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# Cationic aggregation during chromium complexes preparation

## Effects of pH and temperature on $^{51}$Cr-β-glycerophosphate complex structure *

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SUMMARY

*The effects of pH and heating during the preparation of $^{51}$Cr-β-glycerophosphate complex have been studied by electrophoresis, dialysis and ion exchange analysis. When the pH is higher than 4.8 the positive charge of the complex is reversed and it migrates to the catode. The anionic exchange analysis also corroborates this observation of a transition pH at about 4.8. A higher pH and/or heating increases the cationic aggregation with a consequent change in the physicochemical properties of the complex.*

Chromium is an element with unique characteristics due to its coordination tendency. When a Cr (III) salt is dissolved in water, chromium is present as an hexaquo complex $[Cr(H_2O)_6]^{3+}$. This complex is potentially an acid and the equilibrium will de displaced to the right by heating :

$$[Cr(H_2O)_6]^{3+} \rightleftharpoons [Cr(H_2O)OH]^{2+} + H^+$$

Simultaneously the process of olation is promoted leading to the formation of polynucleate complexes consisting of chains of Cr(III) ions linked by bridging hydroxide groups :

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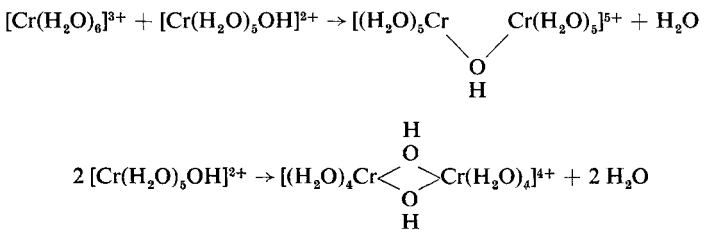
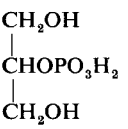
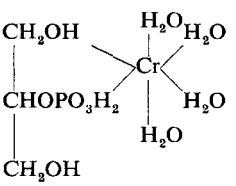


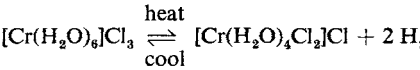
By anion penetration, certain anions can replace some of the OH groups or $H_2O$ molecules, preventing further growth of the dinuclear species through hydroxobridge formation. The number of hydroxy and/or aquo groups displaced by an incoming ion depend on the number of donor groups in the anion and their relative position. In the β-glycerophosphate case, the donating electron groups may be $PO_3H_2$ or the OH groups from the glycerol molecule :



Preliminary experiments with phosphoric acid and glycerol mixture have demonstrated that in such a case, no complex formation is possible because those functional groups present in each individual molecule (OH or $PO_3H_2$) are unable to promote the formation of a ring with the chromium atom as the closing member. On the contrary, the β-glycerophosphate promotes this complex configuration :



A phenomenon of fundamental importance in this complex formation is the anion penetration. Thomas *et al.*[1] explained it as a competition between hydroxide ions or other anions for positions in the first coordination sphere of the metal cation. Coordination groups such as aquo, or anion, also may be replaced by another anion. If a solution of Cr(III) chloride hexahydrate is heated, the bright green (tetra-aquo) form is produced [2] :



In pure water this reaction can be reversed by cooling the solution. If the heating has been performed in presence of sodium β-glycerophosphate the

green color stays after cooling which is an indication that the anion penetration process took place and some chloride ions have been replaced in the inner complex by β-glycerophosphate ions.

The formation of $^{51}Cr$-β-glycerophosphate complex evidently is related to the tendency of Cr(III) to form coordination compounds. In general, is not a unique process involved; on the contrary, hydrolysis, olation, and related processes have a significant effect on the final compound. Therefore, in preparations of this type, the study of critical factors as temperature and pH is very important. This recently developed $^{51}Cr$-β-glycerophosphate complex is a promising tumor localizing agent [3, 4, 5], and the knowledge of its chemistry may considerably improve its usefulness which has been demonstrated by biological studies made using some of the different preparations used in this experimental work [6]. On this basis, the effects of pH and temperature on the nature of the final complex have been studied by electrophoretic, ion exchange and dialysis methods.

## Experimental Part

### Complex preparations

All the complexes used in these experiments have been prepared with a molar ratio Cr (III) : sodium β-glycerophosphate = 1 : 1, according to the following technique : 100 mg of sodium β-glycerophosphate ($Na_2C_3H_5(OH)_2PO_4 . 5\frac{1}{2}H_2O$) dissolved in 1 ml of distilled water were added to 16 mg of Cr(III) (as was $CrCl_3$ with the $^{51}CrCl_3$). The final volume was 5 ml and the pH 3.5. Then, it was heated in a boiling water-bath for 15 minutes. After cooling it down to 8-10° C, the solution was divided into 5 portions. The pH, which had dropped during the heating to 1.2, was adjusted in the following way : 1) to pH 1.5, 2) to pH 4.8, 3) to pH 6.0, 4) to pH 7.0, and 5) to pH 8.2 by adding 1 M $NaHCO_3$. Afterwards, a half of each fraction was heated in a boiling water-bath for 15 minutes. After cooling, the pH, when necessary, was adjusted to its value before heating.

### Electrophoresis.

Samples of each preparation (with and without heating) were analyzed by electrophoresis on Whatman 3MM paper, using 0.02 M Veronal buffer (pH = 8.6) and 0.02 M phosphate buffer (pH = 4.5). Figure 1 shows the migration pattern for each type of preparation after 30 minutes of electrophoresis using a voltage gradient of 15 V/cm.

Under these experimental conditions, any uncomplexed chromium should remain on the starting line when the electrophoresis is performed using pH 8.6 buffer. The migration studies show (Fig. 1) that only when the pH is 1.6 or

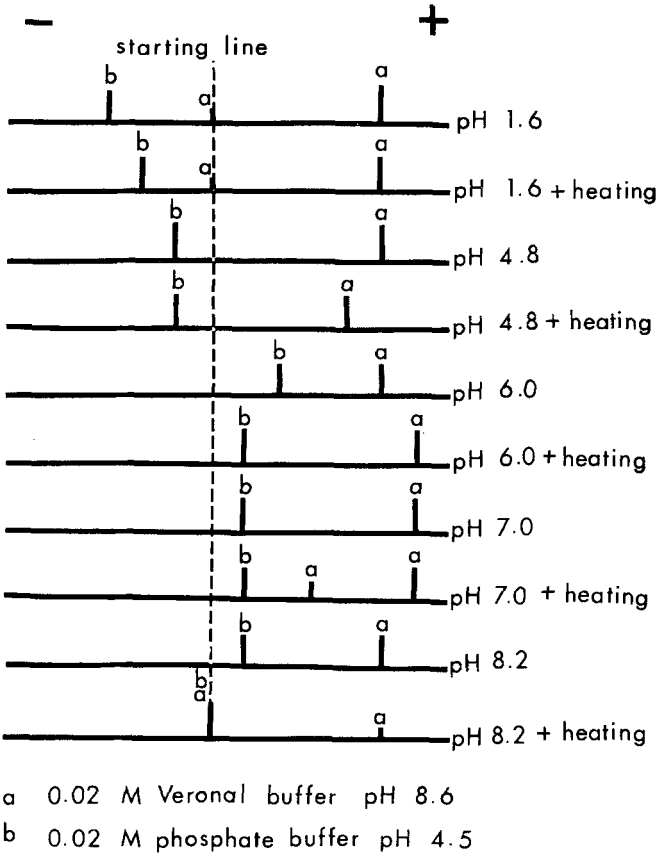



FIG. 1. Electrophoretic migration of different $^{51}Cr$-β-glycerophosphate preparations.

8.2 (with heating) is a non-moving radioactivity noticeable. In the first case it can be related to a free chromic ion precipitated by the basic buffer. This explanation is corroborated by the moiety moving toward the anode when the electrophoresis is done with pH 4.5 buffer. This cationic form migrates faster than the complex at pH 4.8, which also presents a net positive charge as can be seen in figure 1. On the other hand, at pH 8.2 (with heating) the origin of the remaining activity is the oxolation process provoked by heating of the complex at a high pH. On the contrary, in absence of heating the total radioactivity migrates as a catode bound moiety. An interesting observation is that at a pH above 4.8 the radioactivity migrating to the anode disappears. It seems that at a higher pH the positive charge contribution of the chromium ion is neutralized by the olation process, and the final charge is due only to the anionic part of the complex molecule :

$$[Cr(\beta GPA)(H_2O)_4]^{3+} \rightarrow [Cr(\beta GPA)(H_2O)_3OH]^{2+} + H^+$$

where βGPA = β-glycerophosphate ion
as the proton ($H^+$) is neutralized by the increasing basicity, the complex undergoes a further ionization :

$$[Cr(\beta GPA)(H_2O)_3OH]^{2+} \rightarrow [Cr(\beta GPA)(H_2O)_2(OH)_2]^{+} + H^{+}$$

(and so on) until the complex's total electrical charge becomes negative. The negative contribution of the β-glycerophosphate anion to the complex is not considered here. If this electric charge contribution is considered, a less complete olation process is necessary for the total charge reversion.

These experimental results indicate that the transition pH is above 4.8. Also, the fact that the electrophoresis (with buffer pH 4.5 or pH 8.6) shows a similar migration for all the preparations indicates that the complex's charge reversion process is completed above pH 6.0 and is independent of a further pH increase; only the oxolation (provoked by heating) seems to modify the final product.

## Dialysis.

Twenty four hours dialysis against distilled water (with 10 changes of liquid) was performed on samples of each preparation. At the end of the

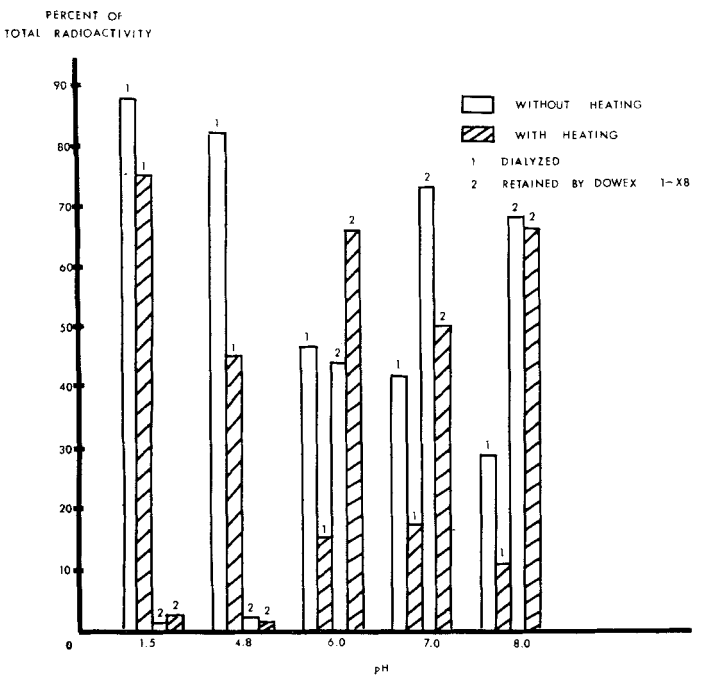



Fig. 2. Dialysis and anion exchange analysis of different $^{51}Cr$-β-glycerophosphate preparations.

experiment, the radioactivity of the dialysis bag contents was measured as was the pooled liquid. The dialysis values (Fig. 2) indicates a higher migration through the membrane at low pH. This migration is reduced as the pH increases and more sharply so when the preparation is heated. This behaviour corroborates the electrophoresis findings of increased olation, which, in this case, originate a higher cationic aggregation with an increase in molecular size.

ANION EXCHANGE ANALYSIS.

Small samples (0.2 ml) of each preparation were passed through small (7 cm × 0.7 cm) anion exchange resin Dowex 1-X8 (chloride form) columns, and eluted 3 times with 1.5 ml of distilled water. Afterwards, the radioactivity was counted on both eluate and resin columns. Figure 2 shows the results as percentages of the radioactivity retained by the resin.

The assumption of a transition pH (about pH 4.8) resulting from the consideration of the experimental electrophoretic study, is reinforced by the anion exchange analysis. Below pH 6.0, practically no radioactivity is retained by the anion exchange resin (less than 3 %). On the contrary, when the pH increases to 6.0, a sharp increase in the anion exchange is observed (between 45 to 61 %). At this point, this high increase and the still greater value observed with a heated preparation is in agreement with the previously stated charge reversion by olation and oxolation of the original complex formed at lower pH.

In conclusion, these experimental findings point out the importance of olation and oxolation processes commonly affecting the nature of covalent complexes, and also indicate the role that certain variables, such as pH and temperature, play in determining the final structure of these complexes during their preparation. Similar behaviour also should be expected in complex preparations involving cations with a defined tendency to undergo cationic aggregation, such as In, Ga, Sc, La, Co, Fe, Ni, Hg and Cu [7].

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