THE MECHANISM OF THE CLARIFICATION OF MUDDY WATER BY *STRYCHNOS POTATORUM* SEEDS.

BY K. SUBBARAMIAH AND BASRUR SANJIVA RAO.
(From the Department of Chemistry, Central College, Bangalore.)

Received November 12, 1936.

Introduction.

THE *Strychnos potatorum* tree (also called the clearing nut tree) belongs to the *Strychnos* family and is found in many parts of India and Burma. The seeds are very hard and non-poisonous. They contain no strychnine, though Brucine is present. The seeds are still used in many rural parts of India for the clarification of muddy water. The use of albumin and casein in the refining of beer and wine is well known and it has been suggested[1] that the clarifying action in the present case is due to the albumin and casein present in the seeds. The mechanism of the clarification has been studied in this laboratory.

Experimental.

It was found that the seeds could be used in the investigation most conveniently in the form of a paste. They were first soaked in water for ten minutes and their outer coat rubbed off on a sand-stone. The seeds were then wiped dry with a filter paper and weighed (correct to a centigram). The paste used in each experiment was freshly made by rubbing the seeds on a sand-stone with small quantities of water.

The presence of alkaloids as cations in the paste was shown by the following experiment. The paste (25 c.c. of 2·5 per cent. concentration) was subjected to electrodialysis in an alundum thimble kept in an outer vessel containing distilled water. An e.m.f. of 150 volts was employed. The electrode in the thimble was of platinum while the other electrode, which surrounded the thimble, was of nickel gauze. The water in the outer vessel gave tests for alkaloid only when the gauze electrode was made the cathode.

The paste was almost neutral. Its pH as determined colorimetrically was 6·9 for a paste of 0·5 per cent. concentration and 6·8 for 5·0 per cent. strength. The protective action of the paste on a red gold sol was determined by Zsigmondy's method and found to be 2–2·5 times as high as that of egg albumin.

The clarifying action of the paste was studied on an aqueous suspension of kaolin (about 1 per cent.), from which the coarser particles had been removed by sedimentation for a few days.

In a series of tall, graduated tubes of equal height (eudiometer tubes of 50 c.c. capacity) quantities of seed-paste varying from 0·01 c.c. to 25·0 c.c. were placed, and the kaolin suspension added to bring up the total volume to 50 c.c. The mixture was well stirred and allowed to stand for 3–4 days. At intervals the volume of clear water in each of the tubes was read off. In all the tubes, the boundary between the clear and the turbid water was noticed to be quite sharp. The results obtained are given in Table A and shown graphically in Figs. 1 and 2. The values indicate that for

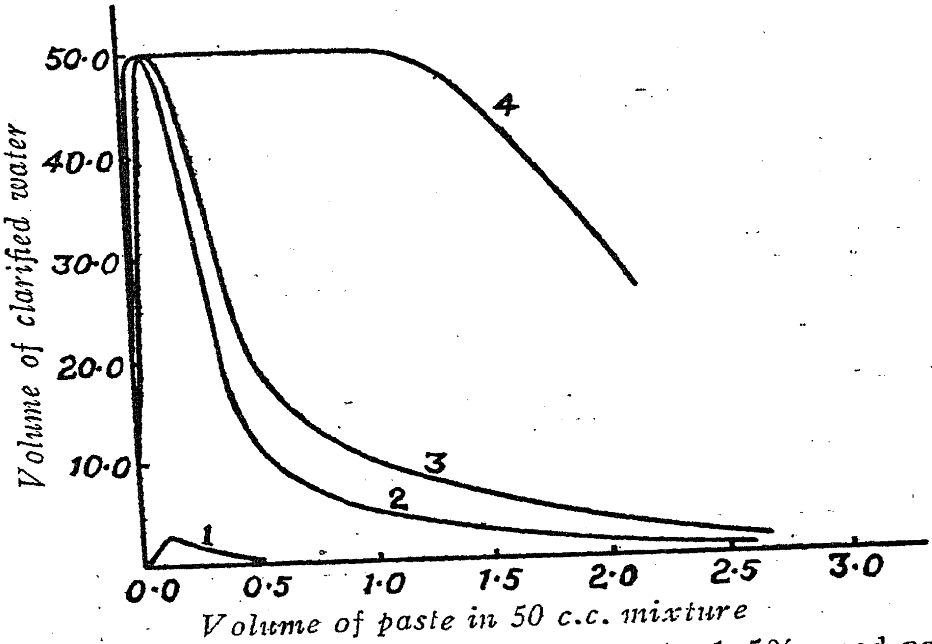

FIG. 1. Clarification of Kaolin suspension by 1·5% seed-paste. Curves 1 = 3 hours ; 2 = 22 hours ; 3 = 46 ; 4 = 96 hours.

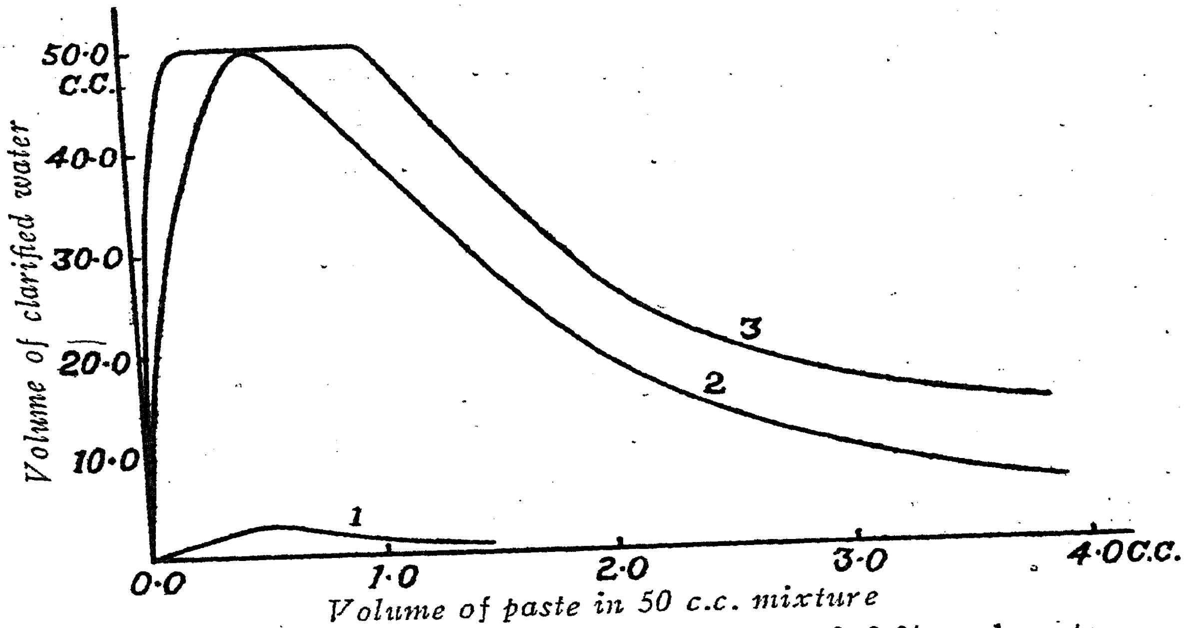

FIG. 2. Clarification of Kaolin suspension by 0·3 % seed-paste. Curves 1 = 3 hours ; 2 = 22 hours ; 3 = 46 hours.

TABLE A.

Clarification of kaolin suspension in water by paste from Strychnos potatorum *seeds.*

Volume of paste in 50 c.c. mixture	1·5 % paste				0·3 % paste			0·06 % paste		
	Volume of clear water after				Volume of clear water after			Volume of clear water after		
	3 hours	22 hours	64 hours	96 hours	3 hours	22 hours	46 hours	3 hours	22 hours	46 hours
0·00 c.c. ..	0·2 c.c.	1·0 c.c.	2·0 c.c.	4·0 c.c.	0·2 c.c.	1·0 c.c.	2·0 c.c.	0·2 c.c.	1·0 c.c.	2·0 c.c.
0·01 ..	0·5	25	40	40	0·2	1·5	8·0	..	..	..
0·05 ..	2·0	35	50	50	0·5	25	40	0·2	10	20
0·10 ..	2·5	50	50	50	1·0	25	50	0·5	15	35
0·50 ..	0·2	12	20	50	2·5	50	50	1·0	30	40
1·00 ..	0·1	5·0	10	40	1·5	40	50	1·5	30	50
2·5 ..	0·1	1·0	2·0	25	0·2	5·0	22	2·5	50	50
5·0 ..	0·0	0·5	1·0	20	0·1	5·0	12	1·5	40	50
10·0 ..	0·0	0·0	0·0	5·0	0·1	0·5	1·5	0·8	15	25
25·0 ..	0·0	0·0	0·0	0·0	0·0	0·1	0·2	0·1	7·0	15

clarification there is an optimum in the concentration (0·003 per cent.) of the seed material. About 24 hours were required for clarification, when the proper concentration was employed. It is of interest to note that the general practice in India is to allow nearly a day for water purification by this process. Excess of the paste actually stabilises the suspension.

To elucidate the mechanism of clarification, experiments were carried out on the kaolin suspension using in place of the seed-paste, the following :

(*a*) solutions of strychnine hydrochloride ;
(*b*) solutions of egg albumin and
(*c*) mixtures of (*a*) and (*b*) in varying proportions.

With strychnine hydrochloride (*a*) (Table B and Fig. 3) it was noticed that the volume of clear water showed a continuous increase with an increase

TABLE B.

Clarification of kaolin suspension in water by strychnine hydrochloride solution.

Volume of clarifying solution in 50 c.c. mixture	0·2% strychnine hydrochloride		0·4 % strychnine hydrochloride	
	Volume of clear water after		Volume of clear water after	
	23 hours	46 hours	23 hours	46 hours
0·00 c.c.	1·0 c.c.	2·0 c.c.	1·0 c.c.	2·0 c.c.
0·01	1·5	2·5	1·5	3·0
0·05	2·0	2·5	2·0	4·0
0·10	2·0	4·0	2·0	4·0
0·50	—	—	2·5	5·0
1·00	2·5	6·0	3·5	15·0
2·5	3·0	8·0	3·5	20·0
5·0	4·0	15	Complete clarification in 4 hours	
10·0	Complete clarification in 90 minutes		Complete clarification in 15 minutes	
25·0	Complete clarification in 10 minutes		Complete clarification in 5 minutes	

in the concentration of the alkaloid. The curves showed an initial small rise, then a range over which an increase in the concentration of the clarifying agent had relatively small effect and finally a third range over which the effect of concentration was very marked. With an increase in the time allowed for clarification, the second range became less pronounced.

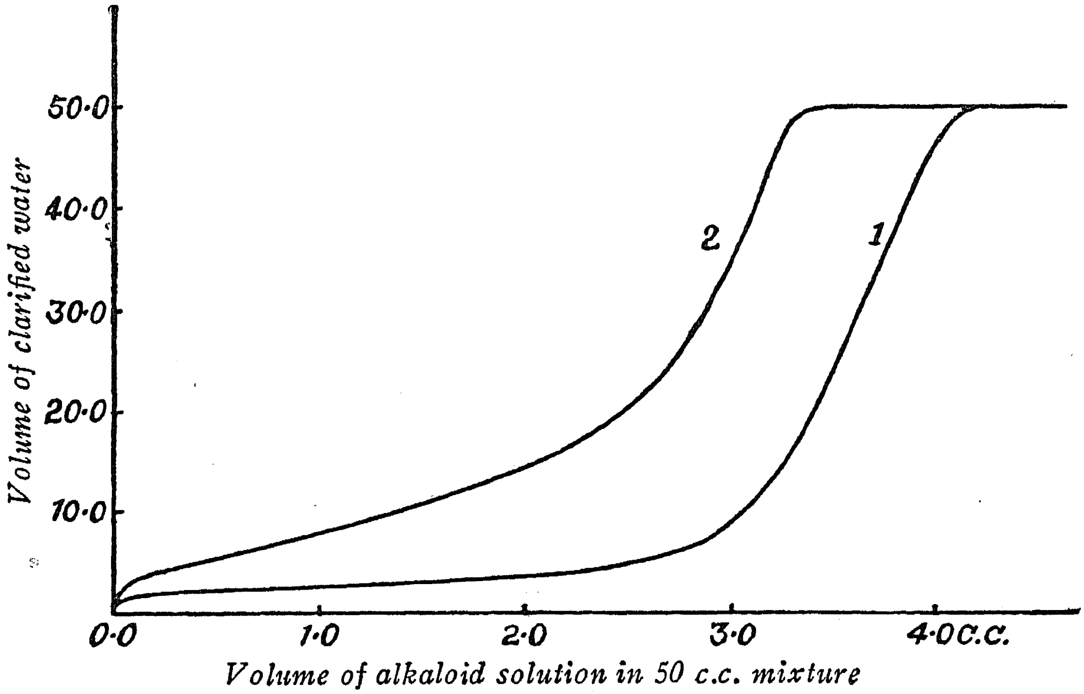

FIG. 3. Clarification of Kaolin suspension by 0·4 % Strychnine Hydrochloride solution. Curves 1 = 23 hours; 2 = 46 hours.

When egg albumin (*b*) was tried (Table C) it was found that there was no sedimentation at all but only a tendency towards greater stabilisation of the suspension.

TABLE C.

Clarification of kaolin suspension in water by 0·2 % *egg albumin solution.*

Volume of albumin solution in 50 c.c. mixture	Volume of clear water after	
	23 hours	46 hours
0·00 c.c.	1·0 c.c.	2·0 c.c.
0·01	1·5	2·5
0·05	1·5	3·0
0·10	1·5	3·0
0·50	1·5	3·0
1·0	1·5	3·0
2·5	1·5	3·0
5·0	1·5	3·0
10·0	1·0	2·5
25·0	1·0	1·5

In experiments (*c*) wherein mixtures of strychnine hydrochloride (0·2 per cent.) and egg albumin (0·1 per cent.) were employed (Table D, Fig. 4), the curve showed a steep rise, a maximum, then a fall and a final rise. The second rise was more pronounced when a longer time was allowed for clarification. On increasing the concentration of albumin (Fig. 5) the second rise disappeared, and the process of sedimentation showed a strong resemblance to that met with when the seed-paste is employed.

For comparative purposes experiments were carried out on the sedimentation of kaolin by barium chloride, with and without the addition of egg

TABLE D.

Clarification of kaolin suspension in water by a solution of strychnine hydrochloride containing egg albumin.

Volume of clarifying solution in 50 c.c. mixture	0·2 % strychnine hydrochloride solution with 0·1 % albumin		0·2 % strychnine hydrochloride solution with 0·2 % albumin		0·2 % strychnine hydrochloride solution with 0·6 % albumin	
	Volume of clear water after		Volume of clear water after		Volume of clear water after	
	21 hours	50 hours	21 hours	50 hours	21 hours	50 hours
0·00 c.c.	1·0 c.c.	5·0 c.c.	1·2 c.c.	2·1 c.c.	1·2 c.c.	2·1 c.c.
0·01	1·5	5·0	1·4	2·5	1·4	2·5
0·05	2·0	6·0	1·5	3·0	3·5	7·5
0·10	2·0	6·0	1·5	3·0	3·0	6·0
0·50	2·0	6·0	2·0	5·0	2·8	6·0
1·00	4·0	12·0	3·0	8·0	1·5	4·0
2·5	2·5	8·0	1·5	3·0	1·5	3·5
5·0	2·5	7·0	1·0	2·0	1·0	2·8
10·0	2·0	9·0	0·2	0·5	0·2	2·0
25·0	4·0	9·0	0·0	0·0	0·0	0·5

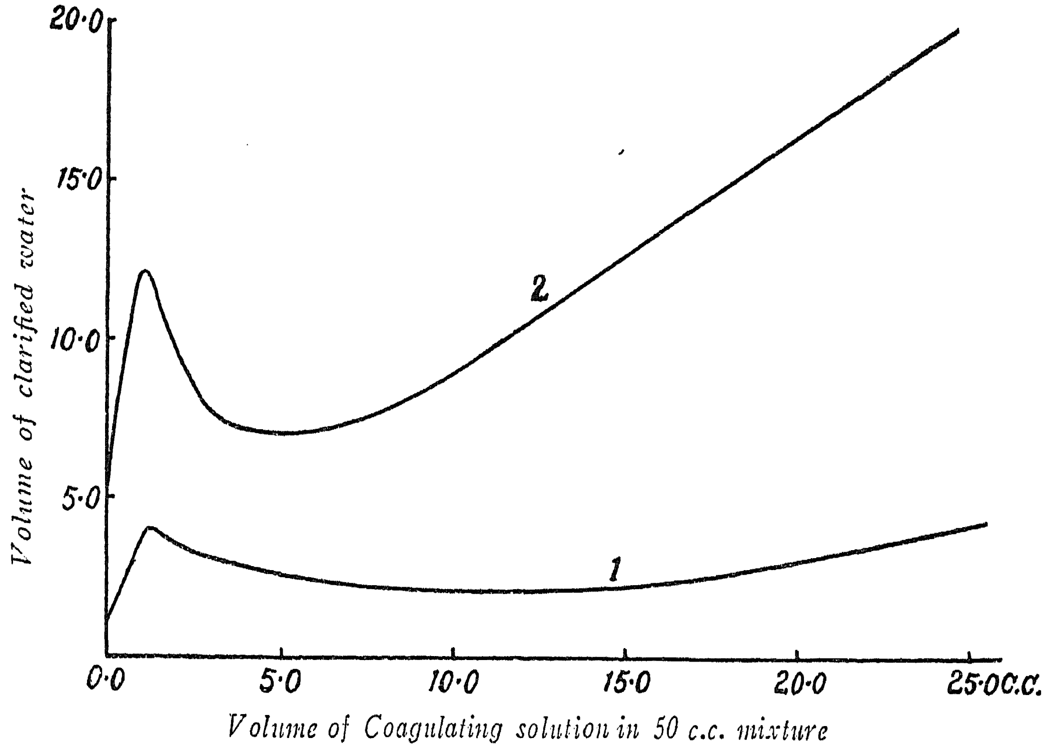

FIG. 4. Clarification of Kaolin suspension by 0·2 % Strychnine Hydrochloride solution containing 0·1 % egg albumin.

Curves 1 = 21 hours; 2 = 50 hours.

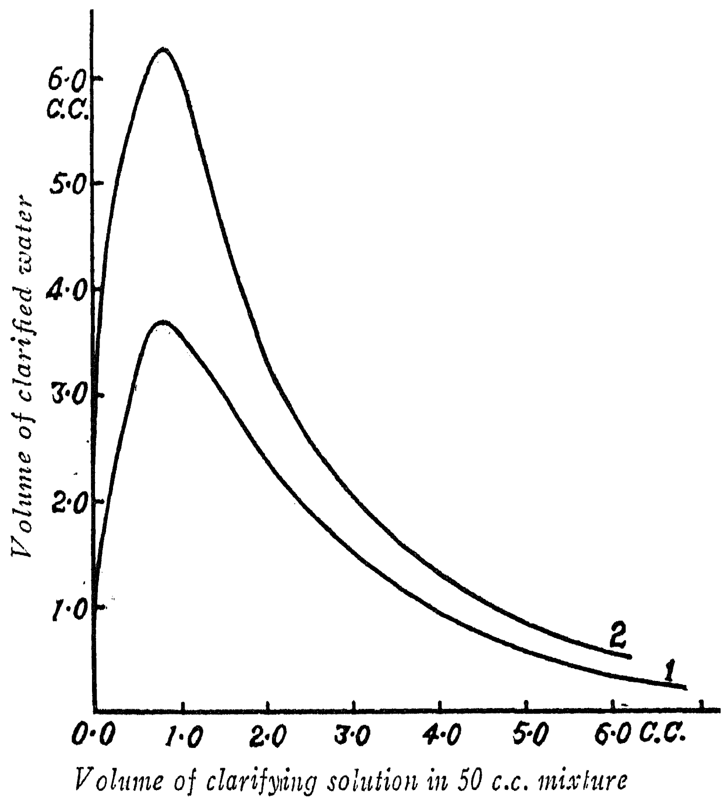

FIG. 5. Clarification of Kaolin suspension by 0·2 % Strychnine Hydrochloride solution containing 0·4 % egg albumin.

Curves 1 = 21 hours ; 2 = 50 hours.

albumin (Tables E and F, Figs. 6, 7 and 8). Barium chloride was selected because alkaloid ions resemble bivalent cations in coagulation processes.[2] The sedimentation effected by barium chloride showed a striking resemblance to that with strychnine hydrochloride (compare Figs. 3 and 6). The combined action of barium chloride and egg albumin resembled that of strychnine hydrochloride and albumin, or with the seed-paste alone (compare Figs. 1, 2 and 5 with Figs. 7 and 8). An attempt was made to study the

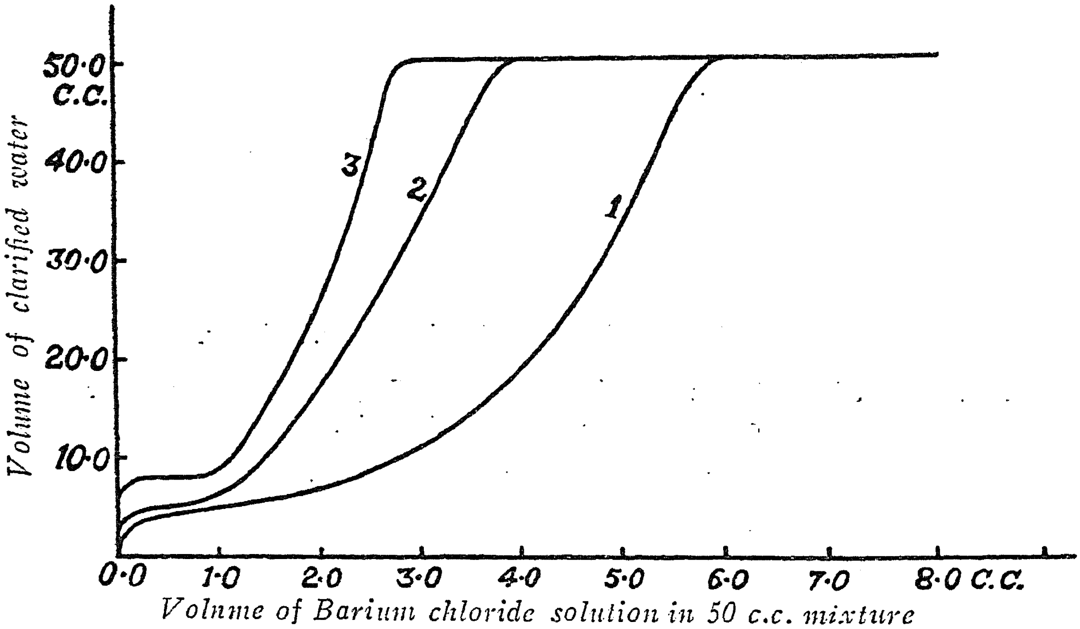

FIG. 6. Clarification of Kaolin suspension by 0·1 % Barium chloride solution.
Curves 1 = 24 hours ; 2 = 52 hours ; 3 = 96 hours.

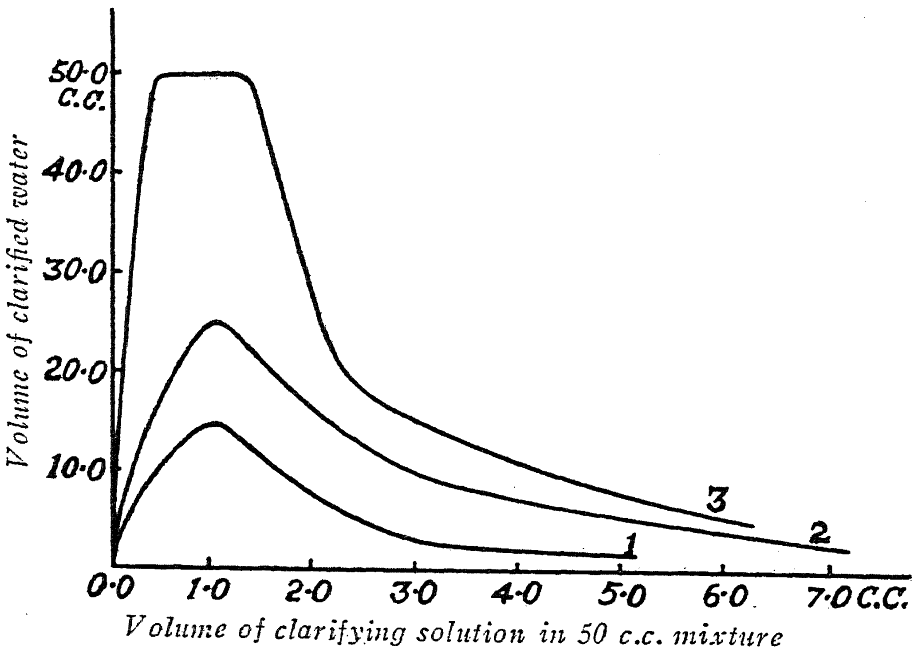

FIG. 7. Clarification of Kaolin suspension by 0·1 % Barium chloride solution containing 0·4 % egg albumin.
Curves 1 = 52 hours ; 2 = 72 hours ; 3 = 96 hours.

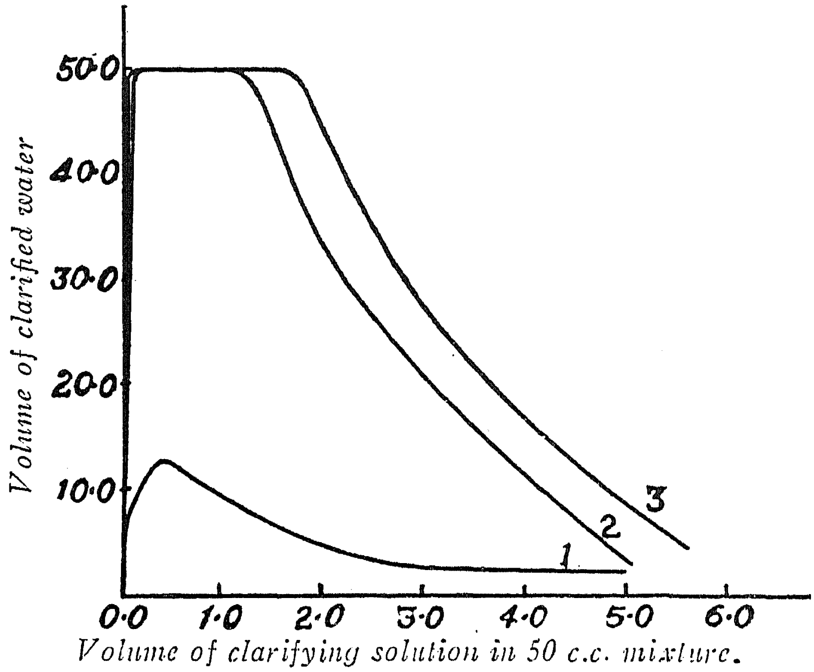

FIG. 8. Clarification of Kaolin suspension by 0·1 % Barium chloride solution containing 1·0 % egg albumin.
Curves 1 = 24 hours ; 2 = 52 hours ; 3 = 72 hours.

TABLE E.

Clarification of kaolin suspension in water by 0·1 % barium chloride solution.

Volume of barium chloride solution in 50 c.c. mixture	0·1% Barium chloride		
	Volume of clear water after		
	24 hours	52 hours	96 hours
0·00 c.c.	1·1 c.c.	2·0 c.c.	4·0 c.c.
0·01	1·5	3·0	4·5
0·05	2·5	4·0	6·5
0·10	3·0	4·0	6·0
0·50	4·0	4·5	7·0
1·00	5·0	6·0	9·0
2·5	8·0	25	40
5·0	25	50	50
10·0	Complete clarification in 12 hours		
25·0	Complete clarification in 30 minutes		

TABLE F.

Clarification of kaolin suspension in water by barium chloride solution containing egg albumin.

Volume of clarifying solution in 50 c.c. mixture	0·1% Barium chloride solution with 0·4% albumin			0·1% Barium chloride solution with 1·0% albumin		
	Volume of clear water after			Volume of clear water after		
	24 hours	52 hours	96 hours	24 hours	52 hours	96 hours
0·00c.c.	1·1 c.c.	2·0 c.c.	4·0 c.c.	1·1 c.c.	2·0 c.c.	4·0 c.c.
0·01	1·5	2·5	6·0	—	—	—
0·05	4·0	5·0	14·0	5·0	25	50
0·10	5·0	6·0	18·0	8·0	50	50
0·50	2·5	6·0	50	12·0	50	50
1·0	2·5	15	50	9·0	50	50
2·5	2·0	4·0	18	3·0	25	50
5·0	2·0	2·0	8·0	2·0	3·0	25
10·0	0·0	0·0	1·0	0·0	0·0	1.5
25·0	0·0	0·5	0·5	0·0	0·0	1·0

sedimentation of kaolin with aluminium chloride in presence of albumin, but the process was complicated by the phenomenon of charge reversal.

The sedimentation of arsenious sulphide sol (0·05 per cent.) by the seed-paste was also tried. The results obtained were similar to those with kaolin (Table G). The curve showed a maximum only at an optimum concentration and not, as in the case of kaolin, over a concentration range. This was probably due to the fact that the arsenious sulphide sol was more unidisperse than the kaolin suspension.

Electrophoretic experiments showed the absence of charge reversal during the clarification. The paste carried a small negative charge. Mixtures of the paste and the arsenious sulphide sol (or kaolin) were also negatively charged at concentrations above and below the optimum.

TABLE G.

Clarification of 0·05% *arsenious sulphide sol by paste from* Strychnos potatorum *seeds.*

ne of seed-paste in 50 c.c. mixture	1·5 % seed-paste					0·3 % seed-paste				
	Volume of clear water after					Volume of clear water after				
	3 hours	24 hours	48 hours	72 hours	96 hours	3 hours	24 hours	48 hours	72 hours	96 hours
0·00 c.c. ..	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.	0·0 c.c.
0·01 ..	0·0	0·1	0·2	0·25	0·3	0·0	0·0	0·2	0·2	0·3
0·05 ..	0·0	0·2	0·3	0·4	0·5	0·0	0·2	0·3	0·4	0·8
0·10 ..	0·2	0·8	2·0	4·0	7·0	0·1	0·2	0·7	1·0	1·2
0·50 ..	0·1	0·2	0·4	0·6	0·8	0·5	1·0	1·4	1·6	2·0
1·0 ..	0·0	0·05	0·1	0·2	0·4	Complete clarification in 25 minutes				
2·5 ..	0·0	0·0	0·05	0·1	0·1	0·0	0·1	0·2	0·3	0·4
5·0 ..	0·0	0·0	0·0	0·0	0·05	0·0	0·05	0·15	0·2	0·3
10·0 ..	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·05	0·1	0·1
25·0 ..	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·05	0·1	0·1

Note :—Paste in 0·006 % concentration has maximum coagulating effect on the arsenious sulphide sol.

The method developed by Usher[3] for investigating rapid coagulation was found to be unsuitable for studying the clarification of arsenious sulphide or mastic sols as brought about by the paste.

Discussion.

The experimental results recorded in this paper indicate that clarification is due to the joint action of the colloids and the alkaloid present in the seeds. The albumin and other colloids present sensitise the suspension and the coagulation is then brought about by the alkaloid ions. The albuminous matter is not able, by itself, to clarify the water.

The seed-paste acts most efficiently when present in a certain optimum concentration. When larger quantities of paste are employed, though the concentration of the alkaloid ion increases no clarification takes place; for, the colloids present in the seed (which sensitise the suspension when present in small quantities) now actually stabilise it. This view is confirmed by the experiments with albumin in presence of strychnine hydrochloride or barium chloride.

Summary.

Clarification of muddy water by paste prepared from *Strychnos potatorum* seeds is due to the combined action of colloids and alkaloids present in the seeds. The albumin and other colloids sensitise the suspension and the coagulation is then caused by the alkaloid ions. The paste, if used in excess, fails to clarify the water. An optimum concentration of paste is therefore to be employed for efficient clarification.

Note.—Some preliminary work on this problem was carried out in this laboratory, under the direction of Prof. B. Sanjiva Rao, by K. N. Nagarkatte, I.C.S., who was obliged to terminate the investigation when he joined the Indian Civil Service.

REFERENCES.

1 Nadakarni, *The Indian Materia Medica*, 1927, 828.
2. Freundlich, H., *Colloid and Capillary Chemistry*, 1926, 420.
3. Usher, F. L., *Trans. Far. Soc.*, 1925, 31, 406.