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 $K_n$ 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.

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.

Poly(N-vinyl-2-pyrrolidone), 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 \times 10^{-6} \text{ mol dm}^{-3} \text{ to } 10 \times 10^{-3} \text{ mol dm}^{-3}, expressed in terms of molecular weight of the polymer and that of dye solution from 1 \times 10^{-5} \text{ mol dm}^{-3} \text{ to } 2.4 \times 10^{-5} \text{ 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 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} \text{ 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 \times 10^3 \text{ cm}^{-1}). The molar extinction coefficient value, \( \varepsilon \) 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 \( \lambda_{\text{max}} \) at 609 nm. But when polymer solution is added, the \( \lambda_{\text{max}} \) is red-shifted to 639 nm with increase in O.D. The absorption spectra \( \lambda_{\text{max}} \) 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/nK_{\text{Free}} + 1/n \quad \text{(1)}

where \( r \) represents the number of moles of bound substrate per mole of polymer, \( C_{\text{Free}} \) the equilibrium or free substrate concentration, \( n \) the total number of binding sites and \( K \) 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 constant 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 \times 10^{-5} \text{ M} to 2 \times 10^{-6} \text{ M}.

The mean value of nK over the whole range of [PVP] is found to be (3.15 \pm 0.40) \times 10^5 \text{ mol}^{-1} \text{ dm}^3 and this has been considered for comparison with other systems.