

Hydrophobic interactions in water soluble cationic polymers studied by fluorescence

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SUMMARY

Evidences of the interaction of an hydrophobic anion (ANS) with various cationic copolymers have been obtained by fluorescence spectroscopy. The fluorescence intensity changes depend on the structure, the cationicity, the copolymer concentration and were interpreted in terms of microenvironment of the fluorescent probe. The enhanced affinity observed was attributed to the contribution of the alkyl substituents of the polyelectrolytes.

INTRODUCTION

Synthetic polyelectrolytes provide convenient models for studying the specific interaction of a small molecule or an ion to a polymer. Fluorescence spectroscopy has been shown to be a useful and a versatile technique for the investigation of the properties of the microenvironment of polymers (1-13) and the association between cationic and anionic polymers (14). The emission intensity of a fluorescent probe depends strongly on the microenvironment. So interaction of a fluorescent molecule with a polymer can be detected when changes in the polarity of the local environment of the probe occur. Fluorescent probes covalently or electrostatically bound to the chain have also been used to investigate the conformational transition of proteins (15-19) and to characterize polyelectrolyte complexes (20).

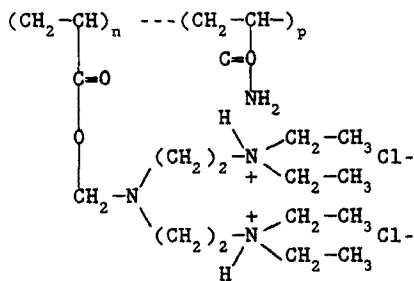
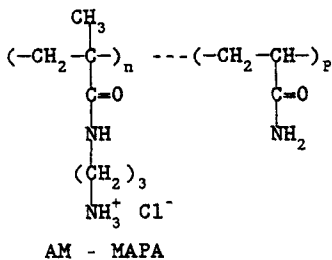
In a preceding paper we reported the synthesis and the characterization of several cationic polyelectrolytes (21) : poly (acrylamide - methacrylamido - propylammonium chloride) (AM - MAPA), poly (acrylamide - 1,1 dimethyl - 3 - diethylamino - propylamide), (AM - DDAPA) and poly (acrylamide - bis (diethylamino-ethyl) aminoethylacrylate) (AM - BDAAE). These copolymers were obtained by radical copolymerization in solution of cationic monomers with acrylamide. They differ by the structure and the environment of the cationic sites.

Poly (trimethyl aminoethyl acrylate) homopolymers (poly CMA) and copolymers with acrylamide have been also synthesized.

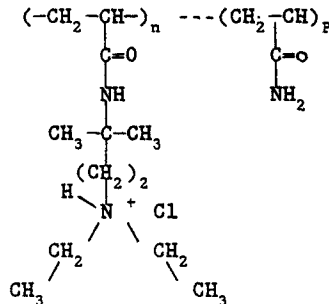
In this work, we study the influence of hydrophobic groups on the interaction of a fluorescent anion on cationic polymers with variable charge density and various ammonium site structure. The 8-anilino naphthalene 1 -sulfonic acid ammonium salt (ANS-NH₄⁺) was used as fluorescent probe. This molecule has a low fluorescence emission in water but shows a strong intensity in organic solvent. The fluorescence of ANS

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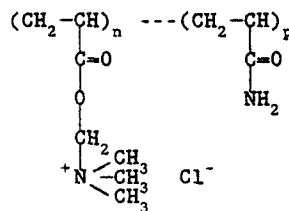
molecule is related to its microenvironment. Two types of excited states



AM - BDAEA



AM - DDAPA



AM - CMA

are involved in the fluorescence process : a non planar singlet state and a planar intermolecular state with charge transfer. The relative population of these two excited states and the wavelengths at the maximum emission depend on the solvent polarity and on the microviscosity (22). In water, the orientation of the molecules induces an excited state with charge transfer, therefore the fluorescence emission is low. An apolar environment deletes partly the orientation of the molecules and promotes the non planar excited state. In pure water or in aqueous macromolecular solutions (e.g proteins or synthetic polymers) the fluorescence spectrum of ANS has two emission states.

EXPERIMENTAL

The synthesis of AM-MAPA, AM-DDAPA and AM-BDAEA copolymers was reported elsewhere (21). The AM-CMA copolymers were prepared according to the method described in the literature (23). The molar weight M_w of homopolymers and copolymers were determined by light scattering. M_w values are in the range $5 \cdot 10^5 - 9.5 \cdot 10^5$.

The fluorescent probe (ANS - NH_4) is a commercial product (FLUKA). Fluorescence experiments were carried out on a spectro fluorimeter FICA model 55 MKII. An excitation wavelength of 350 nm, corresponding to the absorption maximum of ANS molecule in pure water, was used. Measurements were carried out in a thermostated polystyrene cell ($l = 1 \text{ cm}$; $T = 20^\circ \pm 0.1^\circ \text{C}$). Solutions containing variable amount of polymer and constant concentration of ANS were prepared. The polymer concentrations are expressed in cationic monomer units/l.

RESULTS AND DISCUSSION

The fluorescence spectrum of the free ANS, between 380 and 600 nm is broad with a maximum at 506 nm. This maximum is shifted towards the short wavelength (≈ 20 nm) and the emission intensity depends on the ratio C_p/C_{ANS} when a polymer is added to the solution (figures 1 and 2). Differences are observed according to the type of copolymer and the relative intensity I/I_0 dependence with the C_p/C_{ANS} ratio can be correlated with the influence of the ammonium site on the fluorescence emission of the ANS probe. I_0 and I are respectively the intensity of the ANS molecule in pure water and in aqueous polymer solution.

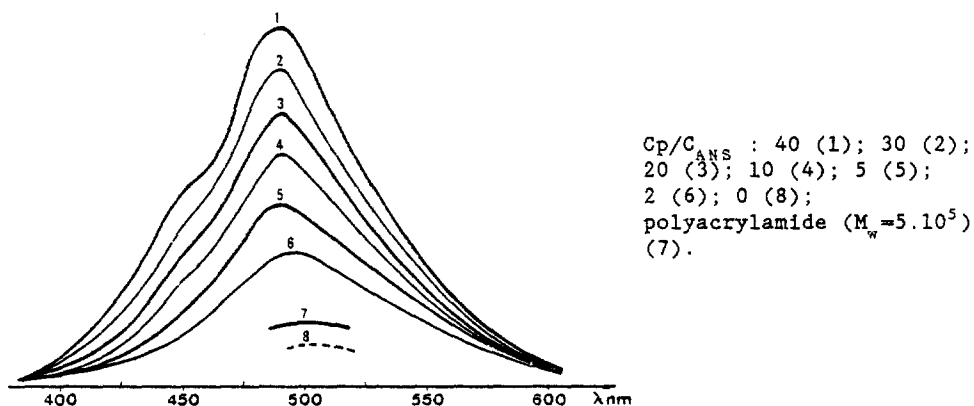


Figure 1 : Fluorescence spectra of ANS in AM-MAPA copolymer solutions. Cationic unit content : 30 % $C_{ANS} = 2 \cdot 10^{-4}$ M.

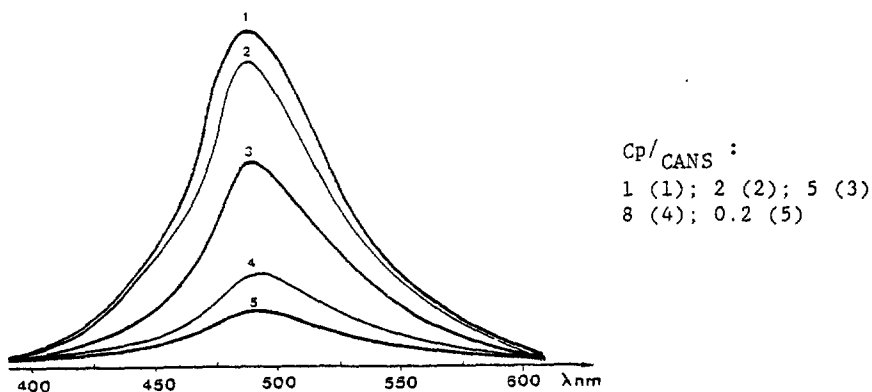
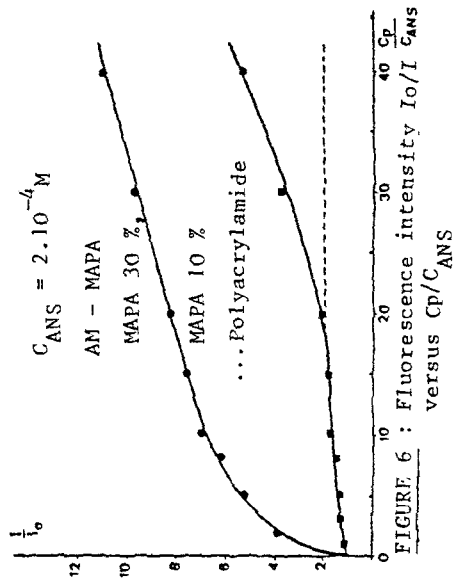
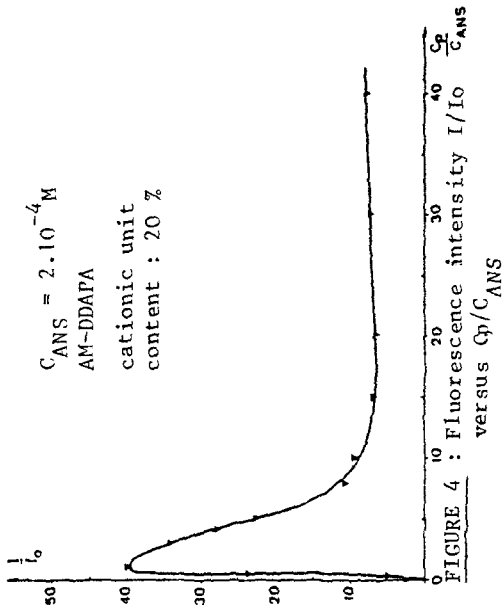
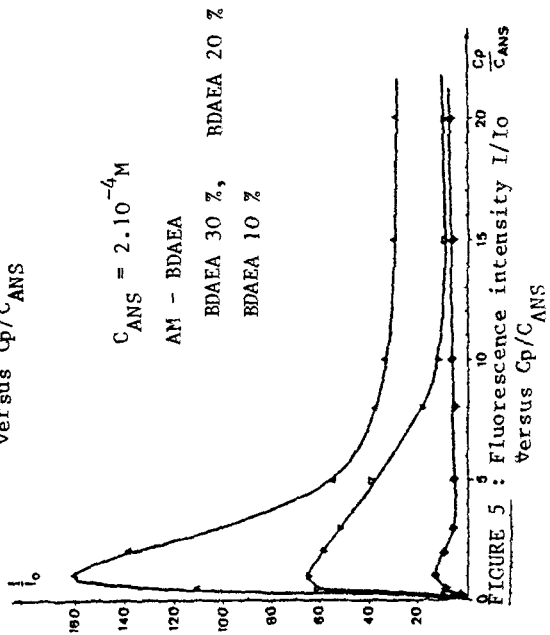
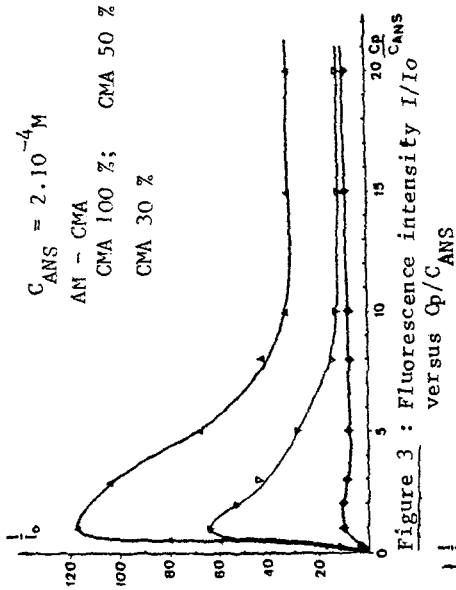


Figure 2 : Fluorescence spectra of ANS ($C_{ANS} = 2 \cdot 10^{-4}$ M) in AM-BDDEA copolymer solutions. Cationic unit content : 20%

The electrostatic interaction between the ANS anion and the ammonium sites of the copolymers can change strongly the fluorescence energy of the excited molecule. The parameters that have an effect on the environment of



the ANS and on the fluorescence process are characteristic of the polymers : charge density, structure of the cationic sites, and conformation of the chain.

The I/I_0 variation versus C_p/C_{ANS} depends on the polymer structure. Two types of curve are observed : the relative intensity I/I_0 goes through a maximum or increases continuously.

Curves with a maximum

The maximum intensity of the fluorescence emission is observed when the C_p/C_{ANS} ratio becomes close to one. (figures 3,4 and 5). The anion interactions with the polymer cationic sites, induce conformational changes, the chain becomes more compact and consequently the environment of the probe is more hydrophobic.

When the polymer concentration is increased so that the C_p/C_{ANS} ratio becomes over one the number of available cationic sites interacting with one ANS molecule increases as also the polarity of its environment because of the chain extension due to the electrostatic repulsions. This conformational change leads to a decrease of the relative intensity I/I_0 until a stabilization occurs for a large excess of polymer.

We observe on figures 3 and 5 a large decrease of the relative intensity I/I_0 with the cationic unit content. It has already been reported that anionic dyes show a larger affinity for cationic polymers when electrostatic and hydrophobic interactions are involved (24) - (26). This behaviour is attributed to cooperative effects all the more effective as the sequence of cationic sites is long.

In addition the interaction of ANS to the polyelectrolyte is promoted by the alkyl groups of the ammonium sites. As the polyacrylamide content decreases, the hydrophobicity of the chain increases.

Curves without maximum

Figure 6 shows the relative fluorescence intensity of ANS in aqueous solution containing AM - MAPA copolymers or polyacrylamide versus the polymer concentration. In comparison with the latter curves, we observe lower relative intensity values. Furthermore the absence of a maximum and the continuous increase of the relative fluorescence intensity with the polymer concentration suggest a loose interaction of the ANS anion with the ammonium sites. Nevertheless the intensity is still higher with AM - MAPA copolymers than with polyacrylamide which has no affinity with the probe and which itself induces a very slight change in the fluorescence intensity of the probe in water.

CONCLUSION

The relative fluorescence intensity changes of the ANS probe dissolved in dilute polymer solution is interpreted by a contraction of the polymer chains when the cationic groups are partially neutralized. The affinity of an hydrophobic anion like ANS for a polyelectrolyte with ammonium functional groups is related to the hydrophobic character of the substituents. We have already studied (27) by potentiometry and viscometry

cationic polymer/dianion systems and found a similar influence.

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