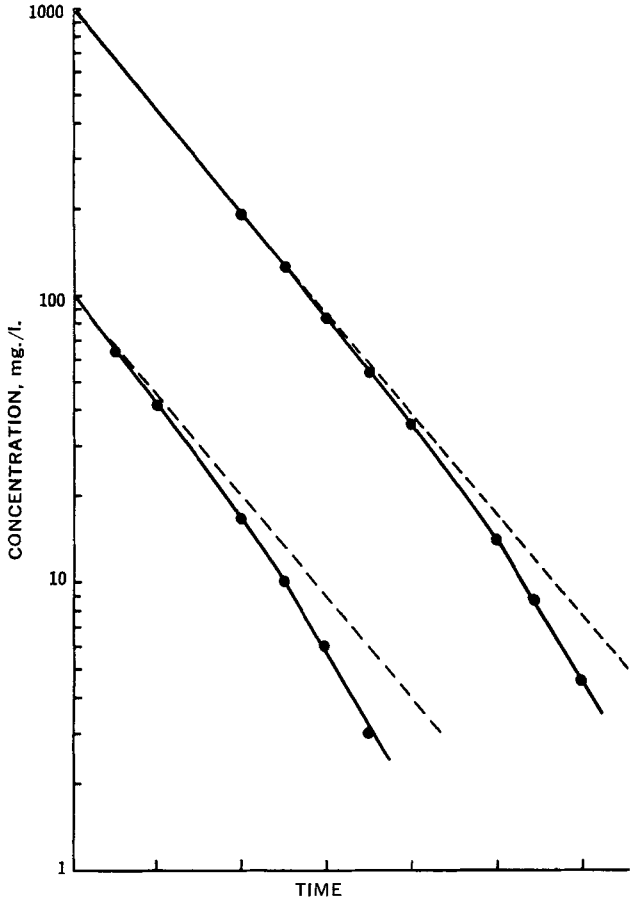

Figure 2—*Hypothetical plasma concentration data* (●) *obtained after intravenous injection of two different doses of a drug which is eliminated by apparent first-order kinetics, if the blank correction is 3 mg./l. too large. The dashed lines represent the correct curve. Note the apparent parallelism of the straight lines fitted to the last three points of each set of data.*

and variability of the blank, relative to the lower range of drug concentrations encountered in the investigation, must be carefully considered in the pharmacokinetic analysis of the data. It is recommended that apparent dose-dependent changes in elimination-rate constants, as shown in Fig. 1, be tested statistically for lack of parallelism of the respective log concentration *versus* time curves *in the same concentration range.* Where plasma concentration data show the pattern presented in Fig. 2, it is best to focus attention on the plasma concentration and/or urinary excretion pattern of the metabolite that is presumed to be subject to capacity-limited formation.

An underestimation of blank values, resulting in higher than correct drug concentration data, has exactly the opposite effects as those described here. Apparent first-order elimination-rate constants may be mistakenly assumed to increase with increasing dose [a type of kinetics that can actually occur due to dose-dependent distributional effects (3)], and a decrease in the slope of log drug concentration *versus* time curves with decreasing concentration might be treated as a linear multicompartment model or be interpreted as suggesting saturation of a renal tubular reabsorption process [a type of kinetics that can, in fact, occur (4)]. Thus, one must be concerned not only with the specificity and sensitivity of an analytical method but also with the possibility of systematic errors in the blank correction.

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Flocculation Theory and Polysorbate 80–Sulfaguanidine Suspensions

Keyphrases □ Flocculation theory—sulfaguanidine–polysorbate 80 suspension □ Sedimentation height—sulfaguanidine–polysorbate 80 suspension

Sir:

Flocculation has been defined as an open network structure formed by aggregated suspension particles (1). Three possible mechanisms by which such a structure can occur are: (*a*) aggregation in the secondary minimum which can theoretically result when the forces of attraction exceed the forces of repulsion (2, 3); (*b*) adsorption bridging—the aggregation of particles whose surface sites are occupied by segments of extended macromolecules; the extended molecules act as bridges between particles (4); and (*c*) chemical bridging—the aggregation by chemical reaction between adsorbed ions extending from the particle surface and media precipitation ions (5, 6).

In a study of the aggregation of a sulfaguanidine suspension with particles wetted by polysorbate 80, it was reported that the addition of increasing amounts of aluminum chloride produced a "flocculated system" which showed a steady increase in sedimentation height (7). A maximum volume was reached, and further additions of salt produced no change in sedimentation height.

Aluminum chloride at the concentrations used in the report could not react with the nonionic surfactant in a manner similar to those interactions that cause flocculation by chemical bridging.

Polysorbate 80 has never, in our experience, shown the characteristics exhibited by macromolecules that produce floccules in suspensions; therefore, it seemed

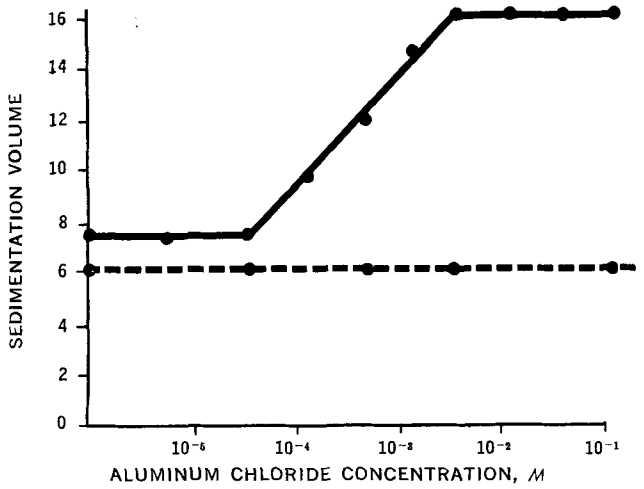

Figure 1—*Aggregation of sulfaguanidine in a suspension containing polysorbate 80 and aluminum chloride. Key:* ●—●, *from Jones* et al. (7); *and* ●- - -●, *this report.*

doubtful that in this experiment aggregation by adsorption bridging would occur.

We do not believe that the theory of long-range forces of attraction, classically believed to be responsible for aggregation in the secondary minimum, applies to the aggregation of hydrophilic particles (5). Since the sulfaguanidine covered with surfactant is a hydrophilic system, no flocculation will occur by mechanism *a*.

What we believed likely to happen is that the dispersed sulfa particles settle as individual entities and/or aggregate by surfactant–water film to surfactant–water film interactions to settle as close packed coagula (1). In either or both cases, the final height could not vary in the manner described in the report.

Since the properties of the system used did not appear to us to be capable of producing a flocculated structure on the basis of any of the three mechanisms enumerated, we studied these same systems for clarification.

Sulfaguanidine NF suspensions in water with polysorbate 80 and aluminum chloride were prepared in the exact manner described in the report (7), with the exception that mixing was done by a magnetic stirrer. The results of our experiments were always a sedimentation to a small volume (Fig. 1). The height did not change over the range of aluminum chloride concentrations shown. The suspensions sedimented in about 3 hr. and the final (*Hu*/*Ho*) was measured after 24 hr. The experimental results reported here indicate that the particles are either in the dispersed state and/or the coagulated state (1).

The system reported to be flocculated, *i.e.*, to increase to approximately twice its minimum (*Hu*/*Ho*) height was found in this laboratory to give only a contant height under all conditions stated in the report. These results support our *a priori* assumption that flocculation cannot take place in this system.

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Carboxyl Protection Using Salt Formation for the Synthesis of Linear Sequential Polypeptides: Synthesis of Poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C Ethyl Ester

Keyphrases □ Polypeptides, linear sequential—synthesis □ Poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester— synthesis □ Carboxyl protection—peptide synthesis □ Immunochemical properties—linear sequential polypeptide

Sir:

The least elaborate approach to carboxyl protection in peptide synthesis is the use of salt formation with such bases as triethylamine, tributylamine, or dicyclohexylamine. This method of protection seems to work best when an activated ester of an *N*-protected amino acid is used for the coupling reaction to amino acids and peptides which were carboxyl protected by salt formation (1, 2).

We have extended this method of protection to the synthesis of high molecular weight linear polypeptides. This is illustrated by a new synthesis of the antigenic polymer poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester (Scheme I).

The previously reported tetrapeptide (3), *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine methyl ester (I), was saponified with 1 equivalent of *N* NaOH to yield the tetrapeptide free acid, *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine (II), m.p. 159–160°, $[\alpha]_D^{25}$ − 10.3° (c 4.23 in dimethylformamide).

Anal.—Calcd. for $C_{35}H_{48}N_4O_{10}$: C, 61.4; H, 7.1; N, 8.2. Found: C, 61.5; H, 7.15; N, 8.1.

Coupling II with pentachlorophenol, using dicyclohexylcarbodiimide, yielded the tetrapeptide activated ester, *N*-carbobenzoxy-*O*-*tert*-butyl-L-tyrosyl-γ-*tert*-butyl-L-glutamyl-L-alanylglycine pentachlorophenyl ester (III), m.p. 185°, $[\alpha]_D^{28}$ − 17.0° (c 1.06 in dimethylformamide).

Anal.—Calcd. for $C_{41}H_{47}Cl_5N_4O_{10}$: C, 52.8; H, 5.1; N, 6.0. Found: C, 52.6; H, 4.9; N, 6.0.