The Gelation of Lead Aldose-cysteinate. The Properties of Gel and the Structure of Lead Aldose-cysteinate

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The authors have studied the preparation and utilization of cysteine and have already reported on the electrolytic preparation of cysteine⁽¹⁾, and on the preparation and properties of glucose-cysteine⁽²⁾. The organo- and physicochemical properties of the metal salts of

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1) J. Mizuguchi, S. Suzuki, F. Takahashi and Y. Kawasaki, Annual Meeting of the Electrochemical Society of Japan, 1958.

2) J. Mizuguchi, S. Suzuki and F. Takahashi, 13th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1960.

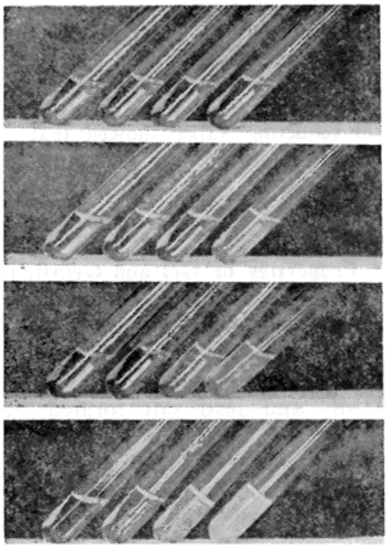

Photo. 1. The solution containing equimolar NaGC and lead acetate.
Concn.; distilled water, 0.01, 0.03, 0.05 M from left to right of test tubes. Temp. 16.5°C

HGC* have now been studied. When a solution containing HGC and lead salt was allowed to stand for some time, it was found to form gel[3], as is shown in photographs attached. This gel is stable, and no syneresis is observed. This gel has a heat-reversible property.

It has been known that the gelation of a solution containing such high molecular substance as agar-agar, gelatin, silicic acid occurs because of the formation of an infinite three-dimentional network in the solution by means of intermolecular cross-linking[4]. Most of the gels of such inorganic substances as hydrous oxides of iron and aluminum have been found to expel liquids.

The gel composed of HGC and lead salt was different from the other gels because of its stability. Furthermore, high molecular compounds were unexpectedly found in this gel.

This investigation was carried out to elucidate the mechanism and kinetics of the gelation of a solution containing HGC and lead salt. In this paper, we report on an extensive study of the properties of the gel and gelation and on the structure of lead aldose-cysteinate.

Experimental

Materials.—HSCs were prepared by the condensation reaction of cysteine hydrochloride and an aldose such as glucose, galactose, mannose, arabinose and xylose in a methanol or aqueous solution after the addition of pyridine[5]. The products precipitated were washed with methanol after filtration.

Apparatus and Procedure. — The turbidity was measured by a Coleman spectrophotometer, universal model. The turbidity was measured from the time a solution of lead salt was added to a solution of HSC. On the basis of these measurements, we got the relative values of turbidity based on the definition that the turbidity of distilled water is 0%. The turbidity measured 3 hr. after the time a solution containing 40 mM of NaGC and 40 mM of lead acetate formed a gel is 100%.

The rigidity of the gel was determined by an apparatus** measuring gel rigidity. The amount of weight which sank into the gel with a velocity of 2 cm./20~40 sec. was adopted as the rigidity of the gel. Each 100 ml. of a solution containing HGC and lead salt was poured into several 100 ml. beakers and allowed to stand at 25°C. The rigidity of the gel was measured at intervals from the time when a solution of lead salt was added to a solution of HGC.

Preparation of Lead Aldose-cysteinates. — *PbGCNO*$_3$—a) 2.83 g. of HGC and 0.84 g. sodium bicarbonate were dissolved in 20 ml. of water; 10 ml. of 1 M lead nitrate was then added. On the addition of 60 ml. of methanol or ethanol before the gelation of the mixture, sticky precipitates soon separated. The precipitates were crystallized in alcohol after an hour or so. The product was filtered and washed with methanol; yield, 5.3 g. (96%).

Found: C, 19.6; H, 3.0; N, 5.1; Pb, 37.3; NO_3, 9.9. Calcd. for $C_9H_{16}NO_7SPbNO_3$: C, 19.6; H, 2.9: N, 5.1; Pb, 37.5; NO_3, 11.3%.

b) 10 ml. of 1 M NaGC was added to 10 ml. of 1 M lead nitrate. The mixture formed at gel after a few minutes at room temperature; then fine crystals separated from this gel. After two days, the product was filtered and washed with 50% aqueous ethanol, and then with ethanol; yield, 5.0 g. The crystalline product melted at 193.5°C with decomposition.

Found: C, 19.7; H, 2.6; Pb, 37.2. Calcd. for $C_9H_{16}NO_7SPbNO_3$: C, 19.6; H, 2.9; Pb, 37.5%.

$PbGCNO_3$ is soluble in water (1.2~1.3 g./dl.) The solution of $PbGCNO_3$ formed a gel when it was allowed to stand at room temperature for a while. $PbGCNO_3$ is insoluble in methanol, ethanol,

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* All the aldose-cysteines and their sodium salts are abbreviated as follows:

Aldose-cysteine	HSC
Glucose-cysteine	HGC
Galactose-cysteine	HGaC
Mannose-cysteine	HMC
Xylose-cysteine	HXC
Arabinose-cysteine	HAC
Various HSC	HSCs
Sodium aldose-cysteinate	NaSC
Sodium glucose-cysteinate	NaGC
Sodium galactose-cysteinate	NaGaC
Sodium mannose-cysteinate	NaMC
Sodium xylose-cysteinate	NaXC
Sodium arabinose-cysteinate	NaAC

3) J. Mizuguchi, F. Takahashi and S. Suzuki, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 1627 (1960).

4) P. J. Flory, "Principles of Polymer Chemistry", Cornell Univ. Press, New York (1953).

5) M. P. Schubert, *J. Biol. Chem.*, **130**, 601 (1939); G. Weitzel, J. Engelmann and A-M. Fretzdorff, *Z. physiol. Chem.*, **315**, 236 (1959).

** A Nikkansui-shiki apparatus measuring agar-agar gel rigidity.

benzene, acetic acid, pyridine, acetone and chloroform.

$PbGaCNO_3$.—A mixture of NaGaC and lead nitrate was prepared in the same manner as has been described in the preparation of $PbGCNO_3$. On the addition of 200 ml. of ethanol, sticky precipitates separated. When it was allowed to stand overnight at room temperature, sticky precipitates were crystallized in alcohol. The product was filtered and washed with ethanol; yield, 4.5 g. (76%).

Found: C, 19.0; H, 3.6; N, 4.6; Pb, 36.4; NO_3, 9.8; H_2O, 3.3. Calcd. for $C_9H_{16}NO_7SPbNO_3 \cdot 2H_2O$: C, 19.0; H, 3.2; N, 4.9: Pb, 36.4; NO_3, 10.9; H_2O, 3.2%.

PbGCOH.—2.83 g. of HGC and 0.84 g. of sodium bicarbonate were dissolved in 20 ml. of water; 10 ml. of 1 M lead acetate was added. On the addition of 300 ml. of ethanol, fine precipitates soon separated. The product was filtered and washed with ethanol; yield, 4.2 g. (75%). The product was dried on phosphorus pentoxide at 100°C under reduced pressure.

Found: C, 21.9; H, 3.3; N, 2.7; Pb, 40.7. Calcd. for $C_9H_{17}NO_8SPb$: C, 21.3; H, 3.4; N, 2.8; Pb, 40.9%.

It was found, from the result of alkaline titration, that the filtrate contained 8.5 mmol. (85%) of acetic acid. PbGCOH is slightly soluble in water. When it was allowed to stand at room temperature for a while, the suspension of PbGCOH formed a gel.

$PbGC \cdot CH_3COO \cdot PbGCOH$.—To a solution in which 2.83 g. of HGC and 0.84 g. of sodium bicarbonate were dissolved in 20 ml. of water, 5 ml. of acetic acid and 10 ml. of 1 M lead acetate were added. A hundred mililiters of methanol and 100 ml. of acetic acid were added before the gelation of the mixture. After a week, precipitates were filtered and washed with methanol; yield, 1.78 g.

Found: C, 22.5; H, 3.0; N, 2.6; Pb, 39.0. Calcd. for $C_{18}H_{36}N_2O_{17}S_2Pb_2$: C, 22.8; H, 3.4; N, 2.7; Pb, 39.3%.

$PbMCNO_3$ and $PbACNO_3$.—It was difficult to obtain pure $PbMCNO_3$ and $PbACNO_3$ prepared in the same manner as $PbGCNO_3$. When the solution containing lead nitrate or acetate and NaMC or NaAC was allowed to stand for a few days, white precipitates separated. The precipitates were found to be crude lead cysteinate from the following results: 1.41 g. of HMC and 0.42 g. of sodium bicarbonate were dissolved in 10 ml. of water; 5 ml. of 1 M lead acetate was added. The mixture was allowed to stand at room temperature for a few days. The precipitates were filtered and washed with distilled water; yield, 1.5 g.

Found: Pb, 61.6; N, 4.1. Calcd. for $PbC_3H_5 \cdot NO_2S$: Pb, 63.3; N, 4.3%.

The determination of the reducing value showed that the filtrate contained 4.4 mM (97%) of mannose.

The Preparation of Lead Cysteinate from Gel.—Fifty milliliters of 0.2 M NaGC was added to 50 ml. of 0.2 M lead acetate. The mixtue formed a gel. An hour after the beginning of the gelation, the gel was heated at 100°C for 6 hr. The gel melted, and then gray precipitates separated. The precipitates were filtered and washed with water; yield 3.1 g. (94%).

Found: C, 11.3; H, 1.6; N, 4.3; Pb, 62.2. Calcd. for $PbC_3H_5NO_2S$: C, 11.0; H, 1.5; N, 4.3; Pb, 63.3%.

The alkaline titration and the determination of the reducting value showed that the filtrate contained 9.7 mM (97%) of acetic acid and 9.5 mM of glucose.

Results and Discussion

Properties of Gel and Gelation.—*Gel-forming HSC.*—A solution containing HGaC and lead salt also yielded gel in the same manner as HGC. However, in the case of HXC and HAC, flocculation occured. A solution containing HMC and lead salt formed neither gel nor fluocculent precipitates.

Turbidity.—The transparent solution containing HGC and lead salt became turbid gel rapidly upon gelation. The turbidity of the gel increased with the lapse of time for a few hours, and then the turbidity approached to almost constant values. The turbidity of this gel was greater than that of agar-agar gel. The results are shown in Fig. 1.

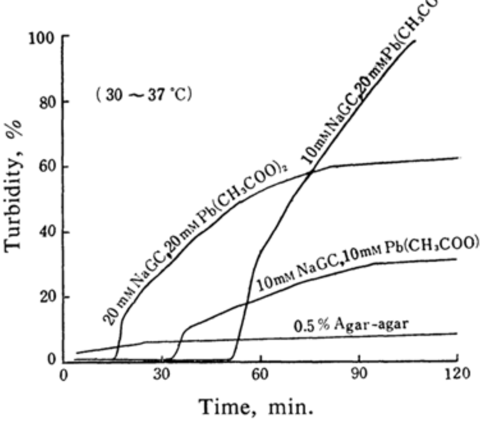

Fig. 1. Development of turbidity.

Rigidity.—The rigidity of the gel increased rapidly with gelation. The rigidity of gel depends on the pH value and the concentration. The rigidity of this gel was smaller than that of agar-agar gel (Figs. 2, 3 and 4).

The Range of pH Values and Concentrations.—The lowest concentration of a solution which was able to yield gel was the 5 mM (0.3%) of a lead glucose-cysteinate solution (Fig. 3). The pH range of the formation of gel was between 3.8 and 11 (Fig. 4). When a solution of NaGC was added to a solution of lead salt in the pH range of 8~11, precipitation occured immediately. The precipitates redissolved gradually, and then the solution set to gel after a certain time, whether precipitates redissolved perfectly or not.

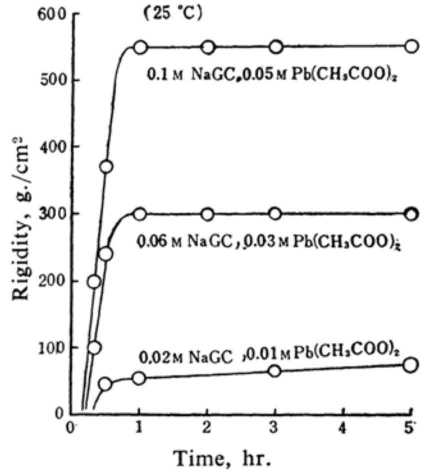

Fig. 2. Development of rigdity.

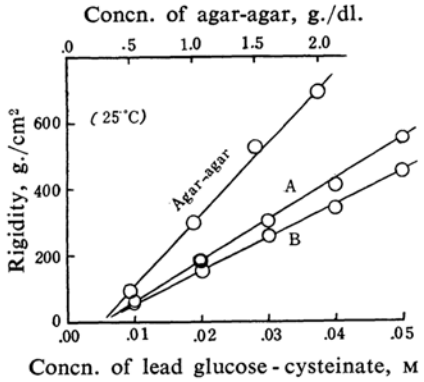

Fig. 3. Rigidity and concentration of gel.

A NaGC : $Pb(CH_3COO)_2=2:1$ (mol. ratio)
B NaGC : $Pb(NO_3)_2=2:1$ (mol. ratio)
Measuring after 3 hr. from the gelation time

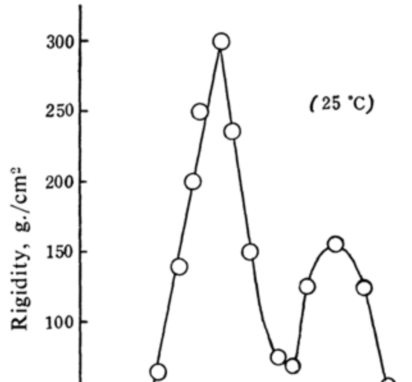

Fig. 4. Rigidity and pH of gel.
0.06 M NaGC, 0.03 M lead nitrate or acetate measuring after 3 hr. from the gelation time.

Heat-reversible Property.—As it was warmed, this gel melted above 70°C. The liquified gel yielded gel containing a small amount of white precipitates when it was allowed to stand at room temperature. The amount of precipitates increased with the lapse of the time of heating. Re-gelation could not occur when the heating of the gel was continued further.

Properties of the Solution before Gelation.—*Chemical Reactions of Lead Ion in Solution.*—Insoluble lead salt was precipitated by the addition of hydrogen sulfide, phosphoric acid, or potassium chromate or iodide into the HGC-lead acetate solution. Lead sulfate, however, was not found to precipitate when sodium sulfate was added.

Stability of Lead Glucose-cysteinate.—The stability constant, $\log k=4.5$, was given from the method of pH titration, as is shown in Table I.

TABLE I. STABILITY CONSTANT

To 50.0 ml. of the solution (4.00×10^{-3} M HGC, 3.79×10^{-3} M $Pb(NO_3)_2$) 0.973 N NaOH solution was titrated. 30°C. Ionic strength 0.1 (KNO_3) $K_{\mathrm{COOH}}=6.6_1\times10^{-3}$, $K_{\mathrm{NH_2}}=8.3_2\times10^{-6}$ (reference value[5]: $K_{\mathrm{COOH}}=7.24\times10^{-3}$, $K_{\mathrm{NH_2}}=4.27\times10^{-6}$)

pH	NaOH, ml.	$\bar{n}$	pA
3.00	0.000	0.41	4.8_5
3.13	0.030	0.45	4.7_3
3.31	0.060	0.50	4.5_7
3.50	0.100	0.63	4.4_8
3.75	0.130	0.72	4.3_4
4.11	0.160	0.83	4.1_6

$pA_{\bar{n}=0.5}\doteqdot 4.5$

Structure and Properties of the Lead Aldose-cysteinate.—*Structure of Lead Aldose-cysteinate.* It was found that the lead aldose-cysteinates isolated ($PbGCNO_3$, PbGCOH and $PbGaCNO_3$), which are the essential materials for gelation, are composed of one atom of lead, one molecule of HSC, and one univalent anion (e. g., NO_3^-, OH^-, etc.).

The infrared spectra of NaGC and $PbGCNO_3$ show peaks at 1600 and 1400 cm^{-1} which arise from the antisymmetric and symmetric carbon-oxygen stretching vibration in the carboxylate ion[6]. The $PbGCNO_3$ bands and lead nitrate bands observed at about 820 and 730 cm^{-1} arise from the deforming vibrations of the nitrate ion, which does not coordinate with the lead atom (Fig. 5).

It was found, from the study of ultraviolet absorption spectra[7], that the sulfur atom and

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[6] D. N. Sen, S. Mizushima, C. Curran and J. V. Quagliano, *J. Am. Chem. Soc.*, **77**, 211 (1955).

[7] The results of ultraviolet spectra will be described in the next paper. F. Takahashi and J. Mizuguchi, This Bulletin, **35**, 1823 (1962).

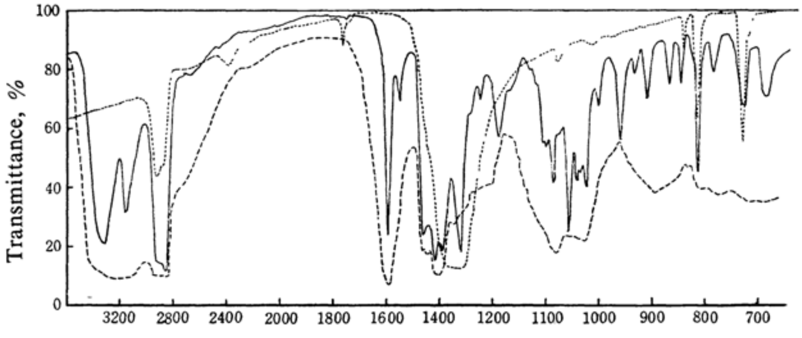
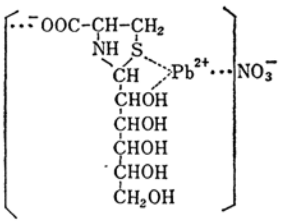


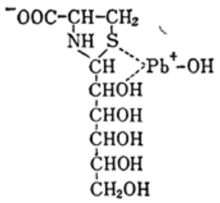
Fig. 5. Infrared spectra (Nujol method). —— $PbGCNO_3$, - - - NaGC, …… $Pb(NO_3)_2$

one hydroxyl group of HGC coordinate with the lead atom in solution.

Based on the infrared and ultraviolet absorption spectra, structural formulae (I, II) were suggested for lead aldose-cysteinate:



(I)



(II)

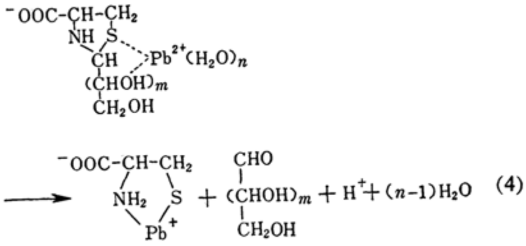
Properties of Lead Aldose-cysteinate. — Pure $PbGC \cdot CH_3COO$ could not be prepared from the solution of NaGC and lead acetate. Judging from this fact, it is presumed that the following reactions occur in solution.

$$NaGC + Pb(CH_3COO)_2 \rightarrow PbGC \cdot CH_3COO + CH_3COONa \quad (1)$$

$$PbGC \cdot CH_3COO + nH_2O \rightarrow [PbGC(H_2O)_n]^+ + CH_3COO^- \quad (2)$$

$$[PbGC(H_2O)_n]^+ \rightarrow PbGCOH(H_2O)_{n-1} + H^+ \quad (3)$$

It is suggested that the lead complex ion of HSC in solution converts to lead cysteinate and aldose as shown in Eq. 4:



(4)

$m=3$ or 4

Summary

When a solution containing HGC or HGaC and lead acetate on nitrate was allowed to stand for sometime, it was found to form a stable gel like agar-agar in the pH range between 3.8 and 11 and with a concentration over 0.3%. The turbidity and rigidity of the gel began to increase rapidly with gelation. A solution containing HMC and lead salt did not form a gel, but precipitated lead cysteinate with the liberation of mannose.

It was found that the lead aldose-cysteinates isolated, which are the essentials materials for gelation, are composed of one atom of lead, one molecule of HSC, and one univalent anion, and that the sulfur atom and one hydroxyl group HGC coordinate with the lead atom, but that the carboxyl group of HGC does not coordinate to the lead atom.

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