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The interaction of *Escherichia coli* and magnesium trisilicate in aqueous suspension

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E. coli interacted strongly with magnesium trisilicate particles in buffer solution with aggregation of the particles and ready removal of bacteria from suspension, an equilibrium being attained between adhering and unattached cells. The relation between $\log_{10}$ (uptake bacteria g^{-1} magnesium trisilicate) and $\log_{10}$ (equilibrium concentration bacteria cm^{-3}) suggested some analogy to the Langmuir type adsorption of solutes. Since both bacteria and particles possessed moderate negative electrical charges of similar magnitude, their interaction is thought to be due to long range additive Van der Waals forces rather than by electrostatic attraction.

Liquid antacid mixtures containing magnesium trisilicate have frequently been reported to be contaminated heavily with Gram-negative bacteria including *Pseudomonas aeruginosa* and that these contaminants may survive for long periods and even grow in the mixtures (Public Health Laboratory Service Report, 1971; Meghji, Buddle & Cherryman, 1971; Pharmaceutical Society's Working Party Report, 1971; McKenny, 1972; Westwood & Pin-Lim, 1972; Fearnley & Ellwal, 1972). During investigations in this department into the survival of micro-organisms in magnesium trisilicate suspensions it was observed that the number of bacterial colonies developing on media inoculated with aliquots of magnesium trisilicate suspension containing *E. coli* was considerably less than expected from the known size of the initial inoculum. However the possible toxicity of magnesium trisilicate was not confirmed by extinction methods of viability assessment nor by the ready growth of *E. coli* on solid media containing high levels of magnesium trisilicate. Thus, the possibility of substantial interaction and aggregation of the bacteria and magnesium trisilicate was investigated and the results obtained are now described.

METHODS AND MATERIALS

Preparation of bacterial suspensions. *E. coli*, NCTC 5933 was grown on Roux slopes of nutrient agar (Oxoid No. CM3) at 35° for 18 h. Cells were harvested, washed three times with quarter strength Ringer solution, and suspensions adjusted to contain the required number (total) of bacteria by reference to calibrated opacity tubes (Wellcome Reagents Ltd.) The suspensions contained 32% non-viable cells determined by comparison of viable counts with total counts made with a Thoma counting chamber.

Interaction of bacteria and magnesium trisilicate. Suspensions of bacteria (25 cm^3) were mixed with Sørensen phosphate buffer (25 cm^3, pH 7·0, 0·067M) and magnesium trisilicate (0·1g) and agitated at 30° for 2 h. A control mixture without magnesium trisilicate was also included. Aliquots were then centrifuged at 2000 rev min^{-1} for

4 min which was found to precipitate the magnesium trisilicate selectively. The numbers of viable bacteria in the supernatant liquids were determined by incorporating diluted aliquots into nutrient agar (Oxoid, No. CM3), incubation at 35° and counting of the resultant bacterial colonies.

Determination of sedimentation rate. After the suspensions of magnesium trisilicate and bacteria had been incubated they were placed in 150 × 24 mm Pyrex tubes and allowed to stand at 30° for 20 min. Aliquots were then withdrawn from a liquid depth of 5 cm and their turbidities measured with an nephelometer (EEL). Their relative turbidities were regarded as a measure of the relative rates of sedimentation of the bacteria-magnesium trisilicate aggregates.

Determination of electrophoretic mobilities. Electrophoretic mobilities of bacteria and magnesium trisilicate in Sørensen phosphate buffer (0·034M, pH 7·0) were measured at 30° in a rectangular lateral glass cell of width 1·0 cm and depth 0·1 cm using platinum electrodes. Measurements were made at the stationary layer. The velocities of the particles or bacteria were measured with reversals of applied field between each measurement.

Determination of surface area and particle size. The specific surface area of magnesium trisilicate was estimated by measurement of the rate of air flow through a compressed bed of powder (Rigden, 1947). Particle sizes of magnesium trisilicate and bacteria were determined microscopically using a calibrated eye-piece graticule, the bacteria being stained lightly with methylene blue.

Magnesium trisilicate was a commercial sample complying with British Pharmacopoeial specifications. It was dried and sterilized by heating at 160° for 1 h and stored in sealed glass containers.

RESULTS AND DISCUSSION

When shaken with magnesium trisilicate in buffer, a high proportion of the *E. coli* interacted with the particles and sedimented readily upon low speed centrifugation, equilibrium between bacteria adhering to particles and those unattached being attained within 90 min. Microscopic examination revealed a progressive and extensive aggregation of the particles with increasing bacterial concentration and some visual evidence of bacteria-particle adhesion. The adhesion of individual bacteria to several particles could explain such flocculation of the magnesium trisilicate.

The bacteria were found to be in the size range 1–2 μm (mean 1·64 μm) in length and 1 μm in width. The magnesium trisilicate had a particle size range of 1–16 μm (mode 5–6 μm) with a specific surface of 1·84 $m^2\ g^{-1}$.

As shown in Fig. 1 a plot of $\log_{10}$ (uptake viable bacteria g^{-1} magnesium trisilicate) and $\log_{10}$ (equilibrium concentration viable bacteria) gave a smooth curve below an equilibrium concentration of 5×10^5 viable bacteria cm^{-3} but above this there was an upwards deflection followed by a marked inflexion at an equilibrium concentration of 6×10^6 viable bacteria cm^{-3}. It is suggested that the bacteria-particle interaction has some analogy to the Langmuir type adsorption of solutes and that the inflexion in Fig. 1 represents a point of "saturation" of available powder surface by the bacteria. The sedimentation rates of the magnesium trisilicate—bacteria suspensions relative to magnesium trisilicate alone increased with increasing cell

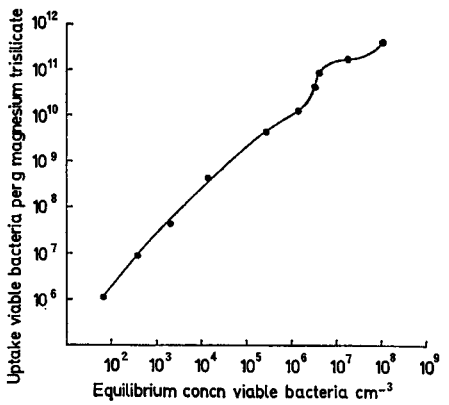

FIG. 1. The removal of *E. coli* from suspension by magnesium trisilicate.

concentrations up to this inflexion but then decreased (Table 1) which might also lend support to the concept of a "saturation" point. If one postulates the interaction of the non-viable bacteria in the suspension in a manner similar to the viable bacteria, then at this "saturation" point approximately 14% of particle surface would be covered by bacteria if each cell occupied a rectangular area of 1·64 μm^2 and they were capable of aligning themselves side by side to form a monolayer. Obviously, because of the small size of the particles, much of their surface would not be available for such a close packing of the bacteria. Similarly any internal pore system in the particles not detectable by the air permeability measurements would be unlikely to be available to the bacteria.

In phosphate buffer magnesium trisilicate particles possessed a mean electrophoretic mobility of $-$ 2·38 $m^2\ s^{-1}\ V^{-1} \times 10^{-8}$ ($-$ 2·26 to $-$ 2·43 $m^2\ s^{-1}\ V^{-1} \times 10^{-8}$) with the bacteria showing a wider range of mobilities from $-$ 1·23 to $-$ 2·33 $m^2\ s^{-1}\ V^{-1} \times 10^{-8}$. Mean zeta potentials and surface charge densities for magnesium trisilicate and bacteria were calculated to be $-$ 28·4 mV and 8580 e.s.u. cm^{-2} and $-$ 19·84 mV and 5536 e.s.u. cm^{-2} respectively for this system. Thus the interaction of particles and bacteria is unlikely to be due to electrostatic attraction and Van der Waals forces are therefore thought to be responsible. In particulate systems of

Table 1. *The effect of* E. coli *upon the sedimentation rate of magnesium trisilicate suspension in phosphate buffer (pH 7.0, 0.034M).*

Total No. of bacteria cm^{-3}	Equilibrium concentration of viable bacteria cm^{-3}	Relative sedimentation rate of magnesium trisilicate–bacteria suspensions
0	0	100
$1{\cdot}35 \times 10^6$	$1{\cdot}30 \times 10^4$	127
$1{\cdot}35 \times 10^7$	$2{\cdot}50 \times 10^5$	147
$4{\cdot}04 \times 10^7$	$1{\cdot}35 \times 10^6$	179
$1{\cdot}35 \times 10^8$	$3{\cdot}04 \times 10^6$	227
$2{\cdot}69 \times 10^8$	$3{\cdot}90 \times 10^6$	294
$5{\cdot}40 \times 10^8$	$1{\cdot}71 \times 10^7$	185

these dimensions the additive contributions of all atoms leads to long range attractive forces, in this case sufficient to overcome the mutual electrostatic repulsion between bacteria and powder.

Micro-organisms have been reported to adhere to a wide range of solid particles, the interaction with aluminosilicate clays and sand receiving particular attention from soil microbiologists (Estermann & McLaren, 1959). The adhesion of kaolinite, montmorillonite and bentonite to bacteria has been attributed to electrostatic attractions between the negatively charged cell surfaces and the positively charged edges of the clay crystals (Lahav, 1962; Marshall, 1968; 1969a, b). Ion-exchange resin beds for the preparation of demineralized water readily adsorb bacteria which grow on nutrients also adsorbed and release high levels of contamination into the eluate. Since bacteria, which generally possess an overall negative surface charge adsorb onto both cationic and anionic exchange resins (Zviaginzeo, 1959) attractive forces in addition to electrostatic forces must be considered. The adsorption of micro-organisms to various filter materials is also reported (Sykes, 1965). It is known that, in their production, inorganic powders such as kaolin, talc magnesium carbonate and magnesium trisilicate adsorb a variety of organic compounds during the washing cycles. Indeed Hugo & Wilson (1971) and Wilson (1972) considered that the growth of *Pseudomonas aeruginosa* in peppermint water could be attributed to nutrients leached from talc used in its preparation rather than to the peppermint oil. Zobell (1943) reported that bacteria adsorbed onto particulate surfaces were often able to grow in media otherwise too dilute to support growth, due to concurrent concentration of nutrients at the particle surface and to inherent protective properties of the surfaces. It is possible that the reported growth and survival of bacteria in antacid mixtures containing magnesium trisilicate might be aided by an adhesion to the particles similar to that described here and by metabolism of nutrients also adsorbed at the particle surface.

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