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# Antimicrobial and antifungal agents derived from clay minerals

## Part V *Montmorillonite and saponite containing some antimicrobial ammonium ions*

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Five antimicrobial ammonium cations were intercalated into two clay minerals (saponite and montmorillonite) by using ion-exchange reaction. About 90% of the cation exchange capacities of the clay minerals were replaced by ammonium ions with a few exceptions. The resultant complexes exhibited antimicrobial activity against both *Staphylococcus aureus* and *Escherichia coli*. After heating to 250 °C, the antimicrobial activity was kept for the complexes with aromatic ammonium ions but not for those with aliphatic ammonium ions. The complexes were treated with deionized water and a 3% aqueous solution of sulphuric acid to examine the elution of the ammonium ions. The elution finished within 1 day, with retention of 80% of the aromatic ammonium ions. Aliphatic ammonium ions remained in smaller amounts.

## 1. Introduction

Zeolites [1] and clay minerals [2] exchanged with $Ag^+$ ions have strong antimicrobial and antifungal activity. It is known that certain ammonium cations have antimicrobial activity [3, 4], so the present authors decided to prepare an antimicrobial clay mineral exchanged with an antimicrobial ammonium ion. The resulting complex may exhibit a high durability as an antimicrobial agent, because ammonium ions may be released slowly from the clay mineral. This work was carried out to develop such a new type of antimicrobial agent. The previous paper in this series (Part 4 [5]) reported work on montmorillonite supported by a silver chelate of hypoxanthine.

## 2. Experimental procedure

### 2.1. Raw materials

The clay minerals used were a montmorillonite (abbreviation: M) and a saponite (abbreviation: S) having a cation exchange capacity (CEC) of 108 and 71.2 meq/100 g, respectively. Further details of the clay minerals have been reported elsewhere [2, 6]. Five antimicrobial ammonium chlorides used were benzalkonium chloride (B), didecyldimethylammonium chloride (D), cetyltrimethylammonium chloride (C), acriflavine chloride (dye content: 90%, m.p. 533 K) (A) and dequalinium chloride (E). Their chemical formulae are shown in Fig. 1.

### 2.2. Preparation procedures

About 3 wt % aqueous sol of the clay mineral was mixed with ammonium hydrochloride of double the molar quantity of the CEC of the sol. The mixture was stirred for a predetermined period at 80 °C to intercalate the ammonium cations derived from their salts. The reaction was stopped after 7 h, at which the interlayer spacing of the resulting complex became

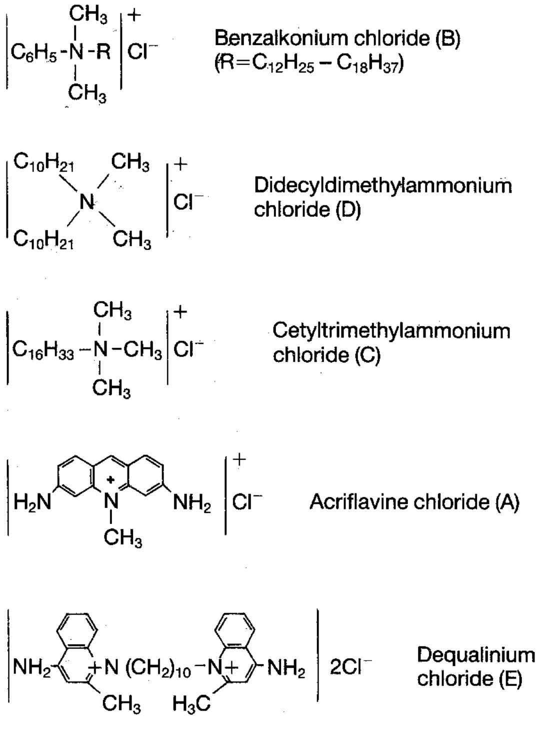

*Figure 1* Chemical formulae of antimicrobial ammonium chloride salts used in this work.

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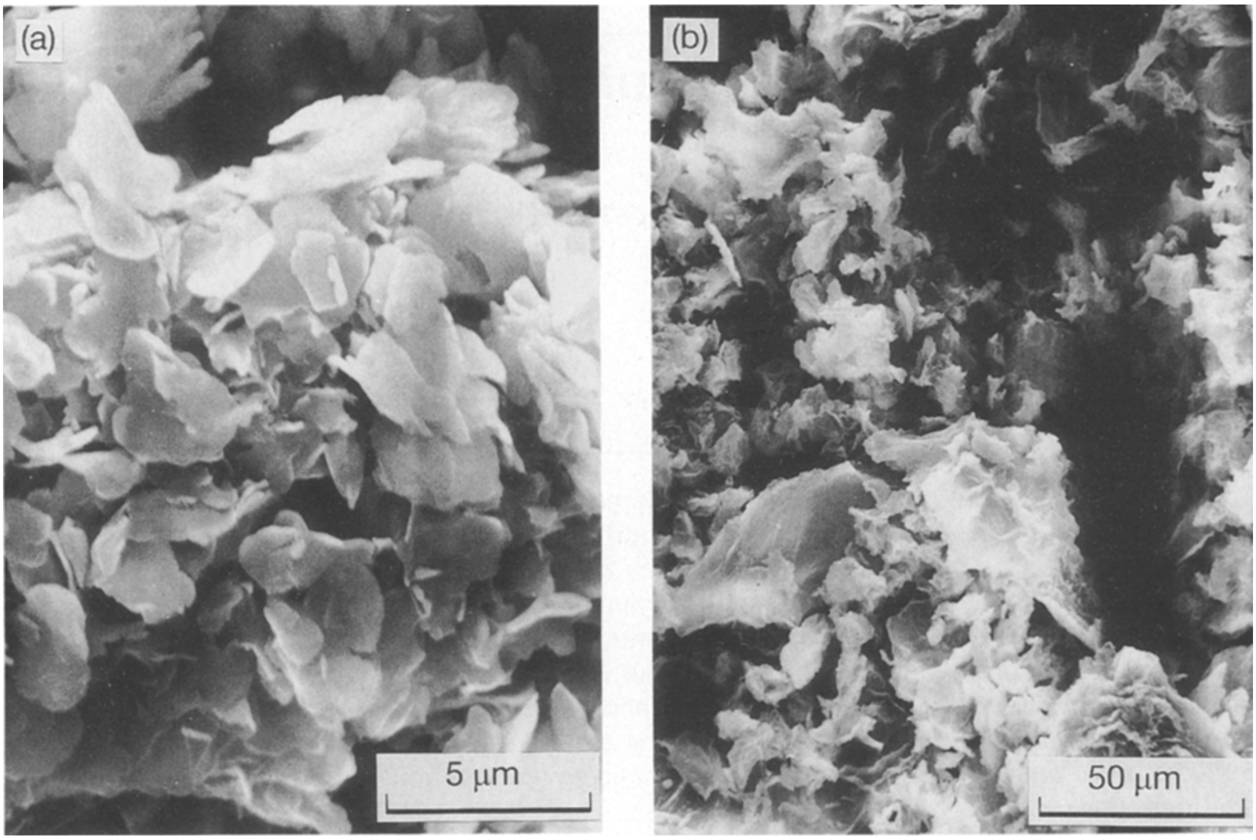

*Figure 2* SEM photographs of (a) BM and (b, c) ES before heating.

constant. The complex was washed with deionized water until the washing was free of chloride, followed by freeze-drying. The resulting complex is denoted by appending M or S after the alphabet letters B, D etc. in Fig. 1. A portion of the complex was heated at 250 °C for 1 h in air to examine its thermostability. The complex after heating is denoted by H, e.g., BSH is the benzalkonium–saponite complex after heating to 250 °C.

## 2.3. Measurements

Powder X-ray diffraction was carried out by using Ni-filtered Cu$K_\alpha$ radiation. The shape and texture of the complex were examined by scanning electron microscopy (SEM). The carbon content was measured by a combustion method using tin particles as a combustion accelerator. I.r. spectra were obtained by the KBr tablet method.

Three complexes were subjected before heating to an elution test. 0.1 g of the complex powder was added to 100 ml of the test solution (deionized water and 3 % $H_2SO_4$ aqueous solution), followed by stirring at room temperature. An aliquot of the solution was taken out occasionally and was subjected to ultrafiltration. The filtrates of AS and ES were used to determine the eluted amounts of A and E ions by using a u.v. spectrometer. The amount of B ion eluted from BS was calculated from the carbon content in BS after the test. Antimicrobial activity of the complexes, before and after heating, was examined against *Staphylococcus aureus* and *Escherichia coli*, of which details were described elsewhere [2].

## 3. Results

Table I shows carbon content of the complex and the exchange ratio (%) of CEC for ammonium ion. The B ion is a mixture of several compounds so that the

TABLE I Carbon content (wt %) and percentage of CEC occupied by ammonium cation

| | BM | CM | BS | CS | DS | AS | ES |
|---|---|---|---|---|---|---|---|
| C content | 17.3 | 17.8 | 19.9 | 14.8 | 12.4 | 9.9 | 17.4 |
| % CEC | 73.3 | 92.9 | 133.0 | 96.1 | 90.6 | 95.8 | 89.1 |

mean molecular structure (R = $C_{15}H_{31}$) was used for the calculation. About 90 % of the CECs of the clay minerals were exchanged by ammonium ions, except for BM and BS.

Fig. 2 shows SEM photographs of ES and BM before heating. BM is a flaky particle. Raw saponite is known to be flaky [6] but ES particles were quite difficult to observe clearly in this work. The shape of the complex was almost the same to that of the clay mineral used, and was not changed after heating to 250 °C.

Fig. 3 shows X-ray diffraction profiles. The (0 0 1) diffraction peaks of the complexes are at lower diffraction angles than those of the original clay minerals, indicating expansion of the interlayer spacings of the clay minerals by intercalation of the ammonium ions. After heating to 250 °C, the peak shifted again to a higher angle, e.g. 1.17 nm of M expanded to 1.78 nm (BM) by intercalation of B ion and then shrank to 1.45 nm after heating to 250 °C (BMH).

Figs 4 and 5 show i.r. spectra of the complexes derived from M and S, respectively. BM, CM, BS, CS and DS showed absorption bands (labelled 1, 2 and 3) which are assigned to $\nu_{as}$ $\nu_s$–C–$CH_3$ and $\delta$–$(CH_2)_n$–, respectively. AS and ES showed different absorption bands 4: ν–NH, 5: ν–aroC–H, 6: ν–aroC=C, ν–aroC=N and 7: ν–aroC=C). After heating to 250 °C, no weakening of the characteristic absorptions in DS, AS and ES occurred.

Table II shows results of the antimicrobial tests. All complexes before heating showed clearly antimicrobial activities against both bacteria, except for AS against *E. coli*. After heating to 250 °C, however, BMH alone was active against both bacteria, and both ASH and ESH showed activity against *S. aureus* alone.

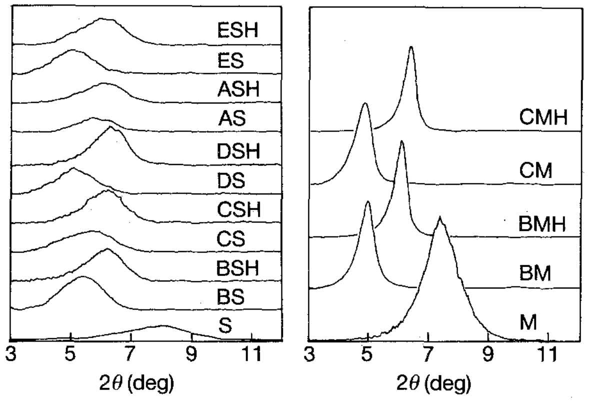

*Figure 3* X-ray diffraction profiles of clay minerals and the complexes before and after heating to 250 °C.

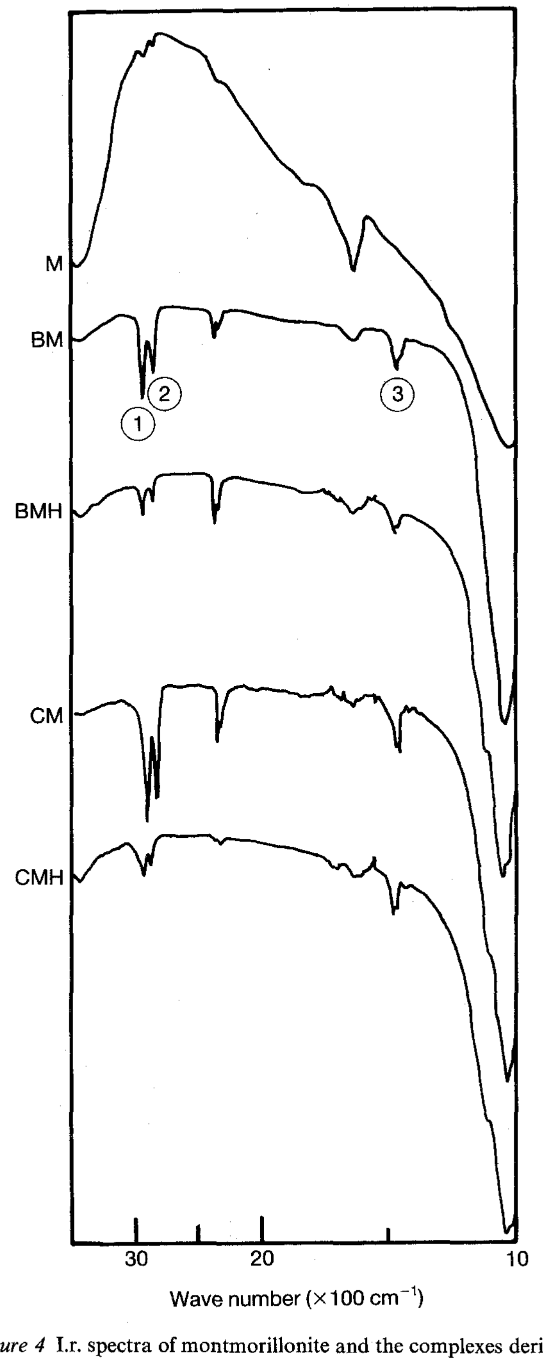

*Figure 4* I.r. spectra of montmorillonite and the complexes derived from it, before and after heating.

TABLE II Antimicrobial activity of complexes[a]

| | BM | CM | BS | CS | DS | AS | ES | BMH | CMH | BSH | CSH | DSH | ASH | ESH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | + | + | + | + | + | + | + | + | – | – | – | – | + | + |
| *E. coli* | + | + | + | + | + | – | + | + | – | – | – | – | – | – |

[a] ( + ) Effective, ( – ) ineffective.

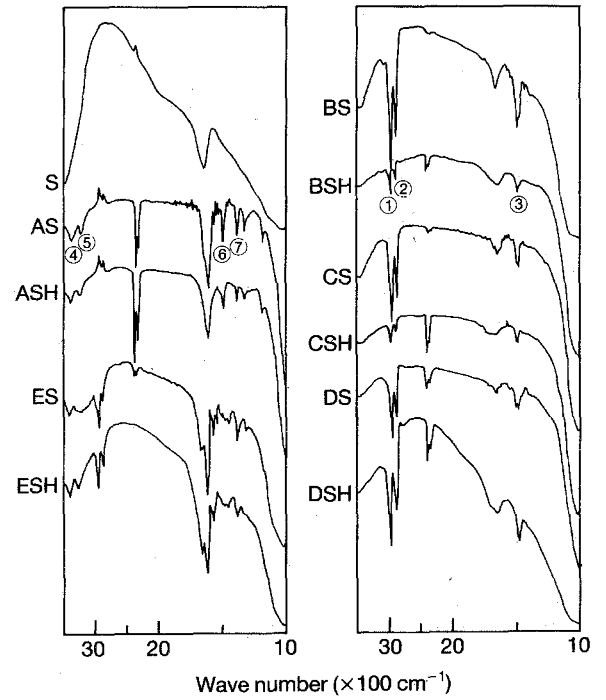

*Figure 5* I.r. spectra of saponite and the complexes derived from it, before and after heating.

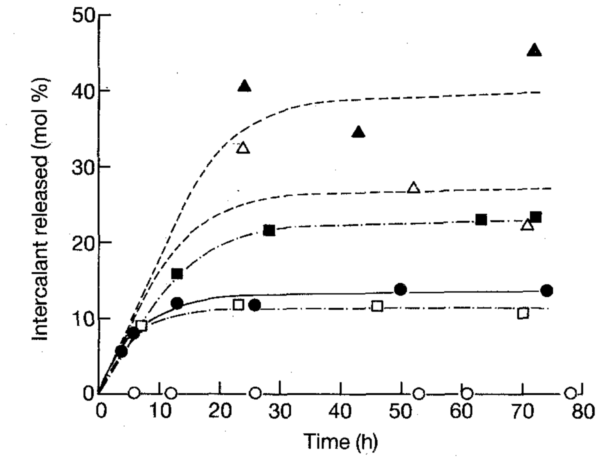

*Figure 6* Relation between soaking time and amount of ammonium ion (mol %) released: -W and -S mean water and 3 % $H_2SO_4$, respectively. (○) AS-W, (●) AS-S, (□) ES-W, (■) ES-S, (△) BS-W. (▲) BS-S.

Fig. 6 shows the results of the elution tests. BS showed some scatter. The ammonium ion was eluted from the complex more rapidly into 3 % $H_2SO_4$ than into deionized water. The elution was finished within 1 day in both solutions. More than 80 % of the aromatic ammonium ions in ES and AS remained after the elution.

## 4. Discussion

The formation of complexes was clearly confirmed by expansion of the interlayer spacing after ion-exchange reaction (Fig. 3). Based on the carbon content in Table I, ca.90 % of the CEC of the clay mineral was replaced by ammonium cation in many cases. The resultant complexes showed antimicrobial activities against both *S. aureus* and *E. coli*. It is concluded that an antimicrobial clay mineral–ammonium cation complex is readily prepared by the cation-exchange reaction technique.

Another interest is the elution rate of the ammonium ion in the complex into the solution. In order to maintain antimicrobial activity for a long time, the ammonium ion must elute slowly (a slow-release technique). As can be seen in Fig. 6, the elution of ammonium ion finished within 1 day with the retention of a large portion of ammonium ion, especially of aromatic ammonium ion, indicating a high antimicrobial durability.

Zeolite containing $Ag^+$ ion is commercially available at present [1]. Differently from zeolite, the complexes prepared in this work were flaky particles which were not changed after heating to 250 °C. This characteristic shape is suitable for preparing films and coatings. A trial to use clay mineral as a reinforcement material of resin is now in progress [7, 8]. Therefore the antimicrobial clay–ammonium complex-reinforced resin may exhibit not only high mechanical strength but also antimicrobial activity. The resin is usually blended with the reinforcement material at lower than 250 °C. Some of the complexes prepared exhibited antimicrobial activity after heating to 250 °C so that they may be available for this purpose.

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