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Interaction of piroxicam with micelles: Effect of hydrophobic chain length on structural switchover

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Abstract

Molecules, whose pK_a values can be easily fine-tuned by their microenvironment, are expected to be profoundly affected by the heterogeneous environments of membranes. Membrane parameters can have a strong effect in choosing a particular structural form of a molecule for incorporation/interaction. A case study has been presented for piroxicam, a non-steroidal anti-inflammatory drug of oxicam group, whose targets are cyclooxygenases, which are membrane active proteins. The structural dynamism of piroxicam is reflected in the ease with which it can switchover or convert from one prototropic form to the other guided by its environment. In this work we have studied the effect of varying hydrophobic chain length and surface charges in fine-tuning the interaction of piroxicam with micelles. Interaction of piroxicam with three types of micelles with identical negatively charged head groups and varying tail lengths viz., sodium dodecyl sulfate (S12S), sodium decyl sulfate (S10S) and sodium octyl sulfate (S8S) shows that there is a shift in the apparent pK_a in the direction that favors the switchover or conversion from the anionic form to the global neutral form. The binding constants of piroxicam with three micelles show a linear dependence on chain length. Interaction was also studied with micelles having oppositely charged head groups and different chain lengths viz., dodecyl *N*,*N*,*N*-trimethyl ammonium bromide (DTAB) and cetyl *N*,*N*,*N*-trimethyl ammonium bromide (CTAB). For micelles having identical chain lengths but oppositely charged head groups viz., S12S and DTAB, pK_a shifts in two opposite directions compared to that in the absence of any surfactant. This is expected when electrostatic force is the only driving force. This case study demonstrates the effect of hydrophobic chain length and surface charges in fine-tuning the equilibrium between different structural forms of piroxicam. Our results also imply that for structurally dynamic drugs like piroxicam the nature of the biomembranes, characterized by different membrane parameters, should play a crucial role in choosing a particular structural form of the drug that will be finally presented to their targets.

Keywords: Piroxicam; Structural dynamism; Membrane parameter; Micellar chain length; Change in pK_a; Optical spectroscopy

1. Introduction

Interaction of molecules, whose pK_a values can be easily altered, is fine-tuned by the parameters of their micro-environment [1–5]. Modulation of pK_a values of a chromophore by sol–gel entrapment has been already demonstrated. The change in the microenvironment achieved in the entrapped sol–gel matrix has been implicated to be the reason behind the modulation of pK_a [2]. Piroxicam [4-hydroxy-2-methyl-*N*-(pyridin-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide], a drug belonging to the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) is a structurally dynamic molecule whose pK_a can be easily altered. Piroxicam is capable of existing in several prototropic forms like the neutral, zwitterionic and anionic forms in solution. The structural dynamism of piroxicam is reflected in the ease with which it can switchover or convert from one prototropic form to the other [6–11]. The neutral and zwitterionic forms are spectroscopically indistinguishable and are together termed as 'global neutral'. The anionic form has spectral properties that are quite distinct from that of the global neutral form and hence spectroscopic techniques can be used to detect them in solution. Target proteins of piroxicam viz., the cyclooxygenases are membrane active proteins [12]. Before

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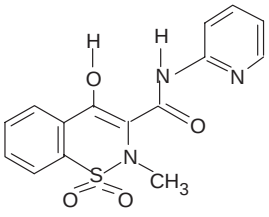
approaching the target proteins piroxicam needs to interact with the membranes. It is therefore interesting to see how different membrane parameters like surface charges, hydrophobic effect, length of the fatty acid chain, etc. would affect the structures of piroxicam. Micelles with their hydrophilic surface and hydrophobic interior serve as simple membrane mimetic system that allows a controlled study of the effect of different membrane parameters on the structural dynamism of ligand molecules. In our earlier studies [7] with oxicam NSAIDs we have shown how surface charge of the micelles guides which prototropic forms of the drugs would be incorporated in the micelles. In this type of incorporation not only a particular prototropic form of piroxicam will be selectively incorporated, but also a switchover of other prototropic forms of piroxicam occur to the one selected for incorporation. The equilibrium of this switchover has been found to be extremely sensitive to surface charge such that, even small modification of the surface charge can fine-tune this equilibrium [8]. The switchover between different prototropic forms could be explained in terms of shift in apparent pK_a values for differently charged micelles. What could not be explained was why for two oppositely charged micelles the shift in pK_a values occurred in the same direction compared to that in absence of micelles [7]. This also indicated that factors other than surface charges, like hydrophobic effect/varying chain length might fine-tune the switchover equilibrium.

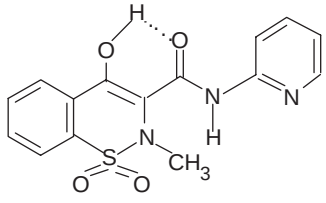
In this work, we have tried to parse the effect of varying hydrophobic chain length from that of electrostatic effect of micellar surface charges on the switchover equilibrium. Three types of micelles with identical negatively charged head groups and varying tail length viz., sodium dodecyl sulfate (S12S), sodium decyl sulfate (S10S) and sodium octyl sulfate (S8S), as well as two types of micelles with identical positively charged head group but different chain length viz., cetyl *N*,*N*,*N*-trimethyl ammonium bromide (CTAB) and dodecyl *N*,*N*,*N*-trimethyl ammonium bromide (DTAB) were chosen for this study. Binding of piroxicam with sodium alkyl series (SNS) was studied using UV–VIS absorption and fluorescence spectroscopy. Our study clearly demonstrates the influence of chain length of micelles (that modulate the hydrophobic interior) on the structural switchover of piroxicam leaving apart the effect of surface charge.

2. Materials and methods

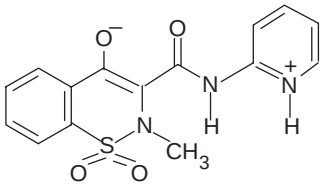
Piroxicam was purchased from Sigma Chemicals (USA) and was used without further purification. Dodecyl *N*,*N*,*N*-trimethyl ammonium bromide (DTAB), cetyl *N*,*N*,*N*-trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (S12S) were purchased from Merck, Germany. Sodium octyl sulfate (S8S) and sodium decyl sulfate (S10S) were purchased from Fluka. Water was glass distilled thrice before use. Stock solutions of piroxicam of concentration 0.5 mM were prepared in ethanol (Merck, Germany) and exact concentration was adjusted by triple distilled water. Each aqueous solution contains a maximum of 6% (v/v) of ethanol. We have changed the pH of the working solutions by adding dil. HCl and/or dil. NaOH to them. The volume of acid (HCl) added to the working solutions is exactly equal to the volume of acid that is



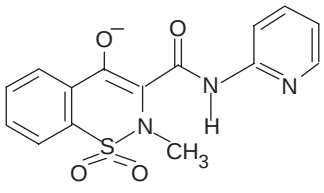
Open conformer of piroxicam



Closed conformer of piroxicam



Zwitterionic form of piroxicam



Anionic form of piroxicam

Fig. 1. Structure of different prototropic forms of piroxicam.

needed to acidify a volume of water equal to the working solution to attain that particular pH. Similar procedure was carried out to make the solutions alkaline by adding NaOH. It should be mentioned that pH of the solution was also checked in the presence of highest concentration of micelles. However, the changes observed were well within the experimental error limit. Solution at pH 5.5 indicates that no acid or alkali was added to the aqueous solutions. Samples were checked for photochemical changes during spectral scan time and no change was found.

We kept the surfactant concentration well above the CMC value during the determination of pK_a to ensure the presence of adequate number of micelles in the solution, so as to keep micellar effect uniform at every pH. The concentration of piroxicam was kept constant at 30 μM for all samples.

Absorption spectra were recorded with Thermo Spectronic spectrophotometer model UNICAM UV500. Baseline correction was done with water before recording each set of data. Fluorescence measurements were performed using Hitachi spectrofluorimeter model 4010. All emission spectra were corrected for instrument response at each wavelength. A 2×10-mm path length quartz cell was used for all fluorescence measurements to avoid any blue edge distortion of the spectrum due to inner filter effect [13]. All measurements were done with freshly prepared samples and at a constant temperature of 298 K.

3. Results and discussion

Piroxicam can exist in different structural forms (Fig. 1). The neutral and the zwitterionic forms are spectroscopically indistinguishable and henceforth will be together termed as global neutral form. This molecule is structurally dynamic and can easily switchover or convert from global neutral to anionic form and vice versa, depending on its immediate environment [7,8,11].

The equilibrium between the global neutral and the anionic form of piroxicam is affected by the environment in solution and is represented by

$$N \rightleftharpoons A$$

where 'N' represents the global neutral form and 'A' represents the anionic form of piroxicam.

Fig. 2 shows the absorption spectrum of piroxicam with increase in the concentration of S12S (Fig. 2a), S10S (Fig. 2b) and S8S (Fig. 2c) at pH 2.42, 2.05 and 1.75, respectively. As will be shown later the apparent pK_a values in the presence of S8S, S10S and S12S are different. For this reason, the pH of (a)–(c) were adjusted at different values so as to be able to demonstrate the inter conversion between only two structural forms. It should be mentioned that this data has not been used for binding calculation. From Fig. 2 it is evident that increase in concentration of an SNS surfactant (N=number of $-CH_2$ group in the hydrophobic tail) leads to an increase in the peak of the neutral form (335 nm) with the concomitant decrease in the peak of the anionic form (363 nm). A clear isosbestic point around 352 nm is seen in all the three figures (Fig. 2a–c). The presence of this isosbestic point clearly points out that there exists an equilibrium between only two species, viz., global neutral and the anion. Increase in SNS concentration leads to a decrease in anionic form with a concomitant increase of the

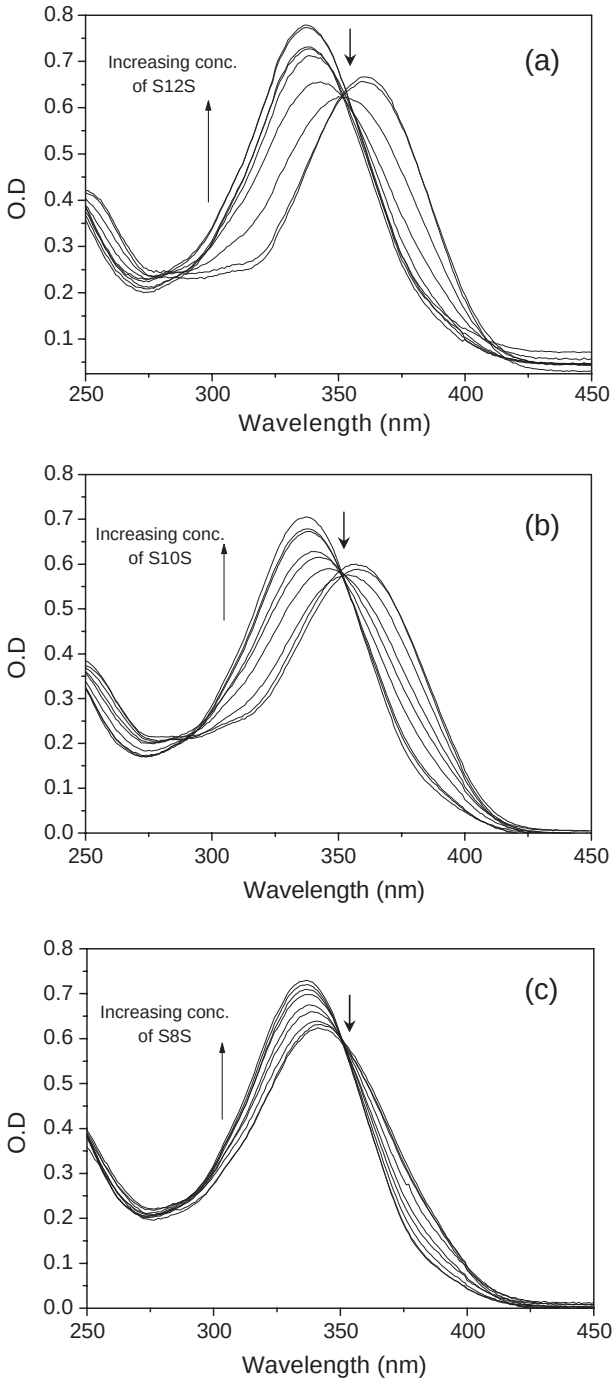


Fig. 2. Absorption spectra of piroxicam with increasing concentration of (a) S12S from concentration 0 mM to 12 mM at pH 2.42, (b) S10S from concentration 0 mM to 45 mM at pH 2.05, and (c) S8S from concentration 0 mM to 160 mM at pH 1.75.

neutral form indicating that it is the global neutral form that is being incorporated in the micelles. The binding of the global neutral form of piroxicam with the SNS surfactants was studied. Fig. 3a–c shows the increase in fluorescence intensity with an increase in S12S, S10S and S8S concentration, respectively, keeping the concentration at 30 μM in all cases. The excitation was kept at 335 nm to selectively excite the neutral form and the pH of the solution was adjusted to 1.5 in all cases to ensure that only the global neutral form of piroxicam is present in the solution. The increase in fluorescence intensity shows that the bound probe fluoresces and has been used to calculate the binding of the global neutral form of piroxicam with micelles of S12S, S10S and S8S. The binding constant with three different micelles has been determined from the fluorescence intensity data following the method described by Almgren et al. [14] using the equation

$$\frac{I_\infty - I_0}{I_c - I_0} = 1 + \frac{1}{K[\mathrm{M}]}$$

where I_0, I_c and I_∞ are the fluorescence intensities of the global neutral form of piroxicam considered in the absence of the surfactant, at any surfactant concentration and at a condition of complete micellization, respectively; K is the binding constant and [M] the micellar concentration. The micellar concentration is determined by

$$[\mathrm{M}] = \{[\mathrm{S}] - CMC\}/N$$

where [S] represent the surfactant concentration and N is the aggregation number of a micellar system. The values of N for S12S, S10S and S8S have been taken as 52, 44 and 38, respectively [11].

The binding constant (K) values have been determined from the slope of the linear plots of $(I_\infty - I_0)/(I_c - I_0)$ against 1/[M] (Fig. 4a–c). Linear regression analysis was done in each case and correlation coefficients have been found to be around 0.98 for all cases. The calculated K values are 1.83×10^5 M^{-1}, 5.56×10^4 M^{-1} and 1.06×10^4 M^{-1} for S12S, S10S and S8S, respectively. The difference among the binding constant values may be attributed to the differences in the hydrophobic interior due to varying alkyl chain lengths as all the three surfactants have the same negatively charged head group. It is observed that the larger the non-polar tail in the sodium alkyl sulfate, the stronger is

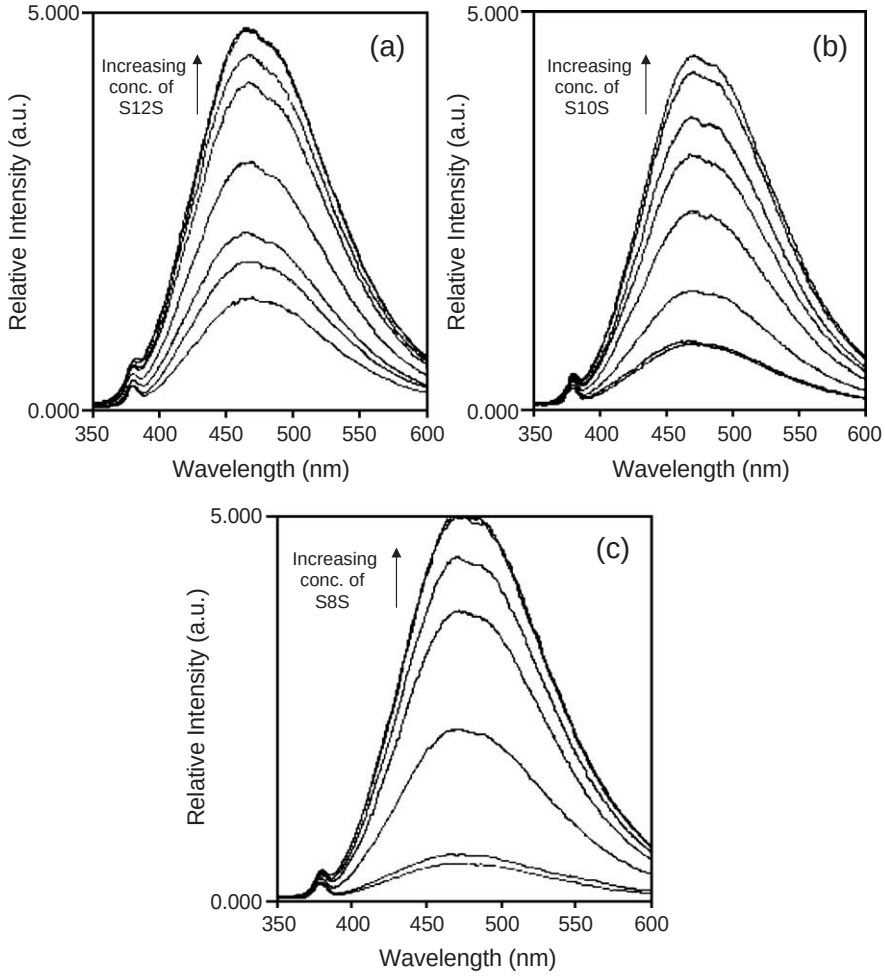


Fig. 3. Fluorescence spectra with increasing concentration of (a) S12S from concentration 0 mM to 12 mM, (b) S10S from concentration 0 mM to 45 mM, and (c) from 0 mM to 160 mM at constant pH (pH 1.5).

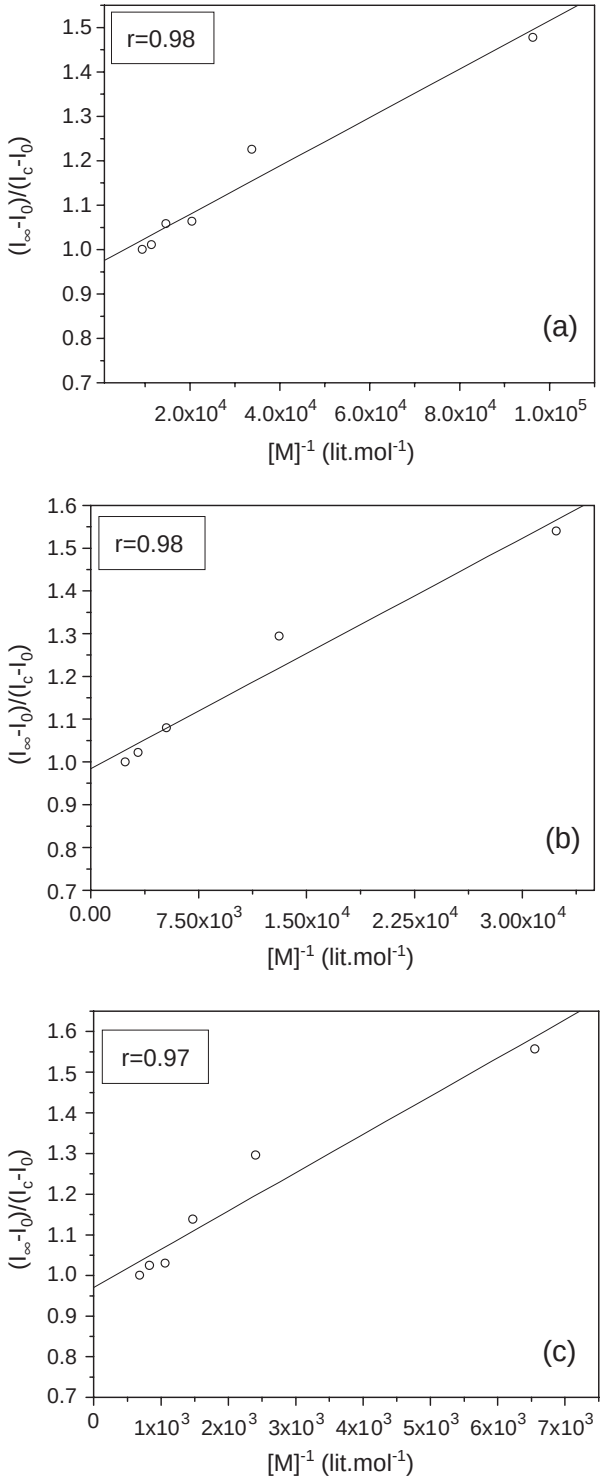


Fig. 4. Plot of $(I_\infty - I_0)/(I_c - I_0)$ vs. $[M]^{-1}$ (where [M] represents the micellar concentration) for (a) S12S, (b) S10S, and (c) S8S, at pH 1.5 where r is the correlation coefficient.

the binding. The log K vs. carbon number (n) for the sodium alkyl sulfates is shown in Fig. 5. The correlation is fairly linear and fits the equation

$$\log K = 1.58 + 0.309n$$

This shows that there exists a direct correlation between complexation and that of the micellar core. The value of the intercept (1.58) refers to the log K at zero carbon number of the hydrophobic tail of the surfactant, i.e., when the interaction with only the head group is considered.

Fig. 2a–c also indicates that the anionic form of piroxicam (A) is being converted to the neutral form with an increase in concentration of SNS surfactant. The switchover or the conversion of one prototropic form to the other occurs depending on the nature of the micelles. In our earlier studies we have shown that the surface charge of the micelles modulates the switchover between 'N' and 'A' [7]. Doping the uniformly charged micelles even with a small amount of oppositely charged head groups was capable of fine-tuning the N and A equilibrium [8]. The underlying cause behind the switchover between two prototropic forms of piroxicam was found to be the change in the apparent pK_a value in the presence of the differently charged micelles [7]. However it could not be explained why both CTAB and S12S micelles having oppositely charged head groups show the change in the apparent pK_a value in the same direction as compared to that in the absence of any surfactant. If electrostatic effect was the only modulating factor of the N and A equilibrium then it is expected that the shift in apparent pK_a values in the presence of S12S and CTAB should be in two opposite directions compared to that in the absence of any surfactant. The shift in the apparent pK_a values in the same direction for S12S and CTAB micelles indicated that factors other than surface charges might also affect the N and A equilibrium. It should be mentioned that S12S has 12-alkyl carbon, whereas CTAB has 16-alkyl carbon. We have measured the apparent pK_a in the presence of S12S, S10S and S8S, where the head group is the same with a negative surface charge and the chain lengths are 12, 10 and 8 CH_2 groups, respectively. The same experiment has been done in the case of CTAB and DTAB micelles also, where the surface charge is positive and the $-CH_2$ chain length is 16

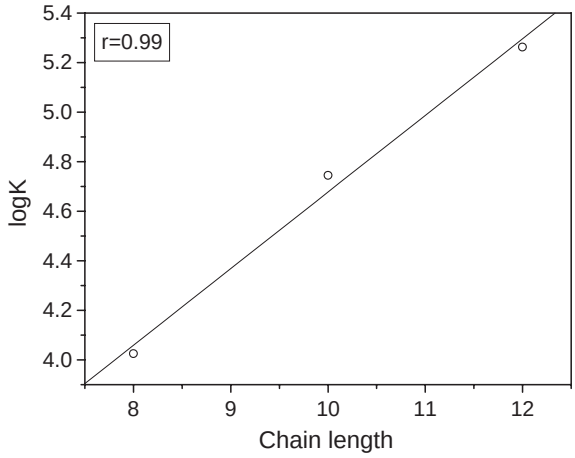


Fig. 5. Profile of log K vs. chain length for the SNS series, where r is the correlation coefficient.

and 12, respectively. The apparent pK_a in the absence and in the presence of different surfactants was determined by measuring the ratio of concentration of the anion to the neutral forms at different pH form with the absorbance values at 363 and 335 nm, respectively. The extinction coefficients used for the anion and the global neutral forms are 2.43×10^4 and 3.52×10^4 $M^{-1}.cm^{-1}$, respectively. The point of inflection is considered as the apparent pK_a of piroxicam in the absence and in the presence of different surfactants (Fig. 6). It should be noted that we have kept the micellar concentration well above the CMC values [15,16] in the full range of the pH variation studies. The concentrations of S12S, S10S, S8S, CTAB and DTAB were kept constant at 12 mM, 50 mM, 150 mM, 1.5 mM and 20 mM, respectively. The apparent pK_a value in the absence of any surfactant is 2.57. For the SNS series, the surface charges are the same, i.e., negative, the apparent pK_a values vary linearly with increasing chain length (Fig. 7). The values are 2.73, 3.04 and 3.42 for S8S, S10S and S12S, respectively. For this reason the pH of (a)–(c) were adjusted so as to be able to see the complete conversion of one prototropic form to the other. Fig. 7 shows that the increase in the hydrophobic chain length would favor the incorporation of neutral form, i.e., the N and A equilibrium will shift to the left. The longer the chain length higher is the hydrophobicity of the micelles. The hydrophobic environment of the micelles preferentially stabilizes the global neutral form and hence micelles with higher chain length support the formation of global neutral form leading to an increase in pK_a value. It will be interesting to compare S12S with DTAB where the surface charges are opposite but the alkyl chain lengths are identical. In this case it is expected that the electrostatic effect would modulate the equilibrium, as the chain lengths are the same. As expected, the shift in the apparent pK_a values is in two opposite directions compared to that in the absence of any surfactant, 3.42 for S12S and 1.99 for

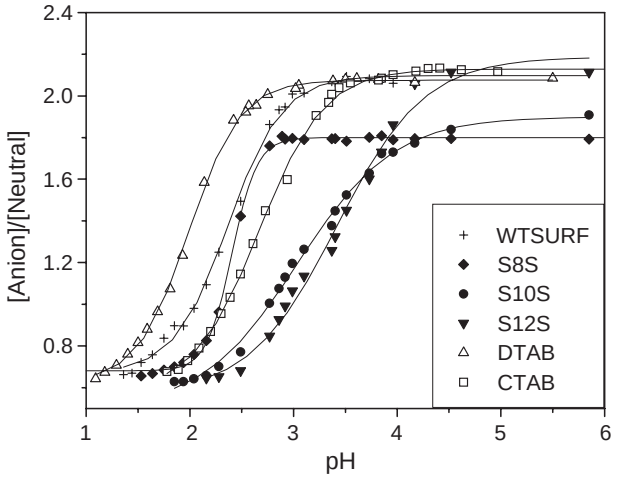


Fig. 6. Trace of [anion]/[neutral] of piroxicam without micelles (+), in the presence of CTAB (□), in the presence of DTAB (△), in the presence of S8S (♦), in the presence of S10S (⋆), in the presence of S12S (▼).

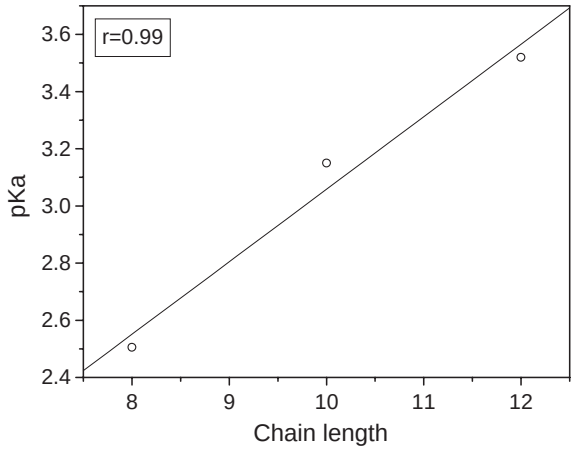


Fig. 7. Plot of pK_a of piroxicam vs. chain length for the SNS series, where r is the correlation coefficient.

DTAB. However, when the chain length is increased as in the case of CTAB, the apparent pK_a is shifted in the same direction as the SNS series, i.e., to a higher value (2.67) than in the absence of any surfactant (2.57).

The above result clearly parses the effect of electrostatics from that of hydrophobic chain length. It also indicates how critical the interplay of these two factors is, in modulating the 'N' to 'A' equilibrium. One can therefore say that even small changes in membrane parameters like hydrophobic effect and electrostatics are important to determine which form of a structurally dynamic molecule will be incorporated in it. Thus the diverse nature of biomembrane in vivo, characterized by their different membrane parameters, should be the decisive factor in choosing which structural form of the drug will be finally presented to its target cyclooxygenases. This also points to the intriguing possibility that the presentation of different structural forms to the cyclooxygenases target might affect their functions. However future studies on this aspect can only answer the above question.

4. Concluding remarks

Our study shows that for a structurally dynamic molecule like piroxicam, the modulation of hydrophobic core of micelles due to variation in alkyl chain length is an important parameter. It not only fine-tunes the switchover equilibrium between global neutral and anionic forms, but also guides the binding of the neutral form that is favored for incorporation in this case.

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