Polymer Bulletin 14, 207–212 (1985)

Polymer Bulletin

Binding of Evans Blue Onto Poly (N-Vinyl-2-Pyrrolidone)

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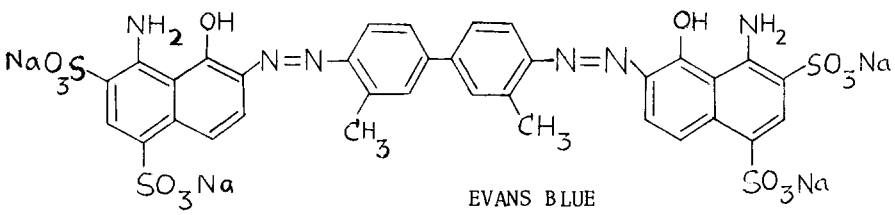
SUMMARY

The binding of evans blue, a bisazo dye used in blood volume determination, onto poly(N-vinyl-2-pyrrolidone) was studied using Klotz's spectrophotometric method. The binding constant nK in 0.01 M phosphate buffer was found to be $(3.15 \pm 0.40) \times 10^5$ mol^{-1} dm^3. Addition of NaCl decreased the binding constant due to competitive binding, whereas urea enhanced the binding due to conformational transition. Analysis showed that electrostatic interaction was the predominant force while hydrophobic bonding was less significant.

I INTRODUCTION

Evans blue (also called T 1824), being used in blood volume determination is physiologically an important substrate. Its role in blood volume determination is wholly due to its high binding affinity to plasma albumin. Many authors (1,2) have made a detailed study of plasma albumin - evans blue system.



EVANS BLUE

6,6'- [(3,3' -dimethyl [1,1'-biphenyl]-4,4'diyl)bis(azo)] bis [4-amino-5-hydroxy-1,3-naphthalenedisulfonic acid]tetrasodium salt.

Poly(N-vinyl-2-pyrroli done) , a water-soluble synthetic polymer shows resemblance to serum albumin in many respects. For e.g., just like serum albumin, this polymer forms complexes with small molecules such as iodine (3) azo dyes (4) and amino acids (5) although poly (N-vinyl-2-pyrrolidone) has been shown to have only about one-third the affinity of serum-albumin (6). In addition to this resemblance, the application of this polymer as blood plasma substitute adds more weight to the study of poly(N-vinyl-2-pyrrolidone) - Evans blue system.

II EXPERIMENTAL

Poly(N-vinyl-2-pyrrolidone)(mol. wt. 25,000) and evans blue were laboratory reagents from S.D.'s laboratory reagent and Loba Chemie, India respectively. Urea and sodium chloride were analytical grade reagents.

All chemicals were used without further purification.

The experiment was carried out in aqueous 0.01M phosphate buffer solution of pH 7 at 30°C. Polymer concentration was varied from 1×10^{-6} mol dm^{-3} to 1×10^{-3} mol dm^{-3}, expressed in terms of molecular weight of the polymer and that of dye solution from 1×10^{-5} mol dm^{-3} to 2.4×10^{-5} mol dm^{-3}. Since the dye has the tendency to adsorb on the walls of the glasswares, they were pre-rinsed to reduce this effect. NaCl and urea were used in the concentration ranges (0.3 - 0.6) M and (0.5-1.0)M respectively. All spectrophotometric measurements were made using UV-Vis specord spectrophotometer.

Klotz spectrophotometric method (7) was followed to study Poly(N-vinyl-2-pyrrolidone) - Evans blue system.

III RESULTS AND DISCUSSION

The dye solution in the concentration range (1-2.4) $\times 10^{-5}$ mol dm^{-3} was found to obey Beer-Lambert's law both in buffer solution and in presence of additives, viz., 0.3 & 0.6 M NaCl and 0.5 M urea. The absorbences were read at 639 nm (wave number = 15.64×10^{3} cm^{-1}). The molar extinction coefficient value, ε in 0.01 M buffer is 41168 M^{-1} cm^{-1}.

III (i) CHANGE IN ABSORPTION SPECTRUM

The absorption spectrum of evans blue (hereafterwards evans blue and poly (N-vinyl-2-pyrrolidone) are denoted as EB and PVP respectively) in buffer solution and in presence of various additives has λ_{max} at 609 nm. But when polymer solution is added, the λ_{max} is red-shifted to 639 nm with increase in O.D. The absorption spectra of EB in the presence of PVP and PVP-Urea are shown in Fig. 1.

Klotz model (8) of binding is found to be applicable for PVP-EB system. According to him,

$$1/r = 1/nKC_{Free} + 1/n \quad \ldots\ldots(1)$$

where r represents the number of moles of bound substrate per mole of polymer, C_{Free} the equilibrium or free substrate concentration, n the total number of binding sites and nK the binding constant. r values in the present work vary from 0.09 to 4.52. A typical set of plots is shown in Fig.2. Since the numerical value of the intercept on the ordinate.i.e., 1/n is very near to zero, a small error in extrapolation to 1/n is reflected largely in the value of n. So it is customary (9) to use nK which can be determined precisely from the slope, rather than the intrinsic binding constant, K.

The binding costant for the system PVP-EB has been measured as a function of [PVP]. Table 1 summarises the nK values obtained for different PVP concentrations in buffer solution and in presence of various additives. In buffer solution, in the absence of any additive, there is no significant change in nK values as [PVP] is varied from 3×10^{-5} M to 2×10^{-6} M. The mean value of nK over the whole range of [PVP] is found to be $(3.15 \pm 0.40) \times 10^{5}$ mol^{-1} dm^{3} and this has been considered for comparison with other systems.

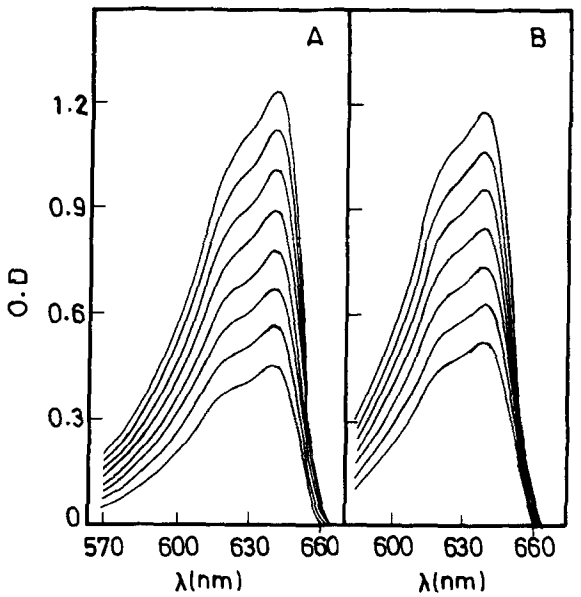


Fig. 1

Absorption spectra of EB in PVP and PVP-Urea systems.
[Buffer] = 0.01 M
pH = 7; Temp.=30°C
A : [PVP] =1 x 10^{-3}M
[EB] = (1-2.4) x 10^{-5} M.
B : [PVP] = 3x10^{-5} M
[EB] = (1.2-2.4) x 10^{-5} M
[Urea] = 1 M

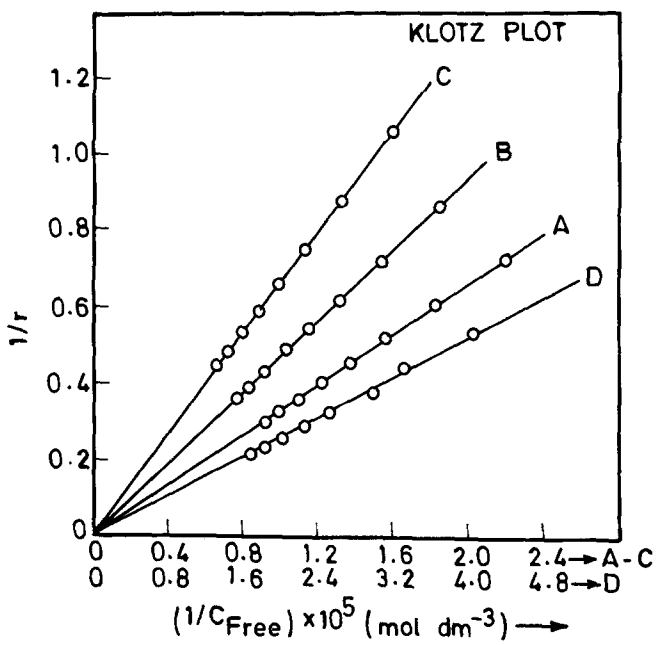


Fig. 2

Klotz plot for PVP-EB system.
[PVP] = 4 x 10^{-6} M
0.01M buffer
pH = 7.0; Temp. = 30°C

A : Buffer only
B : Buffer + 0.3 M NaCl
C : Buffer + 0.6 M NaCl
D : Buffer + 0.5 M Urea.

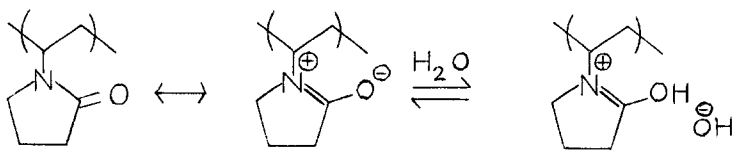
It is evident from the structural formula that EB has a number of groups capable of forming electrostatic interaction, hydrogen bonding, ion-dipole interaction, dipole-dipole interaction and hydrophobic bonding. Thus from the structural view point, EB should have higher binding constant than other azo dyes, methyl orange, butyl orange and orange II and hydrophobic probes, 2-p-toluidinylnaphthalene-6-sulfonate (TNS) and 4-phenylazo-1-naphthol-2-sulfonate (PNS). This can be seen by comparing the binding

constant of EB with the corresponding values of other azo compounds already reported (Table 2).

From the Table 2 it is seen that except Benzopurpurin 4B (BP), all other substrates are having lesser nK values, thus agreeing with our expectation. BP is structurally related to EB. PVP-BP system has larger nK value than does the PVP-EB system. But it is still comparable because BP binding was studied using PVP of higher mol.wt. i.e., 1,75,000 (7 times higher). High mol. wt. PVP has more binding affinity than the low mol.wt. PVP for the same substrate. In this view, we can say that the two systems are having comparable binding constants.

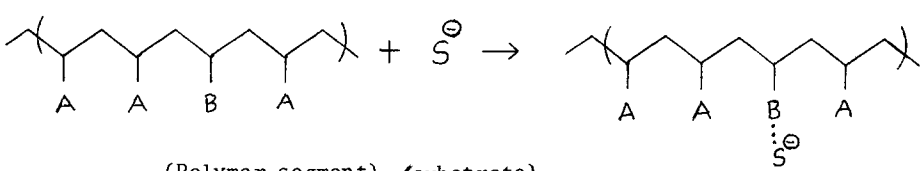
III (ii) NATURE OF INTERACTING FORCES

To explain the strong binding between PVP and EB, let us consider the nature of PVP. Although at pH 7, PVP is a neutral polymer, the tertiary nitrogen in the pyrrolidone ring acquires a positive charge due to the following scheme(I) of equilibrium as proposed by Takagishi and Kuroki(9).



Scheme I Keto-enol tautomerism in pyrrolidone ring.

Even though this equilibrium is unfavourable, we have shown in a previous study (12) the possibility of operation of this equilibrium. A and B type units are the probable binding sites. Since the dye has 4-$SO_3^{\ominus}$ groups, there should be electrostatic interaction between EB and PVP . The binding of one EB molecule through only one -$SO_3^{\ominus}$ group to B unit is shown in scheme II.



(Polymer segment) (substrate)

Scheme II Binding of EB onto PVP

PVP (mol dm^{-3})	nK x 10^{-5} ($mol^{-1} dm^3$)			
	Buffer only	Buffer + 0.3M NaCl	Buffer + 0.6M NaCl	Buffer + 0.5M Urea
3 x 10^{-5}	3.55	0.93	0.66	2.54
1 x 10^{-5}	2.73	1.45	0.92	4.95
4 x 10^{-6}	3.02	2.14	1.51	7.69
2 x 10^{-6}	3.51	2.26	-	-

Table 1 nK values for PVP-EB system. 0.01M buffer pH = 7; Temperature = 30°C.

Table 2

System	nK (mol^{-1} dm^3)	Ref
PVP-EB	3.15×10^5	Present work
PVP-Methyl orange	1.79×10^4	9
PVP-Butyl orange	4.71×10^4	9
PVP-TNS	6.87×10^4	10
PVP-PNS	6.21×10^2	11
PVP-Orange II	7.60×10^4	4
PVP-Benzo-purpurin 4 B	13.0×10^5	4

On the other hand, if dye molecules bind to A type units, since the concentration of binding sites constituted by A type units is very high, the bound dye molecules may get closer to each other. Hence there will be anionic repulsion between the bound dye molecules. This repulsive force will inhibit further binding of dye molecules to the neighbouring A type sites on the polymer chain. In this situation, Klotz model (8) of binding will not be obeyed. But in the present case, we observe a very good linearity in the Klotz plot (Fig. 2). Moreover, the value of r/C_{free} is constant for all polymer and substrate concentrations and hence in the Scatchard plot, i.e., r/C_{free} vs. r plot (Fig. not shown), a straight line parallel to x-axis is obtained. These experimental evidences, thus, support our scheme (II) of binding, i.e., (i) B type units constitute the main binding sites and (ii) out of 4, only one $-SO_3^{\ominus}$ group is involved in binding with $N^{\oplus}$ site, electrostatic interaction being the predominant force. Berman et al., (13) have also shown that in the drag reduction of poly(ethyleneoxide) by some azo dyes including EB, the $-SO_3^{\ominus}$ group is responsible for binding and that the dye molecules bind through only one sulfonate ion, the other anionic sites extending outwardly into the solution.

Aromatic moieties, biphenyl and naphthalene rings may be expected to show hydrophobic bonding to the non-polar portions of the polymer. But these hydrophobic groups are surrounded by strong hydrophilic groups and so the contribution of hydrophobic bonding can be only to a less extent. If hydrophobic bonding were the predominant forces, then on adding urea to PVP-EB complex, the absorption spectrum of EB should have been reverted to its original position, because urea, being a denaturing agent, can release the dye molecules bound through hydrophobic bond formation. But from Fig. 1.B., we see that this is not the case. Moreover urea enhances the binding as is shown by the higher binding constant values given in Table 1. These experimental evidences indicate that in binding, the contribution of hydrophobic bonding is less significant with an indirect support to the operation of electrostatic forces. Previous observation (14) that among the azo dyes, EB is less hydrophobic in nature, also supports this conclusion.

III (iii) EFFECT OF ADDITIVES

From Table 1 it is clear that for a particular [PVP], addition of NaCl decreases the binding constant and that the increase in [NaCl] linearly decreases the latter. It is explained as follows: when [NaCl] is larger, the $Na^{\oplus}$ ions bind to $-SO_3^{\ominus}$ group in the dye molecule and affect the electrostatic interaction between the latter and the $N^{\oplus}$ site in PVP

while $Cl^{\ominus}$ ions exhibit a competitive binding to $N^{\oplus}$ sites in PVP. Thus binding of EB onto PVP is somewhat inhibited, leading to decrease in nK value. Lowering of critical micelle concentration of EB below 1×10^{-4} M in pressence of high [NaCl] and previous study (15) of binding of $Cl^{\ominus}$ ions to PVP support our explanation. Thus NaCl effect is also an evidence for scheme (II) of binding.

Table 1 shows that urea generally enhances the binding. Possible explanation is given below : Urea, being a denaturing agent, induces conformational transition. As a result pyrrolidone side chains of PVP, come closer together, leading to more interaction between PVP and EB, the basic site being the $N^{\oplus}$ one. Gargallo et al., (16) have also recently shown that conformational transition occurs when urea is added to aqueous solution of PVP. As concluded earlier, the urea effect shows the insignificant nature of hydrophobic bonding.

The study of PVP-EB with different experimental techniques is our current research work. We believe that this will give more information about the physiologically important PVP-EB system.

ACKNOWLEDGEMENT

We gratefully acknowledge the financial suppport given by C.S.I.R. New Delhi, India for the present research work.

REFERENCES

1. W.Schwartzkopff and K.H.Hölzer, Clin. Chim. Acta. 5, 845 (1960)
2. F.B.Freedman and J.A.Johnson, Am. J. Physiol. 216, 675 (1969).
3. G.Oster, J. Polym, Sci. 9, 553 (1952).
4. H.P. Frank, S. Barkin and F.R.Eirich, J. Phys. Chem. 61, 1375 (1957).
5. J. Eliassaf, F.Erikson and F.R. Eirich, J. Polym. Sci. 47, 193 (1960).
6. I.M. Klotz and K.Shikama, Arch. Biochem. Biophys. 123, 551 (1968).
7. I.M. Klotz, J. Am.Chem. Soc.68, 2299 (1946).
8. I.M. Klotz, F.Walker and R.Pivan, J.Am. Chem. Soc.68, 1486 (1946).
9. T.Takagishi and N. Kuroki, J. Polym. Sci. Polym. Chem. Ed. 11, 1889 (1973).
10. T.Takagishi, Y.Naoi, and N. Kuroki, J. Polym. Sci. Polym Chem. Ed. 16, 2789 (1978).
11. R.L. Reeves, S.A.Harkaway and A.R.Sochor, J. Polym. Sci. Polym.Chem. Ed. 19, 2427 (1981).
12. M.Maruthamuthu and M.Sobhana, J. Polym. Sci. Polym Chem. Ed. 17, 3159 (1979).
13. S.N. Berman, B.R. Berger and R.J. Leis, J. Rheol.(N.Y.) 24, 571(1980).
14. I.Moriguchi, S.Fushimi and N.Kaneniwa, Chem. Pharm. Bull. 19, 1272 (1971).
15. D.Balasubramanian, B.C.Misra, S.Misra and A.Mitra, Indian J.Biochem. Biophys. 14, 199 (1977).
16. L.Gargallo and D. Radic, Polymer 24, 91 (1983).

Accepted August 8, 1985

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