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Chromic phosphate labelled with ^{51}Cr and ^{32}P-Chromic hydroxide labelled with ^{51}Cr : their nature and radioactive yield

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SUMMARY

Studies were performed on the radioactive yield of preparations of chromic phosphate and chromic hydroxide (as a particulate precipitate and as a true colloid) labelled with ^{51}Cr and ^{32}P.

Using an EDTA solution, and under proposed standard conditions, the degree of polymerization (olation and oxolation) of these compounds was investigated.

Chromic phosphate, labelled with either ^{32}P or ^{51}Cr, has been extensively used in nuclear medicine, and the numerous preparation techniques described [1, 2, 3, 4, 5, 6] have not indicated the nature of the final product, so that considering the coordination capability of the Cr(III) ion, it is unlikely that a unique compound can be obtained.

A simple method of oxidation-reduction for obtaining this compound was previously described by the author [7], and studies have also been carried out on the nature of the final product and the radiochemical yield for both ^{32}P and ^{51}Cr.

This technique is based on the reaction :

$$2\ CrO_3 + 6\ Na_2SO_3 = 2\ Cr^{3+} + 6\ Na_2SO_4$$

but Cr(III) in aqueous solution is always coordinated [8, 9]; it is never present as a simple cation Cr^{3+}. In this case, because of the neutrality of the medium

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and the potential acidity of the aquo-complex $Cr(H_2O)_6^{3+}$, according to the equation

$$[Cr(H_2O)_6]^{3+} \rightleftharpoons Cr(H_2O)_5OH^{2+} + H^+$$

the equilibrium can be displaced to the right by heating. In this way an olation process is initiated. Polynucleated complexes are formed by polymerization, where the chains of Cr(III) ions are bound through hydroxy groups :

$$[Cr(H_2O)_6]^{3+} + [Cr(H_2O)_5]^{2+} \rightarrow [(H_2O)_5Cr\langle O(H)\rangle Cr(H_2O)_5]^{5+} + H_2O$$

$$2\,[Cr(H_2O)_5OH]^{2+} \rightarrow [(H_2O)_4Cr\langle O(H)\rangle Cr(H_2O)_4]^{4+} + 2\,H_2O$$

The monols and diols formed in accordance with these reactions will continue the olation process to form larger aggregates, finally resulting in the precipitation of what is commonly called chromic hydroxide, $Cr(OH)_3$, which in actual fact is a large three-dimensional complex, a hydrate, $Cr(OH)_3.X\,H_2O$.

Since the phosphate ion itself has some coordination tendency, we can expect some competition for Cr(III) between ion phosphate, when present, and hydroxide ion. This phenomenon, called anion penetration or anation, is the replacement of coordinated water molecules, OH^-, organic ligands by anions having some coordinating tendency. Because of this property, the olated compound, either in colloidal dispersion or in the precipitated hydrous form, will contain these anions if present in the solution during the olation. If heating is used to promote olation, another reaction takes place : oxolation.

$$\left[Cr\langle O(H)\rangle Cr\right]^{X+} \xrightarrow{\theta} \left[Cr\langle O\rangle Cr\right]^{(X-2)+} + 2\,H^+$$

This oxolated form presents less reactivity than the olated one.

If the olation is done in a medium containing gelatin, the resulting complexes will combine with the protein, thus avoiding polymerization in larger aggregates, which could ultimately lead to an insoluble hydrate. It has already been suggested that the process depends on coordination with carboxyl groups provided by the aspartic and glutamic acid residues of the protein [10].

Experimentally it was found that freshly precipitated chromium hydroxide dissolves in a 0.1 M EDTA (pH = 5.1) solution (Table I) and, by working on this basis under the standard conditions described elsewhere, it is possible to determine the amount of Cr(III) present in a non-oxolated form (hydroxide) in the presence of chromic phosphate. All this, is on the assumption that the compound also contains some phosphate formed by anion penetration which is more weakly bound than normal chromic phosphate. The experimental results seem to corroborate this assumption.

TABLE I. Solubility in EDTA solution of $Cr(OH)_3$ [a] pre-heated at different temperatures.

Temperature °C	Heating time minutes	Solubilized %
20	15	99.85
40	15	99.86
60	15	45.17
80	15	9.87
100	15	7.13
100	30	6.29
100	60	6.68
100	120	8.10

[a] Prepared according to the reaction : $Cr(NO_3)_3 + 3\ NH_4OH = Cr(OH)_3 + 3\ NH_4NO_3$.

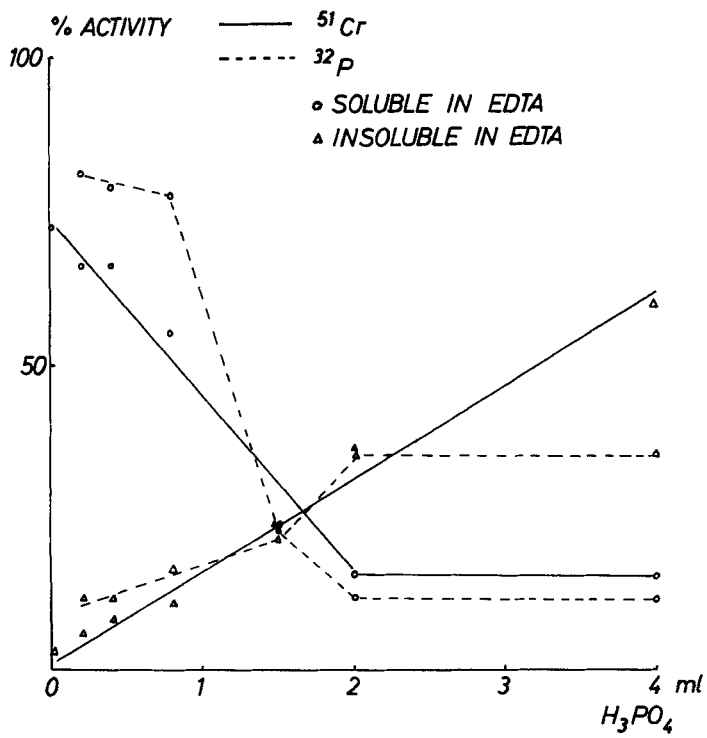

FIG. 1. Effects of increasing amounts of H_3PO_4 in the preparation of particulate chromic phosphate (*B* CrO_3 = 2.5 ml).

FORMATION OF CHROMIC PHOSPHATE

The general technique [11] is to mix a volume *A* of H_3PO_4 solution (10 mg/ml) with a volume *B* of CrO_3 solution (10 mg/ml), both with the activities incorporated. Then 1 ml of Na_2SO_3 (100 mg/ml) is added, bringing the mixture to a volume of 8 ml with distilled water. After heating in a boiling water bath for 15 minutes, it is cooled at room temperature, centrifuged and the precipitate washed three times with distilled water.

For the treatment with EDTA, the precipitate was heated with 7 ml of 0.1 M EDTA (pH = 5.1) solution in a boiling water bath for six hours. Finally, after cooling, it was centrifuged and the activities (beta and gamma) counted in the supernatant as well as in the solution of the precipitate in concentrated HCl.

Fig. 1 shows the values for soluble and insoluble in EDTA, in a test done with a volume *B* = 2.5 ml and values of *A* from 0 to 4 ml.

In the case of ^{51}Cr, when the ratio H_3PO_4/CrO_3 increases the insoluble in EDTA also increases. On the other hand, for ^{32}P the behaviour is similar but the insoluble in EDTA reaches a steady value.

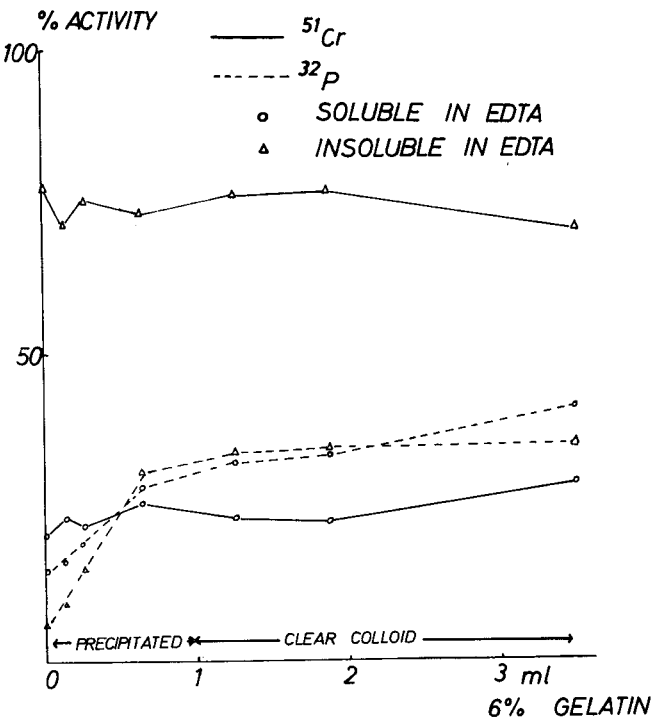

FIG. 2. Effects of the amount of gelatin in the formation of colloidal chromic phosphate (*A* H_3PO_4 = 2 ml and *B* CrO_3 = 2.5 ml).

The use of $A = 0.8$ ml and $B = 2.5$ ml gives the highest yield both in ^{32}P (97.1 %) and ^{51}Cr (67.5 %) incorporated in the chromic phosphate precipitate (Table IV).

Formation of Colloidal Chromic Phosphate

The general technique of preparation is similar to the one used for particulated chromic phosphate, the only difference being that the gelatin is dissolved in the Na_2SO_3 solution before being added. The colloid is purified by dialysis against distilled water until there is no more activity in the water.

Using a volume $A = 2$ ml and $B = 2.5$ ml, it was found that when less than 0.6 ml of 6 % gelatin solution is added, a precipitate is formed. In the clear colloid zone (Fig. 2), the ratio soluble in EDTA/insoluble in EDTA and the yield of both ^{51}Cr and ^{32}P incorporated in the colloid are practically constant.

When the CrO_3 is kept constant, an increase in the H_3PO_4 improves the ^{51}Cr yield and particularly that of the insoluble in EDTA (Fig. 3). Similarly, for the same amount of H_3PO_4 increasing the CrO_3 raises the amount of ^{32}P incorporated as well as the insoluble in EDTA (Fig. 4).

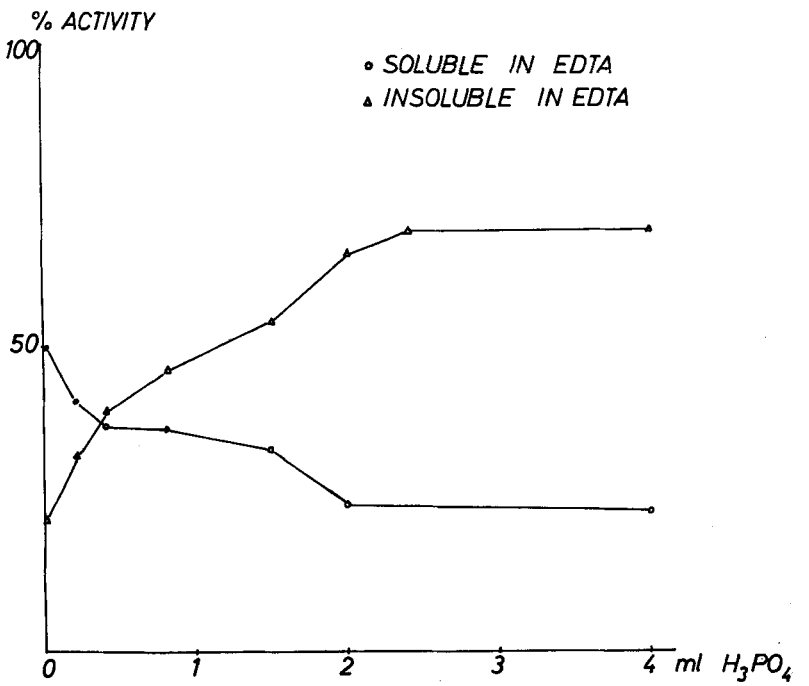

FIG. 3. Effects of increasing amount of H_3PO_4 in the preparation of colloidal chromic phosphate (^{51}Cr) (B $CrO_3 = 2.5$ ml and 6 % gelatin = 2 ml).

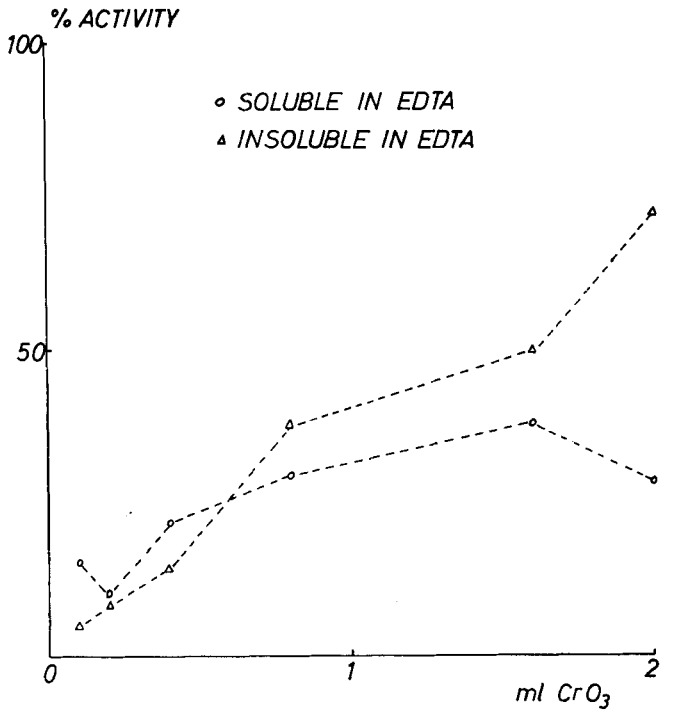

FIG. 4. Effects of increasing amount of CrO_3 in the preparation of colloidal chromic phosphate (^{32}P) (*A* H_3PO_4 = 1 ml and 6 % gelatin = 1 ml).

FORMATION OF COLLOIDAL CHROMIC HYDROXIDE

By using only CrO_3 and Na_2SO_3 with the technique described above it is possible to obtain the precipitation of chromic hydroxide or a colloidal solution (if gelatin is used). The yield of ^{51}Cr incorporated in the colloidal hydroxide (85 %) is practically constant for both soluble and insoluble in EDTA (Fig. 5) and independent of the increase in the amount of gelatin, which only seems to affect the physico-chemical properties. The same preparation carried out at 4° C for 30 hours forms almost exclusively the compound soluble in EDTA (Fig. 5).

EFFECTS OF BLOCKING THE CARBOXYL AND AMINO GROUPS IN THE GELATIN

The carboxyl and the amino groups were blocked using the techniques of Fraenkel-Conrat and Olcott [12] and Philpot and Small [13] respectively. Only the carboxyl blocking showed a slight increase in the fraction soluble in EDTA (Table II).

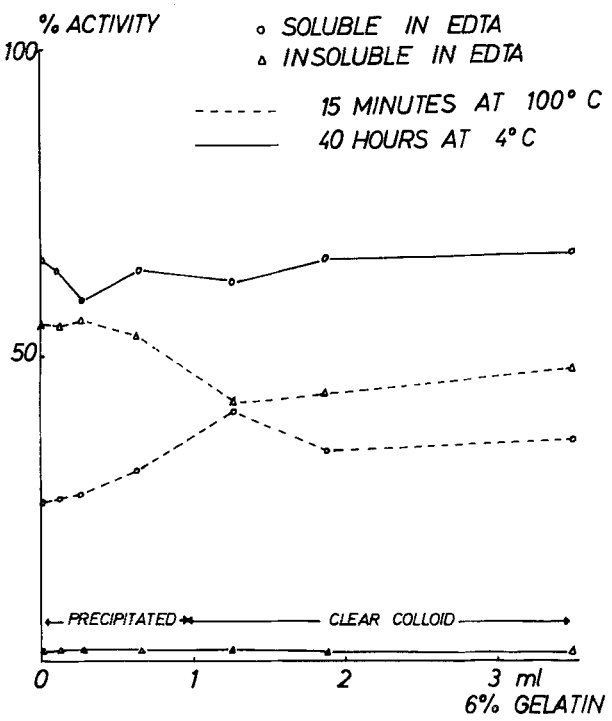

FIG. 5. Effects of increasing amounts of gelatin in the preparation of colloidal chromic hydroxide (*B* CrO_3 = 2.5 ml).

TABLE II. Influence of carboxyl and amino group blocking on the colloidal chromic phosphate preparation.

	EDTA soluble		EDTA insoluble		Unreacted	
	^{32}P %	^{51}Cr %	^{32}P %	^{51}Cr %	^{32}P %	^{51}Cr %
Carboxyl-blocked	20.0	32.0	64.5	67.5	15.5	0.5
Amino-blocked	16.5	27.0	72.0	72.5	1.5	0.5
Control	14.5	22.5	73.0	75.9	12.5	1.6

EFFECTS OF ACETIC ACID

The preparation of both particulate and colloidal chromic phosphate was performed in the presence of acetic acid. A marked increase was observed in the fraction soluble in EDTA (Table III).

TABLE III. Effects of acetic acid on chromic phosphate preparation.

	Type	EDTA Soluble		EDTA Insoluble		Final pH
		^{32}P %	^{51}Cr %	^{32}P %	^{51}Cr %	
+ Acetic acid	Particulate	51.0	56.0	17.0	17.0	4.1
	Colloidal	46.5	59.0	32.0	27.0	4.2
Control	Particulate	22.0	34.5	67.5	76.0	7.6
	Colloidal	15.0	22.5	74.0	76.0	7.7

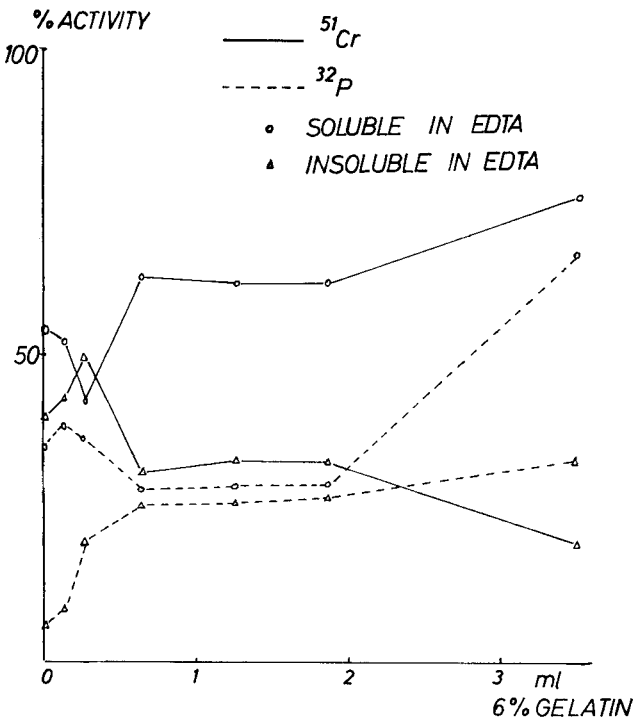

FIG. 6. Effects of increasing amounts of gelatin in the preparation of colloidal chromic phosphate in acetic acid medium (*A* H_3PO_4 = 2 ml and *B* CrO_3 = 2.5 ml).

TABLE IV. Radioactivity yield of different preparations.

Compound	EDTA Soluble		EDTA Insoluble				
	^{32}P %	^{51}Cr %	^{32}P %	^{51}Cr %	H_3PO_4 A ml	CrO_3 B ml	6 % Gelatin ml
$^{51}Cr^{32}PO_4$ particulate	80.3 12.1	57.7 14.3	16.8 35.8	9.8 36.7	0.8 2.0	2.5 2.5	— —
$^{51}Cr^{32}PO_4$ colloidal	23.6	31.5	72.0	67.4	1.0	2.4	1.0
$^{51}CrPO_4$ colloidal	—	26.1	—	73.8	2.0	2.5	2.0
$Cr^{32}PO_4$ colloidal	37.0	—	32.0	—	2.0	2.5	3.5
$^{51}Cr(OH)_3$	—	34.3	—	57.8	—	2.5	—
$^{51}Cr(OH)_3$ colloidal	—	36.2	—	48.0	—	2.5	3.5

The effect of the acetic ion on the preparation of colloidal chromic phosphate was examined by adding 0.1 ml of acetic acid together with the Na_2SO_3. The final pH value was 4.1. The incorporation yield for both activities is much lower and most of the activity appears in the fraction soluble in EDTA (Fig. 6). The same preparation in acetic medium at room temperature, the reaction time being extended to 30 hours, also showed that the colloid presents activity only in the EDTA soluble fraction (Fig. 7).

Effects of the Heating Time

No difference was observed in preparations made with increasing heating times from 15 minutes to 2 hours at 100° C, for both particulate and colloidal chromic phosphate.

Discussion

All these findings are in perfect agreement with the known chromium chemistry. The immediate solubilization in a cold (20° C) 0.1 M EDTA solution (pH = 5.1) of a precipitate of $Cr(OH)_3$ obtained and kept at 4° C, and the increasing insolubility when it was heated for 15 minutes at different

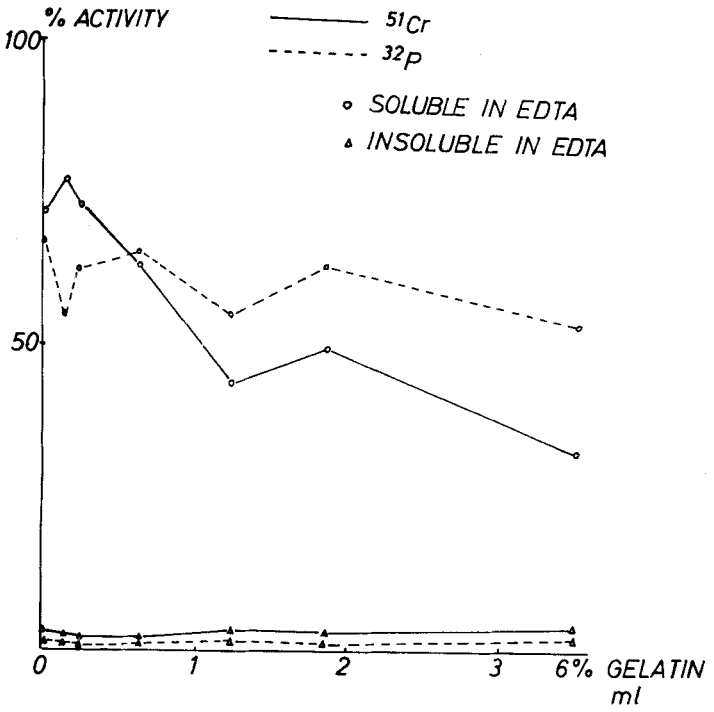

FIG. 7. Preparation as in Fig. 6 but at room temperature for 30 hours.

temperatures (Table I), indicate that the oxolation process starts abruptly above 60° C. Also, when a colloidal $Cr(OH)_3$ is prepared at low temperature, it shows no appreciable olation (Fig. 5).

When the colloidal chromic phosphate is prepared using gelatin, this organic ligand acts similarly to anion penetration by acetate ions. It has been suggested that when olated Cr(III) complexes combine with collagen, this is by a coordination bond with the carboxyl groups [10], this process preventing further oxolation but maintaining the chromium capability to bind phosphate ions. In this case the phosphate ions which have some coordinating tendency are able to perform an anion penetration, replacing the organic ligand.

In general, the effect of the phosphate ions is to increase the amount of the fraction insoluble in EDTA (Figs. 1 and 3), which is an indication that the phosphate is bound to the chromium in a way which excludes subsequent dissolution (deolation) by EDTA.

It should be pointed out that olation and oxolation are widely affected by the presence of different anions, especially when they have a coordinating tendency, such as sulphate and phosphate, and this behaviour could also

affect the solubility in EDTA. Anyway, this technique (treatment with EDTA) can be used in the study of the degree of olation and oxolation of these chromic compounds.

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