to the piecewise function,

$$A = \begin{cases} 0, & if\ c_{TBAU} < CMC \\ (c_{TBAU} - CMC) \times m, & if\ c_{TBAU} \geq CMC \end{cases} \quad (1)$$

where A is the absorbance, c_{TBAU} is the concentration of tetrabutylammonium 10-undecenoate (mol dm^{-3}), m is the slope of the line, and CMC is the critical micelle concentration.

2.2 Effect of Added Hydrochloric Acid

Each solution was prepared by combining appropriate amounts of TBAU and HCl stock solutions, diluting to a final volume, and adding excess OBN. Solution handling and spectrometric measurements followed the same procedure as described in section 2.1. Absorbance data were fit to the function,

$$A = \frac{a}{\left(1 + \left(\frac{c_{TBAU} - {c_{TBAU}}^0}{b}\right)^2\right) \times \left(1 + \left(\frac{pH - pH^0}{c}\right)^2\right)} \quad (2)$$

where a, b, c, ${c_{TBAU}}^0$, and pH^0, are parameters determined during the regression analysis.

3. Results

The experimental results and the regression lines are summarized in Figure 4. In water and up to ¼ equivalents of added HCl, the regression lines show good linearity with well-defined CMCs. With higher equivalents of added HCl, the absorbance was essentially flat and equal to zero.

The CMCs are summarized in Table 1. The first four values show a steady increase in CMC values with added HCl. There was no evidence of micelle formation or solubilization in solutions of 0.5 equivalents of HCl or higher.

Table 1: CMCs for aqueous solutions of tetrabutylammonium 10-undecenoate with added hydrochloric acid.

Equivalents of HCl added	CMC	Standard Error
0	0.029	0.002
0.0625	0.067	0.021
0.125	0.099	0.008
0.25	0.116	0.013
0.5	None observed	
1.0	None observed	

Results of the analysis of oil blue N solubilization as a function of both TBAU concentration and pH are presented in Figure 5. Micelle solubilization is evident at high pH, and clearly absent at low pH.

4. Discussion

The CMC for TBAU without added hydrochloric acid was 0.029 ± 0.002 mol dm^{-3}. This value is in good agreement with the value reported previously [9] from solubilization, 0.031 ± 0.003 mol dm^{-3}, and from conductivity measurements, 0.032 ± 0.004 mol dm^{-3}.

The plots of absorbance versus surfactant concentration in Figure 1 show a distinct trend of increasing CMC with increasing equivalents of hydrochloric acid added. At 0.5 equivalents added and higher, there is no evidence of micelle formation. This indicates a destabilization of the micelle and release of the OBN with added acid. The same effect is observed in Figure 2, as pH decreases, the amount of OBN solubilized by micelles decreases. For any surfactant concentration, at pH values less than 7, most of the OBN has been released. Our results with TBAU are opposite those found for sodium dodecyl

sulfate, where a decrease in CMC with decreasing pH, was found by solubilization, conductivity, and light scattering measurements. [12]

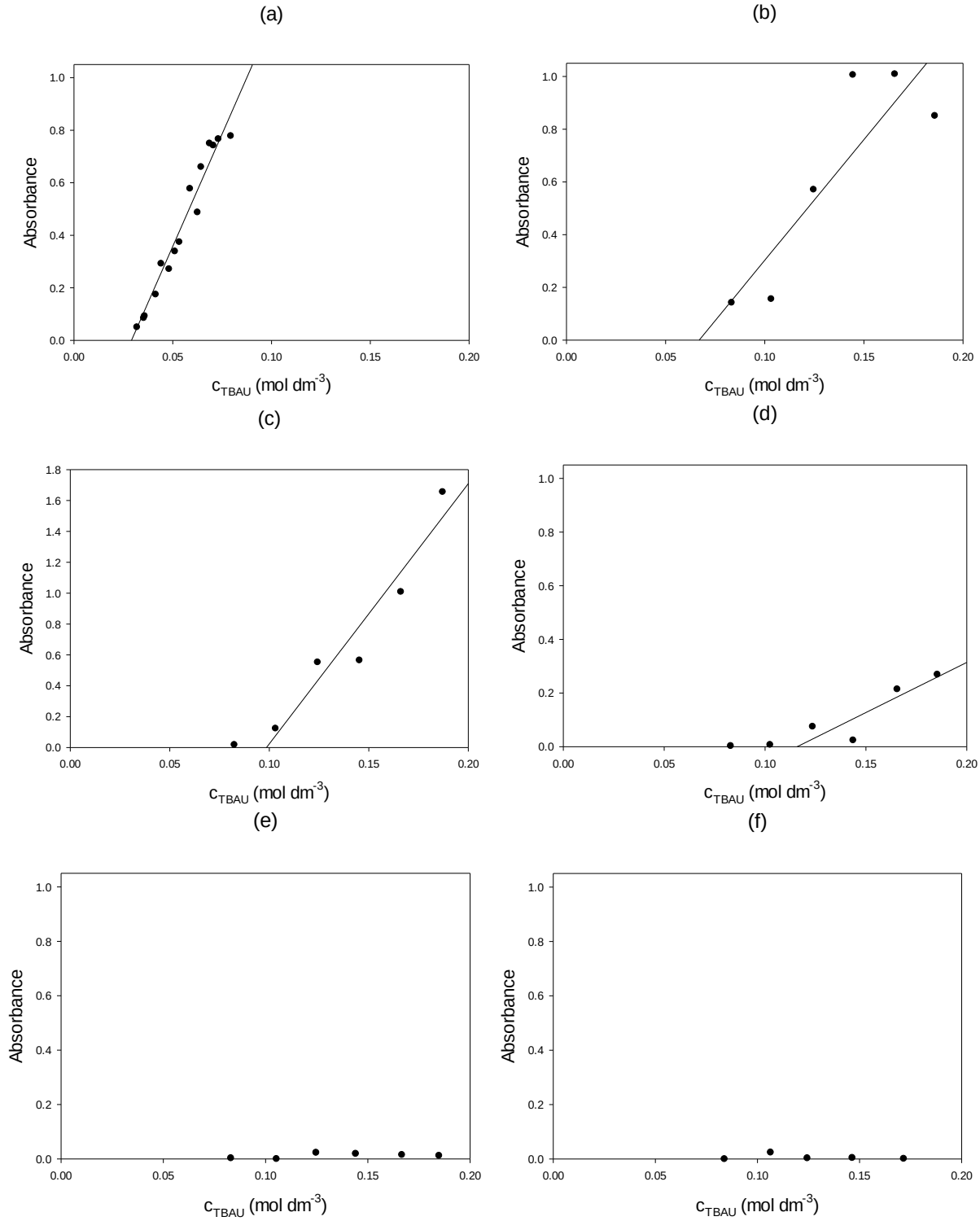

Fig. 4: Absorbance plots of oil blue N solubilized in aqueous solutions of tetrabutylammonium 10-undecenoates: (a) with no added HCL; (b) with $^1/_{16}$ equivalents of HCl; (c) with $^1/_8$ equivalents of HCl; (d) with $^1/_4$ equivalents of HCl; (e) with $^1/_2$ equivalents of HCl; (f) with 1 equivalent of HCl.

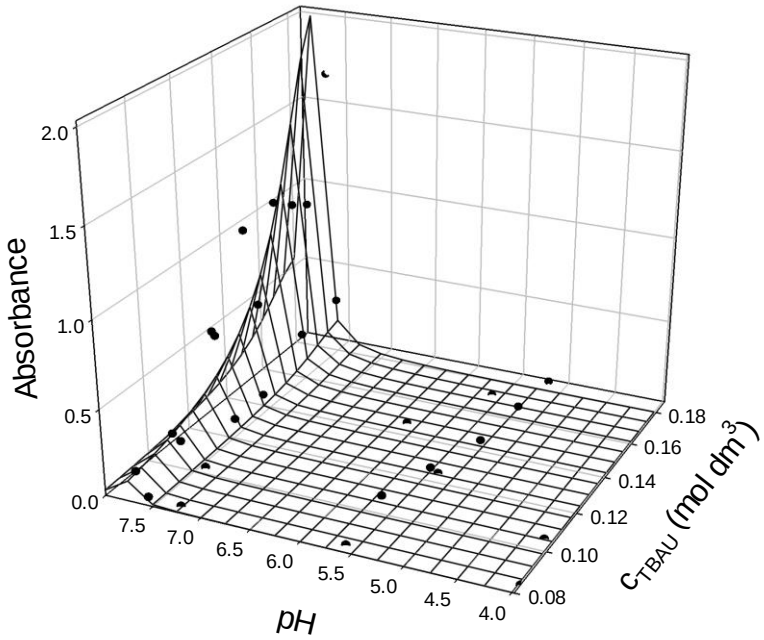

Fig. 5: Three-dimensional absorbance plot of absorbance of oil blue N at 600nm as a function of the concentration of Tetrabutylammonium 10-Undecenoate and pH.

Tetrabutylammonium ions have been shown to have atypical properties in aqueous solution. TBA carboxylate surfactants are highly soluble, do not exhibit clouding in the temperature range 301 – 371 K and form unusually small micelles. [13] They also exhibit an unusually high enthalpy of formation relative to homologous tetra-*n*-alkylammonium carboxylates. [14] In addition, they are the only surfactants capable of forming stable clathrate-hydrates near room temperature. [9], [15], [16]

An attempt to repeat the solubilization experiments with sodium 10-undecenoate resulted in cloudy mixtures rather than solutions suitable for the absorbance measurements. This result is consistent with earlier observations that adding acid to sodium 10-undecenoate micelle solutions yields vesicle formation [17] and the general observation that adding acid to a homogeneous surfactant solutions leads to phase separation. [18].

5. Conclusion

The tetrabutylammonium 10-undecenoate/oil blue n system has been investigated as a model for targeted drug delivery. The system is stable above pH 7 (normal blood pH), but destabilizes as the pH drops below 7. This behavior contrasts with general observation that carboxylate surfactants tend to form vesicles as the pH is lowered [19], and with the specific case of vesicle formation by sodium 10-undecenoate [17]. The fact that TBAU/OBN destabilizes at low pH rather than forming an alternative aggregated phase leads to the complete release of the OBN substrate within a narrow range of pH values. The ability to trigger the release of the substrate with pH is an important feature for targeted drug delivery systems based on the EPR effect. Since many tumors are more acidic than the surrounding healthy tissue, the tumors themselves will trigger the release of the therapeutic agent. In this respect, one of the essential features of targeted drug delivery systems has been captured by the simple model system.

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