The studies reported in this paper do not show conclusively that the colors are due to free radicals. In a few cases addition of water caused a marked decrease in the e.p.r. signal while the color became more intense; a striking example of this phenomenon is 2,6-dimethylphenol. It appears that the colors are due to a mixture of materials, some of which may be free radicals. This is supported by concentration measurements, which showed that the free radicals comprised less than 10% of the parent compound. The changes in color and e.p.r. spectrum with time provide evidence that several reactions are occurring simultaneously in the tests.

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Powdered Particle Interactions: Suspension Flocculation and Caking IV

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The incompatibility of bismuth subnitrate with tragacanth mucilage has been reported in the literature. In this study, the phenomenon is discussed in terms of the chemical reaction that occurs between reactive bismuth sites on the bismuth subnitrate particle surface and the acidic groups of the D-galacturonic acid units of the complex acid polysaccharide present in the tragacanth gum.

An aqueous suspension containing bismuth subnitrate and one of a number of polysaccharide or carbohydrate derivatives such as tragacanth will exhibit a well known incompatibility (1, 2). The substances coagulate on mixing to form soft curd-like aggregates which settle to the bottom of the container. If the compounds are introduced at higher levels of concentration, the aggregates may set to hard, intractable masses.

The phenomenon has been explained (3, 4) as the interaction of positive bismuth or bismuth ions with the negatively charged tragacanth. It has been shown that the incompatibility does not appear in the presence of phosphate, citrate, or arsenate ions (3, 5). The negatively charged phosphate ion was thought to be strongly adsorbed on the tragacanth micelle and thus provided a protective action against the adverse effect of the positively charged bismuth. Nitrate ion was thought to be similarly adsorbed, but due to a weaker protective action could not prevent the occurrence of the incompatibility.

The chemistry of bismuth subnitrate in aqueous suspension has been explored in our recent work (6). The phosphate ion was shown to be a flocculating agent for an aqueous bismuth subnitrate suspension because strong interparticle bonds were formed by chemisorption of the phosphate ions at the surface of the bismuth subnitrate particles. An interaction of this type provides a basis for the discussion of the bismuth subnitrate-tragacanth or similar suspension systems.

EXPERIMENTAL

Materials.—The same lot of a commercial grade of bismuth subnitrate N.F. was used throughout the series of tests. It was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. Two types of tragacanth U.S.P. were used in the work.[1] They will be more simply described as gum or ribbon

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[1] Tragacanth Aleppo Ribbons No. 1 and Fine Powdered Gum Tragacanth Extra Grade No. 1, supplied by S. B. Penick and Co., New York, N. Y.

tragacanth in the balance of this report. Each experiment involving tragacanth was thus run in duplicate—first with the gum tragacanth, then with the ribbon form of the suspending agent. The monobasic potassium phosphate was of reagent grade, supplied by Merck & Co., Rahway, N. J.

Settling Data.—The methods of Ward and Kammermeyer (7) were used to obtain and analyze the sedimentation data. Where necessary, the suspensions were shaken on a Birrell wrist-action shaking machine, model DD.

Suspension Formulation.—Meaningful results could be obtained from suspensions which contained 10 Gm. of bismuth subnitrate and 100 ml. of the suspending medium. The tragacanth was prepared as a stock mucilage and was introduced as required to give a final concentration of 0.1% tragacanth in the suspending medium. The monobasic potassium phosphate was also prepared as a stock solution. This contained 100×10^{-4} meq. of phosphorus per milliliter and was added as required to give the final concentration of 50×10^{-4} meq. of phosphorus per milliliter of the suspending medium. After being prepared, the suspensions were allowed to settle for 5 days before being photographed.

RESULTS

Suspension I.—The bismuth subnitrate was suspended in distilled water and the mixture shaken by hand to disperse the solid evenly. The supernatant liquid was still slightly cloudy after 5 days and the dense, compacted sediment could be resuspended only with difficulty. However, the particles resuspended as individuals in the suspension medium.

Suspension Ia.—This suspension was the same as *Suspension I*, but was placed on the shaker for 24 hours before being allowed to settle. It was visibly comparable to Suspension I in its settling characteristics and resuspended with the same difficulty.

Suspension II.—The bismuth subnitrate was suspended in phosphate solution, promptly became flocculated, and rapidly settled to a bulky sediment. The supernatant liquid was clear and colorless. At the end of 5 days, the sediment was easily resuspendable, with particles in both the flocculated and deflocculated states.

Suspension IIa.—This suspension was the same as *Suspension II*, but was placed on the shaker for 24 hours before being allowed to settle. During this period, it became partially deflocculated and was visibly comparable to Suspensions I and I*a* in its settling characteristics. However, its final sediment height was slightly greater than that of Suspensions I or I*a*, and the solids were a little more easily resuspendable.

Suspension III.—The bismuth subnitrate was suspended in water, and tragacanth was added from the stock mucilage. On mixing, the solids coagulated and settled rapidly to a coarse, lumpy sediment which bulked to a high suspension height. The supernatant liquid was cloudy and failed to clear completely during the 5 days of settling. The sediment was slightly gelatinous and could be easily resuspended in the form of large, coarse aggregates.

Suspension IIIa.—This suspension was the same as *Suspension III*, but was placed on the shaker for 24 hours before being allowed to settle. In settling, the sediment of this suspension bulked to a lesser degree than did that of Suspension III. In addition, it was seen that the sediment was more gelatinous and the shaking had acted to smooth out the suspension and to eliminate the coarse, lumpy appearance of the sediment of Suspension III. The solids were easily resuspendable in the form of small floccules.

Suspension IV.—The bismuth subnitrate was suspended in a medium containing tragacanth and phosphate ion. The order of addition was: (*a*) bismuth subnitrate, (*b*) distilled water, (*c*) phosphate ion, and (*d*) tragacanth mucilage. The solids flocculated and settled rapidly to a bulky sediment. The supernatant liquid was clear and colorless, although not so clear as when phosphate ion alone was the flocculating agent. The sediment had a marked gel structure, but this could easily be broken by shaking. The material was resuspended in the form of fine floccules.

Suspension V.—This suspension was the same as *Suspension IV*, but the order of addition was: (*a*) phosphate solution, (*b*) tragacanth mucilage, (*c*) bismuth subnitrate, and (*d*) distilled water. The solids settled rapidly leaving a cloudy supernatant liquid which failed to clear within 5 days. The sediment showed a marked gel structure; it seemed to be slightly more difficult to resuspend the material than it was to resuspend the solids of Suspensions IV. However, both sediments could be described as easily resuspendable. The floccules of the suspended material were larger than those seen with Suspension IV and settled more rapidly on standing.

Suspension Va.—This suspension was prepared in the same order as *Suspension V*, but the mucilage containing phosphate ion and tragacanth was shaken for 24 hours before the bismuth subnitrate was added. As far as could be seen, Suspensions V and V*a* were identical in their settling characteristics and in their response to resuspension. Any slight difference in their sediment heights may be explained as being due to further hydration of the tragacanth during the shaking period.

Suspension VI.—This suspension was the same as *Suspension V*, but the order of addition was: (*a*) phosphate solution, (*b*) tragacanth mucilage, and (*c*) bismuth subnitrate suspended in distilled water. Suspension VI was quite comparable to Suspensions V and V*a* in its settling characteristics, the only apparent difference being that the final sediment height was slightly greater than that of the other two suspensions.

DISCUSSION OF RESULTS

The study was begun with a suspension of 5 Gm. of bismuth subnitrate in a quantity of 1% tragacanth mucilage sufficient to give 60 ml. of final suspension. Confirming prior work (5), no apparent differences could be seen when the order of mixing was changed. Visual inspection showed that a gelatinous network of flocculated material had formed throughout the suspension. The aqueous medium could be drained from the aggregates; when these were spread on a slide and viewed under low magnification, an open network of gelatinous floccules could be seen. The suspension was prepared again, but was shaken for

24 hours before being examined. The particles were uniformly suspended in a smooth gel, both when viewed macroscopically or under low magnification.

These initial findings showed that the interaction of bismuth subnitrate and tragacanth mucilage was qualitatively similar to the chemical reaction that occurs between bismuth subnitrate and various flocculating anions (6). When the highly viscous tragacanth mucilage was added to a bismuth subnitrate suspension, the bismuth subnitrate could react to set the mixture to a coarse, granular, gelatinous network before complete mixing could be achieved. Twenty-four hours of continuous shaking served to break this network into a more homogeneous gel structure.

The above interaction seemed analogous to the reaction in which bismuth subnitrate was flocculated in a medium containing a large excess of the phosphate ion (6). It was shown that continuous shaking for a 24-hour period would reduce the size of the flocs but would not give rise to deflocculation. On the other hand, a bismuth subnitrate suspension flocculated by a solution containing a relatively low concentration of phosphate ion became deflocculated after 24 hours of shaking. The same type of phenomenon was demonstrated for the bismuth subnitrate-tragacanth mucilage suspension system. An aqueous 4% bismuth subnitrate suspension was reacted with an equal volume of 0.02% tragacanth mucilage to form a flocculated suspension. The solids settled rapidly and bulked to a relatively high suspension height at the bottom of the container. The suspension was then shaken for 24 hours and became deflocculated. After deflocculation, the particles settled slowly, as individuals, to form a dense, compact precipitate with a relatively low sediment height.

Our recent study (6) has shown that a bismuth subnitrate suspension is strongly electrolytic because hydrogen and nitrate ions are released to the suspension medium. The hydrogen ions appear in greater number than do the nitrate ions, and the bismuth subnitrate particle surface becomes correspondingly more basic as it yields this excess number of hydrogen ions to the medium. Bismuth subnitrate or its more basic forms are all quite insoluble, and do not yield trivalent bismuth ions to the suspension medium in an appreciable amount. The possible interaction of bismuth ion with tragacanth mucilage was tested by adding an acidic 0.1% bismuth nitrate solution to an equal volume of 0.1% tragacanth mucilage. There was no visible coagulation or flocculation of the tragacanth. It seems definite that the small amount of ionic bismuth present in a bismuth subnitrate suspension would not give rise to the incompatibility with tragacanth, as has been suggested (3, 4).

Tragacanth has been shown to be a mixture of the salt of a complex acid polysaccharide, a neutral polysaccharide composed principally of L-arabinose, and small amounts of a glycoside and of starch (8). The acidic character of the gum is due to the presence of units of D-galacturonic acid. The D-galacturonic acid units could act somewhat like the acid phosphates in a reaction with the bismuth subnitrate particle surfaces. The official tragacanth mucilage (9) was unsuitable for use in the current studies due to its benzoic acid content. The slightly soluble benzoic acid is also a flocculating agent for an aqueous bismuth subnitrate suspension.

A series of suspensions was prepared in accordance with the formulations described under the heading *Experimental.* Suspensions I and I*a* (see Fig. 1) do not contain a flocculating agent and can be seen to be of the same low sediment height. Thus, a freshly prepared bismuth subnitrate suspension settles to form a compact sediment, and the same end result is obtained either with or without a shaking step. Suspension II illustrates the sediment height obtained by flocculating a bismuth subnitrate suspension with phosphate ion, and Suspension II*a* shows what happens when the same suspension is prepared but shaken continuously for 24 hours before being allowed to settle. Suspensions III and III*a* present the same general picture for the tragacanth-flocculated bismuth subnitrate suspension.

Husa and Plaxco (5), in discussing Schmitz and Hill's work (3) on the bismuth subnitrate-tragacanth incompatibility, suggested that the addition of stabilizing agent to the tragacanth mucilage prior to the final mixing with bismuth subnitrate should afford a greater degree of protection than if the stabilizing agent were first added to the bismuth subnitrate in suspension and the tragacanth mucilage then mixed in to form the final suspension. Husa and Plaxco conducted a few experiments on this point; at the concentrations used, they were unable to show that differences in the order of mixing had significant effect upon the end results. With more dilute suspensions, it has been possible to show differences due to the order of mixing. Four suspensions were prepared, using the three different orders of mixing outlined in Table I.

Suspensions V and V*a* were mixed in the same order and proved to have the same sedimentation characteristics. The only difference between the two was that during its preparation Suspension V*a* was shaken for 24 hours following the mixing of the tragacanth mucilage and the phosphate solution. Hereafter, the two suspensions will be referred to as Suspension V since the shaking step had no apparent effect upon the performance of the suspension formulation.

Suspension IV gave the bulkiest sediment. This seems due to the fact that the bismuth subnitrate

TABLE I.—SUSPENSION NUMBERS WITH ORDERS OF MIXING

Suspension No.	Order of Mixing 1	2	3	4
IV	Bismuth subnitrate	Distilled water	Phosphate solution	Tragacanth mucilage
V and V*a*	Tragacanth mucilage	Phosphate solution	Bismuth subnitrate	Distilled water
VI	Bismuth subnitrate	Distilled water	A mixture of the phosphate solution and tragacanth mucilage	

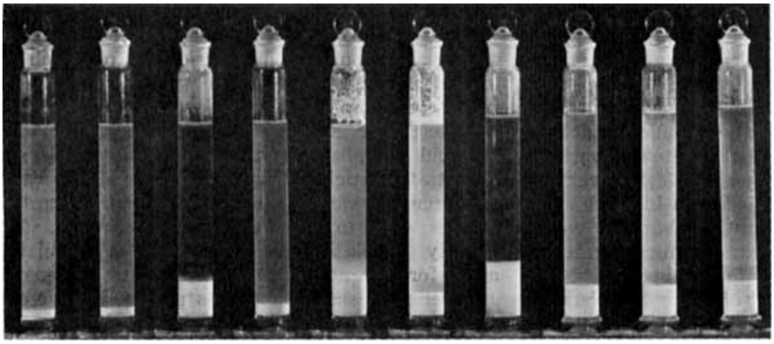

Fig. 1. — Suspensions prepared from the gum tragacanth mucilage.

Suspension No.	I	Ia	II	IIa	III	IIIa	IV	V	Va	VI
Suspension Ht. (as per cent of total suspension volume)	4.5%	4.5%	19.0%	6.0%	20.5%	12.0%	26.0%	15.0%	15.0%	17.5%

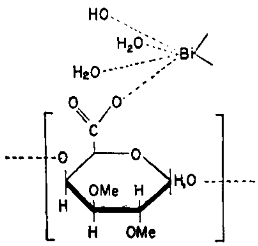

Fig. 2. — Chemical bond between acidic groups of hydrated tragacanth and the hydrated, charged surfaces of the bismuth subnitrate particles.

was first flocculated by the phosphate ion. The flocculated sediment could then be given added volume by the bulking action of the tragacanth without the formation of bonds between the bismuth subnitrate and the tragacanth. The comparable clarity of the supernatant liquids of Suspensions II and IV indicates this to be a proper explanation. In Suspension V, the bismuth subnitrate was placed into a medium containing both phosphate ion and tragacanth. Thus, it could interact with either material, forming bonds with the phosphate ions or with the D-galacturonate units of the tragacanth polysaccharides. It is of interest that the final sediment height of Suspension V was smaller than that of Suspension III, which had been flocculated by tragacanth alone. Two explanations may be offered. First, the gelatinous curd-like aggregates of the tragacanth-flocculated suspension left many open spaces in the sedimented material. These added to the height of the sediment of Suspension III. Second, in Suspension V the dry bismuth subnitrate could interact and become trapped within the floccules before becoming evenly dispersed throughout the suspension. In Suspension VI, some of this lost height was recovered by dispersing the bismuth subnitrate particles more evenly before allowing them to react with the mixture of the phosphate solution and the tragacanth mucilage.

A portion of the experiments were run in duplicate, first with the gum tragacanth and then with ribbon tragacanth. The photographs of Fig. 1 show suspensions prepared from the gum tragacanth mucilage. The ribbon tragacanth series could have been shown and described in the same terms, except that the ribbon tragacanth generally produced bulkier sediments than did the gum tragacanth mucilage.

The results obtained have been explained as being due to the chemical reaction that occurs between acidic groups of hydrated tragacanth and the hydrated, charged surfaces of the bismuth subnitrate particles. The reaction is analogous to that which occurs between phosphate ions and bismuth subnitrate (6). The chemical bond may thus be drawn as shown in Fig. 2.

SUMMARY

The incompatibility that is seen when an aqueous suspension of bismuth subnitrate is mixed with a tragacanth mucilage has been touched upon in many places in the pharmaceutical literature. Most authors have been satisfied to report the phenomenon without discussing the basic reasons for the incompatibility. A few investigators have worked on this aspect of the problem, and their conclusions may be found in the references that have been cited.

In this report, a new interpretation has been made concerning the nature of the incompatibility. The interaction is analogous to the chemical reaction that gives rise to the flocculation of a bismuth subnitrate suspension in the presence of phosphate ion. In the bismuth subnitrate-tragacanth suspension system, the chemical interaction occurs between reactive sites on the bismuth subnitrate particle surfaces and the acidic groups of the D-galacturonic acid units of the complex polysaccharides present in the tragacanth.

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