RHYTHMIC BANDING OF PRECIPITATES (LIESEGANG'S RINGS).

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Rhythmic banding is a common phenomenon in biology. Examples are: the lamellar rings around the Haversian canals in bone; the pigmentation of the iris; the color of butterflies' wings; the growth rings of trees; the agate structure of crystals; the shells of mollusca; the bands of starch grains and of many seeds.

Rhythmic banding of precipitates was first discussed by Raphael E. Liesegang (1), hence the name "Liesegang's rings." He observed that when a solution of silver nitrate is placed on a gelatine gel containing dilute potassium bichromate, rhythmic banding of the precipitate occurs. If the solutions are in a dish or on a plane surface the precipitate is in concentric rings; if in a test-tube, superimposed layers of the precipitate are formed. Numerous explanations have been advanced, but none is generally acceptable. Ostwald's explanation (2), which postulated a metastable condition, was accepted until Liesegang, Bechold (3), and Hatschek (4) proved it untenable.

Bechold (3) advanced the theory that the band precipitate is soluble in the products of the reaction, and new bands are formed only when the concentration of the reaction products is so dilute that it will not interfere. It has been shown, however, that the effect of the by-products is negligible (5).

Bradford (6, 7) in 1916 put forward a theory which would explain ring formation by adsorption. Since then he has published much work to support his views. Holmes (8) has also advanced a theory similar to Bradford's, but attributes the formation to ionic activities, instead of adsorption effects of the colloidal particles of the precipitate. Bancroft (9), quoting Hatschek, states that all theories fail to account for the specific effect of the gel. Bradford,¹ however, accounts for this by saying that the value of N in von Weimann's formula is influenced by the gel. We do not think this explanation is adequate. The explanation given by Bradford and by

¹ Bradford (7), p. 17.
Rhythmic Banding of Precipitates

Holmes, as far as the process of diffusion is concerned, however, seems adequate.

Fischer and McLaughlin (10), on theoretical grounds, think that ring formation is possible only under conditions where a semipermeable membrane is formed by the precipitate. If the precipitate rapidly crystallizes, it is permeable and therefore the precipitation is continuous. If it be temporarily impermeable, rings are formed. If the precipitate first formed is amorphous and "solvatisierte," a ring is formed. Then in time the precipitate becomes "desolvatisierte," solid and permeable, and the penetrating ion passes through. Ring formation is repeated. There is little evidence that the precipitate per se is impermeable, although the precipitate together with the gel may in many cases form an impenetrable layer. This seems to account in some cases for the difference in ring formation in agar and gelatine gels. Where such an impenetrable membrane is formed, however, it remains so, and prevents further ring formation.

Recently, the senior author (11) advanced an explanation of the rhythmic banding of precipitates, which accounts for the effect of the gel. The main points of this explanation are: banding in precipitation is the normal and universal course, but in water solutions, due to secondary causes such as changes in specific gravity, violent combination, concentration, etc., which stir up or obscure the reaction medium, the bands are destroyed as rapidly as they are formed. The functions of gel in banding are: (1) to fix the precipitate where it is formed and thus to keep the rings visible, and (2) to modify the rate of diffusion of the reacting ions so that favorable conditions for banding are provided. This occurs through the influence of the colloid in damping the vibration of the reaction, and in lessening and limiting the rate of diffusion of one ion more than another.

The process of this interrupted growth theory of ring formation, using bichromate gelatine and silver nitrate as a type, occurs as follows: the bichromate in the gelatine is relatively fixed and diffuses slowly. When a solution of silver nitrate is added, silver bichromate (whether chromate is formed is immaterial in this case) is formed and a clear zone, practically free from chromate, results in the gelatine, due to the attraction of the chromate to the silver. This we have called the zone of influence of the silver ion. Beyond the zone the chromate is fixed (relatively) and remains so unless an attraction force is exerted. The silver nitrate now diffuses through the precipitate and through the clear zone until it approximates the chromate in the gelatine sufficiently close to
exert an attraction force which again draws the chromate and forms another ring and clear zone. At the same time the chromate ion attracts the silver ion and the ring is formed where the forces are balanced. When this precipitate is formed there is a zone relatively free from chromate on one side of the precipitate, and a corresponding zone of lessened silver concentration on the other. This leaves a period (season) of no growth, and the new period of growth (ring formation) commences when the silver, which diffuses more rapidly than the chromate under these conditions, passes through the precipitate and the zone of influence and comes again in contact with the chromate ion.

There is some evidence that all chemical action may be periodic; e.g., Ostwald (12) found that if the rate of evolution of hydrogen gas, when HCl acts on one of the allotropic forms of chromium, during the action be plotted as ordinate against time as abscissa, the curve will show alternating periods of rapid and slow evolution. The curve in a measure corresponds to ring precipitation.

The explanation we present does not differ materially from that of Holmes, except that it accounts specifically for the action of the gel. Bradford's explanation is also very similar, but we think he stresses unduly the importance of adsorption. While it is generally accepted that the adsorption of ions from solution by a precipitate in the process of its formation is the rule, there is no reason to attribute more importance to it in banded than in continuous precipitates. Also the factor of adsorption after precipitation we think is overestimated. While Bradford has done much excellent work to support his theory, this work could be used equally well to support any of the other theories. Surface energy always exists, sometimes negative, sometimes positive, and banding may occur in both conditions; adsorption, therefore, cannot be a prime factor in initiating or controlling the reaction. The influence of adsorption would also seem much less than that of the ion, since adsorption varies inversely as the fourth power of the distance, while chemical attraction varies inversely as the square of the distance (13).

Bradford uses the work of von Weimarn (14) to support his theory. In this work von Weimarn shows that the form of the precipitate, i.e. whether crystalline or colloidal, is dependent on the number of crystallization
centers. If in the double decomposition reaction, \( AB + CD = AC + BD \),
the nature of the precipitate \( AC \) will be governed by the formula

\[
N = \frac{P}{L} \times K_{AB} \times K_{CD} \times K_{BD} \times K_{AD} \times Z
\]

\( N \) = the number of crystallization centers, which we may call the disper-
sion coefficient.
\( P \) is the number of equivalents of the precipitate \( AC \) which must be de-
posited out of each liter in order that its concentration be reduced
to
\( L \), the solubility of the precipitate.
\( K_{AB}, K_{CD}, \) etc., are coefficients expressing the complexity of the various
components.
\( Z \) is the viscosity of the reaction medium.

For substances having simple chemical structure, such as we
are dealing with, and which are not associated within the solution,
the above formula may be simplified without significant
error to

\[
N = \frac{P}{L} \times Z
\]

and since von Weimarn found this law to hold in water solutions,
the most important factor in ring formation in gels seems to be
\( Z \), or the viscosity of the gel. This is the factor which Bancroft
states has not been sufficiently considered in other theories.

Reasons for Stating that Banding Is the Normal Method of Precip-
itation.—Experiments cited by Liesegang (15), which we have
confirmed, seem to us to speak against the adsorption theory; e.g.,
if 10 per cent gel, containing \( \text{Na}_2\text{PO}_4 \) (0.5 per cent), be poured
on a glass plate and allowed to dry, definite banding is obtained.
Bechold makes similar claims and also states that freezing water
produces rings (9). This we have also observed. In this case,
unless adsorption be used in a very loose sense, the banding is due
to crystallization. If it is claimed that crystallization is explained
by adsorption, then it has no especial significance in rhythmic
banding.

Rohonyi (16) has shown that gelatine layers, on freezing,
form in rings. Accepting Ostwald's explanation, his results
support it. When tubes with a thin layer of gelatine are frozen,
layers are formed, clear and dark. The clear layers are ice, the
dark, gelatine. These rings are quite similar to Liesegang's.
Again, bands may appear in air, water, or in gels. Holmes (8) quotes an experiment of band formation in a glass tube where air took the place of the gel. We are able to confirm this as follows:

Concentrated HCl was placed in a small flask, and this connected with an upright glass tube 75 cm. long and 2 cm. in diameter. The upper end of the tube was plugged with cotton and a few cubic centimeters of strong ammonia water were dropped on the cotton. On standing overnight practically the whole length of the tube was covered with bands of ammonium chloride.

By rhythmically forcing air into the flask containing HCl (concentrated) so as to change the rate of diffusion, bands are formed on the glass at different places than if the diffusion took place naturally; in fact, by such manipulation, one may place the bands in any desired location. This action resembles that given by Burton and Bell (17), in which rings were formed in gelatin under tension. The gel was stretched longitudinally, the rings were elliptical, the major axis in the direction of the stretching force. In our work, after several distinct single bands were formed, there were zones of numerous very fine bands instead of the single bands. If the NH₃ is drawn down into this clear space, fine bands result.

Again, if solutions of AgNO₃ and KI be placed in an open test-tube and allowed to stand until evaporation occurs, distinct bands may reach to the top of the tube. Also, if a cholesterol in chloroform be placed in a tube and evaporated, definite rings are formed. Slow evaporation of many other solutions will produce rings, which seems due to capillarity and surface tension. This, however, may be quite distinct from the rhythmic banding in precipitation, although capillarity in gels undoubtedly plays a rôle. Perhaps because evaporation rings are of more common occurrence than precipitation rings, they have received less attention.

Dreaper (18) has shown that when precipitation occurs in capillary tubes, preferably set in a horizontal position, and care being taken to avoid stirring, etc., stratified precipitation may be the usual result in water solutions. In this way he prepared bands of lead chloride, lead sulfate, silver salts, barium sulfate, and many others.

We have been able to repeat this with 0.1 N AgNO₃ and 0.05 N K₂Cr₂O₇. If the silver solution be drawn into a capillary tube or small pipette, and introduced with care into the bichromate solu-
Rhythmic Banding of Precipitates

tion, one often finds definite banding. These bands are seen better with a magnifying glass, and are sometimes difficult to prepare. Dr. L. F. Shackell, who first called our attention to this, found that a small bubble of air between the solutions aided ring formation. Again, if the chromate solution be poured on a glass plate and allowed to dry, and a small crystal of AgNO₃ added and moistened carefully, definite ring formation may be seen.

The banding of precipitates, therefore, seems to be a universal phenomenon, and under proper conditions may be visible in all cases. This may appear opposite to the statement of one of us in a previous article (11) where it was said that, if lead acetate be used instead of silver nitrate on a bichromate gelatine gel, no ring formation occurs. Bradford (19) is correct in stating that with proper concentrations, rings of lead chromate in gelatine may be prepared. Many incorrect statements have crept into the literature of rhythmic precipitation, and the reason seems to be that rings in some cases are more difficult to prepare in visible form. But since rings may be rendered visible in practically every precipitation, any statement regarding their presence or absence is relative only. To illustrate further such incorrect statements we may quote the following from Bancroft.² "The reactions between silver nitrate and potassium bichromate give rings or layers in gelatine but not in agar, while lead nitrate and potassium chromate give layers in agar but not in gelatine." Bradford (5) also in one of his latest articles says: "It has been found that neither silver chromate or bichromate form bands in agar gels" and in discussing the theory advanced by one of us, says: "It is not sufficient to explain why bands form in gelatine and not in agar." All such statements require qualification, and some of them have been corrected later by these authors. When we adopt the view that rhythmic precipitation is the normal process, the explanation is more easily understood.

Bands form readily in agar as the following experiments will show.

Mix equal parts of 1 per cent agar and 0.02 N potassium bichromate in a test-tube. When a gel has formed, pour 0.1 N silver nitrate on top. After 12 hours twenty-three bands were counted in a space of 2 cm. beginning 2.5 cm.

² Bancroft (9), p. 259.
from the surface of the gel; these were very delicate and better seen by transmitted light. To show rhythmic banding, time and concentration are important functions as they regulate the rate of diffusion. It is for this reason that the concentration of the penetrating ion is greater. Also the test-tube method may show banding that is not visible on surfaces.

No new chemical process is involved in the formation of rings. The colloid is simply an interfering body, modifying the intensity of the reaction, and calming or damping the reaction field by immobilizing the precipitate where it is formed. In some cases where the gel and the precipitate form an impenetrable membrane, or a membrane that is penetrable with difficulty, no ring formation is visible. If the physical state of the reaction medium remains calm in water solutions, as in the case of the ammonium chloride in air, and in precipitation in water solutions in capillary tubes, ring formation is seen to be the usual process. On plane surfaces precipitation rings are formed in circles somewhat similar to the rings formed by dropping a stone into a pool of calm water. If the pool be turbulent the rings are destroyed or twisted out of their course. Similarly, in an ordinary chemical reaction the local storm, caused by the reacting ions and by the difference in the specific gravity of the product formed and the solvent, disturbs the medium to such an extent that the rings are destroyed.

The opposite effect of a gel can be obtained by adding ether, alcohol, or acetone to the reacting medium. This causes in water solutions a marked increase in the turbulence of the reaction, apparently due to lowering of surface tension. There are cases, however, where the tendency to band is increased by the addition of these bodies to the gel; i.e., where the viscosity is so great that an impermeable membrane is formed. The addition of alcohol, by lessening the viscosity, changes permeability so that banding may occur. Sugar also is known to increase banding in such cases.

Liesegang (15) has recently pointed out a similar phenomenon in the case of silver chloride bands in gelatine. If to a gel containing silver nitrate, sodium chloride solution be added, no banding is visible. The reason seems to be that the penetrating Cl ion moves too rapidly in relation to the Ag ion, which may be partly combined with the gel. The precipitate, therefore, is continuous. If, however, silver bichromate bands are first formed, and then sodium
chloride be added, the chromate bands are replaced by chloride bands. The silver chromate here acts as a “form catalyser.” The reaction involved is that used in the determination of chlorides by Mohr’s method.

Besides the rate of penetration, however, there is another factor of importance, and this is the degree of mobility of the incarcerated ion; i.e., the ion of the gel. The following experiment will illustrate.

Two tubes were made as follows:

Tubé 1. Agar, 0.5 per cent. Tube 2. Gelatine, 5 per cent.
Pb acetate, 0.1 N. Pb acetate, 0.1 N.

When these had coagulated 10 per cent KI was poured on top. After 36 hours the iodide solution had penetrated about the same distance in each, but in the agar there were beautiful rings, while in the gel there was only a suggestion of ring formation.

The difference seems to be due to the nature of combination of the lead acetate in the two cases. In the gelatine it is more intimately combined and less moveable; in the agar it is practically free and moveable, and gets entangled in the agar net only when particles of the precipitate are formed. When, however, precipitation has descended several centimeters in the tube, rings become visible also in the gelatine. This is attributed to a change in the rate of the penetrating ion (I) which is now slower than where the precipitate is continuous. That rate of penetration, or rate of meeting of the ions, is important is readily shown by the methods of preparation of rings. To obtain the best results, the penetrating ion is always in greater concentration than the ion in the gel, e.g. if 0.05 N AgNO₃ be placed in water solution on top of a 5 per cent gelatine gel, containing 0.01 N K₂Cr₂O₇, rings are soon formed. However, if the AgNO₃ be enclosed in 5 per cent gelatine and placed on top, practically no ring formation occurs. Without doubt, if we could, by freezing or otherwise, regulate the rate or meeting, and of the reaction of the ions in water solution, to the same rate as in gelatine, rings would also be visible in the water. Dreaper’s work which we have corroborated substantiates this opinion.
SUMMARY.

A theory of rhythmic precipitation is advanced, which accounts for the action of the gel. This theory accepts the descriptions of the process given by Bradford and by Holmes, which are essentially alike. Precipitation in gels does not differ from precipitation in water; therefore, adsorption is no more important in gels where precipitation is banded, than in water where it is continuous. The function of the gel is to fix the precipitate where it is formed, and to restrict the zone of the influence of the ions, by reducing to a minimum the factors which make for turbulence or diffusion. It is pointed out that any influence that lessens the turbulence of the reaction, makes for rhythmic precipitation.

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