

STUDY OF THE INTERACTION OF Tb(III) WITH DEXTRAN THROUGH
FLUORESCENCE SPECTROSCOPY AND OPTICAL ROTATORY DISPERSION

SANDRA SOARES VASCONCELOS

JUDITH FEITOSA RODRIGUES

Departamento de Química Orgânica e Inorgânica
Universidade Federal do Ceará, Ceará - Brasil.

ABSTRACT - A study of the interaction of Tb(III) with dextran in aqueous solution was performed using fluorescence spectroscopy and optical rotatory dispersion. The results indicate the formation of a complex with the displacement of water from the cation coordination sphere by hydroxyl groups at the second and third carbon atoms of the monomer unit.

INTRODUCTION

Dextran is known to interact with Cu(II), Zn(II), Co(II), Mn(II) and Ca(II) in aqueous solution. These interactions were studied through dialysis equilibrium, UV/VIS spectroscopy, viscosity and optical rotatory dispersion.

The only indication to the formation of the complexes of dextran with rare earth elements in literature is the one of Selye and col. . In this work they reported that the intoxication of rats by letal doses of rare earth chlorides could be prevented by pretreatment with dextran complexes of Fe and Cr.

Terbium(III) is a R.E. ion that presents intense fluorescence in solution. It has been observed that this fluorescence intensity is enhanced when the metallic ion is bonded to polymers^{2,3}.

We describe fluorescence experiments that show that dextran interacts with Tb(III) in aqueous solution.

In our investigations we also used O.R.D. in order to further characterize the complex, since its optical activity in solution depends, among others factors, on the presence of coordinated metallic ion, on the coordination site and on structure and stability of the complex formed.

EXPERIMENTAL

Dextran (M.W. 60.000-90.000) was purchased from NBCo and used without further purification. α -methyl glucoside 99% was obtained from Aldrich Chemical Company. Tb_4O_7 (99%, from Alfa Products) was purified and dissolved in HCl to give $TbCl_3$. Tris (tris hydroxy methyl amino methane) from Merck was used as a non complexing buffer to adjust pH of 6.6. The polymer was characterized by infrared and NMR spectra. The fluorescence intensities (emission 543nm) was recorded by a Perkin Elmer MPF-44B instrument, with and without dextran 0.05M (in monomer residues) by adding small aliquots (10-50 μ l) of $TbCl_3$ 0.2M. The pHs of 3.0 and 4.4 were reached by addition of HCl and the pH 6.6 was buffered with tris 0.03M. The same procedure was used in the titration of α -methyl glucoside 0.05M. Solvent deuteration effect in the intensity of the Tb(III) fluorescence was verified with D_2O from Aldrich Chemical Company with excitation at 290nm and emission at 543nm.

Optical rotation was studied using a Cary 60 spectropolarimeter in the spectroscopy range of 260 to 400nm. Dextran solution (0.05M) in the presence and absence of $TbCl_3$ 0.02M were used. Specific rotation $[\alpha]$ and rotation per polymer unit $[m]$ ($[m] = [\alpha] M/100, M$ is the molecular weight of the polymer unit), was determined. The molecular rotatory displacement $\Delta[m]$ was found as the difference between $[m]$ values of dextran solution with and without metal.

RESULTS AND DISCUSSION

FLUORESCENCE MEASUREMENTS

The emission spectra of Tb(III) ion in presence of dextran and in absence of the polysaccharide are shown in figure 1. The position and shape of the four characteristic bands of the Tb(III) are unaltered by the presence of the polymer. Meanwhile, the fluorescence intensity was considerably enhanced. This enhancement was verified under excitation between 250 and 310nm.

Since the intensity of the Tb(III) fluorescence is pH dependent, the pH effect in the Tb(III) fluorescence intensification in the presence of dextran was verified (table 1). The interaction Dextran-Tb(III), probably responsible for the intensification,

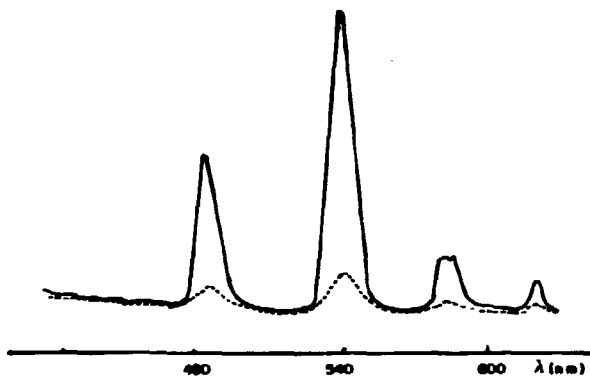


FIGURE 1 - Fluorescence spectrum of Tb(III) in water (---) and Dextran-Tb(III) (—); $\lambda_{ex} = 290\text{nm}$; $5 \times 10^{-3}\text{M}$ TbCl_3 and 0.05M Dextran.

increases with the pH. To confirm these observations we titrated the Tb(III) solution with dextran at 3.0, 4.4 and 6.6 (figure 2).

TABLE 1 - pH effects on the metallic ion enhancement in the presence of dextran; 0.05M dextran, $5 \times 10^{-3}\text{M}$ TbCl_3 ; $\lambda_{ex} = 290\text{nm}$; $\lambda_{em} = 543\text{nm}$.

pH	F^* Tb(III)	F^* Tb(III)-Dex	ΔF
2.9	1.8	7.0	5.2
4.4	3.1	11.5	7.4
5.9	5.3	17.7	12.4
6.9	6.8	31.5	24.7

* Arbitrary units.

A saturation of coordination sites of the polymer as Tb(III) concentration increases is achieved in all the experiments. These results indicate that Tb(III) ions form complex with dextran at pH solution in the range 2.9 - 6.9. Saturation as an evidence of complexation was presented in fluorimetric studies of R.E. ions with hydrolyzed polycrylamide² and transferrin.

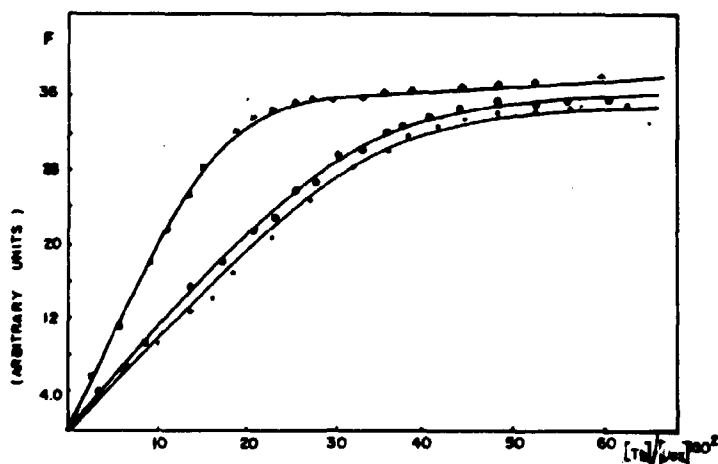


FIGURE 2 - Verification of the pHs effect on the spectrofluorimetric titration of 0.05M dextran with Tb(III); $\lambda_{em} = 543$ nm and $\lambda_{ex} = 290$ nm; pH 6.6 (-), pH 4.4 (o) and pH 3.0 (·).

A displacement of the titration curves to the right, in consequence of an increase in pH, suggest that the interaction dextran-Tb or the complex stoichiometry (molar ratio Tb/Dex) increases with pH. Elmgren demonstrated that the dextran deprotonation occurs in pH superior to 12, what excludes this effect as the one responsible for the possible increase in interaction with the pH. On the other hand, effect of the ionic strength and small modifications in the dextran macromolecular conformation, could be associated with the pH effect in a interaction of predominant eletrostatic character.

As for the variation of the compound stoichiometry, data on figure 2 indicate that Tb(III) coordination is completed when the ration between dextran monomer molar concentration is ≥ 5.6 , ≥ 3.0 and ≥ 2.6 at pHs 6.6, 4.4 and 3.0 respectively. The great difference between the molar ratio Dex/Tb(III) of the complex formed at pH 6.6 and the molar ratio obtained at others pHs could be associated with the Tb(III) hydrolysis. Species such as $[Tb(H_2O)_x(OH)_y]^{+(3-y)}$ ($x+y = 9$), at this pH, might be bonded to the dextran.

A 1:1 stoichiometry was determined for many RE complexes with cyclic polyhydroxyl ligands. The small number of coordinated dextran monomer is probable due to the repulsion that the polysaccharide chain, positively charged by the bonded metallic species offers to new Tb(III) or $[Tb(H_2O)_x OH_y]^{+(3-y)}$ ions

that approach the complex. Another explanation could come from the steric hindrance effects of the dextran ramifications.

Lugovaya and Col. showed that of 340 glucopyranose units, in a concentration of 0.1M dextran in dextran-copper solutions, only 17 ions are combined, a proportion still smaller than the one observed for the dextran-Tb(III) system.

Data in figure 2 together with the Tb(III) ion fluorescence intensity in the absence of the polymer, makes possible to estimate the relative increase in the R.E. ion fluorescence when bonded to the dextran. The complex Tb-Dex fluorescence for 8.0×10^{-3} M Tb(III) is twice more intense than the aqueous $TbCl_3$. A direct excitation mechanism must occur, as suggest by Rodrigues and Galebeck for the polyacrylamide - Tb(III) system.

A solvent deuteration causes, in general, an intensification in the fluorescence of R.E. ions and complexes and this deuteration effect has been used to provide a direct measurement of the number of water molecules coordinated to the metal.

We determined that the ratio of the fluorescence in D_2O and in H_2O (F_{D_2O}/F_{H_2O}) for $TbCl_3$ 0,02M is 16, and for $TbCl_3$ 0,02M in the presence of dextran is 11. Coordinating groups of polysaccharides, probable hydroxide groups, must displace water outside cation coordination sphere, what supresses effectively R.E fluorescence. The asymmetry of the formed complex must also contribute to the intensification of the Tb(III) fluorescence.

Figure 3 compares the fluorimetric titration curves of dextran with its analogue of low molecular weight, the α -methyl glucoside. A larger shift to the left for the polymer curve suggest that Tb(III) interacts more with the polymer.

In small concentrations of Tb(III) a cooperative effect among adjacent groups of the polymer (chain effect) and the ligands groups concentration contributes to a larger interaction Tb(III)-Dextran. Smid and Col. used this effects to explain a larger interaction of metallic ions with polyethers that with its monomeric units.

OPTICAL ROTATORY DISPERSION MEASUREMENTS

Dextran is a dextrorotatory polysaccharide formed of monomers of α -D glucopyranose connected mainly through α 1-6 bonds. From the various possible conformation the more probab

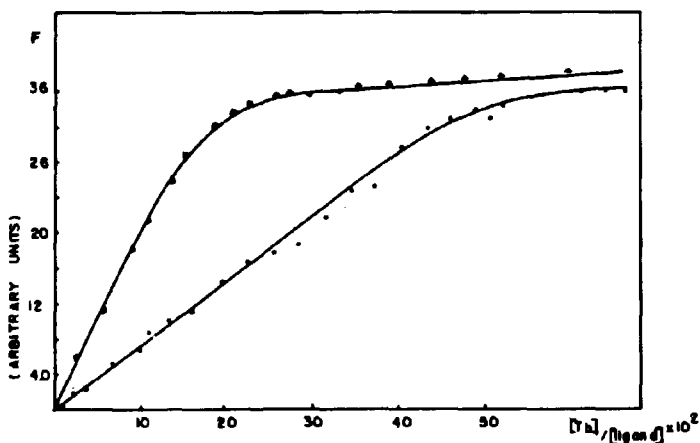


FIGURE 3 - Spectrofluorimetric titration curves of 0.05M dextran (-) and 0.05M α -methyl glucoside (·) with Tb(III) in 0.03M tris (pH 6.6); $\lambda_{ex} = 290\text{nm}$ and $\lambda_{em} = 543\text{nm}$.

for the pyranoside ring is the C-1, (figure 4). This polymer consequently does not have the axial - equatorial - axial sequence of the hydroxyl groups that would be necessary to the formation of the most stable tridentate complex.

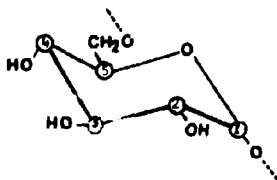


FIGURE 4 - C-1 conformation of glucopyranose units of dextran.

As demonstrated by Reeves⁸ only adjacent OH groups C-2, C-3 and/or C-3, C-4 with projected angles of respectively 60° and 60° are able to form bidentate complexes. The specificity of these angles is reflected in the optical behavior of the resultant complexes, levorotatory complexes are formed in the first case, and for these the molecular rotatory displacement ($\Delta [m]$) is negative. In the second case dextrorotatory complexes are formed since $\Delta [m]$ is positive.

Figure 5 shows typical curve of optical rotation dispersion of dextran in the presence and absence of terbium ions.

The presence of the metallic ions reduces significantly the rotatory power of the dextran confirming the complex formation. Negative values of $\Delta[\alpha]$ (table 2) are determined. The OH groups that take part in the complex formation are the C-2 and C-3 of the monomeric units and a levorotatory complex is formed.

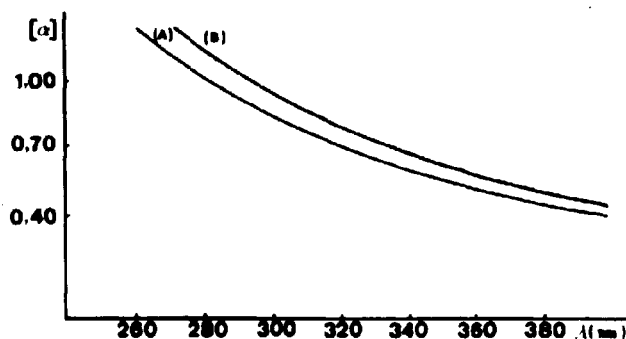


FIGURE 5 - Optical rotatory dispersion curves of; A) 0.05M dextran in the presence of 0.02M $TbCl_3$ and B) 0.05M dextran; 0.03M tris (pH 6.6).

TABLE 2 - Specific optical rotation of 0.05M dextran in 0.03M tris (pH 6.6) in the presence and absence of 0.02M $TbCl_3$ and molecular rotatory displacements ($\Delta[\alpha]$).

λ (nm)	Specific Rotations $[\alpha]$		$\Delta[\alpha]$
	dextran	dextran- $Tb(III)$	
280	1.235 ± 12	1.195 ± 12	-65 ± 20
320	864 ± 12	828 ± 12	-58 ± 20
360	642 ± 12	611 ± 12	-50 ± 20
400	494 ± 12	475 ± 12	-31 ± 20

Similar complexes were obtained by Lugovaya and Col.⁶ with dextran and $Cu(II)$, $Zn(II)$ and $Ni(II)$. At 436nm it was observed $\Delta[\alpha]$ of approximately -40, comparable to the system with $Tb(III)$.

Studies were also made for the α -methyl glucoside; an analogue of dextran with low molecular weight. The specific rotation of the α -methyl glucoside was similar to the polysaccharide indicating that, the optical activity is predominantly due to the monomeric units conformation, with small contribution of the

macromolecular conformation. A negative molecular rotatory displacement smaller than with dextran was also observed for the system.

Results of these investigations therefore indicate the same conclusions of the fluorimetric studies; the interaction of Tb(III) with dextran is higher than the one with its analogue of low molecular weight.

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