XXXIX. ADSORPTIVE STRATIFICATION IN GELS. IV.

By SAMUEL CLEMENT BRADFORD.

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(With Plates VII and VIII.)

DIRECT proof has already been given [Bradford, 1920] of the fundamental rôle of adsorption in the formation of banded precipitates. Unfortunately, owing to its extremely complex nature, mathematical analysis of the phenomenon appears impossible. The adsorbing surface at each band is affected by the mass of the precipitate and by the various factors of von Weimarn's formula, which vary, in an intricate way, with the concentrations of the reaction components. Moreover, notwithstanding statements to the contrary, the diffusion of the hypertonic reagent appears, at times, to be considerably affected by the mass and structure of the precipitate through which it has to percolate. In this connection it is sufficient to compare the extreme slowness of the diffusion of $N/5$ $\text{Pb}(\text{NO}_3)_2$ solution into $N/20$ K_2CrO_4 agar gel, with the rate of diffusion into $N/200$ K_2CrO_4 agar gel of the same solution, which reaches the base of the tube in 14 days. As in the case quoted, band formation does not seem to occur unless the precipitate allows of ready diffusion of the hypertonic reagent. This may be one reason why very colloidal precipitates do not form good bands. But this effect may also be due to the sudden separation of too large a mass of precipitate at one time, owing to excessive supersaturation. The complexity of the phenomenon, generally, is further increased by the influence of the reaction components on the formation of the precipitate, which was first pointed out by Bechhold [1905], and is confirmed in a remarkable way by the experiments to be described. It appears hopeless, therefore, at present, to make exact deductions which can be tested quantitatively.

The precipitate, produced by the interaction of an aqueous reagent on a gel containing a suitable solute, will create a solid boundary surface between the reagent and the solute, at which, by Gibb's law, the concentrations of both dissolved substances will usually increase. If the aqueous reagent is hypertonic to the gel, its concentration at the solid surface will be in excess of that of the solute in the gel, and the reagent will be able to diffuse into the gel. With a hypotonic aqueous reagent the adsorption of the gel solute will usually be greater, so that the aqueous reagent will be precipitated, as it collects at the solid surface, and will be unable to pass into the gel. This is Pringsheim's law. The result, however, depends on the formation of a precipitate. Hitherto, it does not always appear to have been realised that, in

the absence of a precipitate, diffusion can take place in both directions. Coloured reagents make this apparent. When strong ammonium hydroxide is poured on a gel containing copper sulphate the deep blue colour gradually diffuses both ways, until, after 48 hours, the solution and the gel have become the same colour throughout.

Since the separation of the strata depends on adsorption of the gel solute from the region between, it follows that the concentration of the hypotonic reagent must be of primary importance. The distance between the bands should usually be affected more by variations in the concentration of the gel solute than of that of the aqueous reagent. Moreover, adsorption experiments with coloured reagents indicate that the concentration of the gel solute in the direction away from the precipitate may increase very slowly at first, and then quite suddenly, as the maximum concentration of the gel is approached. This would tend to eliminate the influence of the valency of the reaction components in the metastable product, since the critical value, at which precipitation takes place, would be reached at approximately the same point where the sudden rise in concentration occurs. For the same reason this narrow region is the only place where any kind of supersaturation should happen.

These conclusions are subject to modification on account of the inhibiting, or precipitating, action of the various ions present, but they are fairly well borne out by experiment. Four tubes containing: (1) *N*/80 CaCl_2 agar gel, (2) *N*/50 CaCl_2 agar, (3) *N*/70 Na_2CO_3 agar and (4) *N*/20 Na_2CO_3 agar, treated with: (1) and (2) *2N*/1 Na_2CO_3 and (3) and (4) *N*/10 CaCl_2 solutions gave bands at distances (cm.) apart as follows:

(1)	(2)	(3)	(4)
0.57	0.35	0.65	0.15
0.73	0.45	0.65	0.22
1.00	0.55	0.85	0.225
		0.95	0.225
			0.275
			0.225
			0.50
			0.40
			0.55

For a given pair of reagents the figures are roughly inversely proportional to the concentration of the gel.

In another set the tubes contained: (1) and (2) *N*/30 KI agar and (3) and (4) *N*/60 KI agar. These were treated with: (1) and (3) *N*/1 $\text{Pb}(\text{NO}_3)_2$ solution and (2) and (4) *N*/2 $\text{Pb}(\text{NO}_3)_2$ solution. In this case halving the strength of the hypertonic reagent increased the distances of the bands only very slightly, while a corresponding change in the hypotonic reagent caused an approximate doubling of the distance apart of the strata. The inversely proportional effect comes out although the ionic product contains the square of the concentration of the hypotonic reagent.

In a third series the tubes contained: (1) and (3) $N/100$ K_2CO_3 agar and (2) and (4) $N/200$ K_2CO_3 agar. On these were poured 10 cc. each of: (1) and (2) $N/5$ $Pb(NO_3)_2$ and (3) and (4) $N/10$ $Pb(NO_3)_2$ solutions. The following curves, Fig. 1, were drawn freely through points obtained by plotting the distances apart of the bands, as ordinates, against the distances below the surface of the gel of the mid-points between the bands, as abscissae.

Fig. 1.

The very slight difference caused by halving the strength of the hypertonic reagent is apparent, as compared with the much greater effect of a similar change in the gel solute.

Similar reasoning shows that different effects should be obtained according to which reagent of a pair is dissolved in the gel, or to the actual solute chosen to provide a given reaction component. For example bands of lead carbonate should depend on whether the lead salt or the carbonate is dissolved in the gel, and on the particular soluble lead salt and carbonate used. 1 % agar gels containing: (1) $N/100$ K_2CO_3 , (2) $N/100$ $Pb(NO_3)_2$ and (3) $N/100$ Na_2CO_3 were treated with: (1) and (3) $N/10$ $Pb(NO_3)_2$ and (2) $N/10$ K_2CO_3 solutions. The remarkable differences in the bands are shown in Figs. 1, 2 and 3, Pl. VII. The very fine lamination in (2) does not show clearly in the figure. With sodium carbonate as hypotonic reagent the bands are noticeably less regular than with the potassium salt.

It has already been shown [Bradford, 1918] that, since adsorption must depend directly on the specific surface of the precipitate, the formation of bands is determined by the value of N in von Weimarn's formula

$$N = K \cdot \frac{P}{L}.$$

Recently, this expression has been criticised by Buchner and Kalfi [1920]. Their experiments show that, in a given case, the dispersity of the precipitate is proportional to P/L , that is

$$N \propto \frac{P}{L}.$$

They contend, however, that the factor K in von Weimarn's formula is not directly proportional to the viscosity of the reaction medium and to the

degrees of aggregation, chemical (= complexity of structure) and physical, of the reaction components. Thus they obtained precipitates in the gel state with values of P/L varying from 75, 140 and 360 in the case of BaF_2 , CaSO_4 and PbI_2 , to about 10^4 , 10^6 , 10^7 and 10^8 for BaSO_4 , AgCl , AgBr and AgI respectively, and they argue that, while chemical complexity has no exact meaning, the physical aggregation and viscosity cannot be so great as to account for these differing values.

That the chemical complexity of the molecule cannot be ignored is shown by glancing through the pages of Beilstein where its undoubted influence on the form in which a substance occurs may easily be traced. There is, however, an important factor which influences the value of P , which does not seem to have been taken into account hitherto. This is the amount of mixing of the reagents which happens before a precipitate is formed. It can easily be shown that in the case of a substance such as PbI_2 , for example, where

$$H = \text{Pb}^{++} \times \text{I}^{'2},$$

the maximum value of P occurs when two volumes of the iodide solution mix with one volume of the solution of lead salt. In a given case, the degree of mixing before precipitation would certainly be affected by the metastable limit as modified by the various reaction components. The extraordinary effect of H on the form of the precipitate is shown by the experiments with the silver chromates which follow. Thus it will be seen that the value of P may easily differ greatly from that calculated on the assumption of immediate mixing of equivalent volumes of the reacting solutions. It seems probable that the remarkably different values of P/L found by Buchner and Kalfé may be due, very largely, to variations in the corresponding metastable limits.

It must further be noted that L is not the normal solubility of the given substance, but its solubility under the actual conditions of the experiment. Again, the permanency of form of the precipitate may be influenced by the presence of the reaction components, which may tend to produce double salts, or otherwise to peptise the precipitate.

However, these considerations emphasise the difficulty of evaluating the factors in von Weimarn's formula, and it might be preferable to write it

$$N = U \cdot \frac{P}{L},$$

where U is a function, as yet undetermined, of the viscosity of the medium and of the size and structure of the particles in solution, whether due to physical aggregation or chemical complexity. That is to say the formula is, as yet, an incomplete expression of the truth. It is, nevertheless, most useful as a working rule.

It will be seen, therefore, that banded precipitates can be produced only when the specific surface of the precipitate is great enough to cause the exhaustion of the solute from the neighbouring region of gel. Decrease in

N leads to diminution in adsorbing surface until stratification ceases. On the other hand, increase in N may be accompanied by increase in density of the precipitate and slower diffusion of the precipitant through the column of precipitate, until the precipitant cannot permeate the precipitate fast enough to traverse the exhausted region of gel immediately beyond before this is replenished with solute by diffusion from the gel. This is often noticed with lead salts, which, frequently, do not form strata unless the hypotonic reagent is very dilute, usually about $N/200$. When the gel solute is too concentrated, a dense, continuous band of precipitate grows until all the solute has been exhausted from the gel. This generally happens long before the column has reached the base of the tube, as in Fig. 1 of a previous paper [Bradford, 1920] which shows a column of lead chromate nearly complete.

Since the solute in the gel must be sufficiently dilute to allow of its exhaustion from the neighbourhood of the precipitate, with corresponding limitation of the value of P , it is clear that stratification can take place only when L is very small, or when K is large enough to compensate for a somewhat larger value of L . This is why only the more insoluble precipitates readily yield banded structures, and these only within comparatively narrow limits of concentration.

Although it has been argued that the adsorption theory leaves the gel out of account altogether [Hatschek, 1919], it will be seen that the above considerations are sufficient to explain the extraordinary differences in the form of a given precipitate when produced in different gels. The reaction medium, by its viscosity and protective action, profoundly modifies the values of K and P , and, thereby, the specific surface of the precipitate. The nature of the gel is, therefore, of fundamental importance. Various examples of the effect of the gel on the structure of the precipitate have already been discussed [Bradford, 1918]. Two of the most striking cases, and, perhaps, the most well-known, are those of the chromate and dichromate of silver, which form beautiful brownish red bands in ordinary gelatin gels and yield in agar only macroscopic black crystals. It was pointed out, that the absence of stratification in agar must be due to the small specific surface of the precipitate, and that, by increasing this, it should be possible to produce bands in agar similar to those in gelatin. This has been completely realised by experiment. It further transpires that the original Liesegang phenomenon in ordinary gelatin is due to the presence of impurities.

Fig. 6, Pl. VIII, shows the result of treating $N/20$ $K_2Cr_2O_7$, 1 % agar gel with $N/2$ $AgNO_3$ solution. It is a good illustration of the effect of slow diffusion in producing large crystals of a substance which occurs as a finely divided precipitate when the reacting solutions are quickly mixed. The formation of macroscopic crystals of silver dichromate shows that the number of crystallisation centres, N , is much too small. The only criterion for the production of bands is a sufficient increase in N . The application of this principle provides a crucial test for the adsorption theory.

A difficulty in the experimental work is the narrowness of, frequently, both the concentration limits, between which good bands are obtained. For this reason it is possible to make a series of experiments with substances, capable of forming beautiful strata, without obtaining any.

The first experiments were directed towards increase of specific surface by variation in the concentration of the reaction components, or the solubility of the precipitate. The following table shows the result of diminishing the strength of both reagents.

Reagent in the 1 % agar gel	Aqueous reagent	Result
<i>N</i> /50 $K_2Cr_2O_7$	<i>N</i> /10 $AgNO_3$	Red surface layer, clear space 0.25 mm., then small crystals with suggestion of stratification.
<i>N</i> /75 $K_2Cr_2O_7$	<i>N</i> /10 $AgNO_3$	Red surface layer, small crystals arranged distinctly in bands at 1, 4 and 10 mm. below surface.
<i>N</i> /100 $K_2Cr_2O_7$	<i>N</i> /20 $AgNO_3$	Red surface layer, band of microscopic black crystals at 5 mm., part of a red band at 14 mm.
<i>N</i> /200 $K_2Cr_2O_7$	<i>N</i> /20 $AgNO_3$	Red surface layer, band of very small crystals at 1 mm., wide dark red turbidity from 12.5 to 27 mm.
<i>N</i> /300 $K_2Cr_2O_7$	<i>N</i> /20 $AgNO_3$	Red surface layer, band of very tiny crystals at 1 mm., and larger particles from 3 mm.

As the concentrations are reduced, the particles of precipitate grow smaller, with corresponding increase of specific surface, and the tendency to produce bands begins to make itself manifest. The crystals were, however, still much too large. The addition of alcohol further diminished the size of the particles and increased the formation of bands. *N*/20 $K_2Cr_2O_7$ agar gel containing 30 % of absolute alcohol, treated with *N*/10 $AgNO_3$ solution, gave a precipitate in tiny feather-like crystals in distinct layers at 0.5, 2.0 and 3.5 mm. below the red surface layer.

Since the aqueous phase in gelatin gels contains about 0.3 % of gelatin, the effect of adding small quantities of gelatin and gum arabic was tried in a great many experiments. Many of these gave short columns of red precipitate instead of merely surface layers, but after about 1 cm. the colour of the column changed to black with the development of distinct crystals or spherites. With the aid of a lens, however, the red columns were frequently seen to be laminated. By increasing the concentration of the silver nitrate solution to *N*/2, two separate red bands were obtained in *N*/75 $K_2Cr_2O_7$ $\frac{1}{4}$ % agar gel containing 0.5 % of gelatin. Numbers of very beautiful black spherites of $Ag_2Cr_2O_7$ with a greenish lustre were formed below the bands, reaching 0.5 mm. in diameter at the base of the tube. With 2*N*/1 $AgNO_3$ solution and *N*/175 $K_2Cr_2O_7$ 1% agar gel containing 0.06 % gelatin, five bands were formed, the first two red, those below composed of microscopic black spherites. These experiments appeared to show that the red precipitate is not due entirely to the effect of the gelatin. While the column of precipitate remained red it showed very similar banding to the commencement of a column in gelatin. The difficulty appeared to be that the specific surface diminished, as the reagents became more dilute by diffusion, until stratification ceased.

It seemed desirable to find some other way of increasing N . Remembering Bechhold's experiments on the effect of ions on the precipitate, Liesegang's [1914] practice of adding citric acid to washed gelatin was considered. It seemed probable that the trivalent anion of citric acid might tend to prevent the precipitation of negatively charged silver chromate particles, thus increasing the supersaturation and giving a much greater value of P . A series of experiments from this point of view showed that there is a definite concentration of citric acid, or of soluble citrate, for each concentration of chromate, or of dichromate, in the gel, which gives perfectly formed strata with clear interspaces.

Figs. 7 to 12, Pl. VIII, show the effect of adding increasing amounts of citric acid. As the particles grow smaller, the banding gradually appears. At first, while the crystals are still large enough to appear black, only a few layers are formed close together as seen in Figs. 7 and 8. With more citric acid a number of skeletal bands of red precipitate appear, which are much wider apart, but which break up into black spherites lower down the tube, Fig. 9. With the optimum amount of citric acid the red bands are complete and extend right down the tube, Fig. 10. Further increase of citric acid yields a more colloidal precipitate, at first irregularly stratified, Fig. 11, but ultimately separating in large flocks with little trace of stratification, as in Fig. 12. In Figs. 7 to 10 the effect was obtained by decreasing the concentration of potassium chromate while keeping the citric acid constant at $N/300$. The tubes contained respectively $N/88$, $N/107$, $N/125$ and $N/150$ K_2CrO_4 . Fig. 11 had $N/140$ K_2CrO_4 with $N/250$ citric acid, and Fig. 12 $N/100$ chromate with $N/130$ citric acid. The optimum concentration of citric acid for $N/150$ K_2CrO_4 is $N/300$. A similar series was obtained with $N/100$ K_2CrO_4 agar gel by varying the amount of trivalent anion. The optimum concentration was found to be $N/200$. With $N/75$ K_2CrO_4 , $N/125$ citric acid gave the best results.

The agar gel was made by soaking shredded agar for 24 hours in sufficient water to make a 1.25 % gel, afterwards boiling till dissolved and filtering through a Buchner funnel with washed linen. The gel was stored in wide-mouth bottles containing a trace of camphor. The test-tubes used were roughly graduated at 3 cc. and 15 cc. The required amounts of standard solutions of the reagents were added to make the gel of the desired strength, and the liquid made up to the 3 cc. mark with water. The hot agar sol was then filtered into the tube up to the 15 cc. mark, the whole mixed and allowed to set by placing in cold water. This gave a 1 % agar gel. To each tube 3 cc. of $2N/1$ silver nitrate solution were added, very carefully, from a pipette with its nose just above the top of the gel. Without great care, or if the tube was afterwards slightly shaken, the silver solution was liable to run down between the gel and the tube. The standard solutions of K_2CrO_4 and citric acid were made from recrystallised materials, the former by weighing and the latter by titration with carbonate-free alkali and phenolphthalein. It was remarkable that with ordinary stock reagents very different relative amounts of chromate

and citric acid were required. This was assumed to be due to traces of impurities.

The bands produced with citric acid and potassium chromate in agar are much wider apart than in gelatin, and, as they are almost free from secondary white precipitate, the effect is very beautiful. Moreover, they begin to form immediately the silver solution is poured on the gel, which makes them particularly suitable for demonstration. The same recipes give good results in plate preparations and in filter paper.

The effect of citric acid in gelatin was attributed by Liesegang to the hydrogen ion. This does not appear to be the case, since equally beautiful results are obtained by the use of potassium citrate. The bands are now very close together, almost exactly like those in gelatin, but perhaps closer. After about 10 mm. they become granular and blurred, suddenly changing into more colloidal, thicker and wider bands at the base of the tube, just as silver chromate does in gelatin. The optimum concentration for $N/150$ K_2CrO_4 was $N/85$ potassium citrate. By substituting citric acid for part of the potassium salt, the bands became wider apart and lost the blurred granular and more colloidal regions as in Fig. 5, Pl. VII. Wonderfully beautiful bands are produced with potassium citrate and potassium dichromate, which resemble, very closely, those formed in washed gelatin with citric acid. Fig. 4, Pl. VII, shows a 1 % agar gel with $N/100$ $K_2Cr_2O_7$ and $N/55$ potassium citrate and $2N/1$ $AgNO_3$ poured on top. It must be noted, however, that these reagents were not specially pure, or less citrate might have been necessary.

Bearing in mind that the chromates of silver do not form bands in washed gelatin, it seems evident that the different effects obtained with these salts in commercial gelatin and in agar gels are due, rather to mineral impurities contained in the gelatin, than to differences in the action of the gel-substances themselves. This raises the question as to whether the great protective action of commercial gelatin is a property of the pure substance.

The action of the citrate ion in these experiments is artificially to increase the supersaturation of the silver chromate before precipitation takes place, giving a larger P and greater specific surface of the precipitate. By actual cataphoresis, in the presence of gelatin and the reaction components, Mr Hatschek very kindly confirmed the hypothesis that silver chromate particles are negatively charged. This supports the view that the increased supersaturation is due to the trivalent anion. The inhibiting effect of citric acid was demonstrated by experiments on the mutual precipitation of dilute aqueous solutions of K_2CrO_4 and $AgNO_3$ of varying strengths in the presence of different amounts of citric acid. One reagent was added slowly, drop by drop, with vigorous stirring, to the other, mixed with citric acid, until sudden precipitation of the whole excess of silver chromate took place. A number of curves have been obtained showing the relation between the metastable limit and the concentration of citric acid, for different strengths of the reacting solutions. The experiments show that citric acid enormously increases the critical value of the ionic product for silver chromate at which spontaneous precipitation

takes place. Taken in conjunction with Bechhold's observations on the influence of ammonium nitrate in the same reaction, it seems extremely probable that aqueous supersaturated solutions are affected by electrolytes in the same way as colloid solutions. The experiments are being continued.

These investigations must not be taken as supporting the "supersaturation" theory of band formation. It has been shown that the reason why the silver chromates do not form bands in agar or washed gelatin is merely that the particles of precipitate are too large: Precipitates with small specific surface never form bands. The experiments show that, as the size of the particles diminishes, the tendency to stratification appears. The use of soluble citrates is merely a convenient way of increasing the dispersity of the precipitate. To leave no doubt on this point it seemed desirable to continue the experiments in which the specific surface was increased by diminishing the solubility of the precipitate. This time potassium chromate was used, as silver chromate is less soluble than the dichromate. It was immediately found that $N/100$ K_2CrO_4 0.7 % agar gel, containing 30 % of absolute alcohol, treated with $2N/1$ $AgNO_3$ solution gave a good series of narrow bands of Indian red silver chromate, which were very similar to those in ordinary gelatin, but so close together as to require a lens to see them clearly. In this case the precipitate was distinctly granular, like those of lead chromate and iodide. It is clear, therefore, that increased supersaturation is not necessary for the formation of bands. The success of these experiments affords seemingly conclusive evidence for the adsorption theory.

SUMMARY.

Further evidence has been adduced to show that the formation of banded precipitates is due to adsorption of solute from the region between the bands.

Only precipitates with great specific surface form bands.

The specific surface of the precipitate is influenced by the reaction medium, by the presence of ions and particularly of trivalent ions.

By varying its specific surface, a given substance can be obtained in the banded form, or not, as desired.

The occurrence, or non-occurrence, of bands of the same substance in different gels is due to the influence of the reaction medium on the dispersity of the precipitate.

These principles have been tested by application to the well-known cases of silver chromate and dichromate which form bands in gelatin gels and not in agar. The non-occurrence of bands in the latter medium is due to insufficient specific surface of the precipitate. By increasing the dispersity, beautiful bands of both substances have been obtained in agar gels.

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