RESEARCH IN PHARMACY AND MEDICINE
PHARMEDICINE JOURNAL

2026; 3(1):27-36. doi: 10.62482/pmj.xx

Original Article

Micellar Pseudo-Models for Biological Membranes: Spectrophotometric Analysis of Micellar Binding of Antiviral (Oseltamivir Phosphate) and Anticoagulant (Enoxaparin Sodium)

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Received: 2025.10.07; **Revised:** 2025.11.06 **Accepted:** 2025.12.28 **Accepted:** 2026.02.28

Abstract

Introduction: The micellar environment formed by the aggregation of surfactants at a specific concentration, known as the critical micelle concentration (CMC), behaves like a simple biological system and is thus considered a pseudo-model of a biological system. Owing to these properties, which define them as mimetic systems, the microscopic environment created by surfactant micelles is similar to phospholipid membranes, allowing micelles to serve as models for biological membrane systems. Therefore, data on the binding of drugs to micelles is important as it can elucidate the drug's mechanism of action. Due to these interesting and unique physicochemical properties, surfactant micelles are widely used as a simple pseudo-model even for highly complex biomembranes. Therefore, this study aims to investigate the interactions of the antiviral drug Oseltamivir phosphate (OP) and the anticoagulant drug Enoxaparin sodium (ES) with different micellar systems.

Methods: The interactions of ES and OP with anionic, cationic, and nonionic surfactants were examined by the spectrophotometric method (UV-Vis), and their binding constants to micelles were calculated. Tween 20 (nonionic), sodium dodecyl sulfate (SDS) (anionic), and cetyltrimethylammonium bromide (CTAB) (cationic)-surfactants frequently used in pharmaceutical applications as models for mimetic systems-were selected for this study.

Results: The results of this study are expected to provide information about the behaviour of OP and ES in micellar systems that model biological membranes, as well as offer insight into the effectiveness of surfactants used in the improvement of drug formulations.

Conclusions: Given the importance of micellar systems in drug development, these results also offer practical insights for selecting the right pharmaceutical additives to create novel dissolving media for various applications.

Keywords: Drug-membrane interaction, enoxaparin sodium, oseltamivir phosphate, spectrophotometry (UV-Vis), surfactant micelles

1. Introduction

Effectively developed drug delivery systems are crucial for the production of pharmaceutical products that are both safe and effective. The strategic use of surfactants, or surface-active agents, which are amphiphilic molecules with both hydrophilic (loving water) and lipophilic (loving oil) regions, is at the fundamental basis of many of these formulations. They serve a role in drug formulation for processes including solubilization, emulsification, wetting, and improving bioavailability because of their special structure, which lowers surface and interfacial tension. Surfactants are frequently used to stabilize suspensions and emulsions, change the kinetics of drug release, and dissolve poorly water-soluble drugs. However, in a formulation, a drug rarely exists in isolation; it interacts chemically and physically with all excipients, including surfactants (1-7).

The resulting drug-surfactant interaction is a complex phenomenon that dictates the final properties, stability, and therapeutic performance of the pharmaceutical product. These interactions can manifest in various ways, from the partitioning of drug molecules into surfactant micelles to the formation of specific drug-surfactant complexes at interfaces or in bulk solution. Understanding the nature and magnitude of these interactions is crucial for drug development, as even a slight change in the surfactant-to-drug ratio or environmental conditions (e.g., pH, temperature) can significantly alter the drug's thermodynamic activity, solubility, and ultimately, its absorption profile in the body. Therefore, for ensuring the quality, effectiveness, and reproducibility of the pharmaceutical dosage forms, analytical studies and accurate data publishing relating to drug-surfactant physicochemical behaviour are still crucial. Furthermore, the significance of surfactants in the pharmaceutical industry is increased by the restricted water solubility of many active pharmaceutical ingredients (APIs). This is particularly important since a lot of biological activity takes place in the hydrophobic core of membranes or on their surfaces. Surfactants are active in a wide range of environments, including body fluids, bacterial cell surfaces, and animal cell membranes. Furthermore, surfactant micelles can form compartments with distinct properties (hydrophobic and hydrophilic), enabling the selective solubilization and transport of various substances within these compartments. This property makes them suitable for controlled drug transport and release (1,2).

Studies of the physical characteristics of molecules binding to membranes are simplified by surfactant micelles' amphiphilic nature, which enables the use of simple models that mimic biological membrane systems. Accordingly, the physicochemical interaction of a drug and a micelle is considered an approach to modelling the drug's interactions with biological surfaces (1,7-10).

This helps in understanding the more complex issue associated with drug transport across the cell membrane. Estimating the amount of drugs incorporated into membranes can be made possible by determining their binding constants, which relate to the basic molecular interaction between the drug and biological tissues. Based on this information, this study investigated the interactions of nonionic Tween 20, anionic sodium dodecyl sulfate (SDS), and cationic cetyl trimethylammonium bromide (CTAB) surfactant micelles-which were selected as mimetic models with different molecular structures and hydrophobic characteristics-with ES and OP. The present study attempted to identify how ES and OP interact with the examples of mimetic systems include anionic, nonionic, and cationic micelles of surfactants.

OP is an antiviral medication that inhibits the the ability of influenza virus to replicate within body cells by preventing the virus from chemically binding to its host. OP is an antiviral inhibitor that effectively and selectively inhibits the influenza virus's neuraminidase. The IUPAC name of OP is (3R,4R,5S) ethyl-4-acetamido-5-amino-3-(1-ethylpropoxy) phosphate carboxylate of cyclohexene (11). ES is the sodium salt of a heparin possessing a low molecular mass. ES is an anticoagulant that reduces the formation of blood clots. It is used to treat or prevent deep vein thrombosis (DVT), a kind of blood clot that can cause pulmonary embolism. The IUPAC name of ES is 6-[5-acetamido-4,6-dihydroxy-2-(sulfooxymethyl) oxan-3-yl]oxy-3-[5-(6-carboxy-4,5-dihydroxy-3-sulfooxyoxan-2-yl)oxy-6-(hydroxymethyl)-3-(sulfoamino)-4-sulfooxyoxan-2-yl]oxy-4-hydroxy-5-sulfooxyoxane-2-carboxylic acid (12-14).

The interactions were evaluated quantitatively and qualitatively using spectrophotometric

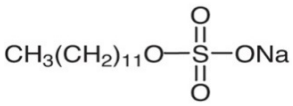
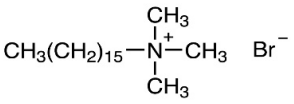
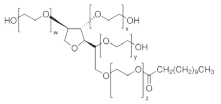
measurements. Based on absorbance and wavelength variations, the Benesi-Hildebrand Equation (15) was applied to determine the binding constants of both drugs to the micelles.

2. Methods

2.1. Materials

OP and ES were supplied by Atabay Kimya Company (Istanbul, Turkey). The chemical structures of surfactant micelles are given in Table 1.

Table 1. The molecular structures and CMC values of SDS, CTAB and Tween 20.

Surfactant	Molecular structure	CMC (mmol/L)
SDS $C_{12}H_{25}SO_4Na$		8.00
CTAB $C_{19}H_{42}BrN$		0.92
Tween 20 $C_{58}H_{113}O_{26}$		0.05

The SDS, CTAB, and Tween 20 used were obtained from Sigma Co. Doubly distilled, high-purity water was used to prepare the solutions. Fig 1 shows the molecular structures of OP and ES.

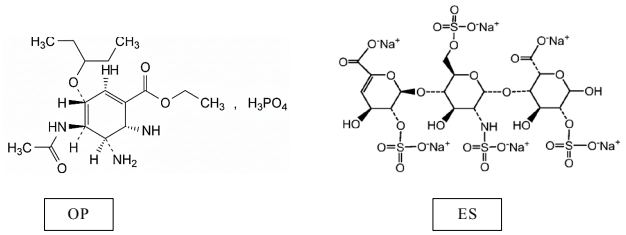


Figure 1. Molecular structures of OP and EP.

2.2. Method

The UV spectra of the fixed concentration of drugs (with and without surfactants) were recorded using a computer-connected Shimadzu UV-2100S double-beam UV-visible spectrophotometer. The instrument utilized a water-jacketed thermostatic cell holder and a matched pair of 1.0 cm path length cuvettes. All measurements were repeated at least three times, and the maximum wavelength (λ_{max}) showed excellent repeatability of ±0.1 nm. The spectrophotometric values reported here represent the mean of at least three measurements performed at 298 K. The relative Standard Deviation (SD) was consistently ≤ 1.1%.

2.3. Quantifying Binding Degree of Drugs using the Modified Benesi-Hildebrand Equation (KB)

The binding constant (K_B) was calculated to quantify the interaction (binding degree) of the drugs OP and ES with the surfactant micelles of SDS, CTAB and Tween 20. The K_B value that describes how a substance (likely a drug or molecule), interacts with micelles is calculated using the pseudophase model. The pseudophase model treats micelles and the surrounding water as distinct compartments or "pseudophases" where the drug molecule can reside. The equilibrium for drug binding to a micelle is represented as:

Drug (D) + Micelle (M) ⇆ D. M

An improved comparison of this interaction degree was obtained using a modified variation of the Benesi-Hildebrand equation (1), which is applicable at high surfactant concentrations (14).

$$\frac{1}{\Delta A} = \frac{1}{\Delta \varepsilon} + \frac{1}{K_B \left[C_M\right](\Delta \varepsilon)} \quad (1)$$

In this equation, $[C_M]$ is the concentrations of the drug and the micelle, where $[C_M]$ equals the total surfactant concentration minus the CMC (C_M= total surfactant concentration – CMC. The terms A and A_0 denote the drug absorbance in the presence and absence of surfactants ($\Delta A = A - A_0$), and $\Delta\epsilon$ is the difference in molar extinction coefficients ($\varepsilon_M - \varepsilon_0$) in the presence and absence of micelles. ε_M is the molar absorption coefficient of drugs when it is completely bound to the micelles. A linear plot of the of 1 /(ΔA) against $1/[C_M]$ confirms that this model and equation are applicable for determining K_B and ε_M. The linearity of the $1/\Delta A$ versus $1/[C_M]$ plot also confirmed that OP and ES binds to the micelles to form a 1:1 complex (8-10).

In this study, the CMC was determined by monitoring the change in the absorption spectrum upon the

interaction of OP and ES, which is indicative of micelle formation. Notably, the CMC values for ionic surfactants in the presence of fixed drug concentrations differed from their CMCs in pure water. However, no such change in the CMC was observed for the non-ionic surfactant, Tween 20.

3. Results

3.1. Micellar binding of OP

OP is a new ester prodrug and neuraminidase inhibitor used to treat influenza types A and B. Since oseltamivir's hydrophobic group causes poor oral absorption, the drug was developed as a phosphate salt to enable oral administration. OP is the salt form of oseltamivir, a small-molecule antiviral prodrug. Its ionization primarily depends on its basic functional group (the amine) and its acidic counterion (the phosphate). The active metabolite, oseltamivir carboxylate, also has a carboxylic acid group with an approximate pKa of 4.3, which significantly affects its properties. The pKa ≅ 7.9 is the most cited value for the amine group of the parent drug itself. In our experimental conditions, the medium pH is above 4.3, resulting in the existence of more stable OP cations. Depending on the concentration range, OP can be easily identified in pharmaceutical formulations at wavelengths of 208.5, 217, and 215 nm. OP, a cationic drug (chemical structure shown in Figure 1), exhibits the maximum absorption band at 209 nm. The absorption spectra for OP within the Lambert-Beer law's linear concentration range in an aqueous medium are plotted in Fig 2.

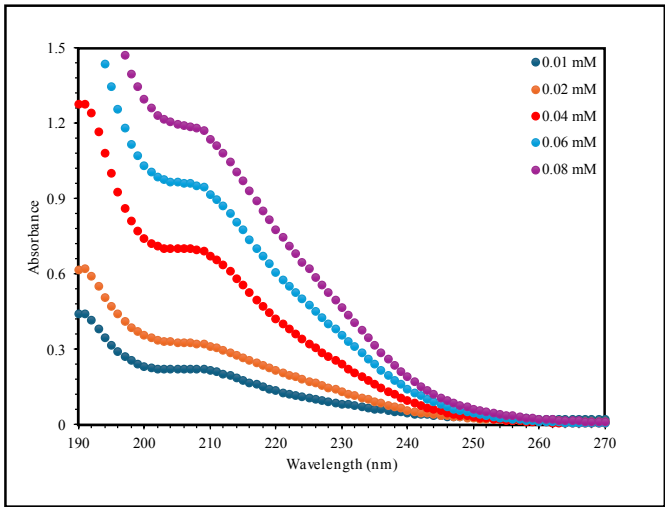


Figure 2. UV-Vis absorption spectra of OP in an aqueous medium at 298 K.

The change in absorbance of a fixed concentration (0.04 mM) of OP at 209 nm was monitored in the present study to analyse how OP interacts with varying concentrations of SDS, CTAB, and Tween 20 (both below and above their CMCs) at 298 K. Figures 3, 4, and 5 show the associated absorption spectra of OP in the presence and absence of CTAB, SDS, and Tween 20 micelles, respectively, to compare their impact on spectral behaviour of OP. In the presence of nonionic surfactant Tween 20, there was no spectral interaction observed with OP below the CMC. Conversely, when the surfactant concentration exceeded the CMC, all studied surfactants caused a progressive increase in absorbance. The only weak interaction observed was between OP and CTAB micelles, due to the electrostatic repulsion.

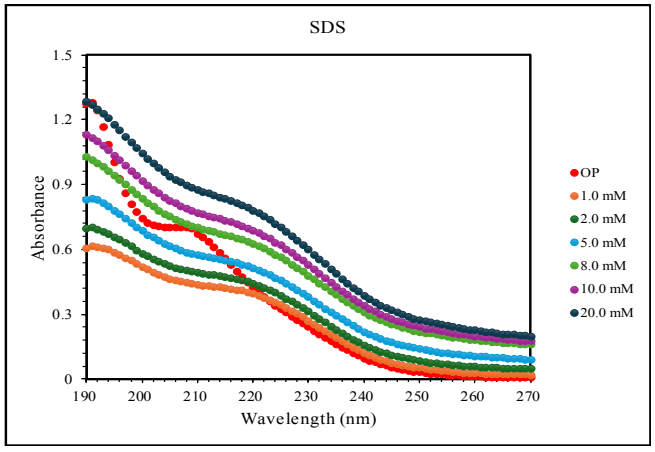


Figure 3. Absorption spectra of OP in various concentrations of SDS at 298 K.

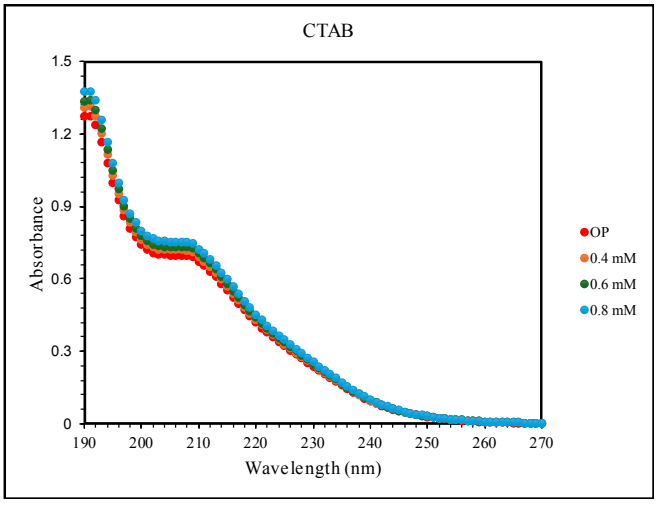


Figure 4. Absorption spectra of OP in various concentrations of CTAB at 298 K.

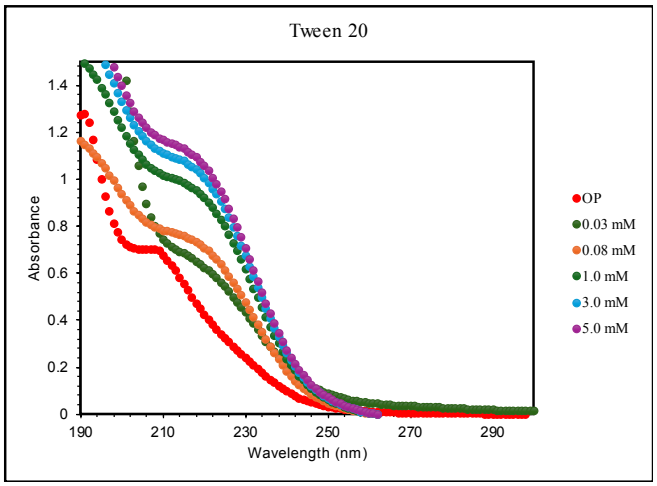


Figure 5. Absorption spectra of OP in various concentrations of Tween 20 at 298 K.

The variation of absorbance values of OP in the presence of Tween 20, CTAB, and SDS was illustrated in Figures 6 and 7. As shown in Fig 7, OP's absorbance dropped sharply as the SDS concentration increased up to 1.0 mM, although its spectral characteristics remained unchanged. This initial decrease in absorbance is evidence that a complex is forming between the OP and SDS molecules. The onset of micelle formation was detected by monitoring changes in the absorption spectrum of OP; this spectral shift served as the basis for determining the CMC in this study (9,16).

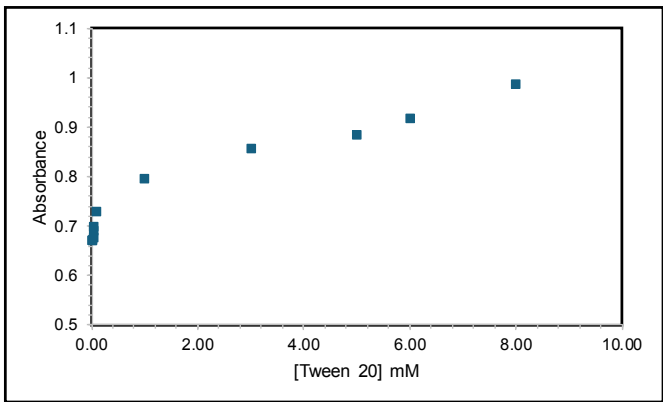


Figure 6. The absorbance change of 0.04 mM OP with the concentrations of Tween 20 at 298 K

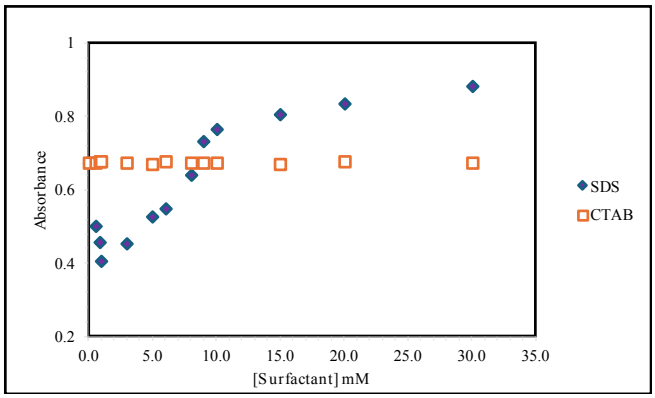


Figure 7. The absorbance changes of 0.04 mM OP with the concentrations of SDS and CTAB at 298 K.

The highly linear plots ($R^2 > 0.99$) of $1/\Delta A$ vs $1/C_M$ for all experiments confirmed that OP binds to the micelles in a 1:1 complex (Fig 8a and 8b). Binding constants (K_B) were calculated using equation (1) and are summarized in Table 2 along with their associated errors and correlation coefficients. Since OP and CTAB did not interact, it was not possible to calculate the binding constant. Overall, the spectral data showed that the affinity of the drug-micelle interaction varied among the surfactants, following the order: Tween 20 > SDS > CTAB.

Table 2. The CMC and calculated binding constants (KB) for the interaction of OP and ES with SDS, CTAB and Tween 20 micelles using UV-vis spectroscopy at 298 K.

	OP			ES		
Surfactant	**K_B (M^{-1})**	**CMC (mM)**	**CMC^{0*} (mM)**	**K_B (M^{-1})**	**CMC (mM)**	**CMC^{0*} (mM)**
Tween 20	1459.70	0.05	0.05	2034.71	0.05	0.05
SDS	89.08	1.0	8.0	-	-	8.0
CTAB	-	-	0.92	215.51	0.8	0.92

*Error limit in KB values is ± 5%. The correlation coefficients (R2) are 0.9988, 0.9987 for OP in the presence of SDS and Tween 20, respectively and 0.9938, 0.9958, for ES, in the presence of Tween 20 and CTAB, respectively. CMC0 values that are consistent with the literature obtained by conductometric measurement in water (17).

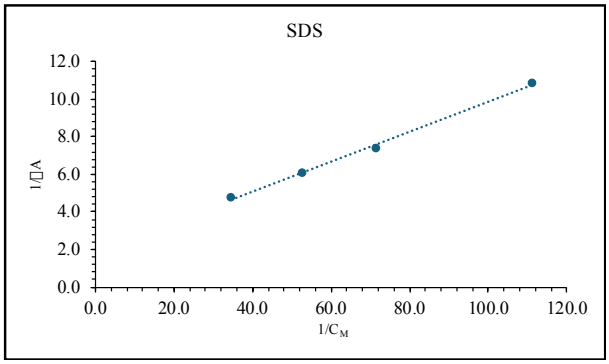


Figure 8a. Linearized Benesi-Hildebrand plot for the binding of OP (0.04 mM) to SDS micelles.

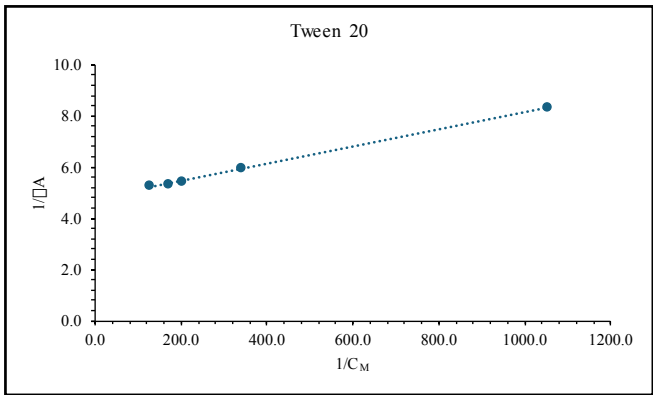


Figure 8b. Linearized Benesi-Hildebrand plot for the binding of OP (0.04 mM) to Tween 20 micelles.

3.2. Micellar binding of ES

ES, chemically a white crystalline powder, exhibits practical solubility in water and free solubility in 0.1 N HCl. Clinically, it is preferred over unfractionated heparin due to its enhanced bioavailability and extended half-life, which enable a reduction in the frequency of subcutaneous administration (12-14). Due to its polymeric structure as a complex mixture of sulfated polysaccharide chains, it does not have a single, defined pKa value, but rather a distribution of values corresponding to its numerous functional groups. Enoxaparin is a strong polyacid, meaning it is highly ionized under almost all physiological conditions. Since ES is a polyanionic molecule, its activity is dependent on its high negative charge density, derived from functional groups that have very low pKa values. These groups are extremely acidic and are essentially fully ionized (as R-OSO_3^-) in aqueous solution across the entire physiological pH range. In our experimental conditions, the medium pH is approximately 5-6, resulting in the existence of more stable ES anions. Within the proper concentration range of the Lambert-Beer Law, the anionic drug ES, the molecular structure of which was shown in Fig 1, was examined in an aqueous medium. The maximum absorption of ES occurs at 230 nm, and the ES spectra of absorption are shown in Fig 9.

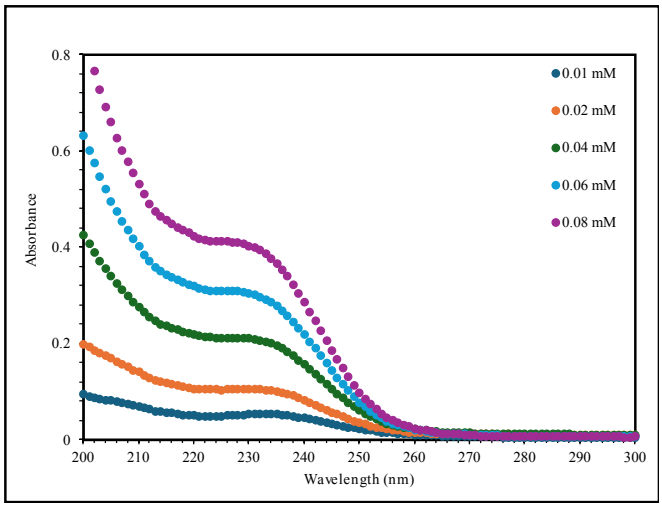


Figure 9. UV-Vis absorption spectra of ES in an aqueous medium at 298 K.

The interaction of a fixed concentration (0.04 mM) of the drug ES with SDS, CTAB, and Tween 20 at 298 K was studied by monitoring the change in the absorbance of ES at 230 **nm** with a wide range of surfactant concentrations both below and above their CMCs. The absorption spectra (Figures 10, 11, and 12) were used to compare the influence of surfactants on the spectral behaviour of ES. Below the CMC, no spectral interaction was observed between ES and the nonionic surfactant Tween 20. However, as the surfactant concentration exceeded the CMC, all three surfactants contributed to a gradual rise in ES absorbance, except for SDS, which showed a small increase. An exception was the weak interaction with SDS micelles, evidenced by a minimal change in absorbance and attributed to electrostatic repulsion. In contrast, a sharp initial drop in the absorbance of ES was seen with CTAB up to 0.8 **mM (**Fig 13), indicating the formation of an ES-CTAB complex without altering the spectral shape. The change in the absorption spectrum of ES was also used as the basis for determining the CMCs of the surfactants. The overall variation in ES's absorbance with each surfactant is detailed in Figures 13 and 14.

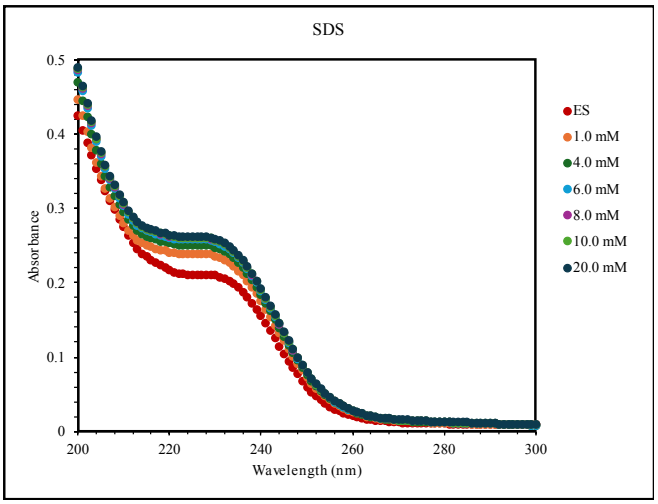


Figure 10. Absorption spectra of ES in various concentrations of SDS at 298 K.

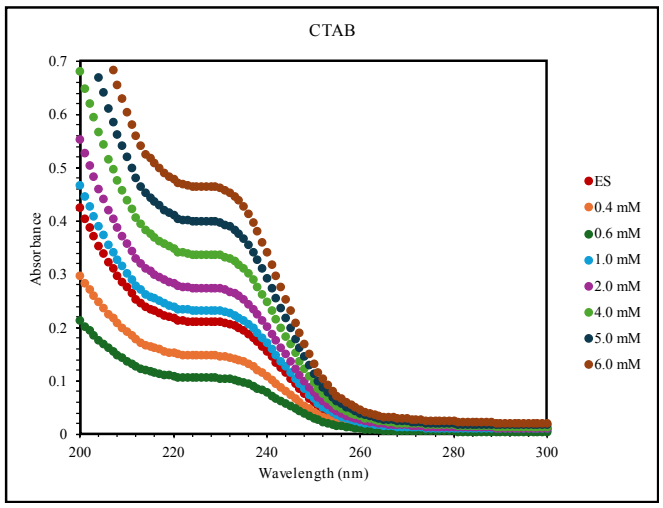


Figure 11. Absorption spectra of ES in various concentrations of CTAB at 298 K.

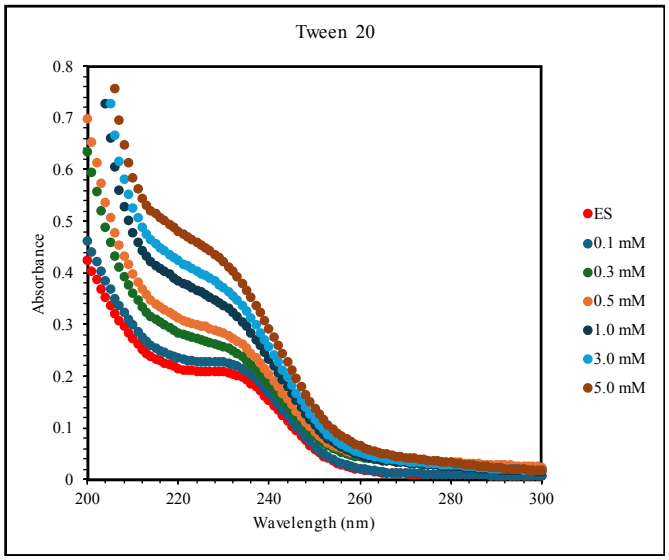


Figure 12. Absorption spectra of ES in various concentrations of Tween 20 at 298 K.

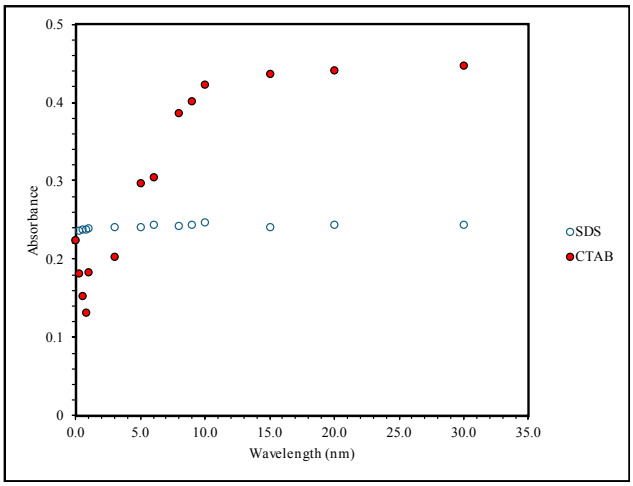


Figure 13. The absorbance changes of 0.04 mM ES with the concentrations of SDS and CTAB at 298 K.

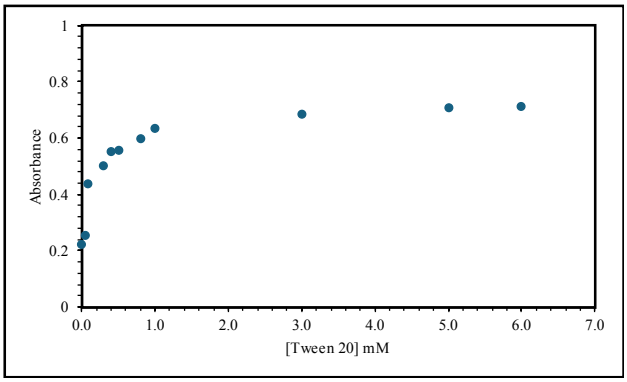


Figure 14. The absorbance changes of 0.04 mM ES with the concentrations of Tween 20 at 298 K.

Based on the highly linear plots ($R^2 > 0.999$) shown in Fig 15a and 15b, the binding of ES to the micelles occurs through a 1:1 complex. The calculated binding constants (K_B) (Table 2) showed that Tween 20 exhibited the strongest affinity, followed by CTAB. Conversely, the lack of interaction between ES and SDS due to the electrostatic repulsion meant its binding constant could not be determined, placing the overall affinity order at Tween 20 > CTAB > SDS.

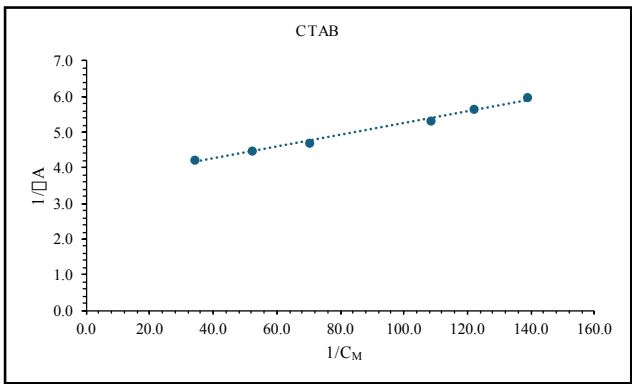


Figure 15a. Linearized Benesi-Hildebrand plot for the binding of ES (0.04 mM) to CTAB micelles.

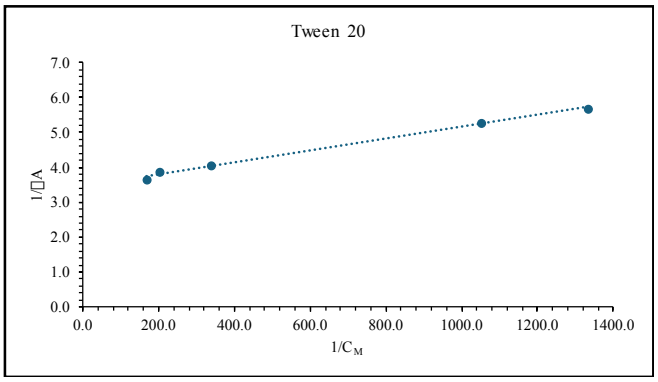


Figure 15b. Linearized Benesi-Hildebrand plot for the binding of ES (0.04 mM) to Tween 20 micelles.

4. Discussion

In the present study, to understand the *in vivo* behaviour of OP and ES, their interaction with different types of micelles was investigated. The results provide valuable information about the character and properties of the binding of the drugs to these structures, which serve as models for various biological components. Fig 16 illustrates the similarities between micelles and biological membranes, which are often referred to as mimetic systems. It can be clearly seen that there is a fundamental similarity between a spherical micelle and a flat lipid bilayer (biological membrane). Both systems self-assemble due to the hydrophobic effect, orienting their hydrophilic head groups toward the water and sequestering their hydrophobic tails away from the aqueous environment. This structural analogy allows micelles to function as mimetic systems for studying drug-membrane interactions and also acting as effective drug carriers.

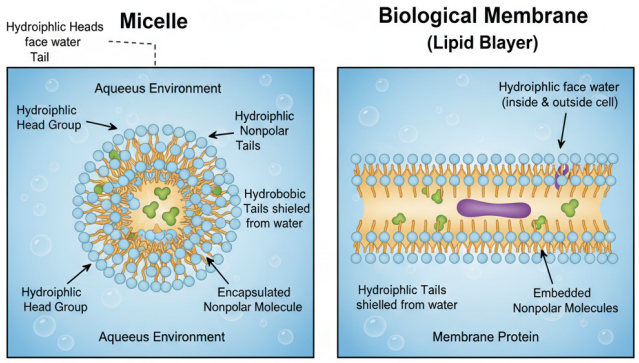


Figure 16. Micelles as mimetic drug delivery systems

In the present study, the observed spectral changes and increased absorbance above the CMC indicate that OP and ES successfully bind to the Tween 20, CTAB, and SDS micelles. Conversely, no binding was detected between the cationic OP and the cationic surfactant CTAB in the same way as anionic ES and anionic SDS. This lack of interaction may be attributed to electrostatic repulsion and inadequate hydrophobic attraction. It was hypothesized that the strongest interaction would occur between oppositely charged drugs and surfactants, given the presence of both electrostatic attraction and hydrophobic forces. This expectation aligns with the observed lack of interaction between ES-SDS and OP-CTAB micelles. Despite this, both ES and OP were found to bind more strongly to the nonionic micelles than to the ionic CTAB or SDS micelles. The superior binding strength is likely due to the nonionic micelles' aqueous polyoxyethylene oxide mantle, which provides a more accommodating environment for drugs compared to the anionic or cationic sites of ionic micelles. The observed variance in the binding constant is mainly a result of the distinctive micellar environments. Specifically, there is a correlation between the local polarity around the micelle and the ethylene oxide (EO) residues of nonionic surfactants, such that a decrease in polarity leads to an increase in the binding constant. The structure of a micelle is characterized by three zones: a nonpolar core of hydrocarbon tails, a Stern layer of head groups, and the diffuse Gouy–Chapman layer containing the majority of counterions. The binding location of the drug molecule is determined by the nature of both the drug and the micelle, resulting in localization within either the nonpolar core or the micelle–water interface. The fact that the maximum absorbance wavelengths of ES and OP don't significantly change upon the addition of surfactants indicates that both drugs are binding at the micelle-water interface (18-20). In this study, we also found that the presence of ES and OP consistently lowered the CMC for ionic surfactants. In case of SDS and CTAB micellar solutions, the addition of both ES and OP exhibited a neutral salt effect, effectively reducing the electrostatic repulsion among the charged head groups. Specifically, when added to ionic micelle solutions, ES and OP acted as a neutral salt, which reduced the electrostatic repulsion between the charged head groups

The chemical mechanisms driving the drug-micelle interactions are governed by a competition between electrostatic forces and hydrophobic partitioning, mediated by the structure of the micelle. In conclusion, the study of the binding affinity of ES and OP with structurally different surfactants (CTAB, SDS, and Tween 20) leads to the following points:

I. The Role of Electrostatic Repulsion

The most definitive mechanism observed is electrostatic repulsion, which prevents strong binding between identically charged components. The anionic drug (ES) exhibited no detectable binding with the anionic surfactant (SDS). The cationic drug (OP) exhibited no detectable binding with the cationic surfactant (CTAB). This lack of interaction confirms that simple electrostatic repulsion, combined with insufficient hydrophobic drive, governs when binding cannot occur.

II. The Superiority of Nonionic Binding

Despite the hypothesis that opposite charges should yield the strongest binding (due to electrostatic attraction), both the anionic (ES) and cationic (OP) drugs bound more strongly to the nonionic surfactant (Tween 20) than to the oppositely charged ionic micelles. The nonionic micelle's structure, specifically its aqueous polyoxyethylene oxide mantle, provides a highly accommodating environment for the drug molecules. This environment is chemically superior for partitioning compared to the more rigidly defined anionic or cationic surfaces of the ionic micelles. A decrease in the local polarity around the nonionic micelle's ethylene oxide (EO) residues directly correlates with an increase in the binding constant, indicating that the partitioning is highly dependent on localized polarity changes within the interface.

III. Binding Location

The exact location of drug incorporation is critical to the mechanism. The micelle structure is characterized by a nonpolar core, a Stern layer (head groups), and a diffuse Gouy–Chapman layer (counterions). The fact that the maximum absorbance wavelengths of ES and OP do not significantly change upon surfactant addition indicates that the drugs are not penetrating deep into the nonpolar core. Instead, both drugs are binding at the micelle-water interface, where they are influenced by both the hydrophobic tails and the surrounding aqueous environment.

5. Conclusion

This study employed spectrophotometric analysis to model the binding behaviour of antiviral (OP) and anticoagulant (ES) drugs with anionic (SDS), cationic (CTAB), and non-ionic (Tween 20) surfactant micelles, thereby simulating drug-membrane interactions at a molecular level. The overall strength of the drug-micelle interaction was found to be greater in nonionic micelles of Tween 20 than in ionic ones. This binding trend directly correlates with the hydrophobic character of the surfactants, i.e., the lower the CMC, the higher the hydrophobicity, confirming that hydrophobic interaction is crucial for the micellar binding, particularly as it enhances the binding constant (K_B) values of OP and ES in ionic and nonionic systems depending on the chemical structure of drugs. Given the importance of micellar systems in drug development, these results also offer practical insights for selecting the right pharmaceutical additives to create novel dissolving media for various applications. Furthermore, by modelling drug-membrane interactions, this study enhances understanding of the behaviour of ES and OP within biological organisms.

Conflicts of interest: The authors declare no conflicts of interest related to this work.

Funding: None

Ethics approval: Not applicable

Acknowledgment

The authors wish to express their gratitude to Atabay Kimya Sanayi ve Ticaret A.Ş. for generously providing the ES and OP drugs.

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Cite this article: Guclu S, Gokturk S. Micellar Pseudo-Models for Biological Membranes: Spectrophotometric Analysis of Micellar Binding of Antiviral (Oseltamivir Phosphate) and Anticoagulant (Enoxaparin Sodium). Pharmedicine J. 2026;3(1):27-36. DOI: 10.62482/pmj.39