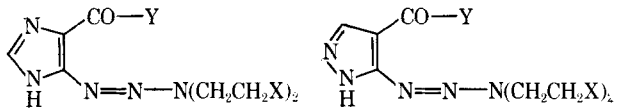

I: Y = $NH_2$; X = Cl
II: Y = $NH_2$; X = F
III: Y = $OCH_3$; X = F
IV: Y = $NH_2$; X = F
V: Y = $OC_2H_5$; X = F

The data in Table I show that the two bis(2-fluoroethyl)triazenoimidazoles (II and III) increased the lifespan of leukemic mice by 50–70% at tolerated doses. By way of comparison, the doses of NSC-82196 reported (1, 2) to be most effective are 300–625 mg./kg. for single-dose treatment and 50–100 mg./kg./day for daily treatment. The data appear to justify the following conclusions: (*a*) the bis(2-fluoroethyl)triazenes are more toxic than is NSC-82196; (*b*) at doses tolerated by the host animals, II and III are less effective than NSC-82196; and (*c*) in the standard L-1210 test system, the increases in lifespan caused by II and III are comparable to those produced by the corresponding dimethyltriazenes of the amide (NSC-45388) (8) and methyl ester (NSC-87982) (5) series.

Both the v-triazole (9) and the pyrazole (10) analogs of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388, DIC) cause significant increases in the lifespan of mice bearing L-1210, and other triazenopyrazole amides and esters have likewise demonstrated activity against L-1210 (7). However, in tests conducted in accordance with the protocols of the Cancer Chemotherapy National Service Center, amide IV displayed minimal activity, whereas the ethyl ester did not significantly increase survival time as a result of either the single-dose or the daily therapeutic regimens. Again, both are more toxic than NSC-82196.

Amides II and IV, like NSC-82196, undergo a change in aqueous solutions to ionic transformation products (11, 12), but the bis(2-fluoroethyl)triazeno derivatives are considerably more stable than NSC-82196. Esters III and V likewise undergo a change, presumed to be the same type. Obviously, the greater toxicity of II–V, in comparison with NSC-82196, may be due to replacement of chloro groups by fluoro groups. It is also conceivable that the lower toxicity of NSC-82196 results in part from its instability. If this is true, the instability may be advantageous.


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## Aggregation Mechanisms in Pharmaceutical Suspensions


**Keyphrases** ☐ Suspensions, pharmaceutical—aggregation mechanism ☐ Flocculation, coagulation—suspensions


*Sir:*

The method of prevention of impaction and caking in pharmaceutical suspensions by controlled flocculation is usually credited to Haines and Martin (1). The work of these authors is, however, sometimes quoted in review articles (2, 3) without reference to the important criticisms subsequently published by Wilson and Ecanow (4) and Ecanow *et al.* (5). We have endorsed (6) some of these criticisms, but have suggested that several generalizations proposed by Ecanow and his coworkers were based on inadequately controlled experiments. The purpose of this communication is to clarify some aspects of suspension theory recently commented upon by Ecanow *et al.* (7), since this area is of considerable importance to the pharmaceutical formulator.

We are grateful to Ecanow *et al.* (7) for amplifying some points in their earlier paper (5), since we had previously found that the almost complete absence of experimental data, such as particle size of the drug and concentration of electrolyte, made an adequate appraisal impossible. Despite the recent criticisms of these authors, we see no reason to retract from our claim that Figure 1 in *Reference 6* demonstrates differences between coagulation and flocculation. Ecanow and coworkers appear to have forgotten that suspensions of drugs in anionic surfactants (1, 8) and cationic and nonionic surfactants (9) in the absence of electrolyte cake on storage. The control suspension described in the uppermost curve of Figure 1 in *Reference 6* caked after ultimate sedimentation. However, we do not consider it semantically or scientifically helpful to refer to this process as coagulation for the following reason. If the particles were slightly smaller, they would remain in permanent colloidal suspension due to Brownian motion

because of their high mutual repulsion. This is the *exact opposite* of coagulation in true colloids. The reason why the particles come together at the base of the container is that they sediment individually and roll over one another until they can go no farther. The repulsion only extends a short distance from the particle surface and is not sufficient to keep them far apart. We prefer to call this process "impaction," thus preserving what Chwala (10) called the "sedimentation paradox." The control suspension of Ecanow *et al.* (7) showed no sedimentation after 7 days due to the presence of glycerol. We would suggest, however, that if it were allowed to sediment, it would impact as did similar suspensions recently described (11). This is another reason why we feel that the inclusion of thickening agents in this type of study may mask important effects.

We naturally accept the statement by these authors that interactions between electrolytes and surfactants are concentration dependent and, if reference is made to our earlier paper (8), it will be found that the interaction of the ammonium salt of the same surfactant was tested under a wide range of electrolyte concentrations. Similar tests were also performed with the purer sodium salt used subsequently (6, 12). Only in much higher concentrations of electrolyte than were used did opalescence occur. We accept, of course, that there is bound to be interaction between ions of opposing charge in solution, whether they are surfactants or simple inorganic ions. Such interactions within the double layer are responsible for the reduction of $\zeta$-potential and subsequent coagulation. Yet, we maintain that this type of interaction should not be called flocculation unless a definite precipitation of the surfactant–metal complex occurs, giving a continuous physical bridge between the particles. This would be analogous to the flocculation of colloids by starch polymer molecules described by La Mer (13). We would remind Ecanow *et al.* (5) that they used the precipitation effect as the decisive criterion for coagulation or flocculation.

We would also like to answer an earlier criticism, by Wilson and Ecanow (4), of the validity of the concept of flocculation (or rather coagulation) of large particles by $\zeta$-potential reduction and van der Waals' attraction. They refer to the statement by Kruyt (14) of the possibility of long-range London–van der Waals' forces between particles of 2–5 $\mu$m. in diameter and the statement that such flocculation has not been experimentally verified.[1] Kruyt was referring to *secondary minimum* flocculation. This is only possible with intermediate concentrations of electrolyte, and we have shown (12, 15) that aggregation in the secondary minimum is unlikely to be the explanation of our results. We have produced calculated energy-of-interaction curves to show that coagulation is more likely in the primary minimum. The depth of this minimum is restricted by the film of surfactant at the solid–liquid interface and makes the suspension still readily redispersible. This is because particle–particle attraction is strong enough to maintain the open structure of the coagulum, although, as Ecanow *et al.* (7) pointed out, such structures can be broken down by centrifuging.

The results of the work by Schenkel and Kitchener (16) on the coagulation of 10-$\mu$m. polymer particles show that, at the highest concentrations of electrolyte, rapid coagulation occurred, forming an open structure. In this case the particles seized on contact, since there was no film of surfactant at the interface.

We suggest that secondary minimum coagulation may be the cause of the effect noted by Ecanow *et al.* with the monovalent ions. Such coagulation would produce very loosely bound aggregates which would settle out more rapidly, but interparticulate forces might not be strong enough to maintain the open structure under the accumulating weight of the growing sediment. The wetting action of the surfactant would still be maintained and would assist the particles to slide over one another.

Calculations and carefully controlled measurements, similar to those previously published (12), may substantiate this point.

We would also like to question the distinction which Wilson and Ecanow (4) and Ecanow *et al.* (7) have made between hydrophobic and hydrophilic particles. The drug particles under consideration are fundamentally hydrophobic and, for this reason, need the presence of an adsorbed layer of amphophilic surfactant to form suspensions. It is important to realize that the stabilizing effect of surfactant films only extends a comparatively short distance from the particle surface (17). For this reason, the diagrams published by Ecanow *et al.* (18) are so far from true scale as to be misleading.

It is accepted that, in suspensions of pharmaceuticals, Brownian motion is less important, but it is not, as Ecanow *et al.* (5) state, negligible. We have calculated (6, 11) that for an aqueous suspension of drug particles, Brownian motion was a greater source of displacement than sedimentation up to a diameter of 2 $\mu$m. For sizes above this value, differential sedimentation rates in polydisperse systems will also cause particle collisions and coagulation. Since, however, glycerol will slow down both Brownian motion and sedimentation, we still consider its use likely to complicate experiments designed to differentiate between aggregation mechanisms. We have also shown (9) that, in some systems, glycerol itself can cause aggregation. We have studied aggregation phenomena in model paracolloidal systems, using a Coulter counter and a digital computer (19–21). It is apparent that much of the theory developed for colloidal systems may, with certain modifications, be applied to dispersions of particles in the 2–10-$\mu$m. regions, this being a size range of peculiar importance to the pharmaceutical formulator. Our results indicate that there is no abrupt particle-size boundary between colloidal and paracolloidal systems but rather a gradual change of properties exists. With regard to the question of period of induction, it is important to stress that coagulation rate is number concentration dependent. Although a suspension may have a small proportion by weight of particles in the region where Brownian motion is important, this can be a significant proportion by number.

We have provided further evidence (12) that coagulation in our suspensions is qualitatively analogous to

[1] Experimental verification of secondary minimum flocculation has been published by Schenkel and Kitchener (16).

that in a lyophobic colloid.

We found that similar coagulation curves could be produced with sodium, calcium, and aluminum salts and that the only real difference was in the position of the vertical region of the sigmoid curve (12). The effect of valency was approximately as would be predicted by the Schulze-Hardy rule. The suspensions coagulated with the calcium chloride were particularly interesting since, with the highest concentration of electrolyte used, a further step in the sedimentation height curve was observed. Compatibility tests between the electrolyte and surfactant showed that, at this concentration, slight precipitation occurred. We interpret this as being perhaps the point of transition between coagulation and flocculation.

Ecanow *et al.* (5, 7, 18) appear to argue that since coagulation and flocculation must produce qualitatively different results and that we merely found quantitative differences, we could not be studying two different phenomena. We find it difficult to answer such circular reasoning. We would still maintain, on the basis of carefully controlled experiments whose results are compared with fundamental theory, that both coagulation and flocculation can produce suspensions that remain free from caking on storage.


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# Effect of Macromolecules on Aqueous Solubility of Cholesterol


**Keyphrases** ☐ Cholesterol, cholesterol-26-$^{14}$C solubility—macromolecule effects ☐ Macromolecular substances—cholesterol solubility effect ☐ Pectin, acacia, dextrans effect—cholesterol solubility ☐ Scintillometry—analysis


*Sir:*

Intravenous administration of dextran solution has been suggested for treatment of hypercholesterolemia and atherosclerosis (1), and numerous investigations have been carried out to determine the effect of dextran on experimental hypercholesterolemia (2–6). Oral administrations of pectin and acacia have been studied for their hypocholesterolemic effects (7–14). The cited reports contain various and conflicting statements concerning the *in vivo* effect, value, and mechanisms of action of macromolecular substances for lowering serum cholesterol. However, there have been no reports of studies conducted to determine the *in vitro* effects of these carbohydrate macromolecules on the solubilization of cholesterol. It was, therefore, considered desirable to study the effect of these macromolecules on the aqueous solubility of cholesterol.

Cholesterol concentrations were determined using a radioactive technique suitable for very low amounts of cholesterol in water. A 10.0-ml. volume of a benzene stock solution, containing 10 mcg. of cholesterol and 0.1 $\mu$c. of cholesterol-26-$^{14}$C/ml., was transferred to a 125-ml. iodine flask, and the solvent was evaporated under a mild stream of nitrogen with constant shaking. A 50-ml. volume of the aqueous solution of the particular macromolecular substance was added to the flask. The dissolution studies were carried out at 30 ± 0.5°, and the test solutions were agitated using magnetic stirrers. At predetermined intervals of time, samples were withdrawn from the system and filtered immediately through a Millipore filtration assembly containing 0.45-$\mu$ filter paper. An accurately measured 0.20-ml. volume of the particle-free filtrate was pipeted into a liquid-scintillation vial. To this was added 15 ml. of dioxane–naphthalene phosphor (15), and the vials were shaken for 30 sec. to ensure thorough mixing. Duplicate samples, along with appropriate standards and blanks, were counted directly using a liquid-scintillation system.[1] The counts per minute involved after 12 hr. were between 150 and 415.

The data from the solubility studies are illustrated in Fig. 1. Each point on the solubility curves represents an average of at least eight determinations. Pectin (0.5%) and acacia (0.5%) solutions significantly increased the aqueous solubility of cholesterol, while slight increases were observed for the solubility of cholesterol in dextran solutions. The results for both high and low molecular weight dextrans[2] were similar.

[1] Unilux II, Nuclear Chicago.
[2] Dextran T70 and T40, Pharmacia Laboratories.