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Studies on the Adsorption Properties of Korean Kaolin IV
The adsorption of bacteria by activated halloysiste

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Abstract □ Studies on the adsorption of four kinds of bacteria, *Staphylococcus aureus, Sarcina lutea, Escherichia coli* and *Serratia marcescens* by activated Korean kaolins have been carried out to innovate utilization as adsorbent preparations. In connection with particle size and size fraction, the adsorption was examined by colony counting and spectroscopy. Korean kaolin was purified from Hadong white species of preminum grade and three size fractions were derived from passage through BS# 100, #200 and #325 mesh sieves, respectively. These were activated at 105° or 280° for three hours and at 550°C for one hours. The results indicated that the adsorbing power of Korean kaolin was superior for *S. aureus* and *S. lutea*, but *E. coli* and *S. marcescens* were not adsorbed by clays. The smaller the particle size, the greater was adsorbing power for Gram-positive bacteria. There appears to be justification for its further investigations as an ingredient in intestinal adsorbent preparations.

Keywords □ adsorption, clays, kaolin, halloysite, Gram-positive bacterium, size fraction, activation.

Kaolin together with other substances, has been utilized[1] for its adsorptive properties in many preparations intended for the treatment of enteric disturbances. Clinically, it is thought to be effective in the treatment[2] of those diarrheal states caused by an agent capable of being adsorbed, including those due to food poisoning, heavy metal poisoning and bacterial or parasitic infections of the intestinal tract. Walker[3], Eisler[4], Gunnison *et al.*[5] and Smith[6] found that kaolin had capacity to adsorb various bacteria. There are also many reports[7-12] on the ability of kaolin to adsorb toxins. As for Korea kaolin has been emported totally from foreign countries even though it has abundant natural resources of halloysite at Hadong area and some other locations. Therefore it is of importance both from economical and scientific view point to investigate the adsorption properties of Korean kaolin. Since it was found that activated halloysite are superior adsorbents[13-17] for strychnine and quinine than other kaolins, the authors decided to investigate the adsorption properties of these Korean kaolin for several bacteria. The result should throw further light on whether activated halloysite of Korea may be useful as an ingredient of intestinal adsorbent preparations.

EXPERIMENTAL METHODS

Material

The sources of the activated halloysite were the same as those used in the study of the adsorption of alkaloids[1,17].

Microorganism

Four bacterial strains were used: *Staphylococcus aureus, Sarcina lutea, Escherichia coli* and *Serratia marcescens.*

Method of Bacterial Adsorption

The bacterium was inoculated on to nutrient agar plate in a Roux bottle and harvested with sterile distilled water after an overnight incubation. Five milliliters of an aqueous suspension of the microorganism under study were introduced into a sterile, screw-capped, tube containing an accurately weighed quantity of clay which had been sterilized in the tube previously by autoclaving. The bacterial-clay suspension was then shaken for thirty minutes and the suspension was permitted to stand for three hours, while the clay settled to the bottom of the tube.

The supernatant was then removed with a

pipette and placed in another strile tube and shaken in order to produce a uniform dispersion of the bacteria. One m*l* aliquot of the supernatant was then removed with a pipette and a bacterial count (colony forming unit: CFU) was made using the standard colony count procedure for the quantitative determination of microorganisms in water. Dilutions were made so that at no time were less than 30 and no more than 300, colonies counted in a single Petri dish. The Petri dishes were placed in an incubator at 37 °C and colony counts were made after twenty four hours. The number of bacteria adsorbed by the clay was obtained as the difference between the bacterial counts in the bacterial-clay supernatant and the control suspension which did not contain any clay.

The other method, spectroscopy was carried out with proper aliquot of the supernatant, the absorbance of which ranged from 0.30 to 0.70 at 600nm. The number of bacteria adsorbed was computed from the absorbance difference between the sample solution and the control solution.

RESULTS AND DISCUSSION

It is apparent from the experimental results in Table I that activated halloysite is capable of adsorbing *Staphylococcus aureus* and *Sarcina lutea.* Under the test conditions, 50mg of activated halloysite adsorbed nearly 80% as many bacteria of *S. aureus* and 55% of *S. lutea* but it did not adsorb the Gram-negative bacteria; *Escherichia coli* and *Serratia marcescens* to any great degree. It is of interest to note that halloysite was found to adsorb only Gram-positive bacteria; *S. aureus* and *S. lutea.* Considering the fact that all the four bacteria are negatively charged[18], it is not understood why only the two gram-positive bacteria are adsorbed by the clays.

Table I. Adsorbing activity of activated Korean kaolin for some bacteria measured by spectroscopic method

Bacterium	% Adsorption[a] by kaolin[b] activated at		
	105°	280°	550°
Staphylococcus aureus	76.7	49.4	39.8
Sarcina lutea	54.8	45.9	22.7
Escherichia coli	2.6	2.5	-1.2
Serratia marcescens	1.8	1.7	-3.1

[a] determined according to the following equation;

$$\% \text{ Absorption} = \frac{(A_t)-(A_k)}{(A_b)} \times 100,$$

where (A_b) is the absorbance (at 600nm) of the bacterial suspension, and (A_t) is the absorbance of test suspension consisting of bacterial cells.
[b] Kaolin was passed through a 100-mesh sieve and activated at different temperatures shown in the table.

Table II. Adsorbing activity of different amount of Korean kaolin for *Staphylococcus aureus*

Amount of Kaolin[a] (mg/5m*l*)	Bacterial concentration after adsorption (CFU/m*l*)	% Adsorption measured by colony counting	Spectroscopy
0[b]	18.1×10^7	-	-
10	9.1×10^7	49.7	49.7
20	5.0×10^7	72.4	77.5
30	3.9×10^7	78.5	82.9
50	2.8×10^7	85.5	87.2

[a] Kaolin was passed through a 100-mesh sieve and activated at 105° for three hours. [b] control suspension without kaolin. CFU: colony forming unit.

It is note-worthy from Table II that whereas 77.5% of the bacteria were removed from the *S. aureus* suspension when it was shaken with 20mg of activated halloysite, 87.2% of bacteria were removed as the amount of clay was increased to 50mg.

Colony counting and spectroscopic method were compared for their adaptability to the study of the adsorption of bacteria by clay. Spectroscopic method gave almost the same results as colony counting method (Table II). However, considering the fact that colony counting is a complicated and time-consuming method, spectroscopic method is highly recommended as a much more simple and rapid procedure for the quantitative determination of the bacterial adsorption by clay in suspension.

In the experiment in which the concentrations of bacteria to clay was changed, the result (Table II) reveals that the adsorption ratio of the bacteria increased only slightly. Table III shows that the adsorption isotherm was not Langmuir type when equilibrium concentrations of bacteria are plotted against the amount of bacterium adsorbed per gram of clay over the range of 9.1×10^7 CFU/m*l*. In this case, every suspended clay particle is thought to behave as though it is a colloidal electrolyte, and in effect as a weak acid macroanion arisen by partial ionization of surface hydroxo groups. Because of its negative charge a given particle closely associates with a swarm of cations. But because the number of

Table III. Adsorbing activity of Korean kaolin[a] for *Staphylococcus aureus* at different bacterial concentrations

Bacterial concentration (CFU/m*l*) in		% Adsorption measured by	
Control suspension[b]	Test suspension[c]	Colony counting	Spectroscopy
9.1×10^7	5.7×10^7	37.6	35.4
18.6×10^7	10.3×10^7	44.6	42.8
26.8×10^7	14.5×10^7	45.9	48.2
51.4×10^7	27.1×10^7	49.5	31.3

[a] Kaolin was passed through a 100-mesh sieve and activated at 105°C for three hours. [b] bacterial suspension without kaolin. [c] bacterial suspension with kaolin (10mg/m*l*). CFU: colony forming unit.

closely associated cations is less than that of cations required for neutrality, net negative particle charge persists.[18] And integral with the cation hull is a shell of associated water as a micell. So a difference in zeta-potential exists between the surface of a clay particle and the bulk of the suspending medium. Usually zetapotentials of stable suspensions range from 15-70 millivolts. Therefore it is assumed that these clays may be capable of adsorbing the bacteria due to the chemisorption with clay at and below the lower limit.

The adsorbing power of clay for Gram-positive bacterium, *S. aureus,* decreases with storage period, as shown in Table IV. It is not understood why the adsorption power was decreased with time. This phenomenon may be partly explained from the assumption that the adsorption might be concerned with the chemical composition and structure of the bacterial cell wall, as Gram stain is. Moreover, the number and arrangement of flagella and the shape of pili of cells might affect the adsorption.

The adsorption of bacteria by clay became greater as the particle size or surface area of clay was increased in case of *S. aureus* (Table V).

In conclusion, Hadong kaolins studied had good adsorption properties for Gram-positive bacteria, but not for Gram-negative bacteria. In case of Gram-positive bacterium, *S. aureus,* halloysite activated at 105°C and fractioned through 3S #325 mesh sieve, was found to be a superior adsorbent to the ones activated at 280° or 550°C. It is clear that not all the four bacterial straining employed in this study are the causative microorganisms in enteric diarrheas, and in this respect, additional studies using some other diarrhea-causing bacteria should be performed. Nevertheless, since activated Hadong halloysite has been shown to be a superior adsorbent for *S. aureus* and for alkaloids[17], there appears to be justification for its further investigations as an ingredient in intestinal adsorbent preparations.

Table IV. Adsorbring activity of Korean kaolin[a] for *Staphylococcus aureus* kept in suspension for different duration at 4°C after overnight incubation

Time(hrs)	0	1	3	6	10	15	24	48	72···	960
% Adsorption[b]	82.8	80.5	77.3	72.7	71.7	69.2	68.0	67.1	66.5···	66.2

[a] Kaolin was passed through a 100-mesh sieve and activated at 105°C for three hours. [b] measured by spectroscopic method.

Table V. Adsorbing activity of size-fractioned Korean kaolins for *Staphylococcus aureus*

Kaolin[a]		% Adsorption measured by	
Size-fraction	Surface area(m^2/g)	Colony counting	Spectroscopy
BS # 100-200	34.6	52.8	55.8
BS # 200-325	38.4	69.5	60.7
BS # 325-	43.8	82.9	80.3

[a] Kaolin was activated at 105°C for three hours, and the suspension contained 30mg of kaolin/5m*l*.

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