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ON THE EQUILIBRIUM PROPERTIES OF POLYELECTROLYTE SOLUTIONS: ISOTHERMAL-CALORIMETRY DATA *

V. CRESCENZI

Dipartimento di Chimica, Università di Roma "La Sapienza", 00185 Roma (Italy)

ABSTRACT

Study of the solution properties of natural and synthetic polyelectrolytes covers a relatively large field of both fundamental and application-oriented interest.

This paper is concerned with equilibrium properties and offers examples of the usefulness of isothermal microcalorimetry investigations for a better understanding of the typical processes taking place in dilute aqueous solutions of ionic polymers, namely, ionization of weak polyacids, interactions between polysaccharidic macroions and divalent counterions, and the binding of certain dyes and drugs by DNA.

INTRODUCTION

This paper presents isothermal microcalorimetry data on dilute aqueous solutions of synthetic and natural polyelectrolytes, almost entirely collected over the last ten years by university researchers in Trieste and Rome, and is not intended to be a comprehensive review of the experimental thermodynamics of ionic macromolecules in solution. Nevertheless, the results discussed seem sufficiently representative of a few, typical calorimetric contributions to this wide field.

Attention will be particularly focussed on the thermodynamics (298 K) of:

(1) proton dissociation (weak polyacids);
(2) divalent counterion binding;
(3) dyes and/or drugs binding by DNA.

POLYELECTROLYTE SOLUTIONS

Direct, accurate determination of changes in thermodynamic state functions accompanying certain typical reversible processes in polyelectrolyte

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solutions is undeniably a valuable achievement. Such processes include, for example: dissociation of interdependent ionizable groups along the chains, binding or chelation of counterions by macroions, and changes in chain shape or conformation when one of the parameters controlling the equilibrium in the polymer solvent system is changed.

All these phenomena have been extensively studied by many workers for a number of synthetic and natural polyelectrolytes, using a variety of techniques that sometimes lead to a more or less straightforward evaluation of the associated free energy changes. However, the important goal of describing accurately how such free energy changes are built up by enthalpic and entropic contributions, e.g., with the aid of direct microcalorimetric measurements, has been achieved in relatively few instances.

Enthalpy of dissociation of synthetic polyelectrolytes [1]

Polycarboxylic acids

Let us first examine the case of four polycarboxylic acids, namely: poly(acrylic acid) (PAA), maleic acid–ethylene copolymer (MAE), maleic acid–propylene copolymer (MAP), and maleic acid–ethyl vinyl ether copolymer (MAEVE), whose chains gradually expand and solvate during the charging process (neutralization with NaOH in water at 298 K). The enthalpy of dissociation data, $\Delta_{diss}H$, are reported in Fig. 1 versus the degree of neutralization ($\alpha = 1$ corresponds to half-neutralization in the case of the maleic acid copolymers).

The enthalpy of the polyelectrolytes becomes more negative as α increases, with the notable exception of MAEVE, for which $\Delta_{diss}H > 0$ over the whole range of values studied so far. Figure 1 clearly indicates that the relationship between $\Delta_{diss}H$ and α greatly depends on the nature of the side chain and/or the structure of the comonomers regularly alternating with the maleic acid residues. Using potentiometric titration, pK_a, data,

$$pK_a = pH + \log\frac{1-\alpha}{\alpha}$$

the total free energy for the removal of a mole of protons from the polycarboxylate chains at each given α value, $\Delta_{diss}G = 2.303pK_a$, was readily calculated. Lastly, the associated entropy change, $\Delta_{diss}S$, was estimated from both the calorimetric and the potentiometric data

$$\Delta_{diss}S = (\Delta_{diss}H - \Delta_{diss}G)/T$$

The results are shown in Fig. 2.

The entropy of ionization is distinctly more negative for PAA than for the maleic acid copolymers when $\alpha < 1$. In the author's opinion, this can be at least partly ascribed to stronger immobilization of water molecules (solvation) during PAA charging.

For a given polyelectrolyte in water, the overall change in heat content of

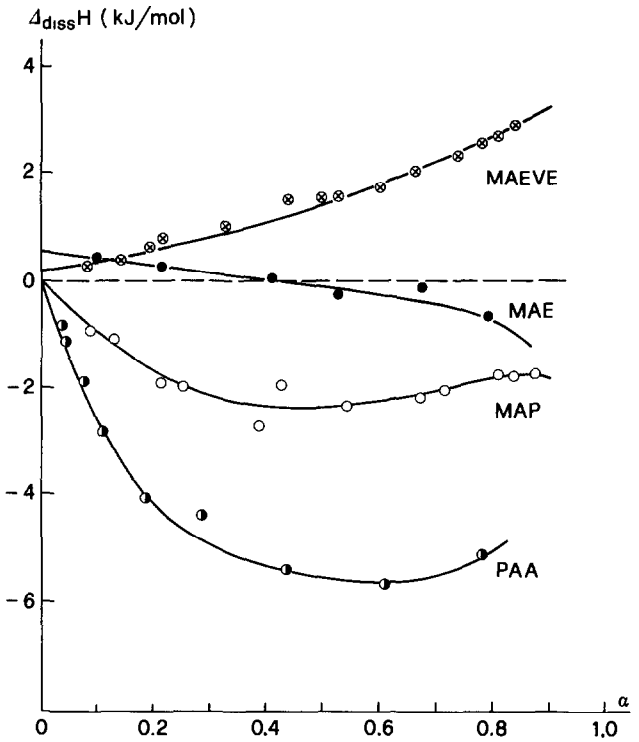

Fig. 1. Dependence of the enthalpy of dissociation, $\Delta_{diss}H$, upon the degree of neutralization, α, for aqueous solutions of: poly(acrylic acid) (PAA); 65.0 moles of charge m^{-3}; maleic acid–propylene copolymer (MAP); 20.0 moles of charge m^{-3}; maleic acid–ethylene copolymer (MEA); 20.0 moles of charge m^{-3}; maleic acid–ethyl vinyl ether copolymer (MAEVE); 20.0 moles of charge m^{-3}.

the system for proton removal from a chain at a given α value may be considered as the algebraic sum of many terms stemming, for example, from the increase in charge density, the associated change in average chain dimensions, and the hydration of ionizable groups and the chain backbone (all of which determine the value of the local effective dielectric constant), as well as changes in the extent of interaction between macroions and counterions (Na^+ in this case).

Likewise, the overall entropy of dissociation will reflect all these phenomena, of which the steadily increasing solvation of the polyelectrolyte with increasing α may be supposed to play a dominant role.

Let us now consider another synthetic polyelectrolyte: poly(methacrylic acid) (PMA). When uncharged, it assumes tightly globular conformations in water that only expand within a critical range of α values to yield open, solvated conformations. This unwinding process is a cooperative conformational transition, usually expressed as:

globule $\overset{pH}{\rightarrow}$ expanded coils

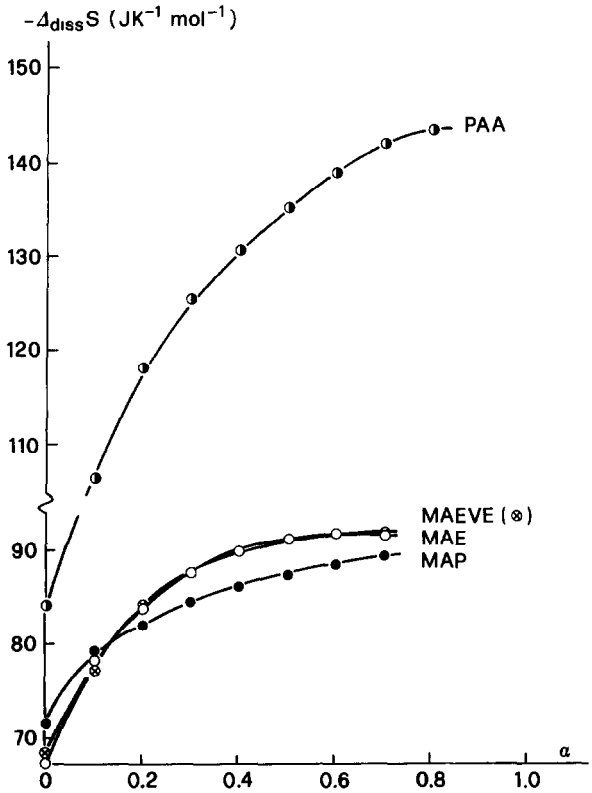

Fig. 2. Entropy of dissociation, $\Delta_{diss}S$, of poly(acrylic acid) (PAA) and of the maleic acid copolymers of Fig. 1 in water at 298 K.

Hydrophobic interactions are regarded as one of the factors responsible for the tight globular state of uncharged PMA chains.

The calorimetric $\Delta_{diss}H$ data for PMA in water at 298 K in Fig. 3 clearly show that its dissociation behaviour is abnormal. This is evidently due to the globule → coil transition.

If this pH-induced transition did not take place, the $\Delta_{diss}H$ values would vary smoothly and continuously with α, as in Fig. 1. The α range within which the transition is thought to occur is also clearly, and concordantly with the calorimetric data, defined by the potentiometric titration plots of PMA. The normalized areas under the peaks in Fig. 3 (dashed baselines) can thus be taken to represent the enthalpy of conformational transition of PMA, $\Delta_c H$. It is interesting to note that the shapes of the curves appear to depend on polymer stereoregularity and/or concentration. However, as indicated in Fig. 3, the $\Delta_c H$ values are nearly independent of these variables within the range of experimental error. The microcalorimetric data for PMA are summarised in Table 1. The values for the free energy change, $\Delta_c G$, associated with the globule → coil transition by means of potentiometric titration under the same experimental conditions are also shown.

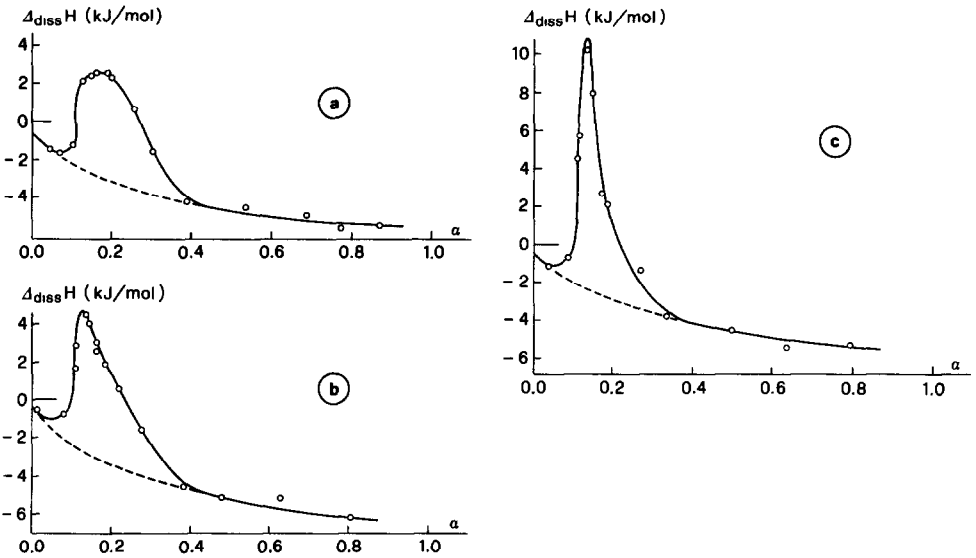

Fig. 3. Dependence of the enthalpy of dissociation, $\Delta_c H$, of poly(methacrylic acid) (PMA) on the degree of neutralization, α, in water at 298 K. The $\Delta_c H$ values have been calculated from the areas under the curves (dashed baselines). (a) Syndiotactic PMA (MW = 9×10^3), $\Delta_c H$ = 0.99 kJ mol^{-1}, polymer concentration = 64.0 moles of charge m^{-3}. (b) Conventional PMA (MW = 3.4×10^5), $\Delta_c H$ = 1.03 kJ mol^{-1}, polymer concentration = 64.6 moles of charge m^{-3}. (c) Conventional PMA (MW = 3.5×10^5), $\Delta_c H$ = 1.0 kJ mol^{-1}, polymer concentration = 20.9 moles of charge m^{-3}.

On the assumption that our $\Delta_c H$ values are not significantly different from the standard-state values and that our $\Delta_c G$ data are reliable, the $\Delta_c S$ values are those given in Table 1.

TABLE 1

Thermodynamics of the pH-induced conformational transition of poly(methacrylic acid)

		$\Delta_c H$ (kJ mol^{-1})	Conc. (mole of charge per m^3)	$\Delta_c G$ (kJ mol^{-1})	Conc. (mole of charge per m^3)	$\Delta_c S$ (J mol^{-1} K^{-1})
(1) 298 K						
in water	C	1.02	64.0	0.77	18.0–65.0	0.84
	C	1.01	21.0			
	S	0.99	64.0	0.77	33.0–65.0	0.71
in 0.5 M NaCl	C	0.53	21.0	0.69	34.0	−0.54
	S	0.51	63.0	0.69	27.0–65.0	−0.63
(2) 318 K						
in water	C	1.52	59.0	0.69	32.0–56.0	2.51
	S	1.44	59.0	0.66	32.0–34.0	2.47

C = Conventional PMA, MW = 3.4×10^5. S = Syndiotactic PMA, MW = 9.0×10^5.

We believe that the observed increase of $\Delta_c H$ with temperature indicates that we are dealing with a hydrophobic-force driven phenomenon, in agreement with earlier studies.

Following the pioneering study briefly described above, attention was directed to several hypercoiling synthetic polyacids, namely (1 : 1) copolymers of maleic acid with hydrophobic comonomers, such as α-olefins and alkyl-vinyl ethers.

Here isothermal microcalorimetry, together with dilatometry, provided valuable, detailed insights into the thermodynamics of proton ionization and pH-induced conformational transitions governed by hydrophobic interactions [2–4].

This short section on the application of microcalorimetry to the study of synthetic polyelectrolytes in aqueous solutions may be concluded with the reminder that a series of accurate heat of dilution measurements have also provided a wealth of information on their thermodynamic behaviour. Heat of dilution and heat of dissociation data, indeed, have laid the theoretical foundation for the study of such solutions [5].

Divalent counterion binding

Ionic polysaccharides

One of the salient properties of ionic polysaccharides in aqueous media is their strong binding of counterions, in particular, divalent metal ions, eventually leading to gelation, depending on polymer and counterion concentration and on temperature. Qualitatively speaking, this is to be expected since the chains of these polyelectrolytes may have a relatively high fixed-charge density. On the other hand, the steric regularity of natural polysaccharide chains and the chemical constitution of their repeating units may add to their "polyelectrolytic" behaviour, certain very interesting features (including counterion binding) that are specifically dependent on macroion structure and conformation.

Binding of Cu^{2+} ions by sodium alginate [6]. The results of calorimetric experiments on the interaction of Cu^{2+} (added $Cu(ClO_4)_2$) with aqueous sodium alginate (in 0.05 M $NaClO_4$ solution) are shown in Fig. 4.

The calorimetric data, as a function of the stoichiometric ratio, $R = (Cu^{2+})/N$ (moles of copper(II) per equivalent of alginate in solution), show that $\Delta_{mix} H$ is always positive and steadily increases with the added Cu^{2+} ion concentration.

The dilatometric results in Fig. 4 were obtained under identical experimental conditions. They demonstrate that there is a net volume increase on binding of Cu^{2+} ions by sodium alginate. Lastly, a few equilibrium dialysis data are reported if Fig. 5 in the usual Scatchard-plot form. They show that a value of 4×10^3 (1/equiv) can be assigned to the apparent equilibrium constant of Cu^{2+} binding on the assumption that it simply involves one fixed

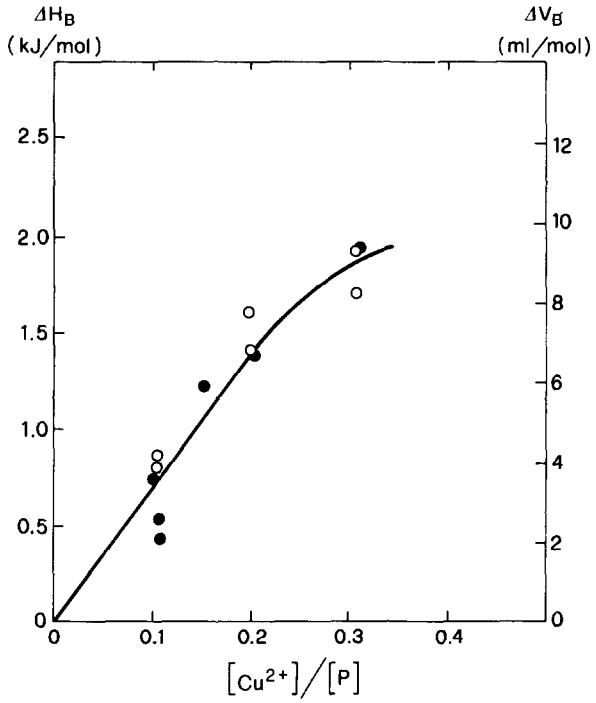

Fig. 4. Enthalpy changes (●) (left-hand scale) and volume changes (○) (right-hand scale) on the addition of $Cu(ClO_4)_2$ to the sodium salt form ($\alpha = 1$) of alginic acid in 0.05 M $NaClO_4$ at 298 K. $[Cu^{2+}]/[P]$ stands for the molar ratio of total copper(II) to polymer repeating units.

alginate charge per Cu^{2+} ion bound and no interdependence between binding sites. It can also be estimated that $\Delta_b G = -20.5$ (kJ per mole of binding reaction), and that in calorimetric and dilatometric experiments at, say $(Cu^{2+})/N = 0.05$, more than 90% of the Cu^{2+} ions are bound to the alginate chains.

The limiting slopes in Fig. 4 give the differential enthalpy ($\Delta_b \bar{H} = 8.4$ kJ mol^{-1}), the differential volume of binding ($\Delta_b \bar{V} = 39$ cm^3, per mole of copper bound), and $\Delta_b \bar{S} = 96.2$ J mol^{-1} K^{-1} (per mole of complex).

In conclusion, Cu^{2+} ions are extensively bound by alginate and this process is entirely entropy driven. The link between the changes in $\Delta_b V$ and $\Delta_b S$ may be readily, although qualitatively, established by assuming, by analogy with similar evidence for other polycarboxylates, that interaction between $-COO^-$ groups and Cu^{2+} ions releases a relatively large number of water molecules from the solvation shells of both species, thus producing a net gain of molecular degrees of freedom. The $(Cu^{2+})/N$ ratios at which this occurs are not higher than about 0.4 since higher Cu^{2+} ion concentrations lead to gel formation and, hence, to experimentally intractable systems.

Binding of Cu^{2+} and Ca^{2+} ions by sulfated polysaccharides [7]. Let us now consider calorimetric data on the enthalpy of Cu^{2+}, Mg^{2+} and Ca^{2+} ion

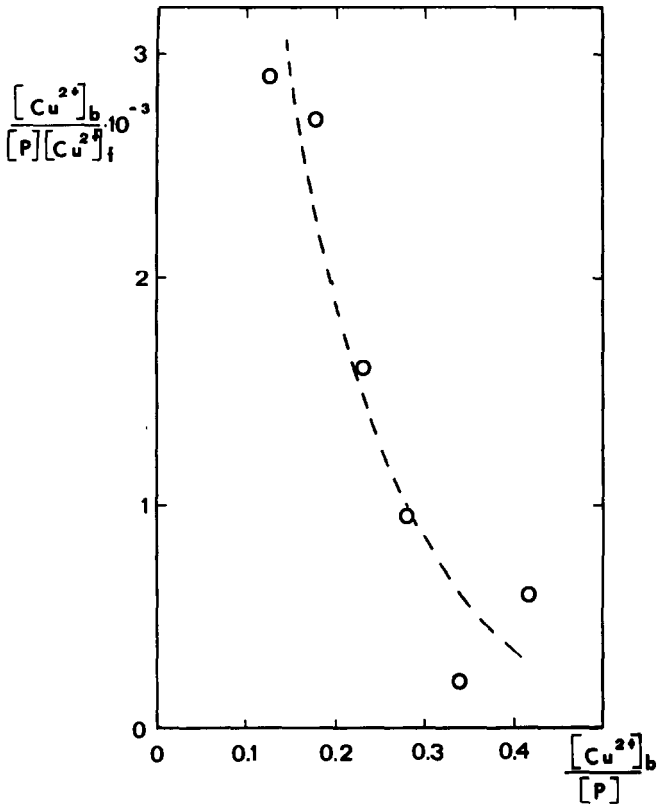

Fig. 5. Scatchard plot from equilibrium dialysis measurements for the binding of Cu^{2+} onto sodium alginate in 0.05 M $NaClO_4$ at 298 K. Polymer concentration, [P], was 12.0 moles of charge m^{-3}. $[Cu^{2+}]_b$ and $[Cu^{2+}]_f$ stand for bound and free copper molar concentrations, respectively.

binding by iota-carrageenan (segments), heparin, and dextran sulfate (one sample with two sulfated groups per glucose residue) in dilute aqueous solution. The anionic charge densities of heparin and dextran sulfate are approximately twice that of iota-carrageenan (one sample having 1.7 sulfate groups per repeating unit, on average) but very different chain architectures. Heparin and iota-carrageenan (segments) appear to have relatively stiff backbones with an essentially regular structure, and are therefore liable to assume an ordered chain conformation in solution. (In heparin, which bears $-SO_3$ and $-COO^-$ groups, there is, in reality, a non-strictly regular substitution: the sample mentioned here had $(COO^-)/(SO_3) = 1/2.3$.) In terms of enthalpy, binding of counterions by iota-carrageenan at very low R values is not too dissimilar from that by dextran sulfate or heparin. If the (M^{2+})/(polysaccharide) concentration ratio values are increased, however, $\Delta_b H$ becomes negative. If one assumes that binding of M^{2+} ions by $-SO_3$ groups along the polysaccharidic chains remains an intrinsically endothermic

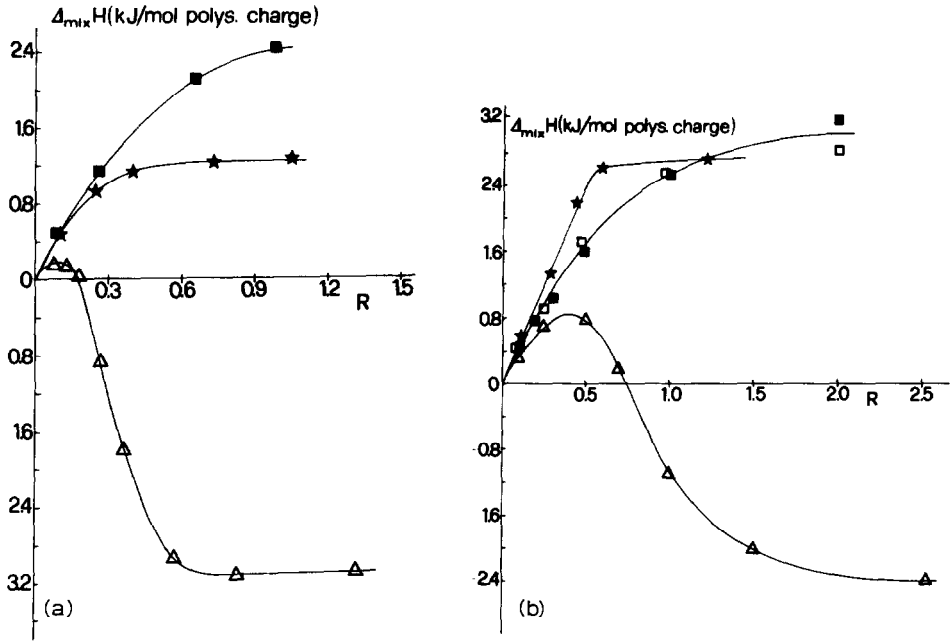

Fig. 6. Enthalpy, $\Delta_{mix}H$, of Ca^{2+} ions binding (a) and of Cu^{2+} ions binding (b), in water at 298 K, by heparin (★), dextran sulfate (average mol. wt. = 10^4; degree of substitution = 2) (□), dextran sulfate (average mol. wt. = 4×10^4; degree of substitution = 2) (■); and iota-carrageenan (segments) (△). R is the stoichiometric ratio between added M^{2+} ions and polyelectrolyte (fixed) concentration, both in moles of charge per m^3. Polymer conc. = 50.0 moles of charge m^{-3}. All heats are corrected for dilution effects.

process (see Fig. 6) for dextran sulfate and heparin, it may be deduced that M^{2+} ion binding by iota-carrageenan promotes an additional, exothermic process, very like a conformational change, via screening of the fixed charges.

For a better illustration of the peculiar features exhibited by iota-carrageenan and to evaluate the enthalpy of the conformational change, the experimental data in Fig. 6 are plotted in differential form, as shown in Fig. 7, which shows that Ca^{2+} ions induce a sharper, more cooperative conformational transition of iota-carrageenan chains that Cu^{2+} ions can achieve. The enthalpy change associated with such a transition, which is assumed to be proportional to the area between the experimental curves and the dashed reference baseline in Fig. 7, turns out to be practically the same, i.e., -4.2 kJ mol^{-1} (polysaccharide), and is independent of the nature of the counterion.

The branched nature of the dextran backbone and its rather irregular distribution of charged ($-SO_3$) groups clearly make dextran sulfate incapable of assuming any ordered conformation on counterion binding. That the same is equally true of the heparin biopolymer, which might be expected to assume ordered conformations, may seem surprising.

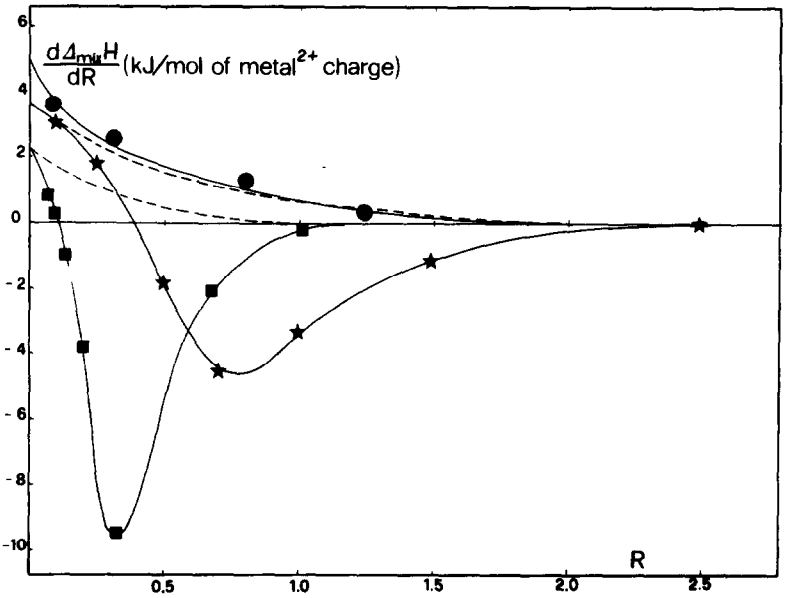

Fig. 7. Differential enthalpy of mixing $d\Delta_{mix}H/dR$ (see Fig. 6) of M^{2+} ions binding by polysaccharides in aqueous systems: dextran sulfate–Cu^{2+} (average mol. wt. = 4×10^4; degree of substitution = 2) (●); iota-carrageenan–Ca^{2+} (■); iota-carrageenan–Cu^{2+} (★); from data of Fig. 6.

Drugs and/or dye binding by DNA [8–10]

A number of papers have appeared on the peculiar features displayed by various ionic dyes in aqueous solutions of polyelectrolytes. Dye binding by biopolymers, in particular, has been closely studied, as it provides an example of small molecule–macromolecule interactions of major importance in biochemistry. Moreover, certain dyes are known to possess antibacterial and mutagenic activities. To summarize the dye binding behaviour by synthetic polyelectrolytes in a very qualitative fashion, one generally speaks in terms of dye bound by electrostatic forces in monomeric form by the macroions (for very low dye-to-polymer concentration ratios) and of "stacking" of bound dye molecules on macroions.

With biopolymers, in addition to these binding mechanisms, very selective interactions may take place with certain dyes through mechanisms involving specific sites along the biopolymer chain and leading, in the special case of DNA, to "intercalation" of dye molecules. A typical example is afforded by ethidium bromide (EB).

The interaction in vitro of antibiotic molecules, such as actinomycin, daunomycin, etc., with DNA has received ever-increasing attention since their complex structure means that the mechanism through which they bind to DNA may involve a complicated array of "interactions".

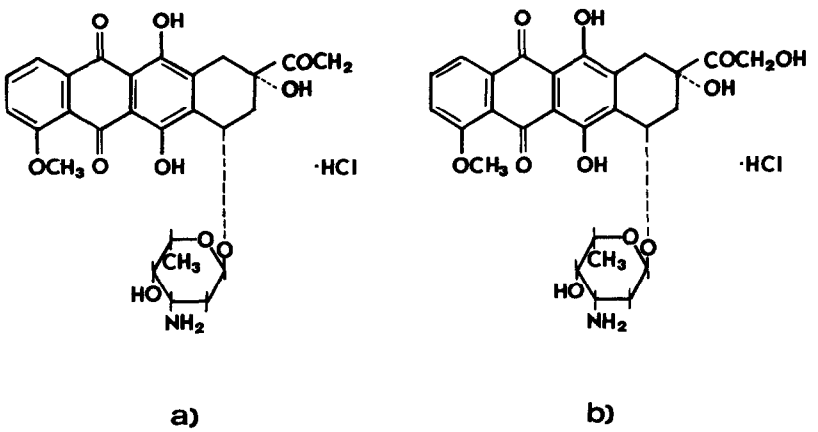

Fig. 8. Structure of daunomycin (a) and adriamycin (b).

The physical chemistry of aqueous solutions of many dyes, e.g., the acridines, is also of interest, since they can form dimers, trimers and higher aggregates by "vertical-stacking", even in very dilute solutions, with concomitant and often dramatic changes in their spectral properties.

Data on the thermodynamics of dimer formation in aqueous solution at 298 K of EB, proflavine (PF), and acriflavine (AF), and the antibiotics daunomycin (D) and adriamycin (A) (Fig. 8), are reported in Table 2.

It is interesting to note that dimerization of adriamycin and daunomycin certainly involves stacking of a pair of molecules with overlap of their fused-ring systems, similar to that of PF and EB. This stacking process involves an enthalpy decrease of 37.7 kJ mol^{-1}, again not dissimilar to that for other aggregating systems.

As to the interactions with DNA of the species in Table 2, convincing spectroscopic, viscometric and ultracentrifugation evidence has been pro-

TABLE 2

Thermodynamics of dimerization in aqueous solution at 298 K

	K_D (M^{-1}) [a]	$-\Delta_D H$ (kJ mol^{-1}) [b]	$-\Delta_D S$ (J mol^{-1} K^{-1})
EB(H_2O)	41	31.8 ± 1.3	75.8
PF(H_2O)	477	26.4 ± 2.5	37.7
PF(0.5 M KCl)	1840	35.6 ± 2.1	58.6
AF	84	29.3	46.0
D	3×10^3	38.1 ± 1.3	58.6
A	1×10^3	40.2 ± 2.5	75.3

[a] K_D values, for the equilibrium: 2(monomer) ↔ dimer, have been obtained from spectroscopic data following the analysis of Schwarz et al. [11].

[b] The $\Delta_D H$ values are calorimetric. EB = ethidium bromide; PF = proflavine; AF = acriflavine; D = daunomycin; A = adriamycin.

vided by several workers that EB and PF when "strongly" bound by native DNA are actually intercalated between adjacent base pairs along the biopolymer double helical chains. This also appears to be true for several other dyes with a suitable molecular structure and fused aromatic rings. "Strong" binding, however, characterized by association constants greater than 10^5–10^6 M^{-1}, is generally limited to molar ratios (R) of bound dye per DNA nucleotide smaller than 0.15–0.20 in dilute aqueous solutions of relatively low ionic strength. At higher R values, where all sites available for intercalation appear to be saturated, weaker binding develops. In this case, additional dye molecules apparently interact with the "surface" of the rod-like macroions of DNA only.

Similar qualitative behaviour has been reported for the interaction of DNA with actinomycin (Act) and D. Intercalation of their aromatic moieties is thought to occur at $R = 0.1$.

The calorimetric data for the heat of intercalation of DNA (native, calf thymus) with PF and EB in 0.015 and 0.1 M phosphate buffer (pH 7) at 298 K show that in the R range within which intercalation may be supposed to take place the average $\Delta_b H$ values are -25.9 and -28.0 kJ mol^{-1} dye bound for EB and -28.0 and -31.8 kJ mol^{-1} for PF, depending on the ionic strength (the fraction of dye bound was derived in each case from equilibrium dialysis experiments).

The results with D indicate that in the R range within which D may be supposed to be intercalated into DNA the enthalpy of interaction is constantly negative (-27.2 ± 2.1 kJ mol^{-1}).

Despite the inaccuracy inherent in each set of measurements, it can be assumed that self-association of PF, EB and D and their "strong interaction" with DNA are characterized by relatively similar heat effects. This can hardly be a mere coincidence. On the contrary, it is original evidence that strong binding of the three species considered with DNA is synonymous with intercalation. Intercalation of an aromatic polycyclic molecule such as PF into the native structure of DNA must involve interruption of one base–pair contact (requiring 21–25 kJ mol^{-1} of contacts, approximately), and establishment of two new contacts between the intercalated molecule and the previously stacked bases. The data thus indicate that intercalation leads to dye–base interactions as energetically favourable as dye–dye interactions per actual surface of contact. It is also important to point out that the data equally show that intercalation as distinct from dye (or antibiotic) dimerization, is also promoted by the entropy (e.g., ~ 41.8 J mol^{-1} K^{-1} for EB intercalation). This can be primarily attributed to the release of water molecules from the hydration sheaths of interacting species (intuitively consistent with intercalation), which would more than compensate for the loss in entropy due to the concomitant increase in DNA chain stiffness, and to crasis.

The case of Act seems more controversial. We have found in fact that this

antibiotic binds to DNA with a positive enthalpy value of about 8.4 kJ mol^{-1}. On the other hand, study of the interaction of DNA with actinomine, an analog of Act containing the phenoxazone moiety, but lacking the cyclic pentapeptide rings, which are replaced by smaller *N,N*-diethyl ethylendiamine side chains, has shown that it is an intercalating agent with an interaction enthalpy, $\Delta_b H$, of -29.3 kJ mol^{-1} (at very low actinomine/DNA concentration ratio). Similar data have been obtained for two of its analogs: 2-amino-3-phenoxazone, $\Delta_b H = -36.0$ kJ mol^{-1}; 2,*N*-methylamino-3-phenoxazone, $\Delta_b H = 33.1$ kJ mol^{-1}.

It therefore appears that when "intercalated" the phenoxazone chromophore behaves almost exactly as other planar ring systems. One might then argue from the calorimetric data that Act is not a "well-behaved" intercalating agent. This is contrary to what is widely believed. At the risk of restating the obvious, one cannot help recalling that thermodynamic information per se can neither rule out nor dictate a mechanism of interaction at the molecular level. This also applies, of course, to the calorimetric data considered in this section, which are basically meant to show that intercalation of a variety of drugs and/or dyes into the double helical structure of DNA is typically an exothermic process (also favoured by the entropy), semiquantitatively correlatable with a reasonable process mechanism.

By contrast, a negative and large enthalpy of binding between a given drug and DNA cannot be considered as unambiguous evidence in favour of drug-intercalation. The situation is well illustrated by the system DNA–netropsin [5], where $\Delta_b H = -38.5$ kJ mol^{-1}, but the interaction mode is non-intercalative [10].

CONCLUDING REMARKS

The aim of this paper has been to provide a few representative examples of calorimetric studies that throw light on the thermodynamics of typical phenomena in aqueous solutions of natural and synthetic polyelectrolytes. There are, of course, many other significant applications of calorimetry, particularly to biopolymer solutions.

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