Poly(sulphopropylbetaines): 4. Binding properties towards reporter anionic probes and local polarity close to the zwitterionic chain in aqueous solution

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The binding properties of a representative poly(sulphopropylbetaine) towards a series of optical (methyl orange (MO) and 2-(4′-hydroxybenzenazo)benzoate (HABA)), fluorescent (8-anilinonaphthalene-1-sulphonate (ANS)) and chemically reactive (6-nitrobenzisoxazole-3-carboxylate (S)) anionic probes have been studied in dilute aqueous solution. The slight ultra-violet spectra modification for MO and HABA, the enhanced fluorescence of ANS and the moderate increase of the decarboxylation rate of S induced by the poly(zwitterion) show that binding of the various anionic probes to the macromolecular chain actually occurs through specific ion–dipole interactions, but that the intrinsic properties of the bound species are only weakly modified with respect to those of the free ones. Dehydration of the bound probe is very limited, and the polarity of its microenvironment close to the polymer chain is analogous to that of methanol. In spite of a relatively weak expansion, the poly(zwitterion) coil does not show microdomains of definite hydrophobicity.

(Keywords: poly(sulphopropylbetaine); aqueous solution; anion binding properties; methyl orange ultra-violet absorption; 8-anilinonaphthalene-1-sulphonate fluorescence; 6-nitrobenzisoxazole-3-carboxylate decarboxylation; polymer microenvironment polarity)

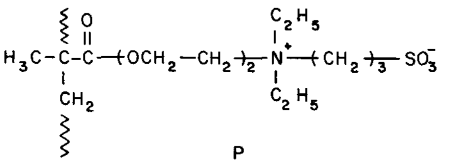
INTRODUCTION

In the two preceding parts of this series, the synthesis[1] and the dilute solution properties[2] of five atactic aliphatic or aromatic poly(sulphopropylbetaines) (P) were described. Because of the strongly dipolar structure of their zwitterionic lateral groups, these linear polymers display a very unusual and unique 'antipolyelectrolyte' behaviour[2-4] characterized by an increasing chain expansion in aqueous solution when increasing the ionic strength in the presence of added salts, and more especially in the presence of highly polarizable 'soft' anions. The major goal of the present work is to analyse these specific anion–dipole interactions on model systems and to quantify the local polarity of the microenvironment of the poly(zwitterion) chain (hereafter referred to as P) through the study of the binding of some reporter anionic probes in dilute aqueous solution. Three complementary topics will be reviewed successively:

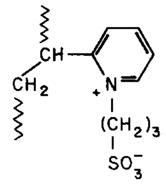
(i) Binding of methyl orange (MO) and of 2-(4′-hydroxybenzenazo)benzoate (HABA), used as optical probes.

(ii) Binding of 8-anilinonaphthalene-1-sulphonate (ANS) used as a fluorescent probe.

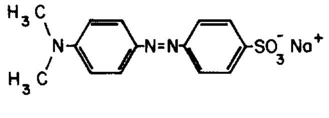
(iii) Binding of 6-nitrobenzisoxazole-3-carboxylate (S), used as a chemical probe (spontaneous decarboxylation).



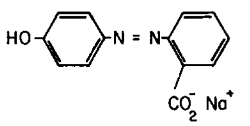
P



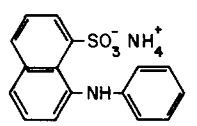
P′



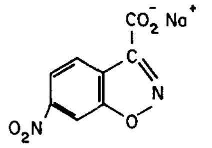
MO



HABA



ANS



S

EXPERIMENTAL

Polymers and probes

The poly(zwitterion) P, obtained by radical-initiated polymerization of the corresponding monomer as detailed elsewhere[1], was used without further fractionation: $\bar{M}_w = 3.23 \times 10^6$, $[\eta] = 1.00\,\mathrm{dl\,g^{-1}}$ in water at 25°C. The various dyes, MO and HABA (sodium salts) and ANS (ammonium salt), of the best reagent grade, were used without further purification. The multistep

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synthesis and purification of S was performed according to literature procedures: the methyl ester of 6-nitrobenzisoxazole-3-carboxylic acid was prepared by condensation of methyl-2,4-dinitrophenyl acetate[5] with $NaOCH_3$ and isoamyl nitrite in CH_3OH[6] and was recrystallized from CH_3OH; 6-nitrobenzisoxazole-3-carboxylic acid was obtained by acid hydrolysis of its methyl ester[7]; finally, titrated aqueous solutions of the sodium salt (neutralization by NaOH) were stored in the frozen state for many weeks without any degradation[8] (u.v. spectroscopy, see below).

Ultra-violet spectroscopy

All the u.v. spectra were recorded on a Cary 2300 apparatus, fitted with an external Haake circulating bath allowing a temperature control of the cell within $\pm 0.1°C$. The 'difference' spectra between MO + P and MO solutions (see 'Experimental Results') were recorded with a slow scanning rate of $0.5\,nm^2 s^{-1}$. The decarboxylation kinetics of the reactive substrate S in aqueous solution (pH = 8.5) at 30°C was monitored by u.v. spectroscopy using the characteristic absorption band of the released 2-cyano-5-nitrophenolate at $\lambda \sim 395$ nm ($\varepsilon = 2570\,M^{-1}\,cm^{-1}$ at $\lambda = 397.5$ nm according to the literature[9]).

Fluorescence spectroscopy

The fluorescence spectra of aqueous solutions of ANS were obtained on a Fica 55 spectrofluorimeter which gives directly corrected spectra for both excitation and emission, using dye concentrations between 1×10^{-5} and 2×10^{-4} M (absorbances were always lower than 0.09 at $\lambda_{max} = 356$ nm); the excitation wavelength was 380 nm in all experiments. Fluorescence emission spectra were recorded in the range 400–600 nm, and the fluorescence intensity (arbitrary units) was measured from the emission area by cutting and weighing.

EXPERIMENTAL RESULTS

Binding of the optical probes

Methyl orange (MO). The transfer of MO from water to a less polar environment results in a significant hypsochromic shift of its visible spectrum which has been repeatedly used to study its binding to proteins[10,11], synthetic neutral polymers[11–16] or cationic polysoaps[8,17] and to detect hydrophobic microdomains in their aqueous solutions.

The addition of increasing amounts of the poly(zwitterion) P to a 1.13×10^{-4} M aqueous solution of MO barely influences its absorption band: $\varepsilon = 2.68 \times 10^4\,M^{-1}\,cm^{-1}$ at $\lambda = 463$ nm for pure MO ($\varepsilon = 2.47$–$2.70 \times 10^4\,M^{-1}\,cm^{-1}$ at $\lambda = 464$–465 nm according to the literature[14–16]) *versus* $\varepsilon = 2.87 \times 10^4\,M^{-1}\,cm^{-1}$ at $\lambda = 465$ nm in the presence of 7.25×10^{-2} M of polymer P. Hwever, these very small effects may be quantitatively analysed when the spectra are recorded in a differential mode[16] using a pure MO reference solution and MO + P solutions of various P content but of constant MO concentration strictly identical to that of the reference. These 'difference' spectra given in *Figure 1* show a minimum and a maximum at about 385 and 436 nm, and the appearance of isosbestic points at about 360, 410 and 515 nm implies the presence of two different species of free and bound MO in the sample solution. Since the polymer itself is transparent in this spectral range, the difference in absorbance ΔA is a simple function of [MO] only:

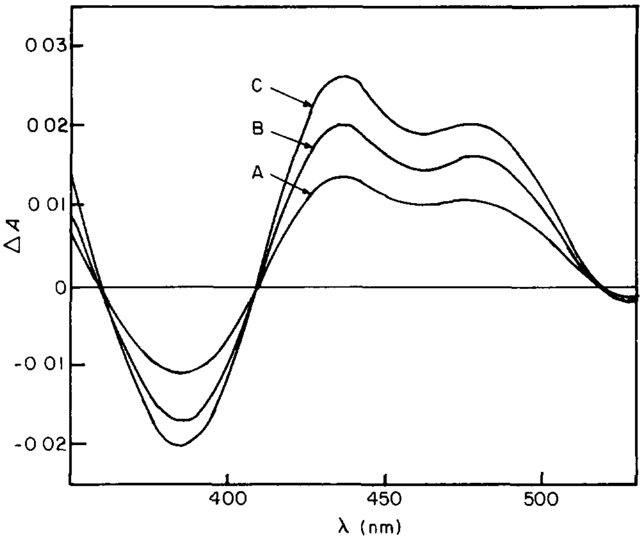


Figure 1 Difference absorption spectra ΔA for MO solutions ($[MO] = 2.37 \times 10^{-5}$ M) in the presence of the poly(zwitterion) P. $[P]_0 = 0.294$, 0.566 and 0.868×10^{-2} M for curves A, B and C respectively

$$\Delta A = [MO]_b \varepsilon_b + [MO]_f \varepsilon_f - [MO] \varepsilon_f$$

But

$$[MO]_b + [MO]_f = [MO]$$

the concentration of total MO in the two cells, so

$$\Delta A = [MO]_b (\varepsilon_b - \varepsilon_f)$$

Here $[MO]_f$, $[MO]_b$ and [MO] stand for the concentration of free, bound and total MO, and ε_f and ε_b for the molar absorptivities of the free and bound MO respectively. Two series of experiments were performed at constant MO concentrations of 4.76×10^{-5} and 2.37×10^{-5} M respectively in the presence of increasing amounts of P, and the variations of absorbances ΔA at $\lambda = 436$ nm were interpreted according to the Benesi–Hildebrand relationship[16]:

$$\frac{[MO]}{\Delta A} = \frac{1}{[P]} \frac{1}{K'(\varepsilon_b - \varepsilon_f)} + \frac{1}{\varepsilon_b - \varepsilon_f} \qquad (1)$$

The experimental data are in good agreement with equation (1), as shown in *Figure 2*, and they lead to a binding constant $K' = 170\,M^{-1}$ and to $\varepsilon_b - \varepsilon_f = 1740\,M^{-1}\,cm^{-1}$ at $\lambda = 436$ nm. The data may be now plotted according to the rearranged form of the Langmuir isotherm suggested by Klotz[18], as shown in *Figure 3*:

$$\frac{[P]}{[MO]_b} = \frac{1}{[MO]_f} \frac{1}{Kn} + \frac{1}{n} \qquad (2)$$

The first binding constant $K_1 = Kn$, which measures the free energy of interaction of the first dye when there are n choices of identical sites on a given chain, is in fairly good

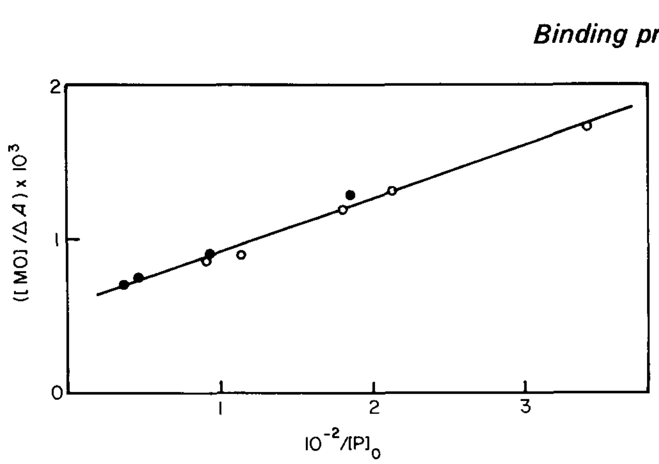


Figure 2 Benesi–Hildebrand relationship for the binding of MO to the poly(zwitterion) P plotted according to equation (1): ○, $[MO]=2.37\times10^{-5}$ M; ●, $[MO]=4.76\times10^{-5}$ M

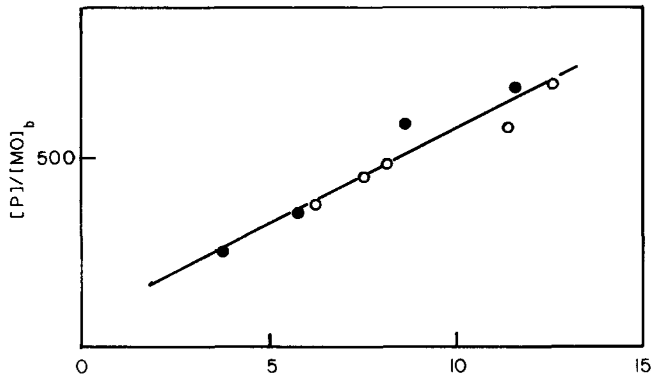
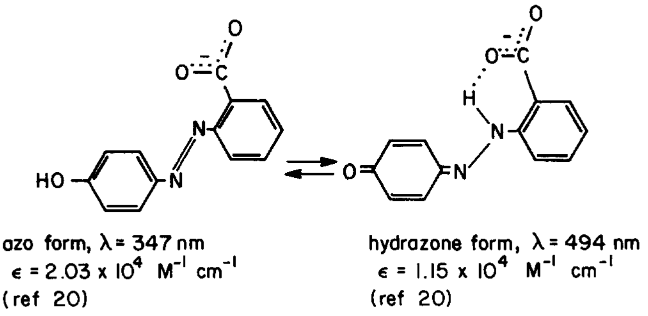


Figure 3 Langmuir isotherm of binding of MO to the poly(zwitterion) P plotted according to the Klotz equation (2): ○, $[MO]=2.37\times{}^{10^{-5}}$ M; ●, $[MO]=4.76\times10^{-5}$ M

agreement with the previous K' value derived from the Benesi–Hildebrand relationship, 200 *versus* 170 M^{-1} respectively. The average number of monomeric units required to bind one dye molecule, $N=n^{-1}$, considered as a measure of the size of the binding site, is about 71. The mean value of the intrinsic binding constant K, which measures the free energy for interaction of a dye with a site and is the same whether the chain contains one or n sites, may be estimated to about $1.3\times10^{4}\ M^{-1}$. The good agreement of the experimental data with equation (2) of the Langmuir isotherm shows the lack of any significant cooperative effects in the binding process, even in the least favourable case where the fraction of occupied sites, f, reaches its maximum value: $f\sim0.28$ for $[P]=0.536\times10^{-2}$ M and $[MO]=4.76\times10^{-5}$ M, corresponding to about 12 dye molecules per zwitterion chain ($\overline{DP}_n\sim3\times10^{3}$).

Finally the u.v. spectrum of bound MO, derived from that of the free species and from the differential spectra previously analysed, shows no significant shift of the wavelength at maximum absorption with respect to that of the free dye ($\lambda_{max}\sim464\pm1$ nm) and only a very slight hyperchromicity ($\varepsilon_b/\varepsilon_f<1.1$).

2-(4'-Hydroxybenzenazo)benzoate (HABA). At pH ~ 6.45 the mono-anion of HABA shows a tautomeric equilibrium between the polar azo form favoured in water and the less polar hydrazone form stabilized by intramolecular hydrogen bonding and favoured in less polar organic media[19] or when the dye is bound to hydrophobic serum albumin[19] or neutral synthetic polymer such as poly(vinylbenzo-crown ether)[20].



The u.v.-vis. spectra of a 1×10^{-5} M aqueous solution of HABA at pH = 6.5 in the presence of increasing amounts of the poly(zwitterion) P show no trace of the hydrazone tautomer. However for [P]/[HABA] ratios higher than 10^{3} the azo band is characterized by a slight hypsochromic shift (λ_{max} progressively decreases from 346 to 339 nm) and a quite strong hyperchromicity (see *Figure 4*). This behaviour suggests that a fraction of the dye is actually bound to the polymer exclusively under its more polar azo form, probably stabilized through specific ion–dipole interactions with the zwitterionic sites.

Binding of the fluorescent 8-anilinonaphthalene-1-sulphonate (ANS)

Fluorescence of ANS in aqueous solution occurs in the range 515–555 nm with a very weak quantum yield of 0.004, but dye transfer from water to less polar environments results in a strong enhancement of the observed fluorescence with a characteristic hypsochromic shift of the emission wavelength[21,22]. As for the previous optical probes, the fluorophore ANS has been extensively used in studies of binding to proteins[21,22], neutral[23–26] or cationic polymers[17].

The addition of increasing amounts of the poly(zwitterion) P to aqueous solutions of ANS induces a very significant enhancement of the fluorescence emission with a maximum wavelength which shifts from about 535 nm for [P]/[ANS] ~ 10 to about 495 nm for [P]/[ANS] ~ 10^{2}–10^{4}. At low and constant dye concentration and in the presence of a large enough and variable excess of P such that only a negligible fraction of the binding sites are occupied, the fluorescence intensity F, in arbitrary units, is given by[26]:

$$\frac{1}{F}=\frac{1}{F'_m}+\frac{1}{K_1}\frac{1}{F'_m[P]_f}\quad\text{with }[P]_f\sim[P]_0\qquad(3)$$

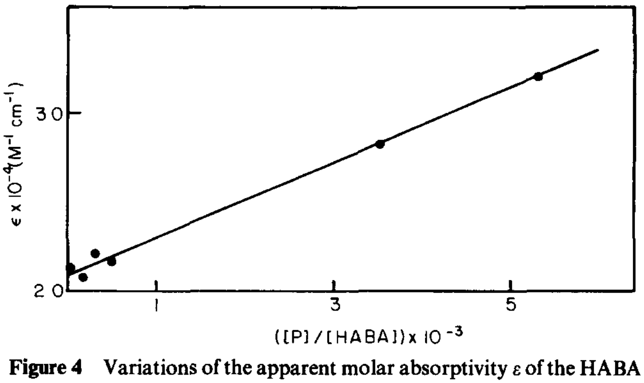


Figure 4 Variations of the apparent molar absorptivity ε of the HABA azo tautomer ($\lambda=346$–339 nm) *versus* poly(zwitterion) concentration at constant dye concentration ($[HABA]_0=1\times10^{-5}$ M) in aqueous solution at pH = 6.5

where $[P]_f$ is the free polymer concentration (in terms of monomeric units), F'_m is the fluorescence intensity when all ANS molecules are bound and K_1 is the first binding constant (see above).

At low and constant polymer concentration and in the presence of a large enough and variable excess of ANS such that only a negligible fraction of the dye is bound, the fluorescence intensity F is given by[26]:

$$\frac{1}{F}=\frac{1}{F_m}+\frac{1}{K}\frac{1}{F_m[\mathrm{ANS}]_f} \quad \text{with } [\mathrm{ANS}]_f \sim [\mathrm{ANS}]_0 \tag{4}$$

where $[\mathrm{ANS}]_f$ is the free fluorophore concentration, F_m is the fluorescence intensity under saturation conditions when all binding sites available are occupied and K is the intrinsic binding constant.

Data related to two series of complementary experiments carried out at constant ANS and P concentrations are plotted in *Figures 5* and *6* according to equations (3) and (4) respectively. They allow one to derive the following values: $K=8.96\times10^3\ \mathrm{M}^{-1}$, $K_1=4.96\times10^2\ \mathrm{M}^{-1}$, $N=K/K_1=18$.

The necessary approximations introduced in equations (3) and (4) may be justified *a posteriori* from the calculated binding constants. For experiments performed at constant ANS concentration, $[\mathrm{ANS}]_0=1\times10^{-6}$ M, the minimum number of available sites is about 5×10^{-5} ($[P]_0=1\times10^{-3}$ M, $N=18$), and the maximum fraction of occupied sites is about 2×10^{-2} in such a way that $[P]_f \sim [P]_0$. In the same manner, for experiments performed at constant P concentration, $[P]_0=1\times10^{-5}$ M, the number of available binding sites is about 5×10^{-7} and, in the least favourable conditions, the maximum fraction of bound ANS is 1×10^{-2} in such a way that $[\mathrm{ANS}]_f \sim [\mathrm{ANS}]_0$. As in the case of MO, the good agreement of the experimental data with both relations (3) and (4) implies that the binding of ANS can be described by a Langmuir adsorption isotherm and is free from any significant cooperative effects, even under conditions where the fraction of occupied sites reaches its maximum value of about 0.68 for $[\mathrm{ANS}]_0=2\times10^{-4}$ M and $[P]_0=1\times10^{-5}$ M, corresponding to about 100 ANS molecules per chain ($\overline{DP}_n \sim 3\times10^3$).

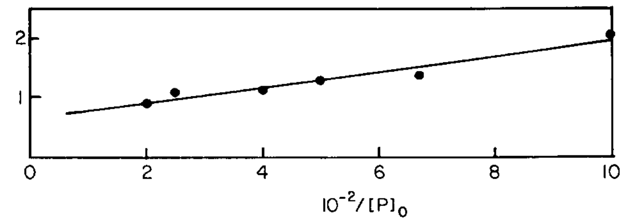


Figure 5 Variations of the fluorescence intensity F *versus* poly(zwitterion) P concentration ($(0.1\text{–}0.5)\times10^{-2}$ M) at constant ANS concentration ($[\mathrm{ANS}]_0=1\times10^{-6}$ M) plotted according to equation (3)

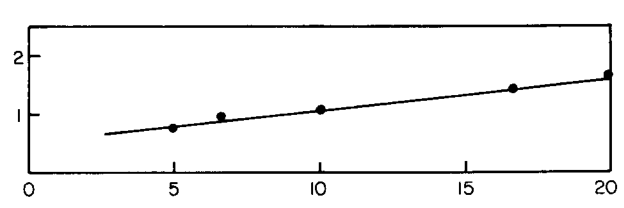
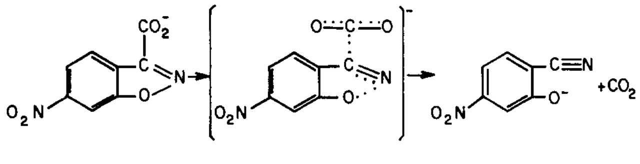


Figure 6 Variations of the fluorescence intensity F *versus* ANS concentration ($[\mathrm{ANS}]_0=(0.5\text{–}2.0)\times10^{-4}$ M) at constant poly(zwitterion) P concentration ($[P]_0=1\times10^{-5}$ M) plotted according to equation (4)

Poly(zwitterion)-catalysed decarboxylation of 6-nitrobenzisoxazole-3-carboxylate (S)

The spontaneous decarboxylation of the carboxylate S into its salicylonitrile salt is a clean unimolecular process which shows an outstanding sensitivity to solvent polarity effects[27]: its first-order rate constant is increased by a factor of 10^8 when transferring S from water to hexamethylphosphoramide, and these polarity effects have been analysed in terms of stabilization or destabilization of the anionic substrate or of its charge-delocalized transition state[28]:



S may thus be considered as a nearly ideal kinetic probe for binding studies, and it has been repeatedly involved in catalysis by micelles[29], neutral[9] or cationic[8,17,30] polysoaps and synthetic bilayer membranes[31]. The S decarboxylation in the presence of a polymer P may be described according to the classical kinetic scheme:

$$\text{products} \xleftarrow{k_f} \mathrm{S} + \mathrm{P} \underset{}{\overset{K}{\rightleftharpoons}} \mathrm{S}\cdot\mathrm{P} \xrightarrow{k_b} \text{products}$$

where k_f and k_b are the rate constants of decarboxylation of the free and of the polymer-bound substrate respectively, and K is the binding constant defined with respect to the concentration of binding sites of size N (N is the average number of monomeric units required to bind one S molecule). In the case where the fraction of bound species remains constant and occupies only a small fraction of the available binding sites ($[P]_0 \gg [S]_0$), the process is pseudo-first-order and the observed decarboxylation rate constant k is given by[9]:

$$\frac{1}{k-k_f}=\frac{1}{k_b-k_f}+\frac{N}{(k_b-k_f)K[P]_0} \tag{5}$$

We performed a series of kinetic experiments in aqueous solution (pH = 8.5) at 30°C using a constant probe concentration, $[S]_0=2.0\times10^{-4}$ M, in the presence of increasingly large amounts of the poly(zwitterion) P, and monitored the appearance of the salicylonitrile salt by u.v. spectrometry at $\lambda=395$ nm (see 'Experimental'). We do not observe any significant change in λ_{max} throughout the decarboxylation process, as already noticed in previous similar studies involving cationic polysoaps[8] or poly(crown ethers)[9]: the possible binding of the released phenate to the polymer may remain limited, or the u.v. spectra of the free and bound species may be similar. In all cases the reaction obeys first-order kinetics up to conversions τ higher than about 0.60 (see *Figure 7*). The simplifying assumptions of equation (5) are fulfilled and, moreover, no retardation arising from possible competitive binding of the released by-product to the polymer, as far as the decarboxylation proceeds, may be observed. The experimental data given in *Table 1* clearly show that the poly(zwitterion) P actually increases the observed rate constant k significantly (runs 3–8), while its

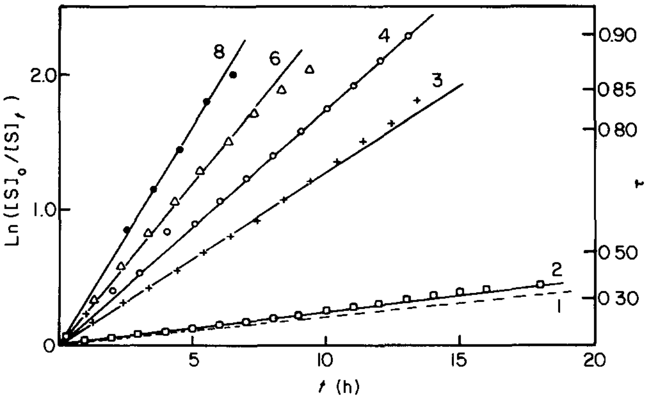


Figure 7 Pseudo-first-order kinetics of S decarboxylation (pH = 8.5, t = 30°C) in the presence of poly(zwitterion) P. The figures on the curves refer to the run numbers in *Table 1*

Table 1 Degree of conversion τ and pseudo-first-order rate constant k of the decarboxylation of S in aqueous solution ($[S]_0 = 2.0 \times 10^{-4}$ M, pH = 8.5) at 30°C in the presence of poly(zwitterion) P

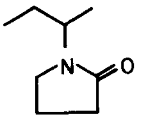
Run	$[P]_0$ ($\times 10^2$ M)	τ	k ($\times 10^5$ s^{-1})
1^a	0	0.63	0.572
2	4.84×10^{-3}	0.64	0.694
3	1.37	0.75	3.64
4	1.74	0.90	4.81
5	2.16	0.81	5.25
6	3.47	0.83	6.89
7	6.08	0.63	8.00
8	7.80	0.83	9.17
9^b	1.97	0.36	0.942
10^b	3.94	0.42	1.07

^{a}Literature values: 0.74×10^{-5} s^{-1} (ref. 27), 0.60×10^{-5} s^{-1} (ref. 17)
^{b}Polymer is poly(2-vinylpyridinium sulphopropylbetaine) of $\bar{M}_w = 0.783 \times 10^5$

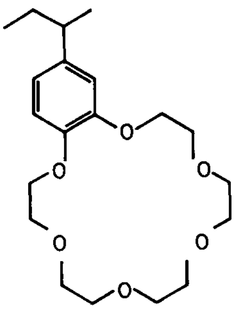
homologue poly(2-vinylpyridinium sulphopropylbetaine) P′ has only a very weak catalytic efficiency (runs 9 and 10): the higher steric hindrance around the zwitterionic site provided by the chain backbone probably prevents efficient binding of the bulky probe to the aromatic polymer. The variations of the pseudo-first-order rate constant k may be readily analysed according to equation (5) (see *Figure 8*), and they lead to: $k_b \sim 1.44 \times 10^{-4}$ s^{-1} and $KN^{-1} = 22$ M^{-1}. Combination of these results with those derived from the kinetic studies in conditions where the probe concentration far exceeds the available binding sites, $[S]_0 \gg [P]_0$, allows one in principle to derive separate values of the binding constant K and the size of the binding site N[8,9]: for our system, unfortunately, the observed rate constant is not increased enough with respect to k_f to be able to perform reliable calculations (run 2 in *Table 1*).

DISCUSSION

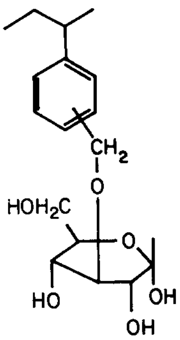
We compare in *Table 2* the first binding constant K_1^* normalized to 10^5 g of polymer (a constant currently used in the literature to take into account the difference of molecular weight of the monomeric units of the different chains) and the characteristic response of the bound probes MO, ANS and S for the poly(sulphopropylbetaine) under study and for some representative synthetic water-soluble polymers: poly(N-vinylpyrrolidone) (PNVP)[12,13,23,24], poly(vinylbenzo-18-crown-6) (PVBC)[9,14,26], poly(D-glucopyranose-3-oxymethylstyrene) (PVBS)[16,25] and a partially laurylated poly(2-ethyl-1-vinylimidazole) (PVIL)[17] considered respectively as highly dipolar chain (PNVP), neutral (PVBC, PVBS) or cationic (PVIL) polysoaps.



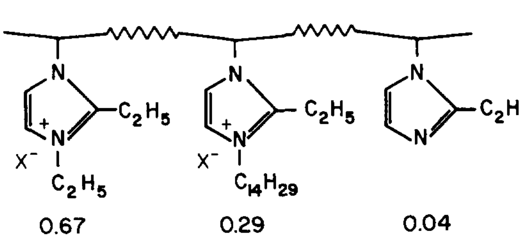
PNVP



PVBC



PVBS



PVIL

In a first approximation if the two most interacting systems PVBC–MO and PVIL–S are considered separately, the binding efficiency of the poly(zwitterion) P is of the same order of magnitude as that of the reference polymers. According to the data related to PVBC[14,26] and PVBS[16,25], the average size of the binding site, N, remains the same for the two aromatic sulphonates MO and ANS: this is not observed for the poly(zwitterion) looking like PVBC for MO and looking like PVBS for ANS, and we have no explanation for such behaviour.

The responses of the probes bound to the poly(sulphopropylbetaine) are not too different from that of the free ones. The λ_{max} of MO absorption is not changed, and that of ANS fluorescence does not show the very characteristic strong hypsochromic shift observed when transferring this fluorophore from pure water to a hydrophobic microenvironment, as in the cases of the polysoaps. The first-order rate constant of the S decarboxylation, k_b, is increased by only a factor of about

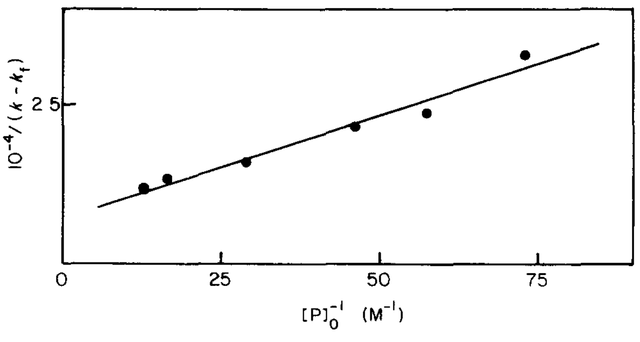


Figure 8 Variations of the pseudo-first-order rate constant k *versus* poly(zwitterion) P concentration ($[P]_0 = (1.37\text{–}7.80) \times 10^{-2}$ M) at constant substrate concentration ($[S]_0 = 2 \times 10^{-4}$ M) plotted according to equation (5)

Table 2 Comparison of the binding of MO, ANS and S to some water-soluble polymers and to the poly(zwitterion) P

	MO				ANS				S			
Polymer	λ (nm)	N	K_1 ($\times 10^{-3}$ M^{-1})	K^* ($\times 10^{-4}$)	λ (nm)	N	K_1 ($\times 10^{-2}$ M^{-1})	K^* ($\times 10^{-4}$)	N	K_1 (M^{-1})	K_1^* ($\times 10^{-4}$)	k_b/k_f
PNVP	470[11]			2.7	480[23]		0.38[a]	3.42[a]				
	475[13]			1.8	480[24]		1.35[a]	12.1[a]				
PVBC	435[15]	85	1.13	33.4	462[26]	80	6.73	19.9	40[9]	8.5	0.251	2300
PVBS	445[16]	20	0.09	3.0	475[25]	20	1.3	4.1				
PVIL	417[17]				476[17]		4.5[a]	12.8[a]		231[17]	6.57	440
P (this work)	464	70	0.18	5.12	495	18	4.96	14.1		22	0.626	25

[a] Approximately estimated values derived from curves given in the corresponding references

PNVP, poly(*N*-vinylpyrrolidone); PVBC, poly(vinylbenzo-18-crown-6); PVBS, poly(D-glucopyranose-3-oxymethylstyrene; PVIL, poly(2-ethyl-1-vinylimidazole) partially laurylated

25, in comparison to 170 for the zwitterionic micelle *N*,*N*-dimethyl-*N*-dodecylglycine[32], 440 for the cationic polysoap PVIL[17] and 2300 for PVBC[9]. The general behaviour of the poly(zwitterion) is more or less similar to that of PNVP. The polarity of the microenvironment of the bound probes may be identified with that of the organic solvents leading to identical responses. Following this simplifying approach the local polarity close to the zwitterionic chain in aqueous solution is slightly higher than that of methanol: λ_{max}(ANS) ~ 476 nm[21] to 492 nm[22] and k(S,30°C) = 2.5×10^{-4} s^{-1} (ref. 27) in this alcohol. On the basis of previous comparisons, the behaviour of the anionic probe–poly(zwitterion) systems in dilute aqueous solution may be rationalized as follows:

(a) Specific ion-dipole interactions involving the zwitterionic lateral groups of the poly(sulphopropylbetaine) are the major driving force for the efficient binding of the low-molecular-weight anionic solutes to the macromolecular chain. It may be emphasized that the local dipole moment of ammonium sulphopropylbetaine is probably very high: $\mu = 27.5$ D for δ-aminobutyric acid in its zwitterionic form in aqueous solution, as a model compound[33]. Hydrophobic interactions are probably quite negligible in spite of the relatively apolar methacrylate backbone of the chain (see below).

(b) In water solution at 25°C (which is quite a θ solvent[2]) the poly(zwitterion) chain shows a weak expansion (the viscosimetric expansion coefficient α_η may be estimated[2] to be only about 1.09 for our sample of very high molecular weight ($\bar{M}_w \sim 3.23 \times 10^6$)), but a very tightly coiled or collapsed conformation similar to that of solvent-impenetrable spheres, as assumed in the case of PVBC[9,15], may be ruled out: $[\eta]_{expt} \sim 1.00$ dl g^{-1} *versus* $[\eta] = 0.018$ dl g^{-1} according to Einstein's law ($[\eta] = 2.5\bar{v}$, with specific volume $\bar{v} \sim 0.740$ ml g^{-1} (ref. 2)). Thus because of conformation reasons as well as structural features (the oxyethylene spacer between the backbone and the zwitterion is already weakly polar and hydrophilic), the development of apolar and hydrophobic binding sites by the folded polymer chains seems very unlikely. The binding of the probes, probably as guest molecules closed to zwitterionic segments, may occur in such a way that the bound species remain exposed to water.

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REFERENCES

1. Monroy Soto, V. M. and Galin, J. C. *Polymer* 1984, **25**, 121
2. Monroy Soto, V. M. and Galin, J. C. *Polymer* 1984, **25**, 254
3. Salamone, J. C., Volksen, W., Olson, A. P. and Israel, S. C. *Polymer* 1978, **19**, 1157
4. Schulz, D. N., Peiffer, D. G., Agarwal, P. K., Larabee, J., Kalades, J. J., Soni, L., Handwerker, B. and Garner, R. T. *Polymer* 1986, **27**, 1734
5. Clinton, R. and Laskowski, S. *J. Am. Chem. Soc.* 1948, **70**, 3135
6. Borsche, W. *Chem. Ber.* 1909, **42**, 1310
7. Lindemann, H. and Cissee, H. *Justus Liebig Ann. Chem.* 1929, **469**, 44
8. Suh, J., Scarpa, I. S. and Klotz, M. *J. Am. Chem. Soc.* 1976, **98**, 7060
9. Shah, S. C. and Smid, J. *J. Am. Chem. Soc.* 1978, **100**, 1426
10. Forbes, W. F. and Milligan, B. *Austr. J. Chem.* 1962, **15**, 841
11. Klotz, I. M. and Shikama, K. *Arch. Biochem.-Biophys.* 1968, **123**, 551
12. Scholtan, W. *Makromol. Chem.* 1953, **11**, 131
13. Takagishi, Y. and Kuroki, N. *J. Polym. Sci., Polym. Chem. Edn.* 1973, **11**, 1889
14. Lok-hei Wong and Smid, J. *J. Am. Chem. Soc.* 1977, **99**, 5637
15. Sinta, R. and Smid, J. *Macromolecules* 1980, **13**, 339
16. Kobayashi, K. and Sumimoto, H. *Macromolecules* 1980, **13**, 234
17. Kunitake, T., Shinkai, S. and Hirotsu, S. *J. Org. Chem.* 1977, **42**, 306
18. Klotz, I. M., Walker, F. M. and Pivan, R. B. *J. Am. Chem. Soc.* 1946, **68**, 1486
19. Moriguchi, I., Fushimi, S., Oshima, C. and Kaneniwa, H. *Chem. Pharm. Bull.* 1970, **18**, 2447
20. Lok-Hei Wong and Smid, J. *Polymer* 1980, **21**, 195
21. Stryer, L. *J. Mol. Biol.* 1965, **13**, 482
22. Turner, D. C. and Brand, L. *Biochemistry* 1968, **7**, 3381
23. Kono, K., Nagai, T. and Nogami, H. *Chem. Pharm. Bull.* 1973, **21**, 366
24. Kirsh, Yu E., Soos, T. A. and Karaputadze, T. M. *Eur. Polym. J.* 1979, **15**, 223
25. Kobayashi, K. and Sumimoto, H. *Polym. J.* 1981, **13**, 517
26. Kimura, K. and Smid, J. *Macromolecules* 1982, **15**, 966
27. Kemp, D. S. and Paul, K. G. *J. Am. Chem. Soc.* 1975, **97**, 7305
28. Kemp, D. S., Cox, D. D. and Paul, K. G. *J. Am. Chem. Soc.* 1975, **97**, 7312
29. Kunitake, T. and Shinkai, S. *Adv. Phys. Org. Chem.* 1980, **17**, 464

30 Yamazaki, N., Nakahama, S., Hirao, A. and Kawabata, J. *Polym. J.* 1980, **12**, 231

31 Kunitake, T., in 'An Introduction to Specialty Polymers', (Eds. N. Ise and I. Tabushi), Cambridge University Press, Cambridge, 1983, p. 191

32 Bunton, C. A., Kamego, A. A., Minch, M. J. and Wright, J. L. *J. Org. Chem.* 1975, **40**, 1321

33 Grunwald, E., Highsmith, S. and Ting-Po, I., in 'Ions and Ion Pairs in Organic Reactions' (Ed. M. Szwarc), Wiley, New York, 1974, Vol. 2, p. 448