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STUDIES WITH COLLOIDS CONTAINING RADIOISOTOPES OF YTTRIUM, ZIRCONTIUM, COLUMBIUM, AND LANTHANUM

1. The Chemical Principles and Methods Involved In Preparation Colloids of YTTrium, Zirconium, Columbium, and Lanthanum

by
John W. Gofman

April 21, 1948

Berkeley, California

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STUDIES WITH COLLOIDS CONTAINING RADIOISOTOPES OF YTTRIUM, ZIRCONIUM, COLUMBIUM, AND LANTHANUM.

I. THE CHEMICAL PRINCIPLES AND METHODS INVOLVED IN PREPARATION OF COLLOIDS OF YTTRIUM, ZIRCONIUM, COLUMBIUM, and LANTHANUM.*

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April 21, 1948
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INTRODUCTION

For a number of investigations, including fundamental studies of radiation effects on living tissues and therapeutic utilization of radioisotopes, it is valuable to have methods for the selective localization of radioisotopes in certain tissues. Finely dispersed anhydrous chromic phosphate has been found useful by Jones, Wrobel, and Lyons[1] in selectively irradiating the liver and spleen with P^{32} beta particles. The present studies, reported in this and the following communication, are concerned with methods for controlled selective localization of colloids (incorporating radioisotopes) in the liver, spleen, or bone marrow, and with an analysis of some of the factors involved in the phenomenon of localization.

Choice of Elements for the Preparation of Colloids.

The elements zirconium, columbium, yttrium, and lanthanum are particularly suitable for the present investigations. First, it is possible to synthesize a variety of types of colloids incorporating radioisotopes of these elements. Secondly, there is available for these elements a large number of radioisotopes of differing nuclear properties. This group includes isotopes of short, intermediate, and long half-life, some of which emit only beta particles, some, only

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gamma rays, and others, both beta and gamma rays. Table 1 gives a description of the nuclear properties of the available radioisotopes.

Table 1

Radioisotopes of Yttrium, Zirconium, Columbium and Lanthanum[5]

Element	$T_{1/2}$	Type of Decay	Energy of Radiations in MEV	
Yttrium			Beta	Gamma
Y^{90}	65 hours	β-; no γ	2.2	None
Y^{91}	57 days	β-; no γ	1.53	None
Y^{88}	105 days	K-capture; γ	None	0.95 1.92
Zirconium				
Zr^{95}	65 days	β-; γ	0.394 (98%) 1.0 (2%)	0.73 (0.92) ?
Zr^{97}	17 hours	β-; γ	2.1	0.8
Zr^{89}	78 hours	β+;(?) γ	1.0	?
Columbium				
Cb^{95*}	90 hours	I.T.,e-,x-rays	e-: 0.22 0.23	(?) (highly converted)
Cb^{95}	35 days	β-; γ	0.15	0.75
Lanthanum				
La^{140}	40.0 hours	β-; γ	0.90 (20%) 1.4 (70%) 2.12 (10%)	0.335 (1%) 0.49 (7%) 0.83 (14%) 1.63 (74%) 2.3 (4%)

Note: None of the isotopes of half life less than 17 hours have been listed. All those described above are available from Oak Ridge or from cyclotron bombardment.

Symbols: β-, electron emission; β+, positron emission; K, K-electron capture; I.T., isomeric transition; e-, conversion electron emission.

The Chemistry of the Preparation of Colloids of Zirconium, Yttrium, and Lanthanum.

Several types of colloids of the elements zirconium, yttrium, and lanthanum, varying in sign of charge, stability toward flocculation by added electrolytes, chemical structure, and particle size, may be prepared. As is shown in the following communication both the disappearance rate of such colloids from the blood stream of animals following intravenous injection and the site of uptake of the colloids can be made to vary through wide limits by alterations in the nature of the colloidal aggregates. The chemical principles involved and the methods used in the preparation of several of the colloids used in this work is given below.

A. Preparation of zirconium colloids of "relatively large" particle size:

1) Zirconium oxide peptized in dilute nitric acid. Owens[2] has shown that freshly precipitated hydrated zirconium oxide may be re-suspended in dilute nitric acid (insufficient stoichiometrically to dissolve the hydrated oxide) to produce positively charged colloidal zirconium oxide. The effective peptizing agent is probably the zirconyl ion produced by the reaction of the zirconium oxide with hydrogen ions.

Preparation: 0.5 ml of 0.1 M zirconium oxychloride solution (containing Zr^{95} or Zr^{89} tracer) is titrated to the phenol red end-point with dilute sodium hydroxide, resulting in the quantitative precipitation of the zirconium. The hydrated oxide precipitate is centrifuged out, the supernate being discarded. The precipitate is suspended in 2.5 ml of 0.02 M nitric acid and then shaken 12 hours in a mechanical shaker to peptize the zirconium oxide. Following this the mixture is centrifuged 3 minutes at ~ 500 G. The supernate from this centrifugation is the final colloidal solution. This sol is visibly cloudy,

demonstrates a bright Tyndall effect, is polydisperse, and of such particle size that the majority of the suspended zirconium is sedimented in ordinary centrifuges at ~ 2000 G. in 60 minutes. Before use in biological experiments this sol is mixed with an equal volume of 10% dextrose solution to render it approximately isotonic.

2) Zirconium phosphate in dilute disodium acid phosphate.

Preparation: 0.1 ml of 0.1 M zirconium oxychloride is mixed with 2.3 ml of 5% dextrose solution and to this mixture is added 0.1 ml of 0.6 M disodium acid phosphate. There results a turbid sol which demonstrates a marked Tyndall effect on illumination. No evident flocculation of the colloid occurs even after 30 minutes of heating at 100°C.

Glucose is present for the purpose of rendering the sol nearly isotonic, since 0.9% saline solution produced immediate flocculation of the colloidal zirconium phosphate. Should foreign ions flocculate the colloid, repeated extraction of the precipitate with the original volume of 5% glucose solution containing disodium acid phosphate (0.024 M) results in colloidal re-suspension of the zirconium phosphate.

3) Zirconium oxide peptized in sodium lactate solution.

0.5 ml of zirconium oxychloride solution is titrated to the phenol red end point with dilute sodium hydroxide, the hydrated zirconium oxide centrifuged out, and the supernate discarded. The precipitate is suspended in 2.5 ml of 0.008 M sodium lactate solution and shaken 12 hours to peptize the hydroxide. Centrifugation for 3 minutes at ~ 500 G. removes the largest aggregates, leaving a supernatant sol that is visibly cloudy and shows a marked Tyndall effect. The mechanism probably operative in production of this type of colloid, namely zirconium-lactate complex-ion formation, is fully discussed in the next section. This colloid is mixed with an equal volume of 10% dextrose solution before being used in biological experiments.

B. Anionic colloids of zirconium and yttrium of varying particle size:

Zirconyl ions form complexes in aqueous solutions with tartrate, lactate, and citrate ions of such stability thermodynamically as to prevent precipitation of the insoluble hydrated zirconium oxide even at relatively high hydroxide ion concentrations. Yttrium ions form complexes having similar properties with citrate ions.

It has been shown by Owens and Thomas[3] that the addition of such salts as sodium citrate, tartrate, or lactate to zirconium oxide hydrosols produces a marked rise in the pH of the hydrosol. This effect is attributed by them to the penetration of the micelles by the organic anions with replacement of hydroxide ions from the micelle, resulting in an increase in the free hydroxide ion concentration in the solution. Owens[2] has interpreted the conversion of positively charged hydrosols of zirconium oxide to negative sols in the presence of added citrate, tartrate, and lactate ions in the light of the above-described complex ion formation, and has shown further that by increasing the concentration of the complexing ion the colloidal aggregates may be broken down to particles diffusible through collodion membranes.

In our study of the zirconium-hydroxide-lactate (or tartrate, citrate) and of the yttrium-hydroxide-citrate systems it has been found that, at a particular pH (e.g. 7.0), by controlled variations of the mole-ratio of complexing ion to metal ion added to the solution, aggregates are obtained from the extreme of rapidly sedimenting macro particles through smaller and smaller particles, to aggregates whose sols (or solutions) no longer give a Tyndall effect. In any one of these preparations, the number of moles of hydroxide ion bound per mole of metal (zirconium or yttrium) varies with the number of moles of complexing ion present. Table 2 gives the titration values (moles hydroxide bound per mole yttrium) for yttrium in the presence of various total quan-

tities of citrate ions (bound plus free) at pH 7. At the mole ratio yttrium/citrate = 2, and at pH 7, one obtains rapidly sedimenting macro particles with 1.4 moles hydroxide bound per mole of yttrium. As the citrate/yttrium mole ratio is raised, the number of moles of hydroxide bound per mole of yttrium decreases sharply, and simultaneously the aggregate size drops from particles sedimenting rapidly, through those sedimenting more and more slowly, then through those whose sols show an intense Tyndall effect without sedimentation at fields of ~ 2000 G. down to those whose sols (or solutions) produce no Tyndall effect. Similar data for the yttrium-hydroxide-citrate system at a final pH of 8.3 (phenolphthalein end point) are given in Table 2.

Table 2

Variations in Chemical Composition and Partidle Size in the Yttrium-Hydroxide-Citrate System with Changes in the Mole Ratio of Yttrium to Citrate at pH = 7.

Total Volume = 10 ml.
Final pH = 7.0 (phenol red endpoint)

	Yttrium (millimoles)	Citrate (millimoles)	Hydroxide (millimoles)	Hydroxide (millimoles)	Appearance of system in the first three hours after titration
	Total present	Total present	Bound	Bound per millimole yttrium	
1)	0.2	0.0	0.03	0.15	----------
2)	0.2	0.025	0.15	0.70	Rapidly sedimenting precipitate
3)	0.2	0.05	0.22	1.1	with clear supernate
4)	0.2	0.07	0.22	1.1	" " "
5)	0.2	0.08	0.22	1.1	" " "
6)	0.2	0.09	0.23	1.2	" " "
7)	0.2	0.10	0.28	1.4	Slowly sedimenting precipitate with supernate showing moderate Tyndall effect
8)	0.2	0.115	0.19	0.95	Very slowly sedimenting precipitate with supernate showing marked Tyndall effect
9)	0.2	0.125	0.18	0.90	
10)	0.2	0.138	0.18	0.90	Intense Tyndall effect in supernate with minimal quantity of very slowly settling precipitate
11)	0.2	0.15	0.18	0.90	Moderate Tyndall effect; no precipitate
12)	0.2	0.20	0.15	0.75	Faint Tyndall effect; no precipitate
13)	0.2	0.25	0.10	0.50	Clear solution; no precipitate
14)	0.2	0.30	0.062	0.31	" " "
15)	0.2	0.35	0.035	0.18	" " "
16)	0.2	0.40	0.013	0.065	" " "
17)	0.2	0.45	0.0084	0.042	" " "
18)	0.2	0.50	0.0056	0.028	" " "
19)	0.2	0.55	0.0016	0.0080	" " "
20)	0.2	0.60	0.00	0.000	" " "

From the data in Table 2 it is seen that on reaching the ratio citrate/yttrium = 3, there is no hydroxide bound by yttrium. As would be expected from equi-

librium considerations this continues to be the case at even higher citrate/yttrium ratios.

Table 3

Variation in Chemical Composition and Particle Size in the Yttrium-Hydroxide-Citrate System with Changes in the Mole Ratio of Yttrium to Citrate at pH = 8.3

Total Volume = 10 ml
Final pH = 8.3 (phenophthalein endpoint)

	Yttrium (millimoles)	Citrate (millimoles	Hydroxide (millimoles)	Millimoles OH/millimole Y	Appearance of solution in the first three hours after titration
	Total present	Total present	Bound	Bound	
1)	0.2	0.0	0.356	1.88	----------
2)	0.2	0.025	0.392	1.96	Rapidly sedimenting precipitate, with faint Tyndall effect in supernate
3)	0.2	0.05	0.344	1.72	Rapidly sedimenting precipitate plus strong Tyndall effect in supernate
4)	0.2	0.075	0.300	1.50	No precipitate, liquid shows strong Tyndall effect.
5)	0.2	0.10	0.278	1.39	No precipitate, no Tyndall effect in liquid
6)	0.2	0.125	0.268	1.34	" " "
7)	0.2	0.15	0.249	1.25	" " "
8)	0.2	0.175	0.246	1.23	" " "
9)	0.2	0.20	0.239	1.19	" " "
10)	0.2	0.25	0.229	1.14	" " "
11)	0.2	0.30	0.232	1.16	" " "
12)	0.2	0.35	0.221	1.10	" " "
13)	0.2	0.40	0.217	1.08	" " "
14)	0.2	0.45	0.218	1.09	" " "
15)	0.2	0.50	0.205	1.02	" " "
16)	0.2	1.00	0.204	1.02	" " "
17)	0.2	4.00	0.188	0.94	" " "

On comparing data in Table 2 with that in Table 3 it is seen that the hydroxide binding to yttrium is much more stable at pH 8.3 than at pH 7.0, so that at the higher pH even with citrate/yttrium ratios of 20/1, there is still approximately 1 millimole hydroxide bound per millimole of yttrium. Further

on comparing the products obtained at any particular citrate/yttrium ratio by raising the pH from 7.0 to 8.3, it is noted that in addition to changing the extent of hydroxide-yttrium binding, the particle sizes are smaller at pH 8.3 than at pH 7.0 (at least through the range of mole ratio of citrate/yttrium from 1:4 to 1:1). Qualitative experiments reveal that on still further raising the pH, (i.e., above 8.3), a region is reached where the aggregate size begins again to increase with pH increase.

It is evident from the above data that it is possible to prepare colloidal sols whose particle sizes may be made to vary through a wide range by altering the citrate/yttrium mole ratio at a particular pH. Or, if a particular citrate-yttrium ratio is chosen, one can vary particle sizes by altering the final solution pH. The aggregates obtained by the second of these procedures are chemically different from those obtained by the first procedure, as is evident from the increased hydroxide binding at pH 8.3 compared with that at pH 7.0. Colloids prepared on the basis of these data may be used to investigate both the effect of particle size and of chemical structure upon such physiological properties as blood stream clearance, rapidity of phagocytosis by elements of the reticulo-endothelial system, and site of uptake of the colloids in vivo.

In the case of zirconyl ions, in a system with hydroxide and citrate, tartrate, or lactate, a set of data similar to those for yttrium may be obtained by varying the ratio of complexing ions to zirconyl ion at any particular pH. One factor which differs is that the reaction between zirconyl ions and hydroxide ions at room temperature and above produces some bonds that, once formed, are very slowly broken by back titration. As a result, care must be exercised in preparing colloids of zirconium to add alkali slowly with adequate stirring, in the cold, to avoid such an occurrence during the process of titrating to the desired final pH value.

For several investigations described in the subsequent communication,

colloidal preparations of zirconium and yttrium prepared according to the above described principles have been used. In the case of both these elements, the colloidal particles thus prepared are much smaller than those in the preparations described in the section on "relatively large particle size" colloids. These latter sols, prepared according to the following directions, show only a faint Tyndall effect, and the particles are non-sedimentible using the centrifugal fields obtainable with ordinary centrifuges (2000 G). Studies are now in progress to obtain a quantitative measure of particle sizes in such preparations as well as information as to particle symmetry and the extent of polydispersity of the sols. For the present the designation of "relatively large" particles and "intermediate" particles will be used without comittment as to actual particle diameters.

Preparation of zirconium-hydroxy-lactate colloid of "intermediate" particle size.

1.0 ml of 0.4 M zirconium oxychloride solution (containing the desired zirconium radioisotope) is mixed with 1.2 ml of 1.0 M sodium lactate solution, 1 drop phenol red indicator solution is added, and the mixture diluted to 6.0 ml with water. This solution is then titrated carefully with 1 M NaOH to the phenol red end point, always maintaining the temperature near 0°C. On heating this solution at 100°C the indicator color reverts to yellow. After 10 minutes at 100°C, the solution is cooled to 0°C and again brought to the phenol red end point with NaOH. The alternate heating and cooling with repeated titration is continued until the end point (color) persists even after 10 minutes of heating. This final solution is then diluted to 10 ml., heated at 100°C for 10 minutes, and cooled. During the course of the titration to the phenol red end point it is imperative to avoid additions of alkali while the solution is warm for reasons mentioned in the general discussion above. Colloids prepared according to these directions maintain their original properties at least over a period

of two months when stored in ordinary rubber-capped pyrex vessels.

Preparation of Yttrium-Hydroxy-Citrate Colloids.

0.5 ml of 0.2 M $Y(NO_3)_3$ solution (incorporating the desired yttrium radioisotope) are mixed with 0.16 ml of 0.5 M sodium citrate solution, one drop phenol red indicator added, and the mixture diluted to 2.0 ml with water. This solution is heated 5 minutes at 100°C. A precipitate will form at this step, or before heating, but the procedure is carried on without separating the precipitate, which later resuspends spontaneously. The solution is then titrated with 1.0 M NaOH to the phenol red endpoint. On reaching the endpoint the solution is heated at 100°C for 5 minutes, cooled in ice water bath, and if the endpoint has faded, is re-titrated with sodium hydroxide. In contrast with the zirconium procedure, this re-titration is necessary only once. The final solution is diluted to 4.0 ml., and then heated 1 hour at 100°C in a rubber-capped pyrex serum bottle. Such colloidal preparations show no change in appearance or biological properties over a period of 4-6 months. These colloids have been heated as long as 8 hours at 100°C without demonstrating any change in properties.

Colloids of lanthanum.

Similar procedures have been utilized to prepare colloids in the lanthanum-hydroxide-citrate system, the qualitative details in this system being similar to those of the yttrium-hydroxide-citrate system, but with differences existing in the thermodynamic stability of the bonds involved. Since we have not yet studied the biological properties of lanthanum colloids, the detailed chemical preparation is not given.

Colloids containing columbium.

In using the radioisotope Zr^{95} (65 day half life), there is the problem of the 35 day Cb^{95} daughter isotope to consider. A chemical separation of the

two isotopes can be made, but, of course, the Cb^{95} will soon regrow in the Zr^{95} preparation. We have investigated the zirconium colloids described in this communication using the Zr^{89} isotope (with a stable columbium daughter), and using equilibrium mixtures of Zr^{95} and Cb^{95}. No quantitative differences in biological behavior have been found, so that for these particular studies it appears that if the Cb^{95} isotope (without carrier) is incorporated into the zirconium colloid it shares the same metabolic fate as does the Zr^{95} isotope. For this reason no separation of these isotopes need be made for the particular purposes described in the following communications.

SUMMARY

1. For studies concerned with selective localization of radioisotopes in the bone marrow, liver, and spleen colloids incorporating such isotopes are useful.

2. Colloids of zirconium, columbium, yttrium, and lanthanum have been chosen for study of selective localization of colloids because of the availability of radioisotopes of a wide range of nuclear properties and because of the comparative ease with which controlled variations in chemical structure and particle size of colloids containing these elements may be made.

3. The methods for preparing zirconium and yttrium colloids of various chemical types and ranges of particle size are described. A discussion of some of the chemical principles of these preparative methods is given.

BIBLIOGRAPHY

1. JONES, Hardin B., WROBEL, Charles, and LYONS, William R. A method of distributing beta-radiation to the reticulo-endothelial system and adjacent tissues. J. Clin. Invest. XXIII (5), 783-788, 1944.

2. OWENS, Harry S. Interpretation of the structure and behavior of anionic and cationic zirconium oxide micelles. Doctoral dissertation, Columbia University, 1935.

3. THOMAS, Arthur W. and OWENS, Harry S. Basic zirconium chloride hydrosols. J. Am. Chem. Soc. 57, 1825-1829, 1935.

4. THOMAS, Arthur W. and OWENS, Harry S. The formation of zirconeate hydrols and their disintegration by certain neutral salts. J. Am. Chem. Soc. 57, 2131-2135, 1935.

5. The Plutonium Project. Nuclei formed in fission. J. Am. Chem. Soc. 68, 2411-2442, 1946.