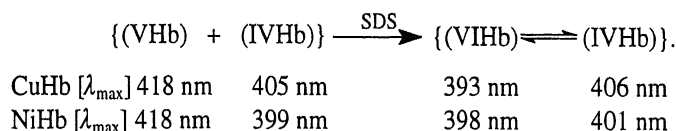similar to that of CuHb–SDS except that the peak position shifted to 398 nm is not substantially different from that of the 4-coordinated one viz 399 nm. The total disappearance of the 5-coordination site occurs in this case also at an SDS concentration of 2×10^{-4} M. Moreover, this 399 nm peak in NiHb initially broadens and blue-shifts to 398 nm which is subsequently red-shifted and sharpened at 401 nm with further increase in the SDS concentrations. These processes are under equilibrium control. The following scheme is proposed to explain the change under the influence of SDS:

$$\{(VHb) + (IVHb)\} \xrightarrow{SDS} \{(VIHb) \rightleftharpoons (IVHb)\}.$$

CuHb [λ_{max}] 418 nm	405 nm	393 nm	406 nm
NiHb [λ_{max}] 418 nm	399 nm	398 nm	401 nm

On the other hand, the CuHb–CTAB interaction interestingly shifts the 405 nm peak to 407 nm with simultaneous reduction in the intensity of the 5-coordination peak leading to its final disappearance at the CTAB concentration of $2 \cdot 4 \times 10^{-4}$ M. This again represents the exposure of the 4-coordinated heme. However, the intensity of the 5-coordinated peak in NiHb at 418 nm is proportionally reduced with the quantitative increasing amounts of CTAB finally disappearing at 3×10^{-4} M, with almost no reduction in the intensity of the 399 nm peak. With further increase in the concentration of CTAB, a minor shift of this 399 nm peak to 401 nm occurs. The subtle differences in the behaviour of NiHb and CuHb towards the surfactant is due to the differences in the T-conformation of these two as evidenced by –SH reactivity experiments reported earlier[25]. Furthermore, for a given Hb, CTAB brings out the sequence of reduction of 5-coordination and finally the exposure of the 4-coordinated heme more clearly than SDS does. In other words, the equilibrium process is slower with SDS than with CTAB. This phenomenon may be due to the higher hydrophobicity of CTAB compared to that of SDS which may be enough to shift the equilibrium such that the 4-coordinated heme from the globin matrix is exposed.

The nature of the SDS/CTAB interaction with FeHbCO [R-state] may be slightly different from that of Cu/Ni Hb [T-state] despite the evidence for metalloporphyrin exposure being detected in both cases. The reconstituted hemoglobins exhibit more extents of heme exposure than FeHbCO, which may be due to the more favorable inter-actions with the T-conformation. The Soret band at 419 nm in FeHbCO corresponding to strongly 5-coordinated [without considering CO bonding] iron undergoes almost a two-fold decrease in intensity with increased SDS concentration. This is further followed by a blue-shift to 410 nm which in turn indicates the tendency of the heme to move to 4-coordination at the SDS concentration of $\sim 4 \times 10^{-3}$ M. However, with CTAB the same 418 nm peak undergoes a greater blue-shift to 401 nm even at lower CTAB concentration $\sim 2 \times 10^{-3}$ M.

The possible reasons for the observed spectral changes in reconstituted hemoglobin on the addition of surfactant are as follows: (i) The 5-coordinated heme is converted to 4-coordinated heme but is still bound to the globin matrix, (ii) the 4-coordinated species in reconstituted hemoglobin arises from the dissociation of the tetramer into dimers or monomers, and (iii) total denaturation of the globin chain followed by heme ejection. The first process can be ruled out on the basis of the observation that in NiHb with increased addition of surfactant, we observe an increase in absorbance of 4-coordinated heme compared to that of 4-coordinated heme in NiHb (figure 3). Similar results are also obtained for CuHb. It may be noted that CuPP and NiPP exhibit bands at 403–408 nm
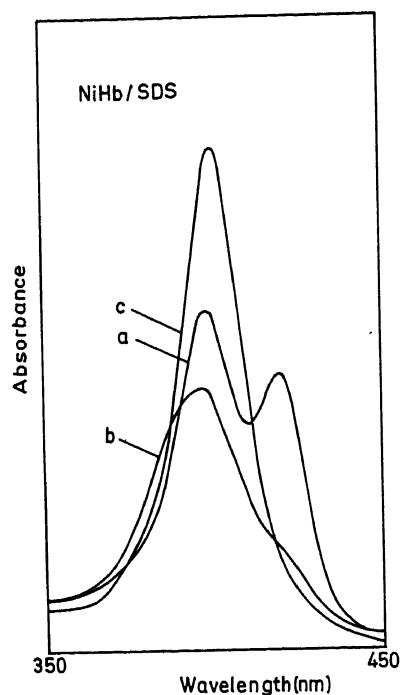
Figure 3. UV-visible spectra in 100 mm phosphate buffer, pH 7·2, for NiHb at different SDS concentration of $2·5 \times 10^{-5}$ (a), 2×10^{-4} (b) and $3·5 \times 10^{-3}$ M (c).

and 401 nm respectively in non-coordinating solvents, representing their 4-coordination character[26,27]. In the present results, the modifications in the metal coordination of metalloproteins in the presence of SDS and CTAB can be correlated to the CuPP and NiPP coordination changes in the respective spectral regions. The second possibility can also be ruled out based on previous CD experimental results[11,13], where it has been reported that the environmental change around the heme group alone involves destruction of the α-helical structure and change in the β-structure. This result even supports the third process. To further confirm this, deliberate additions of NaCl (aggregation favouring agent) to the surfactant–hemoglobin solutions are found to result in precipitation of surfactant components. The residue on redissolution in the buffer when subjected to UV-visible spectrophotometric studies showed the presence of metalloporphyrin in the surfactant media. Hence overall, the third process seems to be the most appropriate description for heme ejection on addition of surfactant.

The extent of heme exposure is proportional to partition coefficient (K) values and their dependence on surfactant concentrations for NiHb and CuHb are evident from figure 4. The higher K values indicate higher extent of heme exposure through stabilization of 4-coordinated heme. These values of partition coefficient show that there is difference in the extent of interaction between SDS and CTAB with NiHb and CuHb. The reactivity is reversed in NiHb compared to that of CuHb. This may be attributed to the different extent of T-state character based on PDS reactivity experiments[19].

Interpretations of these results on the following stepwise metalloprotein–CTAB interactions suggest: (i) The surfactant interactions weaken the proximal histidine [Fe–N_ε] coordinated to the metal centre; (ii) consequently there is a transition from 5- to
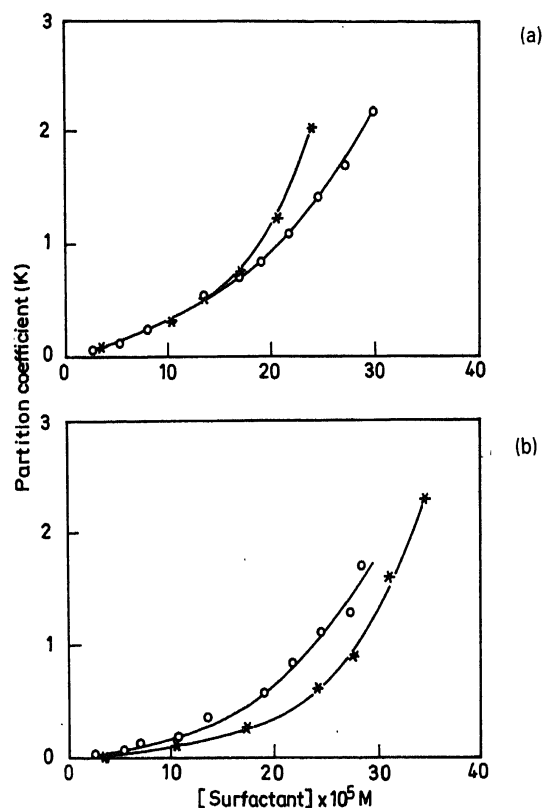
Figure 4. Plot of partition coefficient (K) as a function of SDS (*) and CTAB (O) concentration for (a) NiHb and (b) CuHb.

4-coordination of the porphyrin centre; (iii) the globin envelope of metalloporphyrin then hydrophobically interacts with the surfactant molecules, paving the way for chain unfolding followed by heme extraction into the hydrophobic micellar core. The hydrophobic interactions due to SDS and CTAB perhaps weaken the proximal histidine coordination and this penetrative additive effect may cause the ejection of heme.

4. Conclusions

According to the results of the present study, the interaction of the protein with surfactants and the resultant disappearance of the 5-coordination band are also observed at surfactant concentrations much lower than those for critical micelle formation values. With further increase in the concentration of surfactant, the ejection of heme from the globin matrix followed by stabilization of heme in the micelle media through hydrophobic interaction takes place. Despite these investigations, the specific interactions between surfactant–globin metalloporphyrin have to be rationalized with reference to protein conformational and micellar microstructural changes. Further UV-visible, Raman and EPR spectroscopic studies are under progress to verify the exact nature of such interactions and the consequent changes at different pH conditions.

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