Interaction of Salicylic Acid with Quaternary Ammonium Compounds

By LUCY S. C. WAN

A marked increase in viscosity is produced in solutions of dodecyl, tetradecyl pyridinium bromide, cetylpyridinium chloride, benzalkonium chloride, and benzethonium chloride containing salicylic acid. The viscosity increases with acid concentration to a maximum and then decreases. This viscosity effect is absent in quaternary ammonium compounds which are not surface active. Solutions of alkyl pyridinium and alkyl trimethyl ammonium compounds in the presence of salicylic acid form non-Newtonian systems which are dilatant while the alkyl aryl dimethyl compounds containing the same acid form Newtonian systems. The different rheological behavior is probably due to the number of substituents, R_1 R_2 ... present which are responsible for the surface activity of the molecule and which are attached to the nitrogen atom. The amount of salicylic acid required to produce maximum viscosity is directly proportional to the concentration of the quaternary ammonium compound which exhibits Newtonian flow.

Quaternary ammonium compounds have been found to interact with various substances (1–4). In addition Rodgers (5) has demonstrated the interactions of hexylresorcinol and amaranth with cetylpyridinium chloride and cetyldimethylbenzylammonium chloride while Takruri and Ecanow (6) have studied the interactions of benzalkonium chloride and sodium lauryl sulfate with lipoproteins. In a previous study salicylic acid has been found to be the only one of several substituted benzoic acids to interact with cationic surfactants (7, 8). The interaction is highly specific since related compounds do not behave in like manner and therefore it seems desirable to collect more information about this interaction, in particular the type of quaternary ammonium compound that will exhibit this interaction and also in view of the possible practical applications in pharmaceutical formulations.

EXPERIMENTAL

Materials—Recrystallized salicylic acid m.p. 158.5–159° (British Drug House Ltd.), the quaternary ammonium compounds were benzethonium chloride monohydrate,[1] benzalkonium chloride 92–95% (Farbenfabriken Bayer Co.), dodecyl and tetradecyl pyridinium bromide,[2] cetylpyridinium chloride (E. Merck, Darmstadt), tetradecyl trimethyl ammonium bromide,[3] cetyltrimethylammonium bromide,[4] trimethylphenylammonium chloride, tetramethylammonium bromide, choline bitartrate, carbachol B.P. (E. Merck, Darmstadt), hexamethonium tartrate (May and Baker), decamethonium iodide, neostigmine methyl sulfate, hexamethonium bromide B.P.C. (L. Light Co.), and succinylcholine chloride (Burroughs Wellcome Co.). The critical micelle concentrations of the surfactants as obtained from surface tension measurements using the Du Nouy tensiometer are listed in Table I, those of tetradecyltrimethylammonium bromide and cetyltrimethylammonium bromide are 0.082 and 0.051%, respectively (7).

Apparatus—*Measurement of Viscosity*—Varying amounts of salicylic acid were weighed into a series of 100-ml. volumetric flasks containing the required concentration of the quaternary ammonium compound. The flasks were rotated in a thermostatically controlled water bath at 25 ± 0.1° for 24 hr. Their viscosities were measured using a portable viscometer (Ferranti) placed in a thermostatically controlled water bath at 25 ± 0.5°. The shear rates which ranged from 78.56 to 234.6 sec.$^{-1}$ were increased and then decreased for all viscosity measurements. An interval of 30 sec. was allowed between any two readings.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of salicylic acid on the viscosities of solutions of benzalkonium chloride and benzethonium chloride, respectively. There is a rise in viscosity which increases with additions of the acid until a maximum is reached after which the viscosity then decreases. This fall in viscosity continues until an excess of the acid is present, except in the case of high concentrations of benzalkonium chloride where there is a rise in viscosity before a suspension is formed. The solutions are Newtonian systems, their viscosities being the same when measured at shear rates of 78.56, 117.35, 155.1, 195.9, and 234.6 sec.$^{-1}$ but the suspensions formed are

Table I—Critical Micelle Concentrations of Surfactants at 25°

Surfactant	C.M.C., %w/v
Dodecyl pyridinium bromide	0.039
Tetradecyl pyridinium bromide	0.074
Cetylpyridinium chloride	0.029
Benzalkonium chloride	0.049
Benzethonium chloride	0.063

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[1] Marketed as Hyamine 1622 by Rohm and Haas Co., to whom the author is very grateful for the samples.
[2] Marketed as Morphan DPB and TPB, respectively, by Glovers Chemicals Ltd.
[3] Marketed as Morphan T by Glovers Chemicals Ltd., to whom the author is very grateful for the samples.
[4] Marketed as Cetrimide by Imperial Chemical Industries.

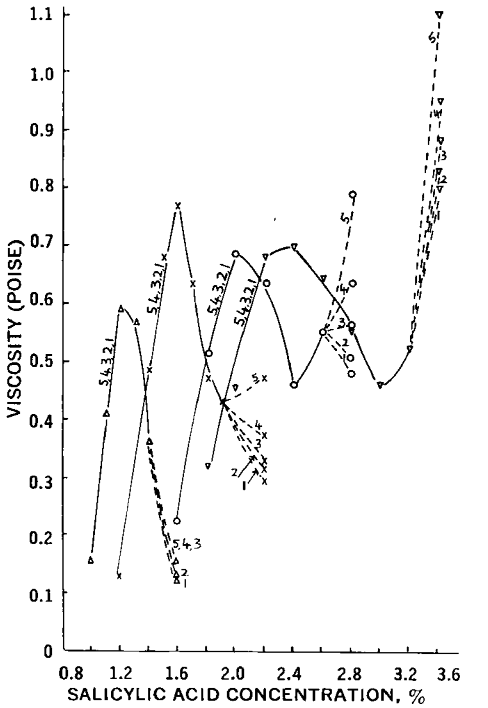


Fig. 1—Effect of salicylic acid on the viscosity of benzalkonium chloride solutions at 25°. Key: Δ, *4%;* ×, *6%;* O, *8%;* ∇, *10%; - - -, suspension. Shear rate:* 1, *234.6 sec.*$^{-1}$; 2, *195.9 sec.*$^{-1}$; 3, *155.1 sec.*$^{-1}$; 4, *117.35 sec.*$^{-1}$; 5, *78.56 sec.*$^{-1}$.

generally non-Newtonian systems. The amount of salicylic acid required to produce maximum viscosity is directly proportional to the concentration of the quaternary ammonium compound (Fig. 3). The ratios of the concentrations of the acid to the quaternary ammonium compound at which maximum viscosity occurs is 1:5 and 1:12.5 for benzalkonium chloride and benzethonium chloride, respectively. Once this ratio has been obtained further additions of the acid reduce the viscosity of the system.

The viscosity effect is also observed in solutions of alkyl pyridinium compounds containing salicylic acid. The increase in viscosity resulting from the interaction of the acid with dodecyl pyridinium bromide is not as marked as that produced by the interaction of the same acid with the corresponding tetradecyl derivative (Fig. 4). In this case the viscosity also increases with increase in acid concentration and then decreases, but when an excess of the acid is added the viscosity remains constant. Solutions of cetylpyridinium chloride gel readily when sufficient salicylic acid is added. If a small amount is present then gelling occurs; gelling is taken as the state in which the viscosity can no longer be determined by the Ferranti viscometer as the dial readings fluctuate during the rotation of the cylinder. These systems are dilatant and can easily be demonstrated by stirring vigorously a solution of cetylpyridinium chloride and salicylic acid which then becomes very viscous but when it is allowed to stand it reverts to a liquid of low viscosity.

In contrast to the alkyl aryl dimethyl compounds, the pyridinium compounds in the presence of salicylic acid form non-Newtonian systems, their viscosities vary with different shear rates (Fig. 4). This behavior is seen with other concentrations of tetradecyl pyridinium bromide solutions studied

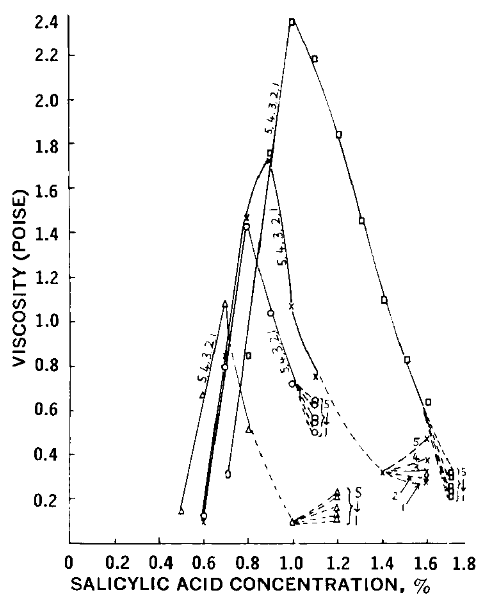


Fig. 2—Effect of salicylic acid on the viscosity of benzethonium chloride solutions at 25°. Key: Δ, *4%;* O, *5%;* ×, *6%;* □, *8%; - - -, suspension. Shear rate:* 1, *234.6 sec.*$^{-1}$; 2, *195.9 sec.*$^{-1}$; 3, *155.1 sec.*$^{-1}$; 4, *117.35 sec.*$^{-1}$; 5, *78.56 sec.*$^{-1}$.

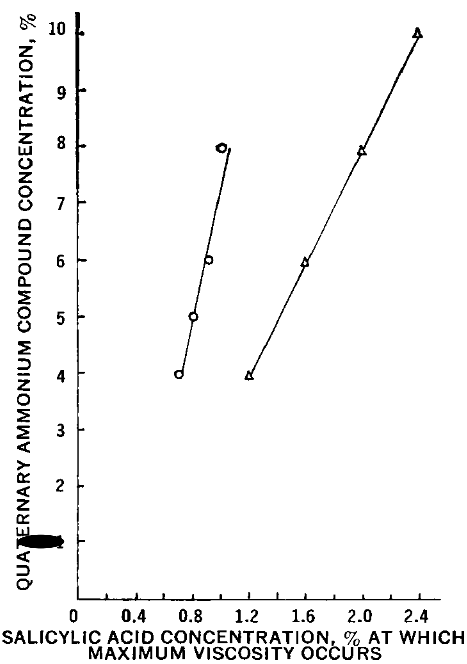


Fig. 3—Relationship between quaternary ammonium compound concentration and salicylic acid concentration required to produce maximum viscosity at 25°. Key: Δ, *benzalkonium chloride;* O, *benzethonium chloride.*

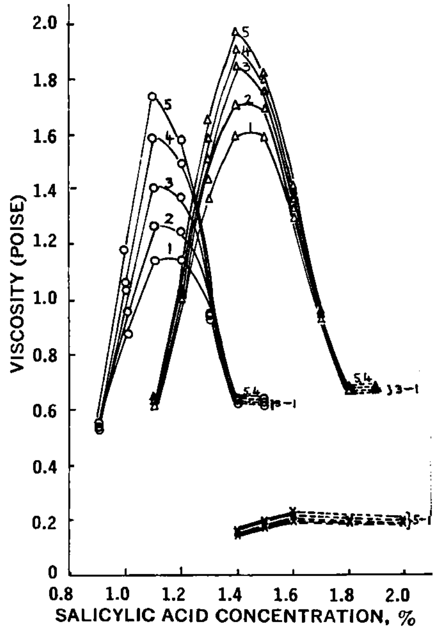


Fig. 4—Effect of salicylic acid on the viscosity of tetradecyl and dodecyl pyridinium bromide solutions at 25°. Key: ○, 3% TPB; Δ, 4% TPB; ×, 5% DPB; - - -, excess salicylic acid. TPB = tetradecyl pyridinium bromide, DPB = dodecyl pyridinium bromide.

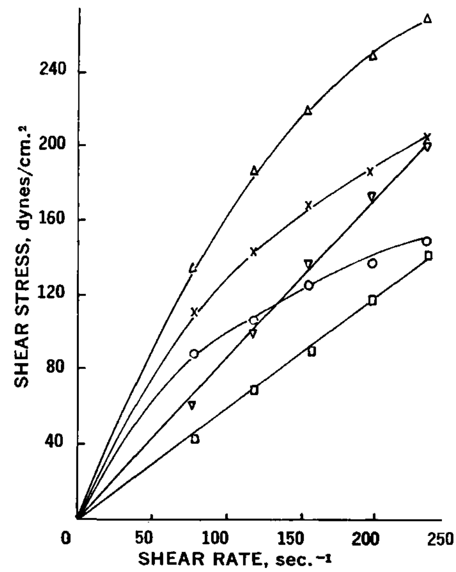
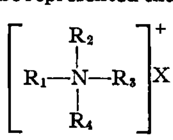


Fig. 5—Flow curves of quaternary ammonium compound solutions containing salicylic acid at 25°. Key: ×, 3% CTAB + 1.0% SA; ○, 3% TTAB + 0.8% SA; Δ, 3% TPB + 1.1% SA; □, 4% BZC + 1.2% SA; ∇, 6% H1622 + 0.7% SA. CTAB = cetyltrimethylammonium bromide. TTAB = tetradecyl trimethylammonium bromide. TPB = tetradecyl pyridinium bromide. BZC = benzalkonium chloride. H1622 = benzethonium chloride. SA = salicylic acid.

such as 2, 5, and 6%. Solutions of the alkyl aryl dimethyl compounds form Newtonian systems in the presence of salicylic acid (Figs. 1 and 2) except when the quantity of the acid present exceeds its solubility in the surfactant and then suspensions are formed which are non-Newtonian dispersions.

Figure 5 shows flow curves of solutions of tetradecyl pyridinium bromide, cetyl and tetradecyl trimethyl ammonium bromide containing salicylic acid. These systems are non-Newtonian and exhibit dilatancy. The flow curves of benzalkonium chloride and benzethonium chloride, however, demonstrate Newtonian flow. Similar behavior is observed with concentrations of the above-mentioned surfactants ranging from 3 to 10% in the presence of varying amounts of the acid. The difference in rheological behavior is probably due to the number of substituted organic groups which confer surface activity on the molecule and which are attached to the nitrogen atom. The quaternary ammonium compounds investigated including those studied previously (7, 8) can be considered to be analogs of the inorganic ammonium salts and their general structure (I) may be represented thus:



I

where R_1, R_2, R_3, and R_4 are organic groups and X is an inorganic radical which in this case is either a chloride or bromide. In one type, R_1 varies while R_2, R_3, and R_4 are kept constant such as dodecyl, tetradecyl, and cetyltrimethylammonium bromide; in a second type R_1 and R_3 are different while R_2 and R_4 are the same, such as benzalkonium chloride or benzethonium chloride. In a third type, a cyclic nitrogen compound replaces the single nitrogen of the former types thus making available in the configuration for R_1 only, such as dodecyl, tetradecyl pyridinium bromide, and cetylpyridinium chloride. Hence the interactions of salicylic acid with the alkyl trimethyl ammonium and alkyl pyridinium compounds represented as $R_1(CH_3)_3N^+Br^-$ and R_1N^+⬡ Br^-, respectively, lead to the formation of Newtonian solutions which may be attributed to the presence of a single substituent responsible for the surface activity of the molecule while the alkyl aryl dimethyl ammonium compounds represented as $R_1,R_3(CH_3)_2N^+$ Cl^- have two substituents and produce Newtonian solutions.

No increase in viscosity is observed when salicylic acid is added to solutions of trimethyl phenyl ammonium chloride, tetramethyl ammonium bromide, choline bitartrate, carbachol, hexamethonium tartrate and bromide, decamethonium iodide, neostigmine methyl sulfate, and succinylcholine chloride. Hence the quaternary ammonium compounds which are not surface active do not demonstrate this viscosity effect. The results obtained indicate that the interaction of salicylic acid with the quaternary ammonium compounds is associated with the presence of micelles as the surfactant concentrations used are well above the critical micelle concentration (C.M.C.) (Table I). When salicylic acid is added it is solubilized by the micelles, the placement of the solute in the micelles is possibly one of adsorption on the micellar surface. The negatively charged salicylate ions will be attracted and adsorbed onto the positively charged micelles in such a manner of arrangement that gives rise to an increase in size and thereby to an increase in viscosity. As more acid is added more salicylate ions will be adsorbed on the micellar surface and this continues till a maximum viscosity is reached. At this stage the micellar surface may have adsorbed sufficient salicylate ions to attain an equilibrium state so that further additions of the acid will tend to upset the equilibrium resulting in a decrease in viscosity. When the amount of acid present exceeds that which can be solubilized by the micelles then the solution or gel becomes a suspension of salicylic acid in the surfactant solution. This change of nature of the system makes it no longer a simple liquid system and this gives rise to non-Newtonian flow which is not unusual with the flow properties of most suspensions. In the case of the alkyl pyridinium compounds the flow properties remain non-Newtonian in the presence of an excess amount of salicylic acid. This is also true for the alkyl trimethyl ammonium compounds studied previously (7).

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Keyphrases

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Rheology—salicylic acid–quaternary ammonium compounds

Effect of Vagal Stimulation on Enterochromaffin Cell Granulation in the Guinea Pig Small Intestine

By MARTIN F. TANSY, ARTHUR S. MILLER, and ARTHUR STEIN

Experiments are presented which study the effect of vagal stimulation on the enterochromaffin cell granulation of the guinea pig duodenum. Concentration of granulated enterochromaffin cells in specially stained intestinal sections is used to determine the response. The results indicate the vagus nerves *per se* and not hydrochloric acid secretion, increased intraluminal pressure, or hyperperistalsis significantly alter the granulation of these cells. The data also suggest that vagal degranulation may occur *via* a noncholinergic mechanism.

The importance of serotonin in the gastrointestinal tract has received considerable investigative attention. Most of the studies, however, have been concerned with its content, fate, and action; whereas, its release has received much less attention. Serotonin release has primarily been concerned with the observations that elevation of intraluminal pressure and/or increased intestinal motility augments the amount of 5-HT released into the intestinal lumen and venous blood (1). It has not, however, been shown that distension or hyperperistalsis of the intestine, kept within physiologic limits, produces in the intact animal a local or systemic discharge of serotonin from the enterochromaffin cells (2).

Argentaffin enterochromaffin cells are distributed throughout the mucosa of the gastrointestinal tract. They contain specific granules which are precipitated by formalin and will stain with silver salts (3). The complete chemistry of the granules is not known, but Barter and Pearse (4) suggest a fully conjugated β-carboline derivative of serotonin may precipitate the silver to give the argentaffin staining reaction. In any event, considerable experimental evidence (5–7) has shown that a decrease in the granularity of these cells parallels the release of serotonin from the gut. This phenomenon provides a convenient

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