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Neutralization of Aluminum Hydroxide Dried Gel

Citrate and Tartrate Inhibition

By MILO GIBALDI and DANIEL MUFSON

Studies were conducted on the neutralization rate of aluminum hydroxide dried gel (AHDG) with HCl as a function of rate of agitation and amount of AHDG. On the basis of these studies it is suggested that the AHDG–HCl neutralization reaction is chemically controlled. Sodium citrate and tartrate were tested as inhibitors of AHDG. Their effect on the neutralization rate was investigated by neutralization, sedimentation, and complexation studies. It was concluded that a dual mechanism is operative to produce inhibition; the AHDG reacts with the salt to produce protons, and this effect, coupled with flocculation, causes a retardation in the rate of change of pH with time.

DESAI *et al.* (1), in 1963, employing the "buffering capacity" procedure, found extensive inhibition of the antacid activity of a large number of commercial antacid products when polypeptides were added to artificial gastric juice. The polypeptides had no effect on the time required to terminate the procedure, but greatly reduced the maximum pH attained. Products containing AHDG were most sensitive to this inhibitory effect.

Gibaldi and co-workers (2) found that the effect of polypeptides on "buffering capacity" could be ascribed to an inhibitory influence on the rate of neutralization. They, therefore, proposed that neutralization rate studies would be as indicative of the activity of a given antacid under different experimental conditions as the more elaborate "buffering capacity" experiments. These workers found that the neutralization rate is strongly dependent on the amount of aluminum hydroxide dried gel (AHDG) employed as well as the amount of inhibiting agent. Inhibition of activity was noted with various proteins, polypeptides, and amino acids. In addition, inhibition was found in the presence of acetate and citrate. Neither of these materials had a perceptible effect on the initial pH of the test solution or on the equilibrium pH. Two possible explanations for the observed inhibition effects, *viz.*, the formation of an *insoluble* AHDG–inhibitor complex and the adsorption of the inhibitor on the surface of the dispersed AHDG particles, were ruled out by the results of their investigations. The authors suggested the formation of a *soluble* complex between AHDG and the inhibitors tested.

In 1933, Tartar *et al.* (3) studied the influence of adsorbed ions on the dissolution of colloidal aluminum hydroxide in HCl. Aluminum hydroxide was allowed to dissolve in HCl in the presence of various salts. The solubility of aluminum hydroxide was determined at the end of 24 hr. at 25°. The data indicated that the greater the concentration of electrolyte, such as arsenate, phosphate, or sulfate, the greater the amount of aluminum hydroxide dissolved.

These authors noted that the "speed" (24-hr. solubility) of the reaction between colloidal aluminum hydoxide and 0.2 *N* hydrochloric acid is increased several-fold by the presence of electrolytes yielding anions of higher valence. It should be pointed out, however, that these workers did not clearly show that the test electrolytes increased the velocity of dissolution since only apparent equilibrium solubility was measured. They proposed that their findings were attributable to the ability of the anions of higher valence to modify the speed of the reaction by influencing the electrical potential at the solid-liquid interface. Tarter (3) also presented evidence which indicated that the change in the "speed" of the reaction was not due to an increase in the surface

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area of the colloid. He notes, in fact, that the reverse is true; phosphate, arsenate, and sulfate ions exert a flocculating effect with an accompanying decrease in surface area.

Thus, a paradoxical situation appears to exist. On the one hand, the anions increase the solubility of the AHDG which should be attended by an increase in the reaction rate. On the other hand, these anions exert a flocculating effect and thereby decrease the effective surface area of the gel. The latter effect would tend to decrease the reaction rate and is in agreement with the recent findings of Desai (1) and Gibaldi (2).

It was the purpose of the present investigation to study the neutralization reaction of AHDG in HCl and to elucidate further the nature of the inhibition of AHDG by various anions. Of interest was the determination of whether the neutralization reaction is diffusion or chemically controlled, as well as the consideration of the role of flocculation and complex formation in the inhibition mechanism.

THEORETICAL CONSIDERATIONS

Neutralization.—Heterogeneous reactions are regulated by chemical effects and by physical phenomena occurring at the interface. When the chemical reaction rate is high, the physical effects become rate-limiting. Three separate steps are necessary for a heterogeneous reaction to proceed: (*a*) the reacting species must diffuse to the interface; (*b*) the chemical reaction occurs at the interface; and (*c*) the reaction products diffuse out into the bulk solution. The slowest of these three steps becomes the rate-limiting step.

Van Name and Hill (4) separated heterogeneous reactions into three classes: (*a*) the chemical reaction proceeds much faster than the diffusion, and the observed reaction rate will be determined by the rate of diffusion; (*b*) the chemical reaction is much slower than the diffusion and is, therefore, the controlling factor; and (*c*) chemical reaction rate and diffusion rate are both of the same order of magnitude, and the observed rate is a function of both.

Rate of agitation has a significant effect on the rate of a diffusion controlled reaction. Wurster and Taylor (5), in a recent review of dissolution rate theory presented the empirical equation,

$$DR = a(AR)^b \qquad \text{(Eq. 1)}$$

relating the agitation rate (AR) and the dissolution rate (DR), where a and b are constants. The value of b for a purely diffusion controlled reaction approximates unity. Its value for a chemically controlled reaction should approach zero.

Davion (6) presented an equation which describes the dissolution of a solid by a diffusion controlled process,

$$\frac{dW}{dt} = \frac{DA}{h}(C_s - C) \qquad \text{(Eq. 2)}$$

where dW/dt is the change in weight of the dissolving solid with time, D is the diffusion coefficient of the solute, h is the thickness of the diffusion layer, A is the area of the dissolving surface, C_s is the equilibrium solubility of the compound, and C is the concentration present in solution at time t. This equation permits one to visualize the effect of agitation on the rate of dissolution. As the stirring rate is increased, the diffusion layer becomes thinner and therefore, the value of h is lowered and the dissolution rate is increased. It may also be observed that for a diffusion controlled reaction the rate of dissolution is dependent on the magnitude of the diffusion coefficient. In addition, the rate of dissolution of a diffusion controlled reaction increases linearly with an increase in concentration of reagent (7). The reaction rate for chemically dependent reactions increases in a nonlinear fashion with increasing reagent concentration and reaches a maximum.

Inhibition.—The reported observations of the effect of anions on the neutralization of aluminum hydroxide are apparently contradictory. If a given salt forms a water-soluble complex with aluminum hydroxide, as has been shown by Zolotukhin (8) for tartrate and citrate, more aluminum hydroxide will be dissolved at equilibrium. This will tend to increase the C_s term in both Eq. 2 and in the equation presented by Zdanovskii (9) for the dissolution rate of a chemically controlled process. Zdanovskii's equation may be written as:

$$\frac{dW}{dt} = \alpha A(C_s - C_y) \qquad \text{(Eq. 3)}$$

where α is the rate constant for the chemical reaction occurring at the interface, C_s is the concentration at saturation, C_y is the concentration in the bulk solution, and the other terms have the same definitions as previously presented. Since the rate of dissolution is proportional to the equilibrium solubility, it would be expected that the formation of a water-soluble complex would increase the dissolution velocity. On the other hand, if aluminum hydroxide is also flocculated by these salts, one would expect a decrease in surface area resulting in a decrease in rate. It would appear that flocculation and complexation are exerting opposite effects on the dissolution velocity.

It has been established that anions decrease neutralization rate (2, 10). This indicates either that the flocculating effect of the anions overshadows its ability to complex aluminum hydroxide, or that dissolution rate cannot be equated with neutralization rate in this system. The latter situation would exist if the hydrated aluminum ion complexed the anion with the subsequent release of protons. This phenomenon would thereby produce a buffer effect. If this were the case, the neutralization rate would not be related to dissolution rate. This type of complexation has been shown by Das and co-workers (11) for the reaction of ferric ions with citric, tartaric, and malic acids.

EXPERIMENTAL

Materials

The various experiments were conducted with aluminum hydroxide dried gel U.S.P., F-1000, lot 194, supplied by Reheis Co., Inc., Berkeley Heights,

N. J. This sample was divided into several portions, and each portion was stored at room temperature in tightly sealed glass jars. All other chemicals were reagent grade and were obtained from Fisher Scientific Co., New York, N. Y.

Methods

Neutralization Studies.—A 1.0-Gm. sample of AHDG was added to a 250-ml. beaker containing 100 ml. of 0.1 *N* HCl well stirred. The test mixture was maintained at 37 ± 1° by immersing the beaker in a constant-temperature water bath. Stirring was effected by an overhead stirrer. Unless otherwise stated, the rate of agitation was 150 r.p.m. The pH changes were recorded with respect to time, utilizing a Beckman Zeromatic pH meter. When inhibitors were used, they were first dissolved in the 0.1 *N* HCl.

Mathematical Treatment of Neutralization Data.—The results obtained from the neutralization experiments were plotted as pH *versus* time. The curves were then evaluated to yield the time of maximum rate of reaction. The maximum reaction velocity occurs at the inflection point of the pH-time curve. The evaluation procedure to obtain the time at which the inflection point occurs consisted of obtaining the second derivative of the pH-time plot and plotting this derivative [Δ (Δ pH)] *versus* time. The time at which the curve crosses the *x*-axis is the inflection time.

Flocculation Studies.—Flocculation studies designed to evaluate the changes in sedimentation rate in the presence of inhibitors were conducted as follows: a 1.0-Gm. sample of AHDG was added to 300 ml. of 0.1 *N* HCl contained in an Andreasen pipet (12). With the breather hole closed, the pipet was shaken vigorously 10 times and placed securely in a constant-temperature water bath at 37 ± 1°. A 10-ml. sample was collected every 2 min. for 10 min. A steady suction was applied until the pipet was filled. The stopcock was then closed and the sample transferred to a previously tared 150-ml. beaker. Pressure was applied to blow out the sample at high velocity. The pressure was repeated several seconds later to insure full collection. The sampling procedure must proceed rapidly. If the stopcock is not closed immediately upon collection of the 10-ml. sample the heavier particles will begin to fall out of the chamber. After withdrawal of the sample the stopcock must be opened slowly so that the liquid remaining in the sample tube can flow into the settling chamber without stirring the suspension.

The suspension in the beakers was evaporated to dryness, allowed to cool, and weighed on a Mettler balance, model H 15. Particular care was taken to prevent overdrying and charring of the organic acid. In the flocculation studies involving the use of sodium citrate and sodium tartrate, 50 ml. of a 2 *N* solution of either salt was added to a sufficient quantity of HCl to yield 300 ml. A correction factor to account for the weight of the salt was applied in each case.

Potentiometric—Complexation Studies.—Solutions containing 0.2 *N* aluminum chloride, sodium citrate, or sodium tartrate, as well as combinations of aluminum chloride and sodium citrate, and aluminum chloride and sodium tartrate were prepared in deionized water by adding 10 ml. of a given 0.2 *N* salt solution to 20 ml. of 0.2 *N* HCl. The resulting solution was titrated with 0.2 *N* NaOH. The pH was monitored with a Beckman Zeromatic pH meter. The solution was stirred during the test by a Teflon-coated magnetic stirrer. Sufficient time was allotted between each addition of alkali to ensure that equilibrium was reached.

Table I.—The Effect of Stirring Rate on the AHDG–HCl Neutralization

Stirring Rate, r.p.m.	Inflection Time, sec.
85	370
120	375
240	360

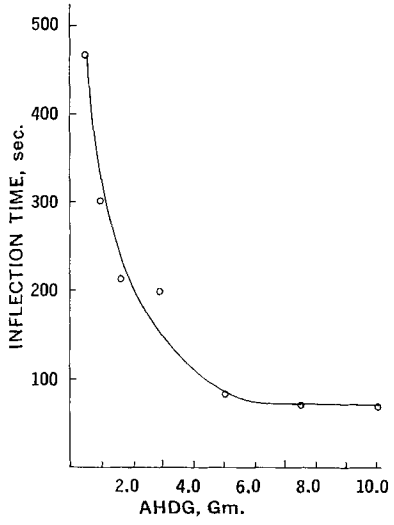

Fig. 1.—The effect of the amount of AHDG on the inflection time of the AHDG–HCl neutralization.

Table II.—The Effect of Various Anions on the Inflection Time (sec.) of the AHDG–HCl Neutralization[a]

Sodium Salt	Concn., eq./L. 10^{-3}	10^{-2}	10^{-1}
Citrate	630	1235	...
Tartrate	695	1020	>1860
Sulfate	...	460	270
Chloride	...	330	...
Acetate	...	320	...

[a] The inflection time for the control was 310 sec.

RESULTS AND DISCUSSION

Mechanism of Neutralization.—In an effort to establish whether the AHDG–HCl interaction was diffusion or chemically controlled, the effect of different agitation rates on the reaction velocity was studied. Experiments were conducted at stirring speeds ranging from 85 to 240 r.p.m. The results of this investigation are presented in Table I. Inspection of Table I indicates that the change in stirring rate had little effect on the neutralization

velocity. Therefore, it may be concluded that the value of the exponent *b* in Eq. 1 is approximately zero.

To further resolve the mechanism of the neutralization reaction, the reactant concentration was varied. AHDG was added to the reaction beaker in quantities ranging from 0.50–10.0 Gm. The results of these experiments are depicted in Fig. 1. It may be observed that neutralization rate is highly dependent upon the amount of antacid employed up to a maximum. Beyond this point (about 5 Gm.), it would appear that the neutralization rate is independent of the amount of AHDG present. It has been established (7) that in chemically controlled reactions, the reaction rate increases in a nonlinear fashion with increasing reagent concentration and reaches a maximum.

In view of the experimental results indicating that the value of exponent *b* in Eq. 1 approximates zero, and that the rate of reaction increases to a maximum with increasing AHDG concentration, it is reasonable to conclude that the reaction is predominantly chemically controlled.

Mechanism of Inhibition.—Several salts were selected to be tested as inhibitors of AHDG, and were tested at several concentrations. The results of these investigations are listed in Table II.

Examination of the data contained in Table II reveals that the order of degree of inhibition exerted by the salts follows the Schulze-Hardy rule. Their descending order of effectiveness as coagulating agents follow their inhibitory activity, *viz.*, citrate = tartrate > sulfate > acetate = chloride. The fact that the experimental results follow this rule supports the mechanism of flocculation as a contributing factor in the inhibition process.

Figure 2 demonstrates the difference in neutralization velocities produced by different concentrations of sodium tartrate. It is typical of the effects observed with sodium citrate.

To confirm the flocculation mechanism for the inhibition of AHDG, sedimentation studies were conducted using the Andreasen pipet. Figure 3 shows that a significantly larger amount of AHDG was collected in the presence of citrate and tartrate as compared to the control. If flocculation did occur, one would expect the citrate- and tartrate-treated AHDG to settle faster due to a greater particle size. When a sample was withdrawn at a preset level there was more AHDG present at that level in the salt solutions. The results obtained from this study correlate to some degree with those obtained from the neutralization studies. It would appear that the salts act to reduce the surface area of the antacid, and thereby produce a concomitant reduction in neutralization velocity.

To investigate the possibility of anion–AHDG complexation with concurrent proton release, during the reaction of AHDG with sodium citrate and sodium tartrate, potentiometric titrations were performed. The results of these studies are shown in Figs. 4 and 5.

The inflection points of the sodium citrate and aluminum chloride curves in Fig. 4 are identical,

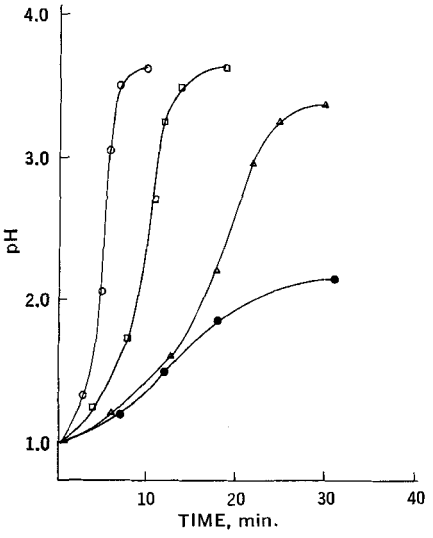

Fig. 2.—The effect of tartrate concentration on the AHDG–HCl neutralization. Key: O, 0.0001 *N*; □, 0.001 *N*; △, 0.01 *N*; ●, 0.1 *N*.

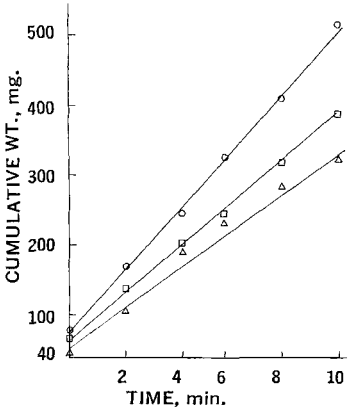

Fig. 3.—Cumulative weight of AHDG collected *versus* time. Key: △, AHDG; O, AHDG plus citrate; □, AHDG plus tartrate.

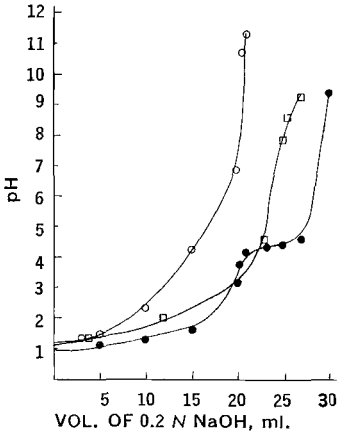

Fig. 4.—Potentiometric titrations of aluminum chloride (●), sodium citrate (O), and aluminum chloride plus sodium citrate (□).

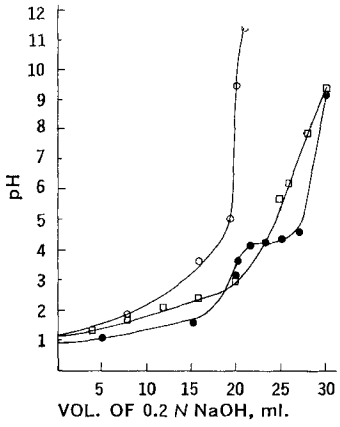

Fig. 5.—Potentiometric titrations of aluminum chloride (●), sodium tartrate (○), and aluminum chloride plus sodium tartrate (□).

both occurring at 20 ml. of alkali. However, the combination of these salts required more base for neutralization; the inflection point was displaced to the right, to about 24 ml. It is interesting to note that although the titration of aluminum chloride alone produced a precipitate of the hydroxide, no precipitate was observed in the presence of citrate or tartrate. This latter phenomenon suggests the presence of a water-soluble complex. In addition, since the system containing the aluminum complex required more alkali to produce neutralization than did either of the salts when tested separately, evidence is provided to indicate that the formation of this complex produces the release of excess protons and thereby decelerates the change of pH with respect to time. Similar results were found for the sodium tartrate–aluminum chloride potentiometric titration (Fig. 5).

In view of these findings we may conclude that a dual mechanism is operative to produce inhibition. AHDG reacts with sodium tartrate or citrate to produce a water-soluble complex with the concomitant liberation of protons. This effect coupled with flocculation causes a retardation in the rate of change of pH with time.

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Cytotoxicity of North Dakota Plants I

In Vitro Studies

By I. A. MUNI, W. H. BHATTI, L. J. SCHERMEISTER, and M. C. VINCENT

In this study, a method is presented for the direct determination of cytotoxicity and antitumor activity of plant materials *in vitro* using HeLa cells. Fifty poisonous plants representing 96 plant parts were tested by this method, out of which 25 plants showed limited cytotoxicity and six plants were found to be highly cytotoxic. This procedure can be used on a large scale prior to *in vivo* evaluation.

THE IMPORTANCE of plant screening for various chemical constituents of medical importance is well established. Plants have provided innumerable drugs which are being used for the prevention or cure of various diseases. This work was directed to the search for new drugs which may find application in the prevention or cure of cancer.

In vitro screening for cytotoxicity based on morphological evaluation was first reported by Biesele and associates (1) in 1952 using nonsynthetic media. Fjelde *et al.* (2) introduced human tumor cell lines for cancer chemotherapy.

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