A STUDY OF POLYANION-CATION INTERACTIONS USING HYDRATED ELECTRONS

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SUMMARY

A new method is described for studying the binding which occurs between the anionic sites of polyanions and the cationic dye, methylene blue (MB^+). The hydrated electron (e^-_{aq}) reacts rapidly with MB^+ according to the reaction $MB^+ + e^-_{aq} \rightarrow MB\cdot$, where MB· represents the methylene blue semiquinone ($k_2 = 2.5 \times 10^{10}\ M^{-1}\ sec^{-1}$). On addition of polyanion, the rate of the e^-_{aq} reaction is markedly decreased, due to neutralization of the positive charge on MB^+ by ion-binding. The changes in rates of e^-_{aq} disappearance can, therefore, be used to study ion-binding. Such ion-binding with cationic dyes is frequently accompanied by spectral shifts in the dye (metachromasia). Measurements of changes in e^-_{aq} disappearance rate demonstrate that the agents which destroy metachromasia in solution, namely addition of sodium chloride, methanol and an increase in temperature lead also to loss of ion-binding.

INTRODUCTION

Ion-binding (Rice and Nagasawa, 1961), where counterions of a polyanion are intimately associated with it, has been demonstrated by a number of

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methods. Measurements of the transference numbers of polyion and counterion using radioactive methods and ionic mobilities (Huigenga, Greiger and Wall, 1950), activity or osmotic coefficients (Nagasawa, Ozawa, Kimura, Kagawa, 1956) and viscosity measurements (Terayama and Wall, 1955) all indicate that some of the counterions are either bound at the negative sites of the polyion or that they are limited in some way to the domain of the polyanion coil. When the counterion is a cationic dye, a spectral shift (metachromasia) frequently accompanies the binding (Lison, 1935) (Michaelis, 1947, 1950). Interaction between adjacent dye molecules is the most common explanation for the spectral shift (Michaelis, 1950, Scheibe, 1938; Kasha, 1959; Bradley and Wolf, 1959; Pal and Schubert, 1962).

It has been found that precipitation of a solid complex can be observed at the point of equivalence of negative sites with cationic counterions (Balazs and Szirmai, 1958). Such complexes, between a range of polyanions and cationic dyes, were shown to become paramagnetic on exposure to light, and this behaviour was attributed to electron transfer between the dye and the anionic site of the polyanion (Balazs, Phillips and Young, 1967). Previously, we and other workers suggested that the metachromatic shift could be in part or entirely due to the loss of spectral symmetry in the chromophoric system as a result of dye-anionic site interaction (Balazs, Phillips and Young, 1967; McKay and Hillson, 1965, 1966). Thus, it is possible that dye-anionic site and dye-dye interactions individually and collectively influence the spectral shift. For this reason it was desirable to seek a method which could be used to distinguish between one or other of these effects, for example, study site-dye interactions alone. In this way, the origin of the spectral shifts whether due to site-dye interactions or dye aggregation effects, or both, may eventually be established. Furthermore, such a method would be of value in the study of other ionic interactions where spectral changes are not observed. In this paper we report a new method of studying the binding which occurs between polyanions and cations, and to illustrate the method we shall limit the discussion to experiments with

one cationic dye, methylene blue. A more detailed account of this work will demonstrate that the method is generally applicable to a wide range of coloured and uncoloured cations.

RESULTS AND DISCUSSION

For the following experiments a commercial sample of heparin (Boots Pure Drug Co. Ltd.) was purified by passing a solution over an OH^- ion exchange resin, eluted as the free acid, neutralized with KOH and finally lyophilized. Before use the methylene blue (Hopkin and Williams) was recrystallized several times from distilled water, and the water content estimated by measuring the loss in weight on vacuum dessication for 24 hr.

The hydrated electron (e^-_{aq}) has a high extinction coefficient (15,800 M^{-1} cm^{-1} at 7200 Å) and may, therefore, readily be observed by optical methods using pulse radiolysis techniques (Keene, 1964; Boag and Hart, 1963; Hart and Boag, 1962). Due to their nucleoplilic character e^-_{aq} react rapidly with methylene blue (MB^+). The fast reaction can be expressed by the electron transfer process $MB^+ + e^-_{aq} \rightarrow MB.$, where MB· represents the methylene blue semiquinone (Keene, Land and Swallow, 1965). The rate constant for this reaction could be measured directly from the decay of e^-_{aq} at 720 mμ, as already described (Phillips, Griffiths and Davies, 1966). Typical semi-log plots for the decay of e^-_{aq} in methylene blue (10^{-5} M) and when heparin and other substances were added are shown in Fig. 1. In order to eliminate any effects due to OH radicals, D-glucose (10^{-2} M) was added as a scavenger, and did not significantly affect the pseudo first-order constant for the e^-_{aq} removal rate. The results are summarized in Table 1.

On addition of heparin, the rate of disappearance of e^-_{aq} is markedly decreased, despite the fact that the MB^+ concentration is unchanged in the solution. This observation indicates that interaction between the anionic sites and the cationic dye leads to a change in electronic configuration over the entire chromophoric system of MB^+ to such an extent that the positive charge is

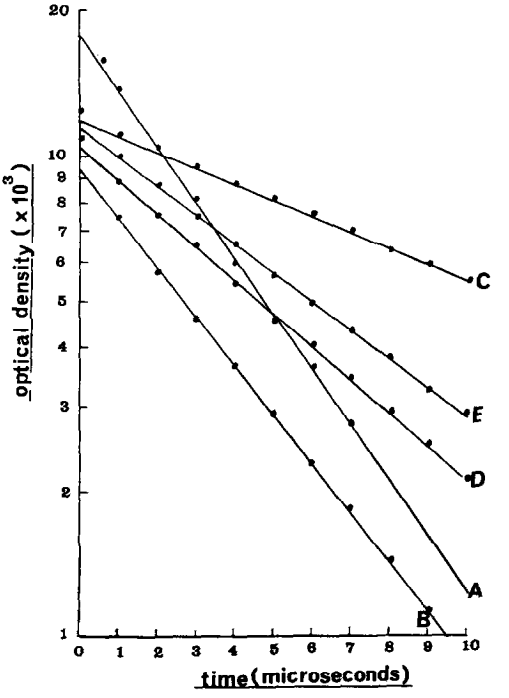

Figure 1. First-order plots for the decay of $e^-_{aq.}$ in aqueous methylene blue (10^{-5}M) systems.

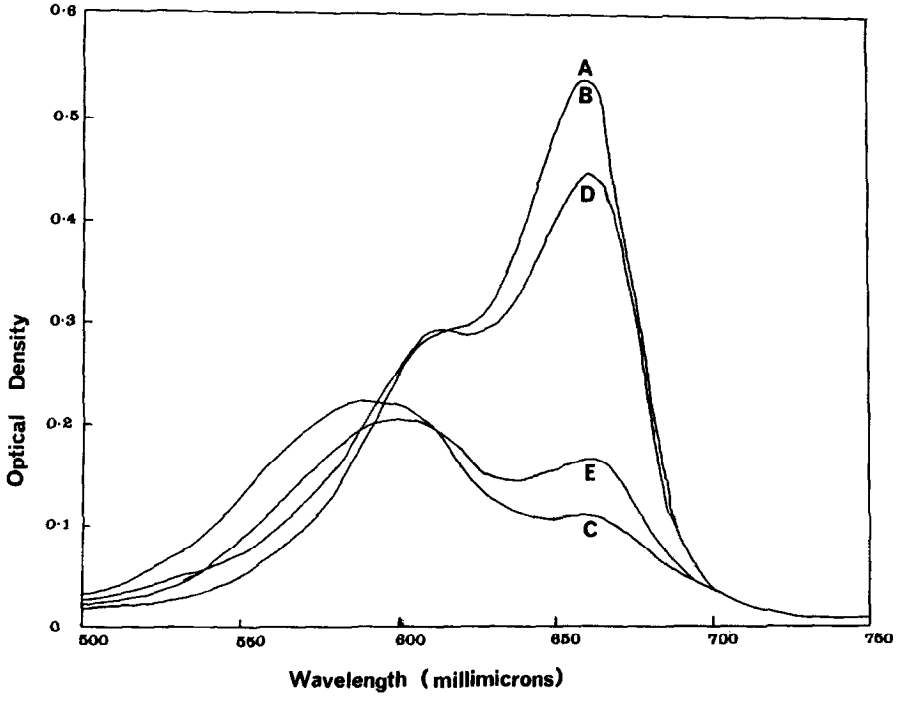

Figure 2. Absorption spectra of methylene blue (10^{-5}M) systems.

In both figures the letters refer to identical systems as follows :-

A MB^+

B MB^+, NaCl 0.1M.

C MB^+, heparin (10^{-3} E/L), D-glucose (1.2×10^{-2}M).

D MB^+, heparin (10^{-3} E/L), NaCe (0.05 M).

E MB^+, heparin (10^{-3} E/L), Methanol (10% v/v).

TABLE 1.

Pseudo first-order rate constants (k_1, 10^4 sec^{-1}) for the decay of e^-_{aq} in methylene blue-heparin systems.

(Methylene Blue) 10^{-5} M.

Added Substances	Without Heparin	Heparin 10^{-4} E/L	Heparin 10^{-3} E/L
	26	11.5	3.1
D-glucose 10^{-2} M	24	1.1	5.4
NaCl 0.1M	23		23
0.05M		15.9	17.5
MeOH 10% v/v		9.24	14.0
$(NH_4)_2$ SO_4 0.5M	46		

partly or wholly neutralized. At a heparin concentration of 10^{-3} equivalent negative sites per litre (E/L) and MB^+ 10^{-5}M the decay rate of e^-_{aq} approaches that which have found for the polyanion alone (k_2 = 2.2×10^7 $mole^{-1}$ sec^{-1}), which represents the lower limit for the removal of e^-_{aq} in these solutions.

It is significant from our results that the agents which destroy metachromasia in solution (Pal and Schubert, 1962), namely addition of sodium chloride, methanol and an increase in temperature also induce a reversal of the e^-_{aq} rate. When sodium chloride (5×10^{-2}M) is added to heparin + D-glucose + MB^+, the e^-_{aq} disappearance rate increases and approaches the value for the free dye. That this maximum value ($k_1 = 2.4 \times 10^5$ sec^{-1}) is not attained reasonably relates to the fact that the metachromatic reaction is not fully reversed at this salt concentration. Metachromasia is eliminated entirely at 10^{-1}M sodium chloride and under these conditions the e^-_{aq} - rate corresponds to that of the free dye. Methanol (10% v/v similarly causes a change in metachromasia and leads also to an increase in e^-_{aq} - rate. The effect of temperature is particularly striking. Increase in temperature from 25-65°, without changing any other parameter, destroys metachromasia and induces a complete reversal in e^-_{aq} - rate from 5.7×10^4 sec^{-1} to that of the free dye. Moreover, this behaviour is reversible during successive cycles of heating and cooling operations. There can be no clearer demonstration that ion-binding is destroyed on increasing the temperature to release free MB^+ which readily reacts with e^-_{aq}. Furthermore, this process is strictly reversible.

It is evident from our observations that loss of ion-binding leads to removal of metachromasia. It does not, however, follow that it is the ion-binding process which is responsible for metachromasia. However, using the pulse radiolysis method, ion-binding can be examined independently of any other process. We have further applied this technique to study the interaction between heparin, hyaluronic acid, DNA, poly(ethylene sulphonate) and poly(styrene sulphonate) and cationic dyes, cetyl pyridinium chloride and the polycations, polylysine and protamine sulphonate. Full details will be published elsewhere (Balazs, Davies, Phillips and Scheufele, in the press).

REFERENCES

Balazs, E.A. and Szirmai, J.A., J. Histochem. Cytochem. 6, 278 (1958).

Balazs, E.A. Phillips, G.O., and Young, M. Biochim. Biophys. Acta, 141,382 (1967)

Boag, J. and Hart E.J., Nature, 197, 45 (1963).

Bradley, D.F. and Wolf, M.K., Proc. Nat'l. Acad. Sci., U.S.A., 45, 944 (1959)

Hart, E.J. and Boag, J., J. Am. Chem. Soc., 84, 4090 (1962).

Huigenga, J.R., Greiger, P.F., and Wall, F.T., J.Am. Chem.Soc., 72, 2636, 4228. (1950).

Kasha, M., Rev. Mod. Phys., 28, 721 (1959).

Keene, J.P., J.Sci.Instr., 41, 493, (1964).

Keene, J.P., Land E.J. and Swallow, A.J., in "Pulse Radiolysis" ed. Ebert, M. Keene, J.P., Swallow, A.J., and Baxendale, J.H., Academic Press, London 1965, p. 277.

McKay, R.B. and Hillson, P.J., Trans. Faraday Soc., 61, 374, 1787, 1800 (1965).

McKay, R.B. and Hillson, P.J., Nature, 210, 297 (1966).

Michaelis, L., Cold Spring Harbor Symposium on Quantitative Biology, X11,131 (1947).

Michaelis, L., J. Phys. Colloid Chem., 57, 5, (1950).

Nagasawa, M., Ozawa, S. Kinura, K., and Kagawa, 1., Mem. Fac. Eng. Nagoya Univ. 8, 50 (1965).

Pal, M.K. and Schubert, M., J. Am.Chem. Soc., 84, 4384 (1962).

Phillips, G.O., Griffiths, W., and Davies, J.V., J. Chem.Soc., (B) 194, (1966).

Rice, S.A. and Nagasawa, M., "Polyelectrolyte Solutions" Academic Press, N.Y., 1961, p. 427.

Scheibe, G., Kolloid-Z., 82, 82 (1938).

Terayama, H., and Wall, F.T., J. Polymer Sci., 16, 357 (1955).