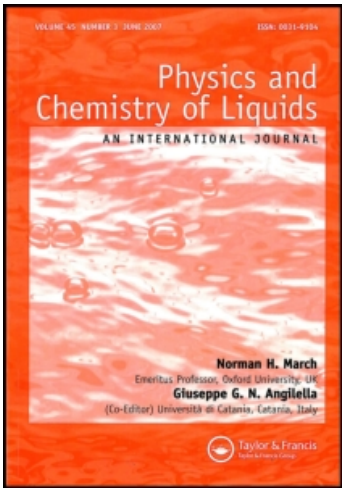


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# STUDY OF INTERACTION OF POTASSIUM DODECATANGESTATO COBALTATE(III) WITH BOVINE SERUM ALBUMIN USING FLUORESCENCE SPECTROSCOPY

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The binding of potassium dodecatangestato cobaltate(III) (PDC) as a water-soluble polyoxometal with bovine serum albumin (BSA) as a major transporting protein of plasma, has been investigated at pH 7.2, 5 mM phosphate buffer, 27°C and various ionic strength by fluorescence spectroscopy.

The results show that the binding of PDC to BSA quenches BSA emission and the Stern–Volmer linear relationship can be applied for the quenching process.

The values of Stern–Volmer constant, $K_{sv}$, which can be considered as a binding constant for formation of 1:1 complex at 0.01, 0.1 and 0.2 M NaCl are $8.56 \times 10^5$, $5.72 \times 10^5$ and $9.60 \times 10^5$, respectively. The interpretation of the results represents that binding affinity depends on both electrostatic forces and conformational stability of BSA. A step-by-step aggregation model, which has been developed by Borissevich *et al*., was used to determine the average aggregation number of BSA, ⟨J⟩, from the fluorescence quenching. The results show that the binding of PDC to BSA does not induce any considerable aggregation in BSA molecules. Therefore, it can be concluded that there are no considerable conformational changes in BSA molecules during its interaction with PDC.

*Keywords:* Bovine serum albumin; Fluorescence; Binding affinity; Polyoxometal

## INTRODUCTION

Polyoxometals are a range of inorganic compounds that have various molecular and electronic structures with analytical, biological, clinical, geochemical and topological applications. In particular, these compounds have attracted attention in view of their potential application in catalysis [1], and as antiviral and antitumoral agents [2]. These complexes exhibited biological activity as a new potent class of anti-HIV drugs [3], and can be used as small individually distinguishable labels for conventional transmission electron microscopy [4]. Interaction of these drugs and their derivatives with

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biological structures is of considerable interest since they have possible medical applications. Polyoxometals should bind to various structures in organisms and this changes their spectral and other physicochemical characteristics. The variation of external conditions, such as ionic strength and pH, can influence the process of the polyoxometal interaction with biological structures. Since albumin is a major plasma protein and binds to a number of drugs altering their pharmocokinetincs [5], the interaction of polyoxometal drugs with albumin is also of interest in order to formulate these drugs into stable, effective and safe forms, that will be clinically useful. However, there is no report on the interaction of polyoxometals with albumin.

In the present work, studies on potassium dodecatangestato cobaltate(III) (PDC), $K_5CoW_{12}O_{40} \cdot 3H_2O$, binding to bovine serum albumin, BSA, have been performed using a fluorescence spectroscopic technique.

## MATERIALS AND METHODS



Potassium dodecatangestato cobaltate(III) was prepared and purified by modifying Baker method [6–8]. BSA (free fatty acid fraction V) was obtained from Merck Chemical Co. and used as received. All of the other chemicals were in analytical grades and purchased from Merck and Sigma Chemical Co. Absorption spectra were recorded on a Shimadzu Uv-160 double beam spectrophotometer. Flourescence measurements were performed with Shimadzu RF-5000 recording spectrofluorophotometer. In all experiments, the PDC and BSA solutions were freshly prepared before spectral analysis. All of the solutions were prepared with double-distilled deionized water. To observe the salt effect on absorption spectra of the PDC, the titration was made by addition of aliquots of the NaCl solution in PDC into a cuvette containing the PDC solution. The concentration of PDC solution was $6.70 \times 10^{-4}$ M.

The interaction of PDC with BSA quenches BSA fluorescenece. For evaluation of this process, the titration of BSA was performed at pH 7.2, 5 mM phosphate buffer by PDC solution. The BSA emission spectra was monitored using excitation at 280 nm. The absorbance of the samples at excitation wavelength did not exceed 0.2, so that the possibility of inner filter effect was eliminated.

Temperature was held constant during all titration experiments at 27°C.

## RESULTS AND DISCUSSION

The Keggin structure of the PDC is shown in Fig. 1. The Co(III) ion is situated in the center of the tetrahedron, and the tungsten atoms are situated in the octahedron centers. PDC has the cobalt ion deeply buried within a shell of $WO_6$ octahedra and is completely shielded from contact with external species [9]. The $WO_6$ shell is stable even under rather strenuous conditions and hence the Co(III) ion in PDC is effectively caged [10].

The Uv/Vis spectra of PDC solution ($6.70 \times 10^{-4}$ M) in the wavelength range from 250 to 350 and 350 to 700 nm are shown in Figs. 2(a) and (b), respectively. The general features of the Uv/Vis spectra are in good agreement with literature and confirm the keggin structure [11]. It consists of three maxima at 270, 290 and 625 nm. The molar absorptivity at these wavelengths at pH 7.2, 5 mM phosphate buffer are $9.73 \times 10^3$,

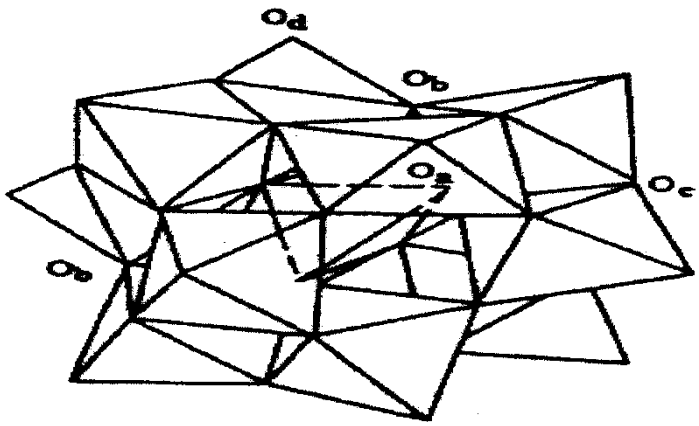


FIGURE 1 The Keggin structure of polyoxometals.

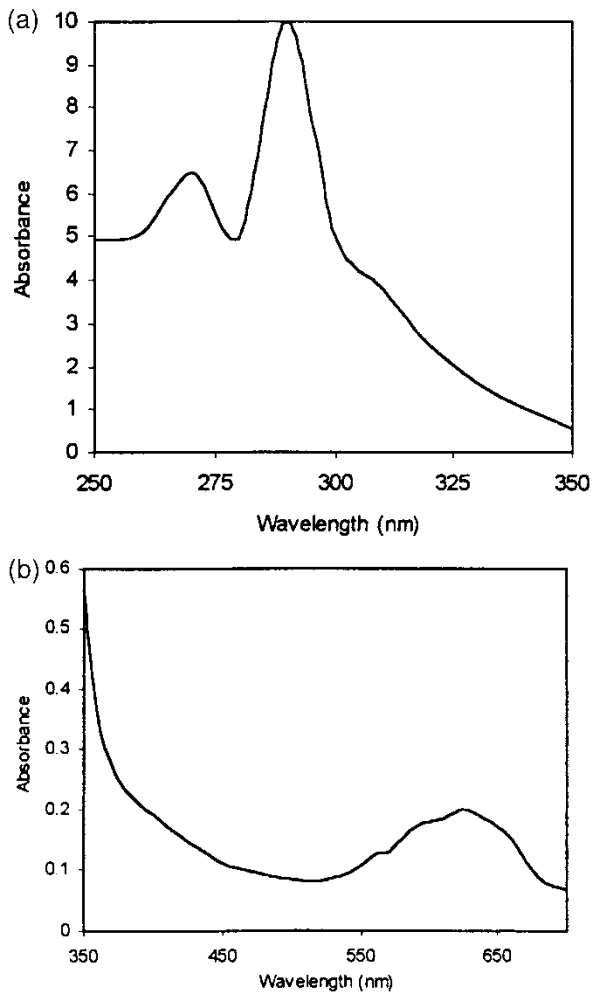


FIGURE 2 Optical absorption of PDC ($6.70 \times 10^{-4}$ M) at pH 7.2, 5 mM phosphate buffer.

$1.47 \times 10^4$ and $297.8\,cm^{-1}\,M^{-1}$, respectively. The Soret band maxima at 625 nm obeys Beer's law over an extended concentration range between $6.70 \times 10^{-4}$ and $4.13 \times 10^{-2}$ M in the buffer solution. From this observation we can conclude that intermolecular interactions between PDC ions in this concentration range are not important. The effect of ionic strength on the Uv/Vis spectra is shown in Fig. 3. It is obvious that the general feature of spectra does not change with increasing ionic strength and just a small uniform decrease in absorbance without any red or blue shift was observed. This represents the structural stability of PDC in this range of ionic strength. However, it was found that the intensity of the spectrum in water was significantly dependent on pH [10]: thus it should be used as a buffer system for doing experiments. The phosphate buffer pH 7.2, was selected as a suitable medium with good coincidency with biological conditions for this purpose. The Uv/Vis spectra of PDC and BSA overlap with each other at wavelength region from 200 to 300 nm. So, it is not informative to do an Uv/Vis titration experiment on the basis of BSA titration by PDC. Furthermore, with respect to low molar absorptivity of PDC at Soret band (625 nm), the titration of PDC solution by BSA solution does not give any significant results. Therefore, Uv/Vis spectroscopy was not a suitable technique for evaluation of the binding process. PDC does not have any emission spectra but the emission spectra of BSA is quenched due to its interaction with PDC. Hence, we used a fluorescence technique for following the binding process. A typical emission spectra of BSA solution upon increasing concentration of PDC is shown in Fig. 4.

For fluorescence quenching of BSA, a Stern–Volmer linear relationship can be applied:

$$F_{max}/F = 1 + K_{sv}[PDC], \tag{1}$$

where $F_{max}$ and $F$ are the fluorescence intensities in the absence and presence of PDC as a quencher, respectively. $K_{sv}$ is the Stern–Volmer constant.

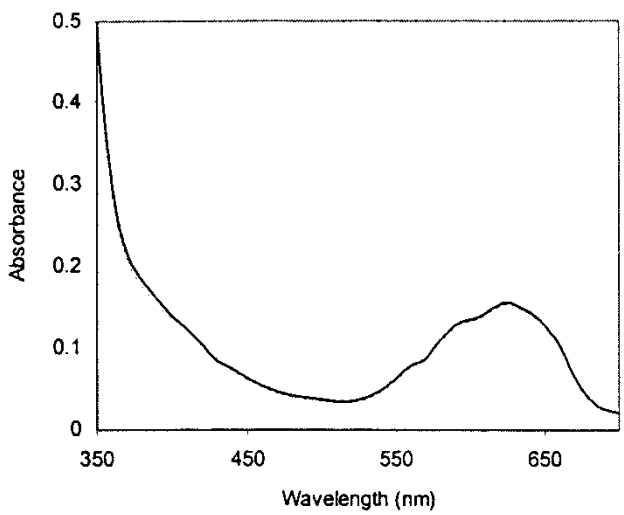


FIGURE 3 Optical absorption of PDC ($6.70 \times 10^{-4}$ M) at pH 7.2, 5 mM phosphate buffer, (–) 0.1 M NaCl, (. . .) 0.2 M NaCl.

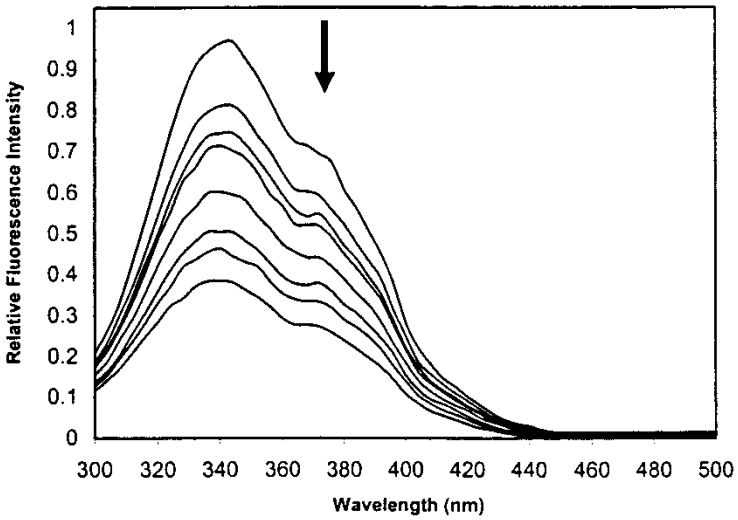


FIGURE 4 Corrected emmision spectra of BSA. 2.2 ml of BSA solution ($3.03 \times 10^{-6}$ M) was placed in cell and various micro liter amount of PDC solution ($3.33 \times 10^{-6}$ M) were added from 0, 100, 200, 300, 400, 500, 700, 900, 1100, 1150 and 1200 in each consecutive step. $\lambda_{ex} = 280$ nm, $\lambda_{em,max} = 310$ nm , band slits are 3 nm and 5 nm for excitation and emmision wavelenghts, respectively.



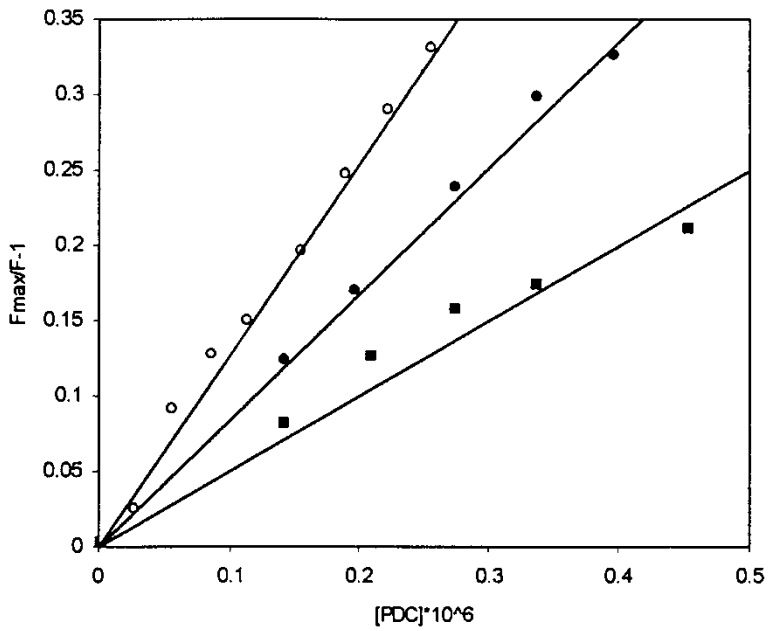


FIGURE 5 Stern–Volmer plot for fluorescence quenching of BSA by PDC. (●) 0.01 M NaCl, (■) 0.1 M NaCl and (○) 0.2 M NaCl.

The linear Stern–Volmer plot shown in Fig. 5, indicates that Eq. (1) is applicable for the present system. Titration experiments were conducted at a $[BSA]_0/[PDC]_0$ molar ratio up to 6.0. $K_{sv}$ could be approximately regarded as binding constant [13–15]. The values of $K_{sv}$ at various ionic strengths are recorded in Table I.

TABLE I The values of Stern–Volmer constant and average aggregation number of BSA due to its interaction with PDC at pH 7.2, 5mM phosphate buffer and various concentrations of NaCl

| [NaCl] M | $K_{sv}$ ($M^{-1}$) | ⟨J⟩ |
|---|---|---|
| 0.01 | $8.57 \times 10^5$ | 1.23 |
| 0.10 | $5.72 \times 10^5$ | 0.65 |
| 0.20 | $9.60 \times 10^5$ | 1.64 |

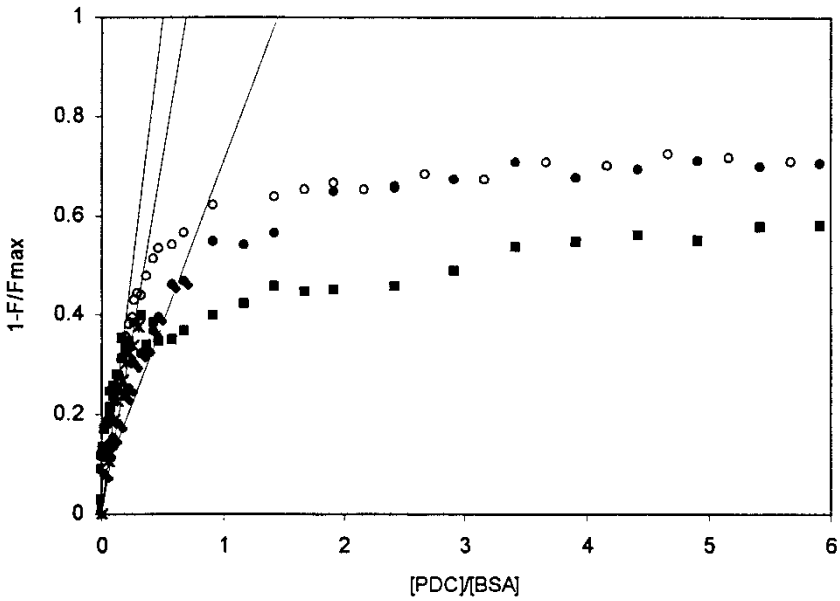


FIGURE 6 Determination of average aggregation number of BSA in the presence of PDC. (●) 0.01 M NaCl, (■) 0.1 M NaCl and (○) 0.2 M NaCl.

One of the major problems in application of drugs is the protein aggregation which is usually induced by drugs. By a similar method developed by Borrisevich *et al.*, for the determination of average aggregation number ⟨J⟩ of BSA in the presence of porphyrin [13], it is possible to determine ⟨J⟩ of BSA in the presence of PDC. It has been shown that:

$$1 - F/F_{\max} = \langle J \rangle [PDC]_0/[BSA]_0 \tag{2}$$

Figure 5 shows the variation of $(1-(F/F_{\max}))$ *versus* $[PDC]_0/[BSA]_0$. The deviation from Eq. (2) at higher $[PDC]_0$ concentrations is due to the presence of free PDC molecules in the solution. The ⟨J⟩ values calculated from the slope of the lines in Fig. 6 are listed in Table I.

## CONCLUSION

Based on our data we can conclude that PDC bind effectively to BSA and therefore, BSA is a suitable drug carrier for PDC. The quenching of BSA solution by PDC indicates that PDC probably binds to the site I of BSA located in subdomain (IIA) where trp (214) was located.

A Stern–Volmer linear relationship can be applied for quenching of BSA by PDC. $K_{sv}$ can be taken as a binding constant, the values of $K_{sv}$ in Table I showing that the minimum affinity of binding is at 0.1 M NaCl. This indicates that the interaction depends on both electrostatic forces and the conformational stability of the BSA molecule. The values of $\langle J \rangle$ show that binding of PDC does not induce any considerable aggregation in BSA molecules. Due to the fact that the aggregation of proteins depend on the extent of protein–protein interactions, it can be concluded that binding of PDC to BSA does not induce any considerable change in the conformation of BSA molecules.



### *Acknowledgement*

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