On the Calcium Phosphate Banding in Silicic Acid Gel.

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In regard to the formation of rhythmic precipitate of calcium phosphate in other medium than a silicic acid gel, several reports have been published. There have been the studies of Dogadkin,⁽¹⁾ Hatschek,⁽²⁾ and Schemjakin⁽³⁾ concerning the formation in a gelatine gel and those of Schemjakin on the phenomenon in an agar-agar gel. As to the rhythmic precipitation of this substance in a space between two pieces of glass and in capillary spaces without the aid of any gels, the studies of Doyle and Ryan⁽⁴⁾ and Wo. Ostwald⁽⁵⁾ can be mentioned. But the banding in a silicic acid gel have never been reported. Therefore, the present author has tried an experiment in order to know whether the Liesegang phenomenon of calcium phosphate could be caused or not in the silicic acid gel. And it was certified that calcium phosphate produced an extremely prominent rhythmic precipitate even in a silicic acid gel and that the banding

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(1) *Kolloid-Z.*, **40** (1926), 33.

(2) *Kolloid-Z.*, **27** (1920), 225; *Biochem. J.*, **14** (1920), 418.

(3) *Kolloid-Z.*, **50** (1930), 58.

(4) *Brit Chem. Abstract*, A, **1929**, 1144; *Proc. Roy. Irish Acad.*, B, **38** (1929), 435.

(5) *Kolloid-Z.*, **40** (1926), 144.

was closely related to the hydrogen ion concentration of the medium or a silicic acid gel. The experimental result will be described in the following.

Although calcium salts were used as an inner electrolyte and sodium phosphate made diffuse into the gel in all the cases hitherto reported, whichever the inner electrolyte might be, calcium salts or sodium phosphate, as favourable a result was obtained in a silicic acid gel as in other gels. There was a marked difference between the silicic acid and other gels. Use of sodium phosphate as an inner electrolyte had rather a strong point that preparation of gel needed no special care. In use of calcium salts as an inner electrolyte, acetic acid, sodium silicate and calcium salt should be mixed in the order and the mixture stirred up rapidly. Unless these were mixed in this order or unless the mixture stirred up rapidly, white turbidities, which seemed to calcium silicate, were formed, partially coagulated, so that no transparent gel could be obtained. Use of sodium phosphate and the likes as an inner electrolyte, on the contrary, needed no special care. But in either case where a phosphate solution was added to diffuse into the gel containing a calcium salt or where a calcium salt solution was poured on the gel containing a phosphate, a rhythmic precipitate with very clear bands was obtained.

The following can be mentioned as one of the most favourable conditions. That is, for dissolving phosphate in a gel, mix 1 c.c. of a 8% solution of secondary sodium phosphate with 10 c.c. of a 0.75 N acetic acid and then add 10 c.c. of a 1.06 density solution of sodium silicate to it and allowed to stand to gelatinize. After the gel set firmly, a 2 mol solution of calcium nitrate was superimposed on it.

For dissolving calcium salt in a gel, after mixing the sodium silicate solution ($d = 1.06$) with 10 c.c. of 0.75 N acetic acid add 1.0 c.c. of a 10% calcium chloride solution to the mixture, while stirring up. After the gel set firmly, a 8% solution of secondary sodium phosphate was poured on it.

The right of Fig. 1 shows the bands formed by the former method and the left, those obtained by the latter method. Fig. 2 shows Liesegang rings of calcium phosphate formed on a glass plate by the former method.

The effect of the hydrogen ion concentration, inner electrolyte and its concentration, outer electrolyte and its concentration, concentration of gel, and, moreover, the effect of aging on the formation of this rhythmic precipitate have been examined, and the results are described in the following lines.

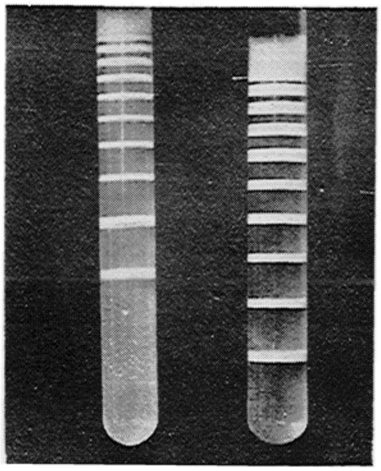

Fig. 1.

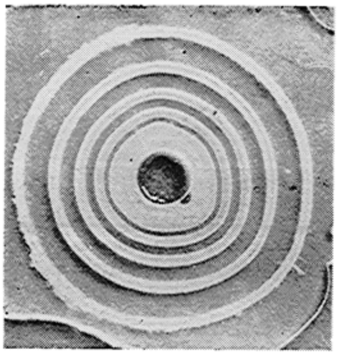

Fig. 2.

Effect of Hydrogen Ion Concentration. It has already been mentioned that the hydrogen ion concentration has a very marked effect on the formation of these bands. At first, the case where calcium chloride was used as the inner electrolyte will be described. If the hydrogen ion concentration was low in the addition of calcium chloride to a silicic acid sol, a partial coagulation occurred and produced a curdy precipitate; accordingly it was impossible to prepare a uniform gel. This is probably caused by the fact that the calcium salt was acted simultaneously by an amount of sodium silicate, which remained undecomposed, in the process of the formation of silicic acid by the action of acid, which was small in quantity.

In employment of a sol containing a little more concentrated acid, for instance, a sol which was prepared by mixing 0.66 N acetic acid and an equal volume of sodium silicate solution of 1.06 density, an uniform gel could be obtained. By diffusion of sodium phosphate into the gel, however, a long zone of white precipitate was formed, which was not rhythmic.

In a case where an acid was more concentrated, that is, in a case where a gel made from 0.75 N acetic acid and an equal volume of sodium silicate ($d = 1.06$) was used, the rhythmic precipitate could be formed. When the concentration was further more increased, the bands were hardly formed. In a gel composed of 1 N acetic acid and an equal volume of sodium silicate ($d = 1.06$), two or three incomplete bands were only formed on the contact surface of the diffusing solution and the gel.

In short, the formation of rhythmic bands was only possible in the gels which were made from 0.7–1 N acetic acid and an equal volume of a sodium silicate solution ($d = 1.06$). Especially 0.75–0.8 N acetic acid gave a favourable result. This can be seen in Table 1.

Table 1. Na_2SiO_3 ($d = 1.06$); $CaCl_2$ 0.08%; Na_2HPO_4 0.5 mol.

Conc. of acetic acid added (N)	Number of bands formed	Remarks
0.50		
0.66		A continuous zone of precipitate.
0.70	3	After a continuous precipitate as long as 4 cm., extremely thick bands were formed.
0.75	10	Typical rhythmic precipitate.
0.80	13	Typical rhythmic precipitate.
0.90	(15)	The bands were fragmental and incomplete but stood close.
1.00	(3)	Fragmental.

Equal volumes of Na_2SiO_3 and acetic acid were mixed. This result is in the case where the gel was filled to a height of 8 cm. of a test-tube of 1 cm. in internal diameter and 14 cm. in length.

The results were the same, where secondary sodium phosphate was used as the inner electrolyte. When a sodium silicate solution ($d = 1.06$) was mixed with an equal volume of 0.5, 0.6, and 0.7 N acetic acid respectively, the uniform gel could generally be obtained. When a calcium salt was allowed to diffuse into them, however, there formed only a zone of completely even white precipitate. When the acid or hydrogen ion is further more concentrated, the rhythmic precipitation may occur. As the concentration of acetic acid increased, the number of formed bands gradually decreased. No band was formed by the used of 2 N acetic acid. The results are given in Table 2.

Calcium phosphate was precipitated as the primary, secondary or tertiary calcium phosphate according to the value of pH of the gel in which the precipitation occurred. Therefore the primary salt can probably form no rhythmic bands on account of its solubility; while the secondary or tertiary salt can produce the rhythmic precipitate. When pH of the gel is high, the tertiary salt only formed, and it precipitates rather unrhythmical because a salt of too poor solubility, gives generally no rhythmic bands. Judging from these facts, the absence of rhythmic precipitate in this case is acknowledged.

Table 2. Na_2SiO_3 ($d = 1.06$); Na_2HPO_4 0.4%; $CaCl_2$ 30%.

Conc. of acetic acid (N)	*p*H	Number of bands formed	Remarks
0.60	8.98		Continuous precipitate formed.
0.70	7.72		,,
0.75	7.04	9	
0.80	6.01	7	
0.90	5.50	4	
1.00	5.30	3	
2.00	4.67	0	No precipitate formed.

The gel was made by mixing the equal volumes of sodium silicate and acetic acid. As much of the gel was taken as to fill to a height of 6 cm. of a test tube of 1.2 cm. in internal diameter and 11 cm. in length.

Inner Electrolyte and the Effect of Its Concentration. As already mentioned, calcium salts as well as sodium phosphate are usable as an inner electrolyte. At first the case of calcium chloride will be described. The experiment was conducted in a gel which was made from 0.75 N acetic acid and sodium silicate solution of 1.06 in density, containing calcium chloride in various composition from 0.004 to 5%.

The rhythmic precipitate was formed in any gels containing calcium chloride in higher concentrations than 0.017%. But when the amount of calcium chloride exceeds 5%, then a 0.5 mol solution of secondary sodium phosphate was hardly diffused into the gel and a stopper of precipitate was only formed on the surface contacting the gel. Yet generally, when calcium chloride was more concentrated, though all the other conditions were the same, the formed bands were large in number and stood close. If the concentration was beyond a certain limit, the bands increased their thickness, but the number of bands rather decreased. This was probably because by the osmotic pressure of the electrolytes in the gel. The diffusion became difficult into such a gel which contained so much inner electrolyte. The results are given in Table 3.

As much of a gel was taken as to fill to a height of 8 cm. of a test-tube of 1 cm. in internal diameter and 0.5 mol secondary sodium phosphate solution was allowed to diffuse in it. When calcium chloride was too much concentrated, the band of calcium phosphate which was formed on the contact surface of the gel and diffusing agent was broken by the osmotic pressure of calcium chloride solution. Then a new precipitate

Table 3. Outer electrolyte: Na_2HPO_4 0.5 mol.

Conc. of inner electrolyte $CaCl_2$ (%)	Number of bands formed	Remarks
5.00	0	Dendrites grew in the diffusing solution.
2.50	2	
1.50	8	The bands formed in the lower part of gel were fragmental.
0.50	9	
0.30	9	
0.15	11	
0.08	10	
0.03	6	
0.017	2	
0.010	0	
0.004	0	

The gel was made by mixing equal volumes of Na_2SiO_3 ($d = 1.06$) and acetic acid (0.75 N). The asterisk in the subsequent tables shows that such a gel was used.

was formed there and it grew in dendrites in the solution of secondary sodium phosphate as the diffusing solution. This seemed to be ascribed to the same cause as that the silicates of cobalt, nickel, and manganese, etc. grew in dendrites in a sodium silicate solution which is known as a "chemical garden" and that the copper ferrocyanide grew in a saccate form in a potassium ferrocyanide solution. An example is given in Fig. 3. It was a matter of course that this could be produced also when a sodium phosphate solution was added slowly to a concentrated solution of calcium chloride, or solid calcium salt.

When gels were prepared in the same conditions, except for employment of sodium phosphate as the inner electrolyte instead of calcium chloride, so far as the experiment is conducted in the range of 0.0125–1.25%, the gels containing the sodium phosphate in higher concentrations than 0.038%, always gave very clear rhythmic precipitates. If the inner electrolyte was small in concentration, the stopper of precipitate which was formed first of all, was large and the distances between the successive bands were large. Contrary to the case where calcium chloride was used as the inner electrolyte, if the inner elektrolyte was small in concentration, each band was rather thick. This was not because the precipitate was separated in abundance, but because the locality of the deposition was sharp in either side, having a certain width. The relation between the concentration and the number of formed bands is given in Table 4, where each band was as thick as 5 mm. in the case of the least concentrated inner electrolyte.

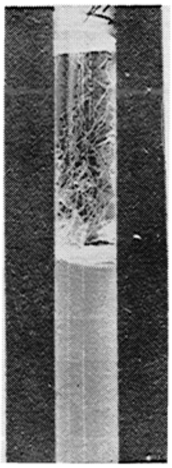

Fig. 3.

Table 4.* Outer electrolyte: $CaCl_2$ 30%.

Conc. of inner electrolyte Na_2HPO_4 (%)	Number of bands formed
1.25	13
1.08	12
0.87	10
0.54	8
0.13	5
0.07	3
0.038	2
0.0125	0

In experiment with the inner electrolyte of such other salts than secondary sodium phosphate, as primary potassium phosphate or tertiary sodium phosphate, 0.5 c.c. of each 0.5 mol primary or tertiary salt was added to each 10 c.c. of the sol composed of sodium silicate ($d = 1.06$) and an equal volume of 0.75 N acetic acid, in order to obtain a gel. In the gel containing the primary salt, the precipitate was formed rhythmically, while in the gel containing the tertiary salt, a continuous precipitate was obtained. But the rhythmic precipitation was recognised by adding half the quantity of the tertiary salt or by using 0.8 N acetic acid in preparation of the gel.

From the measurement of the pH in these cases, the rhythmic precipitation was possible in the gels of lower pH values than about 7.6. Therefore, it is not an important factor that which salt should be added, the primary, secondary, or tertiary one but the hydrogen ion concentration of the gel is an essential factor. The formation of the rhythmic precipitate was possible in the case where the pH was about 5.3–7.6; that is where the inner electrolyte was a mixture of the primary and secondary salts. Therefore it is supposed that the white bands formed are chiefly composed of the secondary calcium phosphate.

Outer Electrolyte and the Effect of Its Concentration. A sol was made by mixing 0.75 N acetic acid with an equal volume of sodium silicate solution ($d = 1.06$) and as much of calcium chloride was added to the sol as to become 0.1% in concentration. After the sol set to a gel, secondary sodium phosphate solutions of various concentration were poured on the gel. The experimental results are given in Table 5.

In this case, as much of the sol was taken as to fill to a height of 8 cm. of a test-tube of 1 cm. in inner diameter. The more concentrated sodium phosphate solution gives the more closeness of the bands and the more number of the bands.

Table 5.* Inner electrolyte: $CaCl_2$ 0.1%.

Conc. of outer electrolyte Na_2HPO_4 (%)	Number of bands formed.
8.74	13
5.82	11
2.91	9
1.96	4
0.87	1

Calcium chloride solutions of 5 to 30% were poured on the gel which was prepared in the same manner as the above and contains 0.5% of secondary sodium phosphate. In any case, the clear rhythmic precipitate was produced. The relation between the concentration and number of formed bands is given in Table 6.

Table 6.* Inner electrolyte: Na_2HPO_4 0.5%.

Conc. of outer electrolyte $CaCl_2$ (%)	Number of bands formed.	Remarks
30	8	These three agree completely and no difference is recognized among them.
25	8	
20	8	
15	8	The bands were close in proportion to the concentration of outer electrolyte.
10	8	
5	7	

In the case where the calcium chloride solutions of higher concentrations than 20% were used, no difference was recognized in the closeness and the total number of bands. This was a characteristic result. When the concentration was lower than 15%, the total number of formed bands was large in the case of the highly concentrated outer electrolyte, and consequently the closeness of bands increased. This is a common rule in the formation of rhythmic precipitate.

Next, the effect of diffusion of different electrolytes into the fixed inner electrolyte was scrutinized. At first, primary potassium phosphate, secondary and tertiary sodium phosphate solutions of an equal concentration were respectively allowed to diffuse into silicic acid gels which were made from a sodium silicate solution ($d = 1.06$) and an equal volume of 0.75 N acetic acid, containing calcium chloride.

According to the experiment with gelatine gel, when the primary salt is allowed to diffuse in, no rhythmic precipitate is formed. In the silicic acid gel, there are the substances which act as a buffer solution on the hydrogen ion concentration. Therefore, it may form a rhythmic precipitate. When the primary salt was diffused, as a matter of course, the bands were formed at large intervals and the number of formed bands also was extremely small.

The diffusion of secondary sodium phosphate gave the most typical rhythmic precipitate, as already described. With tertiary sodium phosphate the rhythmic precipitate was formed, but the silicic acid gel was gradually peptized by the added solution of strong alkaline. On the other hand, the bands of calcium phosphate which had once been formed, received no effect by the alkalinity of the solution and remained unchanged, though they fell down slowly by their own weight to the level where there was still unpeptized gel. In the part where the peptization had not yet occurred, the rhythmic precipitate was successively formed, so that those bands piled up one after another.

Solutions of chloride, bromide, iodide and nitrate of calcium of the equal concentration made respectively diffuse into gels containing sodium phosphate as the inner electrolyte; the rhythmic precipitates were formed at the same distances and closeness, and scarcely any essential difference was found among them.

Effect of the Concentration of Gels. The acetic acid solution of an adequate concentration was added so as to be roughly $p\mathrm{H} = 6$ to the sodium silicate solutions of an equal volume, whose densities were respectively 1.06, 1.08 and 1.10. In any cases of the gels, the formation of the rhythmic precipitates could be observed. In the gel from the silicate solution of the density of 1.10, each stratum of precipitate somewhat blurred on either face and hardly grew into a clear band. Accordingly, the gels of as low concentrations as possible, are adequate for the present purpose of producing the rhythmic precipitate.

Effect of the Presence of a Third Substance. It was already known that the presence of a certain substance in the gel indifferent to the precipitation reaction, often had an effect on the formation and the properties of rhythmic bands. Hatschek, moreover, pointed out that in formation of the rhythmic precipitate of calcium phosphate in gelatine, some marked abnormalities were caused by the kinds of gelatine and impurities in it. The present author mixed the solutions of cane sugar, glucose, urea, potassium bromide and common salt, with the outer electrolyte in order to examine their effect as the third substance. As much of secondary sodium phosphate was added as to become 0.4% in concentration, to the sol which was made by mixing a sodium silicate solution ($d = 1.06$) and an equal volume of 0.75 N acetic acid. After the gel set firmly, the solution of outer electrolyte was put on it which had been prepared by mixing an equal volume of 2 mol calcium nitrate solution with cane sugar (1 and 2 mol solution), glucose (1 and 0.5 mol solution), urea (1 and 0.5 mol solution), sodium chloride (1 mol solution), and potassium bromide (1 mol solution), respectively. Pringsheim⁽⁶⁾ states that when two salt solutions diffuse into a gel in opposite directions the reaction does not proceed beyond a thin film if the solutions are isotonic. A hypertonic solution continues to diffuse into a hypotonic and any precipitate formed is deposited in the latter. In accord with this idea, indifferent substances are often added to the solution on the top of the gel in order to ensure reaction below the gel surface. In the present case, however, when a third substance is added to the outer electrolyte, the increase in the diffusion velocity, which is determined by the osmotic pressure of the electrolyte will be very small, so that the effect on the formed rhythmic precipitate is negligible.

Next, the effect of a third substance added to the inner electrolyte was tested. The solution of a third substance was mixed with acetic acid solution to obtain a 0.8 N acetic acid solution and an equal volume of sodium silicate solution ($d = 1.06$) was poured into it. Then as much of secondary sodium phosphate was further added to the mixture as to be 0.4% in concentration. After gelatinized this sol to a firm gel, 2 mol solution of calcium nitrate was added to diffuse into it. When some organic compound (cane sugar, glucose and urea) was added as the third substance, at first, the bands were somewhat closer, but later rather thinner than those in the cases without the addition of the third substances.

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(6) *Z. physik. Chem.*, **17** (1895), 473.

When, however, potassium iodide or bromide for instance, was added in the inner electrolyte (0.3 mol in the gel), some different results have been obtained. The first three or four bands could be produced although, whose particles were coarse, while those lower than the fourth or fifth bands composed of so coarse particles, that no distinct bands could be observed. The fact may be explained as follows. When an organic matter such as cane sugar, is added, the outer electrolyte is checked to diffuse into the gel by the osmotic pressure of the substance in the gel. Therefore, the concentration of the diffusing electrolyte inside the gel suffers a sharp gradient, so that the bands will deposit relatively close in the part contacting the diffusing solution, and afterward more thinly than the case where no third substance is added. The abnormalities caused by the presence of inorganic salts may be imputed to the fact that calcium phosphate has generally high solubility in the solutions of some other salts. An excess of the diffusing ion is readily brought forth in the vicinity of the part contacting the diffusing agent, so that the bands are formed. But tolerably deep inside of the gel, the minute change of solubility has a great effect on the velocity of separation of the precipitate. In the case of a high solubility, the separation of the precipitate is slow, and their particles are coarse; accordingly, the precipitate will deposit in abnormal bands.

Effect of Aging. Scott Blair, for the first time, reported that the rhythmic precipitate of silver chromate in a gelatine gel was greatly effected by the aging of the gel before superimposing the diffusing electrolyte.(7) The present author ascertained that this change of gel by the age was not really caused by the change of the structure of the gel.(8) Koenig,(9) on the other hand, advocated the effect of aging even in silicic acid gels, and reported that the abnormal bands were brought forth in an aged gel. But it was already mentioned that the normal rhythmic precipitate of copper chromate could be formed in a gel, which had elapsed a very long time.(8) It seemed, therefore, that the aging has no effect on bands in the case where there is no mutual action between the gel and inner electrolyte, especially in silicic acid gels. This view was confirmed by the rhythmic precipitates of calcium phosphate. The experiments have been conducted as follows. Five c.c. of a 0.5 mol sodium phosphate solution was added to 100 c.c. of a sol, which had been prepared by mixing sodium silicate solution ($d = 1.06$) and an equal volume of 0.75 N acetic

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(7) *Phil. Mag.*, **49** (1925), 90.
(8) Isemura, this Bulletin, **8** (1933), 108.
(9) *J. Phys. Chem.*, **24** (1920), 466.

acid, and the mixture was separated into 12 test-tubes and let gelatinized. These test-tubes were kept in a thermostat at 25°C. and an equal volume of 0.5 mol calcium nitrate solution was poured on gels at 1, 3, 12 hours, 1, 2, 3, 4, 5, 9, 10, 12 and 15 days respectively after the setting of the gel. All these test-tubes made quite the same rhythmic precipitate, and cannot be noticed any difference among them. Thus the aging effect, generally, is not observed, provided the inner electrolyte does not react with the gel.

Summary.

(1) The formation of rhythmic precipitate of calcium phosphate in a silicic acid gel was described.

(2) The formation of this rhythmic precipitate was strongly affected by the hydrogen ion concentration of the gel.

(3) The effects of the concentrations of inner and outer electrolytes, and gel on this rhythmic precipitate, were studied.

(4) When the concentration of the inner electrolyte ($CaCl_2$), was sufficiently great, no diffusion of the outer electrolyte into the gel took place but reversely calcium salt diffuse into the outer electrolyte solution and dendrities were formed in the outer solution.

(5) The effect of the existence of a third substance in the gel and in the diffusing electrolyte, were also investigated. The existence of it in a diffusing electrolyte had almost no influence but the existence in a gel had some effects, especially in the case where bromide and iodide of potassium were used as the third substance.

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