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Adsolubilization studies on collagen fibres with 2-Naphtol as model substance

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Abstract

Adsolubilization has been defined as the incorporation to solid-water interfaces of molecules that do not adsorb spontaneously to such interfaces, but can be incorporated through an interaction with an adsorbing surfactant molecule. Surfactants are incorporated by a process of self-assembly onto the solid in the form of micelle-like aggregates, known as *hemimicelles* and *admicelles*. Admicelles can incorporate, also by a process of self-assembly, other molecules that are scarcely adsorbed or not adsorbed by themselves in the solid, in a way similar to water insoluble compounds that are solubilised in micelles. This particular and important solubilization phenomenon has been termed surface solubilization, surfactant mediated adsorption, coadsorption, and more recently *adsolubilization* to differentiate it from the classic *solubilization* based on micelles. The aim of this work was to study the adsolubilization phenomena on collagen fibres using 2-Naphtol as a model substance. The collagen fibres were previously treated with an anionic surfactant under mild acidic aqueous conditions to form the admicelles.

The following parameters have been studied: kinetics of the adsolubilization process; surfactant type: sodium dodecyl sulphate and sodium dodecylbenzene sulphonate; influence of pH; presence of electrolytes and polarity of the medium, and adsolubilization isotherms at 10, 20, 25, 30 and 35 °C. Thermodynamic parameters such as enthalpy change, entropy change and free energy change were calculated by applying the van't Hoff equation for the adsolubilization of 2-Naphtol.

Keywords: fibrous protein, hide powder collagen, anionic surfactant, 2-Naphtol, admicelle, adsolubilization, isotherm, hydrophobicity, thermodynamic parameters.

1. Introduction

The adsorption phenomenon of surface active agents (surfactants) at the solid/liquid interface has a broad significance from the academic and technological points of view [1]. Surfactants are incorporated by a process of self-assembly onto the solid in the form of micelle-like aggregates, known as *hemimicelles* and *admicelles* [2]. In this process, the main contribution to the standard free-energy change is entropic, which underlines the importance of hydrophobic interactions [3]. One important characteristic of the admicelle formation is that surfactant molecules interact with the solid above a critical admicelle concentration (CAC), which is considerably lower than the critical micelle concentration (CMC) exhibited by the surfactant in aqueous solution [4]. Admicelles can incorporate, also by a process of self-assembly, other molecules that are scarcely adsorbed or not adsorbed by themselves in the solid, in a way similar to water insoluble compounds that are solubilised in micelles. This particular and important solubilization phenomenon has been termed surface solubilization, surfactant mediated adsorption, coadsorption, and more recently *adsolubilization* [2,5] to differentiate it from the classic *solubilization* based on micelles [6]. According to Treiner [7], the adsolubilization phenomenon can be defined in a broader sense as the incorporation to solid-water interfaces of



molecules that do not spontaneously adsorb to such interfaces, but can be incorporated through some type of interaction with an adsorbing surfactant molecule. The adsolubilization phenomenon has opened

up a variety of applications in separation, reaction processes, surface engineering and nanotechnology [8].

To date, research on adsolubilization has been mainly performed with inorganic oxide substrates such as silica and alumina [4,8] and some studies have been carried out on synthetic polymers such as polyester for the development of an antimicrobial treatment on this material [9].

In the field of the natural fibers, the adsolubilization process has been practically limited to the research and application of the phenomenon on some cellulosic materials [10,11,12,13]. However, the investigation of the adsolubilization properties of fibrous proteins, such as collagen, has aroused little attention despite their academic and technological interest.

As regards adsolubilization on collagen, the work of Maldonado et al. [14] should be mentioned. These authors studied the hydrophobic effect in the interaction of ionic and non-ionic surfactants in a sequential treatment on hide powder collagen. They assumed a two step process: a *primary adsorption* of the anionic surfactant that takes place through an electrostatic bonding at the basic residues of the protein that act as centres of adsorption. This bonding is reinforced by the hydrophobic interaction between the apolar chain of the anionic surfactant and the hydrophobic residues of the protein. This primary adsorption would correspond to the creation of hemimicelles and admicelles that in a *secondary adsorption* allows the incorporation of different molecules through an exclusively hydrophobic interaction akin to the aforementioned adsolubilization process. The existence of this secondary adsorption was demonstrated by the adsorption of non-ionic surfactants on hide powder collagen previously treated with an anionic surfactant [14]. In a recent study Maldonado et al. [15] demonstrated that the adsolubilization phenomenon may take place on fibrous collagen with organic molecules with different structures and molecular weights.

The aim of this work was to study the adsolubilization phenomena using collagen fibres treated with an anionic surfactant, and 2-Naphtol as a model molecule. The following parameters have been studied: kinetics of the adsolubilization process; surfactant type: sodium dodecyl sulphate and sodium dodecylbenzene sulphonate; influence of pH; presence of electrolytes and polarity of the medium; adsolubilization isotherms at 10, 20, 25, 30 and 35 °C. Thermodynamic parameters such as enthalpy change, entropy change and free energy change were calculated by applying the van't Hoff equation for the adsolubilization of 2NP. This work contributes to new fundamental understanding for the incorporation to collagen-water interfaces of organic compounds that do not spontaneously adsorb to such interfaces, but can be incorporated through the interaction with an adsorbing surfactant molecule.

2. Materials and methods

2.1. Hide powder collagen.

The adsorption experiments were performed on hide powder collagen (hp) supplied by the British Leather Confederation (BLC, Northampton, UK). The hide powder, obtained from bovine hide, met the specifications of the Official Hide Powder of the Society of Leather Technologists and Chemists (SLTC). The batch supplied had the following characteristics: ash content 0.36%, pH 6.5, moisture 12.5% and point of zero charge of pH 6.6-6.8. Hide powder collagen is a highly porous and hydrophilic material with a specific surface area of 475.2 m²/g, which was determined in a Q5000SA Dynamic Vapour Sorption (DVS) Analyzer from TA Instruments by applying the GAB model [16].



2.2 Chemicals.

The anionic surfactants used were sodium dodecylbenzene sulphonate (SDBS) (Aldrich-Sigma, ref. 28995-7) and sodium dodecyl sulphate (SDS) (Sigma-Aldrich ref. L4509) and 2-Naphtol (2NP) (Aldrich, ref. 185507) as a model substance for the adsolubilization process. Fig. 1 shows the chemical structure of the chemicals used. All chemicals were commercial products of analytical grade and were used as received.

2.3. Experimental methods

Adsorption and adsolubilization experiments were carried out in a batch mode consisting in a heterogeneous system with the surfactants and 2NP in aqueous solution and the hide powder (hp) in suspension. Two alternative preparations were used depending on the experiment: a) the direct treatment or *one step method* where the hp was treated with the surfactant and 2NP in a sole batch, and b) the so-called *two steps method* where the hp was treated with the surfactant in a first batch followed by a drying step and carrying out the adsolubilization of 2NP in a new batch. According to our experience, both methods give similar adsolubilization results. The two steps method allows to work with 2NP concentrations a little bit higher and to carry out the study of the adsolubilization process without possible interferences.

One step method

The hide powder (hp), the surfactant SDS and 2NP were used according to the following rate:

$$1 \text{ (g hp)} : 0.5 \text{ (mmol SDS)} : x \text{ mmol 2NP} : 400 \text{ (g water)}$$

Unless otherwise stated the pH was adjusted to 2.9-3.0 with HCl 0.1 N and shaken for 3 hours at 25 ± 0.2 °C in a water bath. The analysis of SDS and 2NP was carried out as stated in the two step method.

In these conditions, the adsorption of SDS on hide powder was equal to or higher than 95%. The initial concentration of SDS is lower than its CMC.

Some experiments were carried out without surfactant to evaluate the adsorption of 2NP on hide powder. As observed in figure 3 there was some adsorption of 2NP in the absence of surfactant:

Two steps method

The hide powder (hp) and the respective surfactant were prepared according to the following rate:

$$1 \text{ (g hp)} : 0.4 \text{ SDS or } 0.5 \text{ SDBS (mmol surfactant)} : 50 \text{ (g water)}$$

and shaken for 1 hour at 25 ± 0.2 °C in a water bath. After adjusting the pH with 0.1 N HCl, agitation followed for 2 hours. The material identified as SDS-hp and SDBS-hp, respectively, was filtered and subsequently dried by lyophilisation and stored in airtight container until it was used. The amount of SDBS in the filtrate was determined spectrophotometrically in a Shimadzu apparatus, model UV-240 using the peak at 223.5 nm as that of maximum absorbance whereas the amount of SDS was determined by measuring the methylene blue index [17].

At the following step, aliquots of the surfactant treated hide powder were shaken for 3 hours with 2NP at 25 ± 0.2 °C and pH 2.9, according to the following rate:

$$1 \text{ (g surfactant-hp)} : x \text{ (mmol 2NP)} : 400 \text{ (g water)}$$

At the end of this step, an aliquot of the liquid was taken and the 2NP was determined spectrophotometrically in a Shimadzu apparatus, model UV-240 using the maximum absorbance peak of 327.5nm.



The quantity of 2NP adsorbed or adsolubilized (Q_e) was calculated from the difference between the initial and final concentration according to the following equation:

$$Q_e = (C_0 - C_e)V/W \quad (1)$$

where Q_e is the mass of solute sorbed per unit mass of sorbent in mmol/g hp, C_0 is the initial concentration of solute, C_e is the concentration of the solute at equilibrium, both in mmol/L, V is the volume of the solution in liters and W is the mass of hide powder in grams

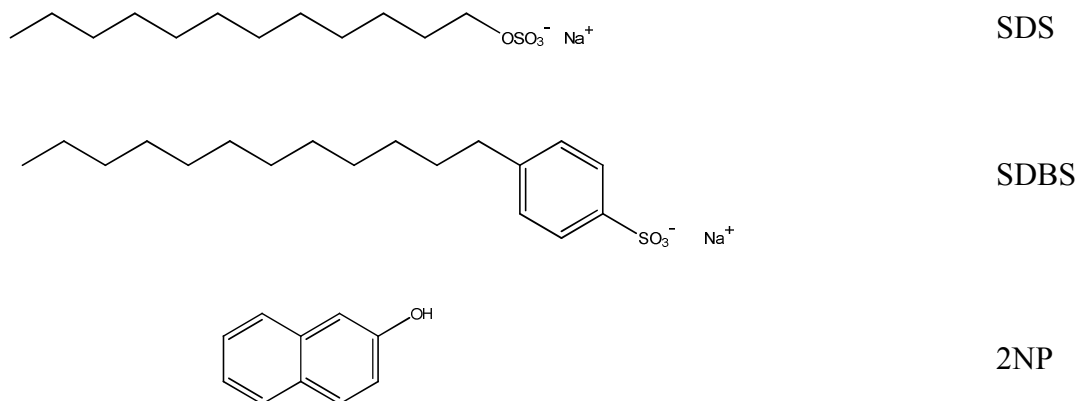


Figure 1 Chemical structure of the studied molecules.

3. Results and discussion

3.1 Kinetic study

The kinetic study of adsolubilization of 2 NP was carried out with SDS-hp substrate in a two steps method. Figure 2 shows that the sorption of 2NP rapidly increased with increasing sorption time and that equilibrium was practically reached after two hours treatment. A treatment time of 3 hours was established in this study to ensure that equilibrium was reached.

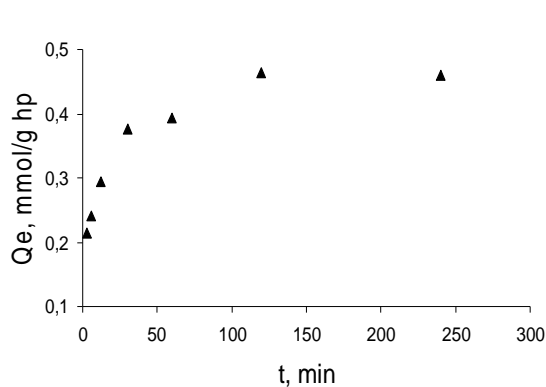


Fig. 2 Adsolubilization kinetic of 2NP onto SDS-hp at pH 2.9, C_0 3.9 mmol/L and 25°C.

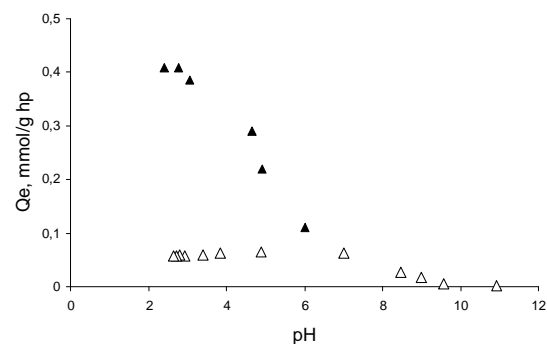


Fig.3 Adsolubilization of 2 NP onto SDS-hp as a function of pH (▲). Adsorption of 2 NP in the absence of surfactant (△)



3.2 Effect of pH

Figure 3 shows the adsolubilization values of 2NP as a function of pH in a one step treatment with an offer of 0.5 mmol SDS/g hp and an initial concentration (C_0) of 3.5 mmol/L of 2NP. A considerable increase of adsolubilization of 2NP was observed when the acidity of the aqueous medium also increased. The maximum 2NP adsolubilization took place at a pH value of 2-3, i.e., when the maximum adsorption of the anionic surfactant occurred. Figure 3 also shows the adsorption of 2NP in the absence of surfactant.

This adsorption was practically constant at acid pH, but it was sensibly reduced at alkaline pH due to ionization and consequent increase in solubility of 2NP in water.

3.3 Adsorption and adsolubilization isotherms.

The adsorption of a substance (sorbate) from one phase to the surface of another (sorbent) in a specific system leads to a thermodynamically defined distribution of that substance between the phases when the system reaches equilibrium, that is, when no further net adsorption occurs. The common manner to depict this distribution is termed *adsorption isotherm* that shows the amount of substance adsorbed per unit weight of sorbent, Q_e (mmol/g sorbent), as a function of the residual equilibrium concentration of sorbate in the solution phase, C_e (mmol/L), at constant temperature. When the adsorption occurs through an adsolubilization process we term it adsolubilization isotherm.

Figure 4 shows the adsorption isotherm of 2NP onto hide powder collagen (hp) and the adsolubilization isotherms of 2NP onto SDS-hp y SDBS-hp in a two step method. As observed, the relationship between the equilibrium concentration of 2NP in the solid phase (Q_e) and in the liquid phase (C_e) presented linearity and could be defined by the simple distribution coefficient. In this case, the adsorption data were fitted to a linear adsorption isotherm as described in Eq. (2).

$$Q_e = K_d C_e \quad (2)$$

where K_d is the distribution coefficient or partition coefficient.

The adsolubilization capacity of 2NP was similar with both surfactants taking into account that the amount of surfactant in hp was higher in the hp treated with SDS (offer 0.5 mmol/g hp) than with SDBS (offer 0.4 mmol/g hp).

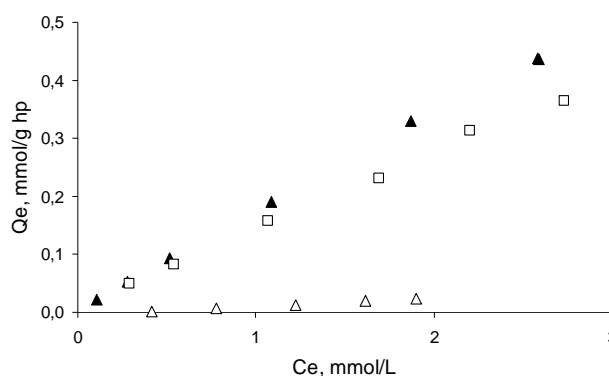


Fig. 4 Adsolubilization isotherms of 2NP on SDS-hp (▲) and SDBS-hp (□), and adsorption isotherm of 2NP (△), at pH 2.9 and 25°C.

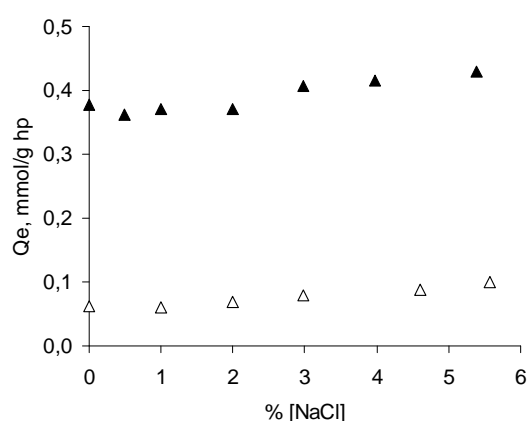


Fig. 5. Adsolubilization of 2NP on SDS-hp (▲) and adsorption of 2NP on hp (△) as a function of the ionic strength at C_0 3.0 mmol/L, pH 3.0 and 25°C.



3.4 Influence of the ionic strength

The values of the adsolubilization of 2NP onto SDS-hp as a function of NaCl concentration in a one step method are plotted in figure 5. A trend towards a slight increase in the adsolubilization of 2NP for increasing NaCl concentration in the treatment solution was observed. A similar behaviour took place in the adsorption of 2NP onto hp in the absence of surfactant.

3.5 Influence of the medium polarity

Water is the medium where hydrophobic interaction is favoured, any other medium reduces or even impedes that this interaction takes place [3]. The adsolubilization of 2NP onto SDS-hp as a function of ethanol concentration in the one step method is plotted in figure 6. As observed, a steep drop in the 2NP adsolubilization with increasing ethanol concentration took place due to the hydrophobic nature of the bond.

The adsorption of the anionic surfactant SDBS is also plotted in figure 6 for comparison purposes. In this case, the adsorption values were relatively less affected by the presence of ethanol. This is due to that the interaction between SDBS and protein is also ionic and this bonding type is favoured by the presence of solvents that are less polar than water. The drop in the adsorption values was not so marked as in the case of 2NP.

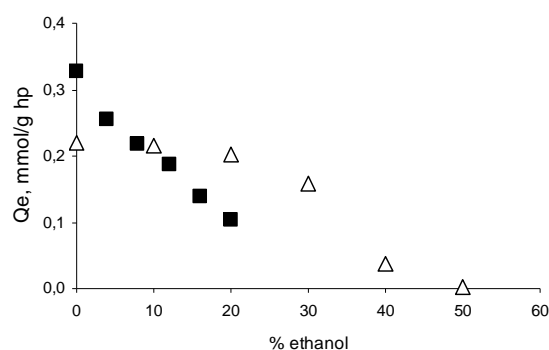


Fig. 6. Adsolubilization of 2NP onto SDS-hp as function of the ethanol concentration, Co 2.6 mmol/L, pH 2.9 and 25 °C (■), and adsorption of SDBS onto hp as reference (△)

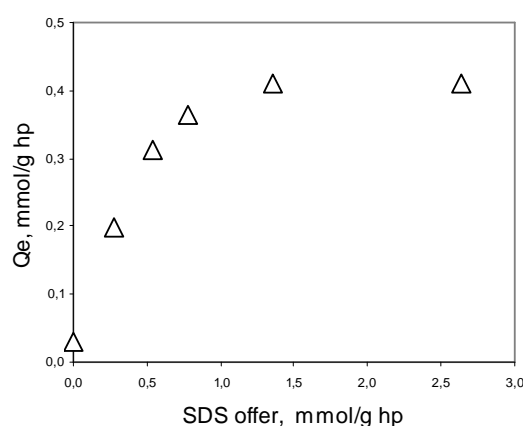


Fig. 7. Adsolubilization of 2NP: as a function on the initial SDS offer (△), Co 2.8 mmol/L, pH 2.6 and 25°C.

3.6 Influence of the initial SDS offer

Figure 7 shows the adsolubilization of 2NP as a function of the initial offer of SDS following the one step method. The initial offer of SDS varied from 0 to 2.6 mmol/g hp. The maximum adsolubilization of 2NP was achieved from an initial SDS offer of 1.5 mmol/g hp.

3.7 Influence of temperature. Thermodynamic parameters.

Figure 8 shows the adsolubilization isotherms of 2NP onto SDBS-hp at temperatures of 10, 20, 25, 30 and 35 °C. The experiments were carried out in accordance with the two step method at a pH of 2.9. The isotherms were fitted to a linear model and determination coefficient values (R^2) higher than 0,99 were obtained. The thermodynamic distribution coefficient (K_d) showed that the adsolubilization of 2NP decreased with increasing temperature (Table 1).



Thermodynamic parameters such as free energy change (ΔG°), enthalpy change (ΔH°), and entropy change (ΔS°) for the adsolubilization of 2NP were calculated using the Eq. (3) and the van't Hoff Eq. (4), which relates the thermodynamic distribution coefficient (K_d), with temperature.

$$\Delta G^\circ = -RT \ln K_d \quad (3)$$

$$\ln K_d = -\Delta H^\circ/RT + \Delta S^\circ/R \quad (4)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (5)$$

where R is the universal gas constant (8,31 J/(molK)) and T is the temperature expressed in kelvins.

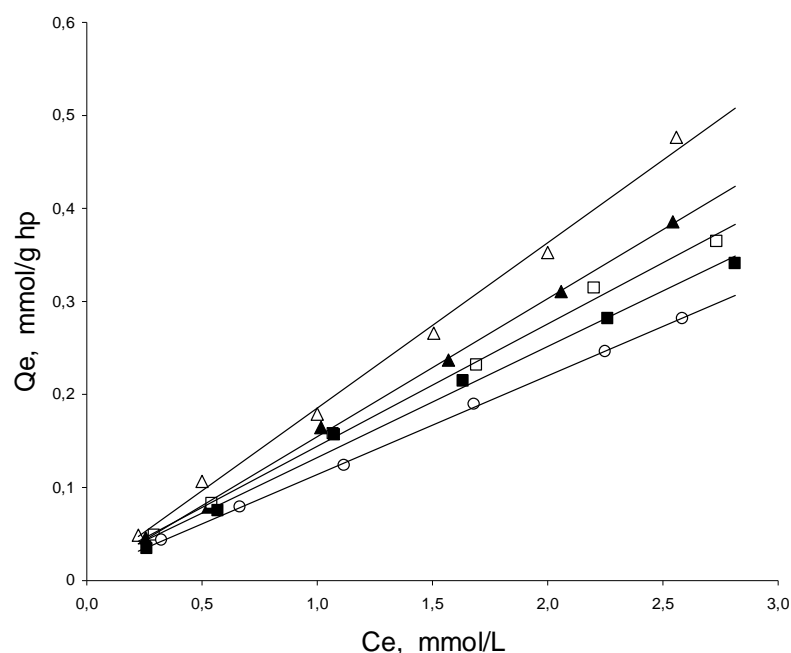


Fig. 8. Adsolubilization isotherms of 2NP onto SDBS-hp at: 10 (Δ), 20 (\blacktriangle), 25 (\square), 30 (\blacksquare) and 35 °C (O).

According to the van't Hoff equation (4) a plot of $\ln K_d$ as a function of $1/T$ yielded a straight line (figure 9). The values of ΔH° and ΔS° obtained from the slope and intercept of the plot are summarized in Table 2. The negative value of ΔH° (-15.03 kJ/mol) indicates the exothermic nature of the adsolubilization process. The negative value of ΔS° (-9.86 J/molK) might be associated with the adsolubilization of 2NP to the sorbent, which resulted in a decreased in freedom degree of the system during the process. The negative value of the free energy change (ΔG°) indicates the spontaneity of the adsolubilization process. In order to check the validity of the results, the values of ΔG° were also calculated by Eq. 5. [18]. These values are also shown in Table 2.

Table 1 Adsolubilization isotherms of 2NP at different temperatures			
T (K)	Linear sorption isotherm	K_d (L/kg)	R^2
283.2	$Q_e = 0.1777C_e + 0.0078$	177.7	0.9960
293.2	$Q_e = 0.1484C_e + 0.0066$	148.4	0.9989
298.2	$Q_e = 0.1315C_e + 0.0131$	131.5	0.9970



303.2	$Q_e = 0.1195C_e + 0.0125$	119.5	0.9929
308.2	$Q_e = 0.1056C_e + 0.0083$	105.6	0.9995

Table 2 Values of thermodynamic parameters for the adsolubilization of 2NP onto SDBS-hp

T(K)	ΔH° (kJ/mol)	ΔS° (J/molK)	$T\Delta S^\circ$ kJ/mol	$\ln K_d$	ΔG° (1) (kJ/mol)	ΔG° (2) (kJ/mol)
283.2	-15.03	-9.86	-2.79	5.181	-12.20	-12.24
293.2			-2.89	5.000	-12.19	-12.14
298.2			-2.94	4.879	-12.10	-12.09
303.2			-2.99	4.783	-12.06	-12.04
308.2			-3.04	4.660	-11.94	-11.99

(1) $\Delta G^\circ = -RT \ln K_d$; (2) $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$

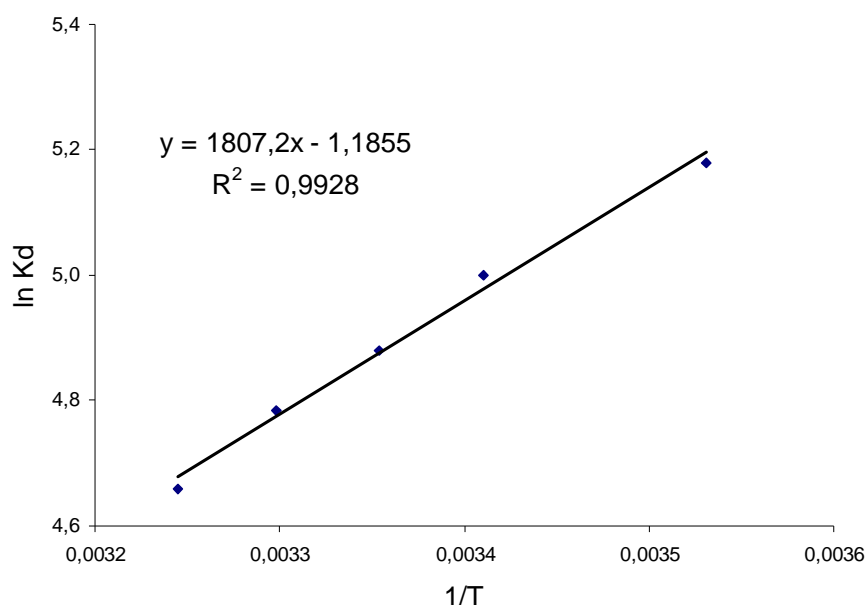


Fig. 9. Van't Hoff plot for the adsolubilization of 2NP onto SDBS-hp

4. Conclusions

The adsorption of an anionic surfactant onto hide powder collagen in an aqueous acidic medium allows the aggregation of other organic molecules that in the absence of the surfactant are scarcely or not adsorbed in the protein, in a process called adsolubilization. The experiments carried out in this work have shown that the adsorption of the anionic surfactants SDS and SDBS, respectively, on hide powder collagen allows the adsolubilization of 2-Naphtol that is scarcely sorbed by itself in the protein. The experiments carried out in presence of ethanol confirm the hydrophobic nature of the adsolubilization process. The sorption isotherms revealed that in the case of 2NP the adsolubilization phenomenon can be viewed as a partition of this compound between the core of the adsorbed surfactant and the aqueous phase, defined by a constant distribution coefficient or partition coefficient, K_d .

The negative value of ΔH° (-15.03 kJ/mol) indicated the exothermic nature of the adsolubilization process. The negative value of the entropy change ($\Delta S^\circ = -9.86$ J/molK) might be associated with the



adsolubilization of 2NP to the sorbent, which resulted in a diminution in freedom degree of the system during the process. The negative values of the free energy change (ΔG°) revealed the spontaneity of the adsolubilization process.

This research opens up new ways of incorporating organic molecules of very different characteristics onto collagen-water interfaces through the interaction with an anionic surfactant. This could be useful both in leather manufacturing and to achieve a better exploitation of the residues generated by the industry such as shavings, which could be used in the remediation of organic contaminants from waste waters.

However, further research is warranted to gain a greater understanding of the mechanism of the adsolubilization process.

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