# 242. Micellar Solubilization of Biopolymers in Hydrocarbon Solvents III. Empirical Definition of an Acidity Scale in Reverse Micelles

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*Summary*

This paper deals with the problem of defining, and measuring, the pH inside the water pool (which we define as $pH_{wp}$) of reverse micelles, *i.e.* micelles formed by surfactants dissolved in apolar solvents in the presence of minimal amounts of water. The conceptual and experimental difficulties are discussed, and it is argued that no absolute determination of $pH_{wp}$ is possible, mostly because water in the water pools of reverse micelles is a new solvent, for which no standardization of acidity is available. The problem can be approached only on the basis of an empirical acidity scale.

An empirical acidity scale for water pools in reverse micelles of bis (2-ethylhexyl) sodium sulfosuccinate (AOT) in isooctane has been defined by measuring the $^{31}P$-chemical shifts of phosphate buffers. The chemical shifts in bulk water were compared to those found in reverse micelles under the assumption that the *pK* of phosphate ion is the same in the two systems. It was found that in most cases there was little difference (less than 0.4 pH units) between $pH_{wp}$ and the pH of the starting buffer in bulk water (which we define as $pH_{st}$). However, this difference between $pH_{wp}$ and $pH_{st}$ may become much larger in certain cases.

The difference ($pH_{wp}-pH_{st}$) is measured under a variety of conditions, and this permits the determination of an operational acidity in the micelle water pools as a function of the $pH_{st}$ with which the aqueous micelles are prepared. The significance of such data for interpreting the behaviour of enzymes confined in the micelles water pool is discussed. Based on the $pH_{wp}$ scale, the apparent $pK_a$ of phenol-red and 4-nitrophenol were determined in reverse micelles containing different buffers and different water content. The $pK_a$ values obtained were rather sensitive to changes of both these factors, which was taken to signify that organic dies have only a very limited applicability to measure the acidity of the water pools of reverse micelles.

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**Introduction.** – Much interest has been shown recently in solubilizing various enzymes in reverse micelles consisting of bis (2-ethylhexyl) sodium sulfosuccinate (AOT) in isooctane containing various amounts of water [1–7]. A common method of preparing such solutions is to inject an aqueous stock solution of enzyme into the AOT/isooctane. In this way, enzymatic activity is retained, the amount of which depends also on the pH of the solution. If one wants to understand the behaviour of enzymes in reverse micelles, one has than to face the question of the relationship between the pH of a solution before injection into reverse micelles and the acidity within the water pool of these reverse micelles. Furthermore, recent results with liver alcohol dehydrogenase (LADH), lysozyme, and α-chymotrypsin have shown that their enzymatic activities vary with the water content of the reverse micelles even when buffers of identical pH (before injection into reverse micelles) are used [5–7]. Thus, one should also clarify how the acidity within the resulting micellar water pools varies when different volumes of a given buffer are injected into reverse micelles.

The question of the acidity in the water pool of reverse micelles has been already addressed using different techniques. The most common is to inject into reverse micelles various organic dyes whose absorption spectra depend on pH and observe the shift of these spectra with respect to the injected water solution [8–11]. *Menger & Yamada* [2] used a glass electrode to measure the electropotential of various reverse micellar solutions. *Fujii et al.* [12] used NMR, spectroscopy to measure the pH-dependent $^{31}$P-chemical shifts of phosphate buffers injected into reverse micelles.

All these data clearly show that the acidity within the water pool can be, depending upon conditions, rather different from that expected for a normal water solution. This is not surprising, if one considers the high charge density in the water pools caused by the ionized surfactant heads. Thus, the question of the acidity in the water pools is only one aspect of the more general question of the structure and properties of water confined in a reverse micelle.

In this regard, it is well established that the structure of water within reverse micelles is significantly different from that of bulk water. *Wong et al.* [13] used NMR. spectroscopy to study the structure of water in AOT reverse micelles. They found that the $^{1}$H-chemical shift of water depends on the water content of the system. Similar results were obtained by *Wells* [14] who demonstrated that the IR. spectrum of water within reverse micelles was different from that of bulk water. *Menger et al.* [15] determined the hyperfine coupling constant and line widths of three spin labels in AOT reverse micelles by using ESR. spectroscopy. Their data indicated that the local polarity and viscosity of the micellar water pools were different from that of bulk water. Moreover, the local polarity and viscosity depended on the water content of the reverse micelles. Also the fluorescence yield of 2-toluidinylnaphthalene-6-sulfonate (TNS) in reverse micelles of low water content resembled that seen in methanol [10]. This observation was taken to imply that the polarity of micellar water pools at low water content resembles that of methanol.

All these observations can be summarized in a simple, but important statement: that the water pools within reverse micelles is a different solvent than bulk water.

If this is true, then the acidity scale one uses to describe the water pool within reverse micelles must be different from the acidity scale used in bulk water (*i.e.* pH). Thus, a simple comparison of the $pK_a$ of any given acid in bulk water to micellar water pools makes no more sense than a comparison to the acidity (or «$pK_a$») in solvents such as methanol or liquid ammonia.

From these considerations it also follows that an absolute measure of the «pH» inside the micelles cannot be obtained. One can get a reading by utilizing a glass-electrode, but the meaning of this figure is uncertain, since the glass electrode is not (and probably cannot) be calibrated in the new solvent. One can use a dye as a measure of the local acidity in the water pools, but, since the p*K* of the dye cannot be determined independently (an assumption on either pH of pK is necessary), this measure is also deprived of intrinsic validity.

It is also clear that different «pH»s of the water pools would be obtained if different dyes (and generally different methods) are used as reference.

The question of the acidity in the water pools can, at least for the time being, be approached only empirically, *i.e.* assuming an arbitrary standard for the acidity scale.

This communication describes the use of NMR. spectroscopy for measuring the $^{31}$P-chemical shifts of phosphate buffers at various pH values. The chemical shifts of phosphate buffers in reverse micelles are compared to those in water (no AOT or isooctane present) to empirically define an arbitrary acidity scale. This scale relates the pH of buffers before injection into reverse micelles to the apparent acidity after formation of reverse micelles, under the (unprovable) assumption that the p*K* of phosphate ions ($H_2PO_4^- \rightleftarrows HPO_4^=$) is the same. Although any other reference (in principle any dye) can be used to build an arbitrary acidity scale in the water pools once an assumption such as this is being made, there are several advantages in the use of $^{31}$P-chemical shifts, as it will be discussed later on in this paper.

**Results and Discussion.** – First, let us define certain terms. The water content will be expressed in terms of $w_O$, which is defined as the ratio of the molar concentration of water to that of AOT, *i.e.*

$$w_O = \frac{[H_2O]}{[AOT]}$$

As shown by other workers [16] [17], it is this ratio, and not the absolute amounts of water or AOT which determines the size of the reverse micelles. We will also use the term «water pool» which was defined by *Menger et al.* [15] as the water within the reverse micelles. This is as opposed to bulk water which refers to an aqueous environment containing neither AOT nor isooctane. Finally we define $pH_{st}$ as the starting pH of an aqueous buffer before injection into AOT/isooctane and $pH_{wp}$ as the acidity within the resulting water pool of the reverse micelles. Thus, $pH_{st}$ refers to the commonly used pH scale in bulk water (as measured directly by a glass electrode), whereas $pH_{wp}$ refers to an empirically defined acidity scale.

The empirical method used in this communication is similar to that reported by *Fujii et al.* [12]. This method is based on the fact that the $^{31}$P-chemical shift of phosphate depends on the pH of the phosphate solution. Thus, the $^{31}$P-chemical shift is determined for a series of phosphate buffers both before and after injection into reverse micelles. As seen in *Figure 1* there is a small, but noticeable, difference

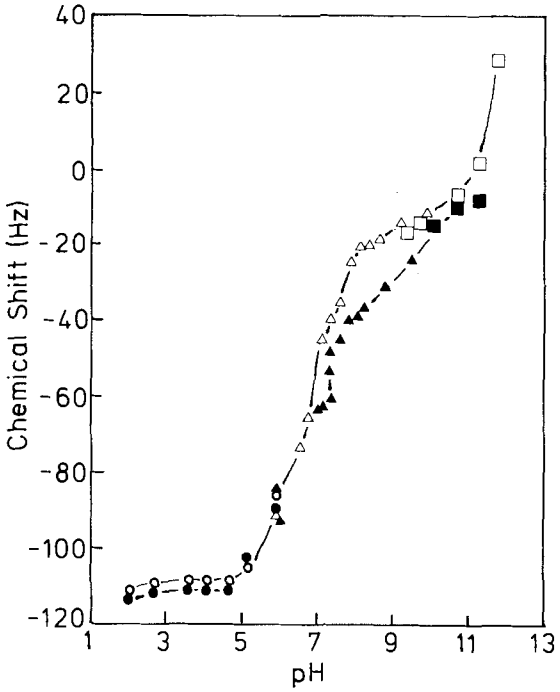


Fig. 1. *Dependence of $^{31}$P-chemical shifts of phosphate on the pH of the buffer solution.* The $^{31}$P-chemical shifts of various phosphate buffers in bulk water (open symbols) or in reverse micellar water pools (closed symbols) is plotted against the $pH_{st}$. Circles represent 0.1 M citrate/0.1 M phosphate, triangles represent 0.1 M phosphate, and squares represent 0.1 M glycine/0.1 M phosphate [AOT] = 100 mM, $w_0$ = 12.7.

in $^{31}$P-chemical shifts of phosphate buffers before *vs.* after injection into reverse micelles. Similar results were obtained when pyrophosphate buffers were used as seen in *Figure 2*. This difference between $^{31}$P-chemical shifts in micelles *vs.* bulk

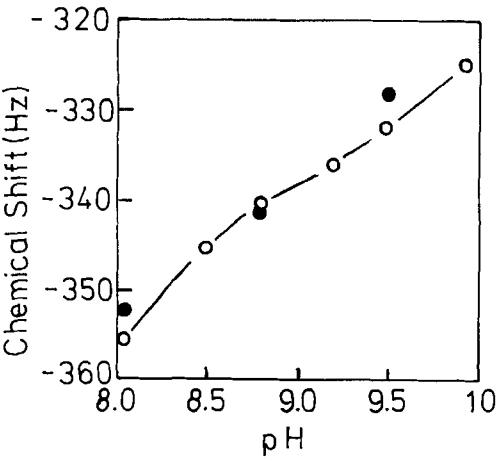


Fig. 2. *Dependence of $^{31}$P-chemical shifts of pyrophosphate on the pH of the buffer solution.* The $^{31}$P-chemical shifts of various pyrophosphate buffers in bulk water (open circles) or in reverse micelles (closed circles) is plotted against the $pH_{st}$ AOT and $w_0$ as in *Fig. 1.*

water depends on the buffer used. In general, this difference in chemical shifts is less when a stronger buffer (*i.e.* more resistant to changes in pH in bulk water) is used. Thus, pyrophosphate ($pK_a = 8.2$) is a stronger buffer at pH 8.8 than is phosphate ($pK_a = 7.2$) and the difference between the $^{31}$P-chemical shifts in reverse micellar water pools *vs.* bulk water is less in pyrophosphate at pH 8.8 (compare *Figures 1* and *2*).

The $^{31}$P-chemical shifts may be used to define an arbitrary acidity scale in reverse micelles as follows. The $pH_{wp}$ for a buffer which had any given $pH_{st}$ can be determined by comparing the $^{31}$P-chemical shift in the water pool to the standard curve of $^{31}$P-chemical shifts in bulk water. Thus, if a phosphate buffer after injection into reverse micelles has the same $^{31}$P-chemical shift as phosphate in bulk water at pH 7.5, then $pH_{wp}$ of the phosphate in water pool is 7.5. As explained in the introduction, the underlying assumption to define such $pH_{wp}$ is that the p$K$ of phosphate ions is the same in the water pools and in bulk water. Using this method, the $pH_{wp}$ for several buffers after injection in micellar water pools was calculated. The «pH jump», *i.e.* $pH_{st} - pH_{wp}$, is plotted against $pH_{st}$ in *Figure 3*. The «pH jump» depends upon the buffer used, and is less when a stronger buffer is used.

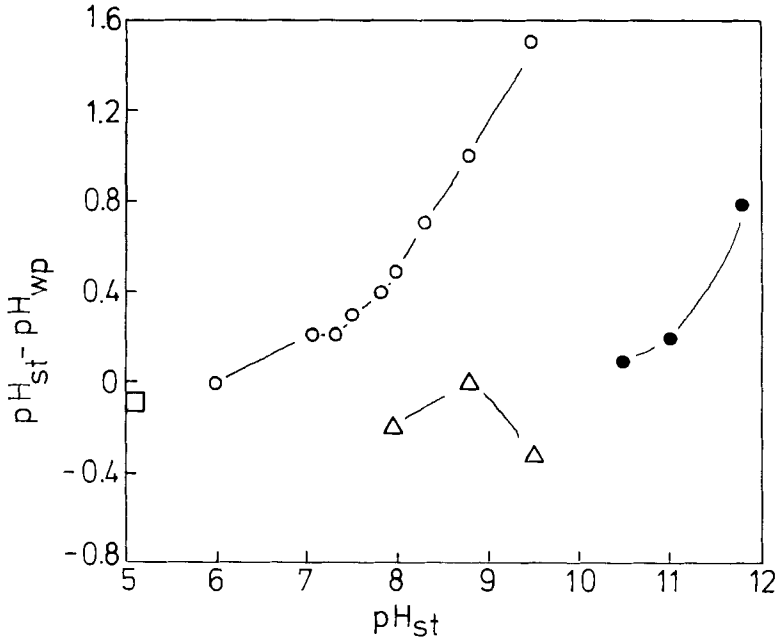


Fig. 3. *«pH Jump» as a function of $pH_{st}$.* The differences between $pH_{st}$ and $pH_{wp}$ (*i.e.* «pH Jump») is plotted against $pH_{st}$ for the following buffers: 0.1 M phosphate/0.1 M citrate (□); 0.1 M phosphate (○); 0.1 M pyrophosphate (△); 0.1 M phosphate/0.1 M glycine (●).

As suggested by *Fujii et al.* [12], it is possible that the detergent itself may partly cause the discrepancy between the $^{31}$P-chemical shifts in reverse micelles *vs.* bulk water. To measure this effect due to detergent, the $^{31}$P-chemical shift of a

P-atom whose chemical shift is independent of pH (in bulk water) can be measured. For example, the α-phosphorus of adenosine triphosphate (ATP) is independent of pH [18] and for this reason was used by *Fuji et al.* as an internal standard [12]. The chemical shift of trimethylphosphate (TMP) is also independent of pH, and as such, it can also be used to determine the effects of detergent. The chemical shifts of the α-phosphorus of ATP and of trimethylphosphate in different solvents are shown in *Table 1*. Note that the chemical shift of the α-phosphorous of ATP

Table 1. *Measurement of effect of micellar environment alone on $^{31}$P-chemical shifts*

| Compound | Solvent | Chemical shift ($H_z$) |
|---|---|---|
| P of ATP | Water | −530.932 |
| P of ATP | 100 mM AOT $w_0 = 8.9$ | −522.11 |
| P of ATP | 100 mM AOT $w_0 = 12.7$ | −527.3 |
| Trimethylphosphate | 100% Trimethylphosphate | 0[a]) |
| Trimethylphosphate | Water | +0.74 |
| Trimethylphosphate | 100 mM AOT $w_0 = 8.9$ | −13.2 |

[a]) All chemical shifts are compared to trimethylphosphate which is arbitrarily assigned chemical shift of zero.

is somewhat higher in reverse micelles than in bulk water, but the opposite is true for TMP. Thus, different internal standards respond differently to the micellar environment, and therefore a correction for the detergent effect based on such measurements would not improve the reliability of the acidity scala. Moreover, in the pH regions where $pH_{wp}$ can be estimated most precisely, any correction of chemical shifts due to detergent effects will have little effect on the estimated $pH_{wp}$ (This is because correction due to detergent effects would change the chemical shift by no more than 13 Hz which would alter $pH_{wp}$ by only 0.1 pH unit).

For these reasons, all our data on $^{31}$P-chemical shifts in reverse micelles are not corrected for possible detergent effects.

More generally, it should be noted that the precision with which $pH_{wp}$ can be determined depends on the pH. In bulk water, the $^{31}$P-chemical shift changes sharply with respect to pH in the region of pH 6–8, and less so in the region of pH 3–5 (for this reason, *Fig. 3* shows no data below pH 5). In the region of pH 8–9.5, the $^{31}$P-chemical shift of pyrophosphate changes more sharply, so that the $pH_{wp}$ of pyrophosphate can be estimated to greater precision than the $pH_{wp}$ of phosphate buffers in the same region.

Since more phosphate can be solubilized in solutions containing higher concentrations of AOT, most reverse micelles were prepared with 100 mM AOT (thus, less time was required for measuring each spectrum). It was important for us to compare these results with data obtained in 50 mM AOT, since most of our enzymatic activity data have been obtained under such conditions [5–7]. Thus, the $^{31}$P-chemical shifts of a few different phosphate buffers were measured in both 50 and 100 mM AOT with $w_0$ being held constant. As seen in *Table 2*, there is no appreciable difference, especially if one bears in mind that a change of 0.1 pH unit in this range of pH values causes a change in $^{31}$P-chemical shift of about 20 Hz.

Table 2. *Effect of varying AOT concentration on $^{31}P$-chemical shifts*

| AOT mM | $pH_{st}$ | Chemical shift (Hz) | AOT mM | $pH_{st}$ | Chemical shift (Hz) |
|---|---|---|---|---|---|
| 50 | 7.0 | – 60.3 | 100 | 7.5 | – 42.0 |
| 100 | 7.0 | – 57.4 | 50 | 7.7 | – 39.7 |
| 50 | 7.2 | – 57.4 | 100 | 7.7 | – 38.0 |
| 100 | 7.2 | – 53.5 | 50 | 8.4 | – 32.4 |
| 50 | 7.5 | – 44.1 | 100 | 8.4 | – 33.3 |

In every case, $w_0 = 12.7$ and phosphate concentration within the micelles was 100 mM.

The $^{31}P$-chemical shifts of identical phosphate buffers were determined as a function of $w_0$. As seen in *Table 3*, there is no appreciable difference in the chemical shifts of either phosphate or pyrophosphate between $w_0 = 8.9$ to 14.8. Measurements of chemical shifts beyond such $w_0$ range were not possible, due to

Table 3. *Effect of varying $w_0$ on $^{31}P$-chemical shifts*

| $w_0$ | $pH_{st}$ | Chemical shift (Hz) | Phosphate Concentration (mM) |
|---|---|---|---|
| 8.9 | 7.0 | – 57.4 | 100 |
| 12.7 | 7.0 | – 57.4 | 100 |
| 8.9 | 7.5 | – 44.1 | 100 |
| 12.7 | 7.5 | – 42.0 | 100 |
| 12.7 | 7.5 | – 50.0 | 42 |
| 14.8 | 7.5 | – 51.5 | 42 |
| 12.7 | 8.4 | – 39.7 | 42 |
| 14.8 | 8.4 | – 39.7 | 42 |

In every case, AOT concentration was 100 mM.

the exceedingly long accumulation time and/or to the poor solubility of phosphate buffer in reverse micelles. It is however to be expected that the «pH-jump» would be smaller at larger water content. The concentration of phosphate buffer within the reverse micellar water pool does affect the observed $^{31}P$-chemical shift, as seen in *Table 4*. The difference between $pH_{st}$ and $pH_{wp}$ is greater at lower concentration

Table 4. *Effect of varying phosphate concentration on $^{31}P$-chemical shifts*

| Phosphate mM | $pH_{st}$ | Chemical shift (Hz) | $pH_{wp}$ |
|---|---|---|---|
| 42 | 7.5 | – 50.0 | 7.0 |
| 70 | 7.5 | – 48.5 | 7.1 |
| 100 | 7.5 | – 42.0 | 7.2 |
| 42 | 8.4 | – 39.7 | 7.4 |
| 70 | 8.4 | – 36.8 | 7.5 |
| 100 | 8.4 | – 33.3 | 7.7 |

In every case, $w_0 = 12.7$ and AOT concentration was 100 mM.
The buffer concentrations are referred to the water pool, since we expect all salts to be only soluble in the water microphase.

of phosphate. This is consistent with the observation *(Fig. 3)* that the «pH jump» ($pH_{st} - pH_{wp}$) is less for stronger buffers.

Several data of *Table 2–4* have been repeated in the presence of lysozyme (10 to 50 $\mu$M, overall concentration), in order to test for the possible influence of the guest macromolecule on the $pH_{wp}$. No influence has been observed.

**Determination of apparent p$K_a$ of organic dyes.** – Using the values of $pH_{wp}$ as defined by $^{31}$P-NMR., one can determine an apparent p$K_a$ of organic dyes. For example, the UV./VIS. absorption spectra of phenol-red and 4-nitrophenol vary with pH in bulk water and with $pH_{wp}$ in reverse micelles. Thus, one can measure the absorption due to the basic form of these dyes at various $pH_{st}$ values and determine the apparent p$K_a$ according to routine procedure [19] also in reverse micelles. This value of p$K$ is based of course upon the assumed value of the p$K$ of phosphate ions in the micelles.

Phenol-red in reverse micelles is well-behaved with respect to a standard [19] p$K_a$ analysis (*i.e.* log $\alpha$ *vs.* $pH_{wp}$ yield straight lines, $\alpha$ being the fraction of ionized dye). The apparent p$K_a$ of phenol-red depends on the buffer present within the reverse micelles, and is about 9.9 in glycinephosphate and 11.0 in pyrophosphate (p$K_a$ in bulk water is 7.8). Similar results are obtained for 4-nitrophenol as seen in *Table 5* (p$K_a$ in bulk water is 7.1). This is in agreement

Table 5. *p$K_a$ of 4-nitrophenol in reverse micelles*

| Buffer | $w_0$ | p$K_a$ |
|---|---|---|
| 0.1 M Glycine/0.1 M Phosphate | 8.9 | 11.5 |
| 0.1 M Glycine/0.1 M Phosphate | 13.3 | 11.0 |
| 0.05 M Glycine/0.05 M Phosphate | 13.3 | 11.4 |
| 0.025 M Glycine/0.025 M Phosphate | 13.3 | > 12 |
| 0.025 M Glycine/0.025 M Phosphate | 25.4 | 11.2 |

with the results of *Menger & Saito* [15], who reported that the p$K_a$ of 4-nitrophenol depends on the buffer used. They found an apparent p$K_a$ of about 11.6 in phosphate *vs.* 7.7 in imidazole within AOT reverse micelles. Moreover, similar results were obtained by *Fujii et al.* [12] who also used $^{31}$P-NMR. to define an acidity scale within reverse micelles. They found that the apparent p$K_a$ of phenol-red (in a different reverse micellar system) was higher than the p$K_a$ in bulk water. In conclusion, previous results [11] [12] [15] agree with those reported here in that the p$K_a$ of an organic dye is different in reverse micelles, and does depend on the buffer used.

It is perhaps important to point out again that this p$K$ difference holds in our case despite the assumption that the p$K$ of phosphate ion is the same in water and in reverse micelles. This apparent discrepancy may be due to the specific interaction of the organic dye with the surfactant molecules. In fact, *Menger & Saito* have already suggested that 4-nitrophenol has a different degree of interaction with AOT when phosphate as opposed to imidazole buffer is used [15].

All this information can be taken to strengthen the notion that organic indicators cannot be reliably used to measure the acidity of the water pool of reverse

micelles. By the same token, the measurement of their p*K* in the water pool is probably deprived of any clear physical meaning. The p*K* value obtained assuming the same pH in the water pools as in bulk water is a number which reflects the interactions of the dye with the micelle components, interactions which in turn depend upon the chemical nature of the dye, the micellar structure, and the type of salt present in the water pool.

**Concluding remarks.** – The arguments developed at the end of the previous section make clear why an organic dye would not be a suitable standard for an empirical scale of acidity in the water pool of reverse micelles. The standard chosen must meet certain operational and structural prerequisites, and this is why the use of phosphate ions is a good choice. In fact, both $H_2PO_4^-$ and $HPO_4^{--}$ due to their high hydrophylicity and relatively small size, and to their negative charge, are with all likehood confined in the water pool, with no tendency to adhere to the hydrophobic parts of the surfactant, nor to their negatively charged heads. Also, the $^{31}P$-chemical shifts are not affected, or only sparingly, by AOT concentration or water content.

It has been made clear in this paper that «pH» and «p*K*» in reverse micelles, as measured on the basis of an arbitrary standard, do not have the precise meaning as for a bulk water solution. For example, the observation that $pH_{wp} = 7$ does not automatically mean that $[H^+]_{wp} = 10^{-7}$ (this would be only if the p*K* of phosphate in the water pools happens to be the same as in bulk water). These limits notwithstanding the data obtained in this work, particularly those relative does not automatically mean that $[H^+]_{wp} = 10^{-7}$ (this would be only if the p*K* of to the «pH-jump» (*i.e.* $pH_{st} - pH_{st}$), are very important to interprete the behaviour of enzymes confined in the water pools of reverse micelles. Thus, in the case of horse liver alcohol dehydrogenase, α-chymotrypsin, and lysozyme [5–7], it can be now ruled out that the change in activity as a function of $w_0$ is due to a trivial pH change in the water pool occurring as a result of changing the water content. Moreover, based on this empirical acidity scale, the changes of activity of α-chymotrypsin and lysozyme upon changing $w_0$ at constant $pH_{st}$, can be attributed to changes in the p*K* of the enzyme's group (s). Needless to say, the evaluation of the p*K* of certain of the enzyme group(s) relies not only upon our basic assumption (that the p*K* of the phosphate ion is the same in bulk water and in the micelle water pools) but also upon the assumption that such enzyme groups in the micelle experience the same water environment as the phosphate ion. This second assumption cannot be taken always for granted, and should be checked separately for each enzyme (*e.g.* by spectroscopic techniques, see [5–7] [16]). The question of why the p*K* of certain enzyme's groups change as a function of $w_0$ eludes the frame of the present work, but it touches upon a problem which is basic for better understanding the terms «pH» or «p*K*» in reverse micellar water pools. This is the problem of water structure in such systems some aspects of this question are presently under investigation in our group.

The discussion with Prof. *C. Vernon* (University College, London) has been very helpful to the Authors.

## Experimental Part

*Materials.* Adenosine-5'-triphosphate (ATP), phenol-red, trimethylphosphate (TMP), and puriss grade isooctane were from *Fluka AG* (Buches, Switzerland). Bis (2-ethylhexyl) sodium sulfosuccinate (AOT) was from *Merck* (Darmstadt). It was purified before use by treatment with activated charcoal as described previously [13] and stored in vacuum under $P_2O_5$.

*Measurements.* $^{31}$P-NMR. spectra were measured with a *Bruker* WH 90 spectrometer at 36.43 MHz. The following setting of the pulse unit were employed: spectral width 3012.048 Hz, repeat time 0.679 s, pulse width 4.5 $\mu$s, and from 700 to 2000 accumulations per spectrum (depending on sample concentration). The absorption spectra were recorded on a *Beckman* DB-GT spectrophotometer.

The technique of the preparation of reverse micelles is described in our previous papers [4] [5] [16].

## REFERENCES

[1] *Martinek, K., Levashov, A.V., Klyachko, N.L. & Berenzin, I.V.* Dokl. Akad. Nauk, SSSR (Engl. edition) *236,* 951 (1978).
[2] *Menger, F.M. & Yamada, K.* J. Amer. Chem. Soc. *97,* 909 (1979).
[3] *Dozou, P., Keh. E. & Balny, C.* Proc. Natl. Acad. Sci. (USA) *76,* 681 (1979).
[4] *Wolf, R. & Luisi, P.L.* Biochem. Biophys. Res. Commun. *89,* 209, 217 (1979).
[5] *Meier, P. & Luisi, P.L.* J. Solid Phase Bioch., in press (1980).
[6] *Barbaric, S. & Luisi, P.L.* Manuscript submitted for publication (1980).
[7] *Grandi, C., Smith, R.E. & Luisi, P.L.* Manuscript submitted for publication (1980).
[8] *Nome, F., Chang, S.A. & Fendler, J.H.* J. Chem. Soc. Faraday Trans. *1,* 296 (1976).
[9] *Fendler, J.H. & Fendler, E.J.* Catalysis in Micellar and Macromolecular Systems, Academic Press, New York (1975).
[10] *Wong, M., Thomas, J.K. & Grätzel, M.* J. Amer. Chem. Soc. *98,* 2391 (1976).
[11] *Levashov, A.V., Pantin, V.I. & Martinek, K.* Kolloid. Zhurn. *41,* 453 (1979).
[12] *Fujii, I., Kawai, I. & Nishikawa, H.* Bull. Chem. Soc. Japan *52,* 2051 (1979).
[13] *Wong, M., Thomas, J.K. Grätzel, M.* J. Amer. Chem. Soc. *98,* 2391 (1976).
[14] *Wells, M.a.* Biochemistry *13,* 4937 (1974).
[15] *Menger, F.M. & Saito, G.* J. Amer. Chem. Soc. *100,* 4376 (1978).
[16] *Bonner, F.J., Wolf, R. & Luisi, P.L.* J. Solid-Phase Biochem., in press.
[17] *Eicke, H.F. & Rebak, J.* Helv. *59,* 2883 (1976).
[18] *Cohn, M. & Hughes, T.R., jr.* J. Biol. Chem. *235,* 3250 (1960).
[19] *Albert, A. & Serjeant, E.P.* Ionization Constants of Acids and Bases, Wiley Pres, New York, N.Y., p.73. (1962).