XXVIII. THE RELATION BETWEEN THE MICROSCOPIC APPEARANCE OF PRECIPITATED CALCIUM OXALATE AND THE OPACITY OF ITS SUSPENSIONS.

By J. HOLKER.

From the Department of Pathology, The University, Manchester.

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IN the course of an investigation of the opacity of suspensions resulting from the complex interactions of electrolytes, suspensoids and emulsoids, it became advisable to study the factors governing the opacity of suspensions of relatively simple substances. It was decided therefore to begin with a crystalline organic substance, and for this purpose calcium oxalate seemed to be a suitable substance since by reason of its high degree of insolubility it offered a considerable range in the concentration of its suspensions. Moreover since the main research was concerned with organic substances capable of yielding insoluble products of hydrolysis, it was advisable to choose for the present investigation an organic acid of known chemical composition and crystalline appearance. It was thought that by comparing the microscopic appearance of the precipitate with the curve obtained by plotting the opacity of the suspension against the concentration, information would be gained which might be of use in studying the factors involved in producing the opacity of more complex suspensions. Such complex suspensions are the rule in serological investigations, and it seems to me that the problems of immunology will only be solved by approaching the subject from the physico-chemical standpoint. Then after analysing the simpler phenomena it should be possible to build up a framework on which to mould the more complex reactions.

The apparatus used for measuring opacity was that recently described by the author [1921,1]. The method of preparing the suspensions of precipitate was as follows. Into each of two dry test-tubes of resistance glass of about 80 cc. capacity were pipetted 25 cc. of known strengths of calcium chloride and potassium oxalate respectively. The two reagents were then thoroughly mixed by pouring a definite number of times from one tube into the other. The tube containing the 50 cc. of mixed reagent was then allowed to stand a predetermined time at room temperature so as to allow the turbidity to become constant.

The time necessary for attaining equilibrium was determined by plotting the opacity against the time. By this means it was found that in no case did

it take longer than half-an-hour for the opacity to become constant. With very dilute solutions however there was a lag before the precipitate appeared, and the more dilute the reagents, the longer the lag. This lag was no doubt due to the fact that in very dilute solutions the crystals are very small in size and very few in number. As is well known small particles of a precipitate dissolve more readily than large ones. For example, Hulett [1901] found that particles of calcium sulphate 0.0003 cm. in diameter had a solubility of 18.2 millimoles per litre, while with particles 0.002 cm. in diameter the solubility was 15.33 millimoles per litre. With more concentrated reagents the velocity of growth of the crystals became more and more rapid, and moreover there developed between the initial steep slope and the final level line of equilibrium a series of oscillations. The more concentrated were the reagents, the more pronounced became the oscillations.

In the next series of experiments 25 cc. each of equimolecular solutions of calcium chloride and potassium oxalate were mixed together in the manner described above, and, after allowing the mixture to stand at room temperature for half-an-hour until equilibrium was attained, the opacity was measured and plotted against the net concentration of the reagents. For instance, equal volumes of $N/200$ reagents produced a turbidity of 88; the latter figure was therefore plotted against a net concentration of $N/400$. Six or more experiments were made for each concentration of the reagents and the mean of them taken. At the same time the appearance of the precipitate under the high power with the microscope was examined, and the crystals drawn as nearly as possible to scale. The opacities of the equimolecular mixtures are plotted in Fig. 1, curve A. A summary of the drawings corresponding to these experiments is shown in Fig. 2.

According to the well-known law for the solubility product of a saturated solution of a difficultly soluble substance, excess of either of the ions of calcium oxalate will diminish the solubility of the calcium oxalate and so correspondingly increase the amount of the precipitate. It was decided first to test the effect of excess of potassium oxalate on the form of the curve.

For this purpose 25 cc. of $N/10$ potassium oxalate were mixed with 25 cc. of varying concentrations of calcium chloride from $N/10$ downwards. The results are plotted in Fig. 1, curve B. The microscopical results were similar in general character to those shown in Fig. 2, but differed in points of detail.

In the next series it was decided to test the effect of excess of calcium ions. For this purpose therefore 25 cc. of $N/10$ calcium chloride were mixed with 25 cc. of varying concentrations of potassium oxalate from decinormal downwards, the results being plotted in Fig. 1, curve C. The microscopical appearances were similar to those shown in Fig. 2.

It will be seen from Fig. 1 that the opacity concentration curves for the precipitation of calcium oxalate may be divided into three parts, viz. a short initial lag, a longer intermediate zone which increases in steepness to a maximum, and a very long final zone which decreases in steepness until the line

Fig. 1.

<p>Concⁿ of Reagents 1.0 millimoles Opacity 16</p>	<p>Concⁿ of Reagents 1.5 millimoles Opacity 30</p>	<p>Concⁿ of Reagents 2.0 millimoles Opacity 53</p>	<p>Concⁿ of Reagents 2.5 millimoles Opacity 88</p>
<p>Concⁿ of Reagents 3.6 millimoles Opacity 192</p>	<p>Concⁿ of Reagents 4.4 millimoles Opacity 266</p>	<p>Concⁿ of Reagents 5.5 millimoles Opacity 310</p>	<p>Concⁿ of Reagents 6.6 millimoles Opacity 420</p>
<p>Concⁿ of Reagents 10 millimoles Opacity 564</p>	<p>Concⁿ of Reagents 7.28 millimoles Opacity 728</p>	<p>Concⁿ of Reagents 2.0 millimoles Opacity 827</p>	<p>Concⁿ of Reagents 25 millimoles Opacity 948</p>

Fig. 2. Equimolecular solutions.

becomes nearly straight. As I have recently shown [1921, 2] that the opacimeter used measures the total surface of the particles scattering light, and that the opacity concentration curve is a straight line, in those cases where the particles remain all of the same size and shape in all concentrations, some explanation was required for the type of curve obtained with calcium oxalate. On studying the microscopic appearances of the precipitates corresponding to the zones in the curves, it was found that in the initial lag the crystals were very few in number and very small in size, and that they tended to be spherical in shape. The small number and size of the particles therefore accounted for the low turbidity in this zone. The ultimate cause of the low turbidity was no doubt the fact that small particles, having a relatively large surface, tend to redissolve in order to diminish the surface energy to a minimum. At a certain critical point in the curve represented by the beginning of the intermediate zone, the crystals begin to increase in size and number with increasing concentration of reagents. In this zone then the growth is mainly by surface condensation with the production of crystals of more or less typical structure. The growth in size goes on until the crystals have attained maximum size; this point being represented in the curve by a second critical point which marks the end of the intermediate zone and is accurately defined as the point of change in direction of the tangent to the curve. Immediately beyond the second critical point the crystals tend to be formed of spherical aggregates. With still higher concentrations of reagents the spherical aggregation becomes less pronounced and is finally replaced by a non-geometrical polar aggregation which becomes more and more pronounced. It is this increasing aggregation beyond the second critical point which is responsible for the continually decreasing steepness of the curve in the final zone.

The effect of excess of oxalate or calcium ions is well shown both in the curves and in the appearance of the precipitates. The curves bear out the solubility law in that excess of either oxalate or calcium ions leads to an increase in turbidity of the suspension. In the initial zone the lag with excess of oxalate ions is very short, while that with excess of calcium is intermediate in length. In the intermediate zone, the steepness with excess of calcium is very marked while that with oxalate is intermediate in degree. The maximum steepness or rate of increase of turbidity with respect to the concentration of the reagent is strikingly shown by the values for the tangents to the curves at the second critical points.

	Maximum steepness of the curves
1. Equimolecular solution ...	1.32
2. Excess of oxalate ions ...	1.70
3. Excess of calcium ions ...	5.26

It would appear that excess of calcium ions is more favourable for the production of typical crystals of calcium oxalate, but even in this case there is a gradual transition in the type of crystal from the beginning to the end of the intermediate zone.

SUMMARY.

1. The turbidity of a suspension of calcium oxalate produced by the interaction of calcium chloride and potassium oxalate takes a certain time for the development of equilibrium.

2. With dilute solutions there is a lag before any turbidity appears.

3. With concentrated solutions there is a period of oscillations of the turbidity before equilibrium is established.

4. The turbidity concentration curve for equilibrium turbidities is not a straight line, but shows a short initial flat lag, a longer intermediate zone of increasing steepness, and a long final zone of diminishing steepness.

5. Two critical points in each of the curves represent the beginning and end of the intermediate zone. The first critical point represents the point at which the crystals have attained a size at which they do not tend to redissolve. It also represents the point of first appearance of the typical "envelope" crystal. The second critical point represents the point at which the typical crystals reach their maximum size. Beyond that point the crystals diminish in size but still increase in number. The second critical point also indicates the onset of aggregation of crystals, with consequent diminution in the rate of increase of turbidity.

6. Excess of either oxalate or calcium ions increases the opacity.

REFERENCES.

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