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# Factors affecting the Adsorption of Chlorhexidine Gluconate by Hydroxyapatite[1,2]

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In order to assess the suitability of chlorhexidine for dental use, we investigated the effects of various factors such as temperature, pH, inorganic ions ($F^-$, $Ca^{2+}$, $Sr^{2+}$, $Na^+$) and polymers (mucin, bovine serum albumin, polyvinylpyrrolidone) on the adsorption of chlorhexidine gluconate (CHG) from aqueous solution by hydroxyapatite (HAP). The adsorption isotherms were of Langmuir type at low equilibrium concentration. The amount adsorbed was of the order of $10^{-7}$ mol/g, and was a little higher at 10° than at 37°. With the addition of inorganic ions such as $Ca^{2+}$, $Sr^{2+}$ and $Na^+$, the amount of CHG adsorbed decreased. These results indicated that the adsorption involves electrostatic forces, and is a physical adsorption. Fluoride had an interesting effect on the adsorption. When the concentration of $F^-$ was low (0.01 M), the amount adsorbed was higher, while when the concentration was high (0.1 and 0.25 M), the amount adsorbed was lower. This phenomenon might be a result of the formation of $CaF_2$ on the surface of HAP. Polymers such as mucin and bovine serum albumin increased the adsorption of CHG by HAP. This might be due to the strong binding of CHG with these polymers. On the other hand, polyvinylpyrrolidone had no significant effect on the adsorption.

**Keywords**——chlorhexidine gluconate; hydroxyapatite; adsorption; Langmuir's equation; temperature; pH; inorganic ions; mucin; bovine serum albumin; polyvinylpyrrolidone

Many theories have been presented concerning the mechanism of caries formation, that is, acid decalcification, protein dissolution, sulfatase action, protein dissolution-chelation and so on.[3] Among these theories, the acid decalcification theory has been supported by many researchers. According to this theory, hydrocarbons in food residues remaining on the surface of teeth are fermented by bacteria present in the mouth, and the resulting acids attack inorganic substances of the enamel surface, leading to the formation of caries.

Recently, Löe *et al.* reported[4] that a mouth-wash containing chlorhexidine prevented the growth of dental plaque and calculus, which are major factors in the formation of caries, as a result of its antimicrobial activity against *Streptococcus mutans*. There are several reports concerning the effects of chlorhexidine on dental plaque, dental calculus, gingivitis, and so on.[5-10] However, there are very few reports on the prevention of caries by means of drugs in the pharmaceutical field. In the present study, we attempted to investigate the effects of various factors such as temperature, pH, inorganic ions and polymers on the adsorption of chlorhexidine gluconate (CHG) by hydroxyapatite (HAP) in order to clarify the mechanism of adsorption.

## Experimental

**Materials**——An aqueous solution of 20% chlorhexidine gluconate (CHG)[11] was supplied by Sumitomo Chemical Ltd. Very pure hydroxyapatite (HAP) $Ca_{10}(PO_4)_6(OH)_2$ was purchased from Seikagaku Kogyo Ltd. The powder X-ray diffraction pattern and infrared (IR) absorption spectrum of HAP were identical with those of powdered human enamel reported by Wei *et al.*[12] The molar ratio of calcium to phosphorus was 1.57. HAP was used after passage through a 200-mesh sieve. Polyvinylpyrrolidone K-90 (PVP) and gastric mucin were purchased from Wako Chemicals Ltd., and used without further purification. Bovine serum albumin powder, fraction V (BSA), was purchased from Sanko Junyaku Ltd. Other chemicals used were of reagent grade.

**Batch Procedures for Determination of the Amount of Chlorhexidine Gluconate Adsorbed by Hydroxyapatite**——Four hundred mg of HAP and 5 ml of $2.14\times10^{-4}$—$1.00\times10^{-2}$ M CHG in aqueous solution containing an appropriate concentration of an additive, if necessary, were put into a 10 ml test tube covered with aluminum foil and kept for 24 hr at 10° and 37°. The suspended sample was centrifuged at 3000 rpm for 10 min, and the concentration of CHG in the clear supernatant was determined. The adsorption was found to reach equilibrium after 24 hr, and thus the sampling was done at 24 hr after incubation.

**Quantitative Determination of Chlorhexidine Gluconate**——CHG was determined by the ultraviolet (UV) absorption method at 257 nm using a Hitachi 124 spectrophotometer.

## Results and Discussion

### Adsorption Isotherms and Effects of Temperature and pH on Adsorption

Figure 1 shows adsorption isotherms of CHG by HAP from aqueous solution at 10° and 37°. The amount adsorbed at 10° was a little higher than that at 37°; this indicates that the adsorption enthalpy was low and that the adsorption is a physical adsorption. The adsorption isotherm at equilibrium concentrations higher than $1.5\times10^{-3}$ M were of multi-layer adsorption type. The adsorption isotherms at lower equilibrium concentrations were of Langmuir type,

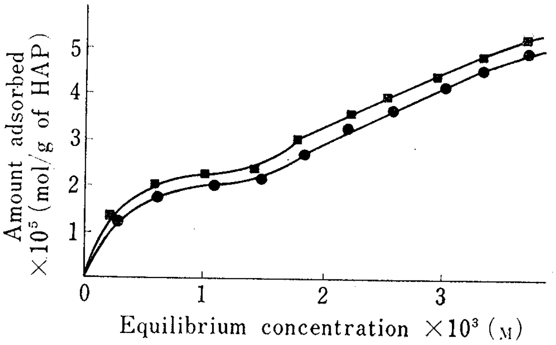

Fig. 1. Adsorption Isotherms of Chlorhexidine Gluconate (CHG) on HAP from Distilled Water at 37° and 10°

-●-37°; -■- 10°.

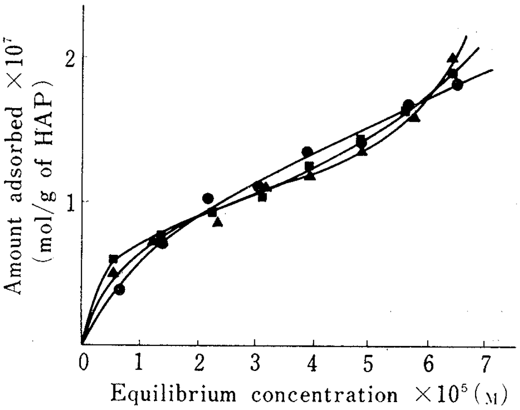

Fig. 2. Effect of pH of Phosphate Buffer Solution on the Adsorption of Chlorhexidine Gluconate by HAP at 37°

-■- pH 6.0; -●- pH 7.0; -▲- pH 8.0.

Table I. Effect of Inorganic Ions on the Adsorption of CHG by HAP from Aqueous Solution at 37°

| Additive | | $a\times10^7$ | Ratio |
|---|---|---|---|
| No additive | | 4.15 | 1.00 |
| NaF | 0.01 M | 4.51 | 1.09 |
| | 0.10 M | 2.83 | 0.682 |
| | 0.25 M | 1.76 | 0.424 |
| $CaCl_2$ | 0.001 M | 3.07 | 0.740 |
| | 0.01 M | 0.41 | 0.10 |
| | 0.10 M | 0.59 | 0.14 |
| $SrCl_2$ | 0.001 M | 3.04 | 0.733 |
| | 0.01 M | 0.43 | 0.10 |
| | 0.10 M | 0.35 | 0.084 |
| NaCl | 0.01 M | 1.71 | 0.412 |
| | 0.10 M | 0.81 | 0.20 |

*a*: the amount of CHG adsorbed when the entire surface is covered by a monolayer (mol/g).

and they were analyzed according to Langmuir's equation to determine the amount adsorbed.[13] The amount adsorbed was of the order of $10^{-7}$ mol/g.

Figure 2 shows the effect of pH on the adsorption of CHG by HAP. There was not much difference in the adsorption curves in the pH range from 6.0 to 8.0. The surface of HAP becomes unstable and dissolves at pH below 5.0. Therefore, adsorption experiments could not be carried out at pH below 6.0.

### Effect of Inorganic Ions on Adsorption

Table I shows the effects of various inorganic ions on the amount adsorbed as calculated by means of Langmuir's equation. The amount adsorbed increased a little with the addition of 0.01 M NaF, while it decreased at higher concentrations of NaF. This might be interpreted as follows: when the concentration of NaF was low (0.01 M), fluoride ions were adsorbed on HAP and the negative charges on the surface of HAP increased. Rölla *et al.* reported[14] that the amount of protamine, a basic amino acid, adsorbed by HAP increased upon treatment of HAP with 1 mM NaF, but the amount adsorbed decreased upon treatment with more than 0.02 M NaF. When the concentration of fluoride is more than 0.02 M, $CaF_2$ is reported to be formed on the surface of HAP as follows:[3]

$$Ca_{10}(PO_4)_6(OH)_2 + 20NaF \longrightarrow CaF_2 + 6Na_3PO_4 + 2NaOH$$

$CaF_2$ was found not to adsorb CHG under the same conditions as HAP. Therefore, in this case also, $CaF_2$ was presumably formed on the surface of HAP at higher concentrations of NaF, and the adsorbed amount of CHG decreased. To investigate the effect of $Ca^{2+}$, which is one of components of HAP, $CaCl_2$ was added to the solution. As shown in Table I, the amount adsorbed decreased significantly with the addition of $CaCl_2$. This result is consistent with the results on the adsorption of protamine by HAP[14] and of the cetylpyridinium ion by HAP.[15] The reason why the amount of CHG adsorbed by HAP decreased in the presence of $CaCl_2$ was considered to be that positive charges on the surface of HAP increased due to adsorption of $Ca^{2+}$ and chlorhexidine ions were repelled from the surface of HAP.

The adsorbed amount of CHG also decreased with the addition of $SrCl_2$ and NaCl, as shown in Table I. Nakagaki *et al.* reported[16] that various ions such as $Mg^{2+}$, $Ba^{2+}$ or $Ca^{2+}$ decreased the amount of cetylpyridinium ions adsorbed by HAP. According to them, these ions were preferentially adsorbed on the surface of HAP and thus cetylpyridinium ions were repelled from the surface of HAP. The effects of $SrCl_2$ and NaCl in this experiment might be interpreted in the same way as the results of Nakagaki *et al.*

Since $CaCl_2$, $SrCl_2$ and NaCl decreased the amount of CHG adsorbed by HAP, the adsorption might involve electrostatic forces (physical adsorption).

TABLE II. Effect of Polymers on the Adsorption of CHG by HAP from 1/30 M Phosphate Buffer Solution at pH 7.0 and at 37°

| Additive | $a \times 10^7$ | Ratio |
|---|---|---|
| No additive | 2.45 | 1.00 |
| Mucin 0.001% | 3.16 | 1.29 |
| 0.002% | 3.22 | 1.31 |
| 0.004% | 3.64 | 1.49 |
| 0.008% | 4.48 | 1.83 |
| PVP 0.5% | 2.57 | 1.05 |
| 1.0% | 2.01 | 0.820 |
| 2.0% | 3.18 | 1.30 |

*a*: the amount of CHG adsorbed when the entire surface is covered by a monolayer (mol/g).

## Effect of Polymers on Adsorption

Table II shows the effects of various polymers on the adsorption of CHG by HAP. Mucin, which is a main component of mucilaginous substances in the saliva, substantially increased the amount of CHG adsorbed by HAP. The main components of mucin are acidic sialoglycoproteins. The teeth or tissues in the human mouth are covered with mucin,[17] and proteins in the saliva were reported to be adsorbed by HAP.[18,19] Schiött *et al.* also reported[20] that chlorhexidine was bound to pellicles on the surface of teeth and to proteins in the saliva. Therefore, the increase in the amount adsorbed in the presence of mucin might be interpreted as follows: sialoglycoproteins of mucin were adsorbed by HAP at first, and then chlorhexidine ions were bound to the sialoglycoproteins on the surface of HAP.

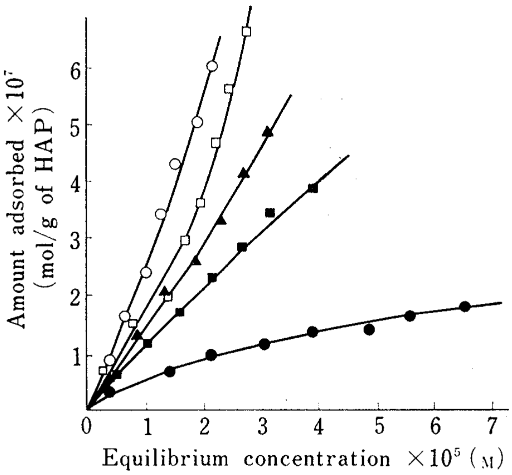

Fig. 3. Effect of Bovine Serum Albumin on the Adsorption of Chlorhexidine Gluconate from 1/30 M Phosphate Buffer Solution (pH 7.0) by HAP at 37°

BSA; -●- 0%, -■- 0.05%, -▲- 0.10%, -□- 0.20%, -○- 0.40%.

Figure 3 shows the effect of BSA on the adsorption of CHG by HAP. Adsorption isotherms deviated from Langmuir's adsorption type in the presence of BSA and the amount adsorbed of CHG increased with the concentration of BSA. Proteins such as BSA were reported to be adsorbed easily by HAP,[21,22] and chlorhexidine can bind to these proteins.[18,19] Therefore, a large part of adsorbed CHG might be bound to BSA which was itself already adsorbed on the surface of HAP. This might inactivate the antibacterial activity of CHG, but further investigations are desirable.

As shown in Table II, PVP, which is a typical water-soluble polymer, unexpectedly had no significant effect on the adsorption in this concentration range. The molecular weight of PVP used was about 360000, and PVP was expected to have some effect on the adsorption. The reason for this result is not clear and should be studied later.

There have been very few pharmaceutical reports concerning dental drugs, and these basic *in vitro* studies may be useful in connection with the practical use of CHG as a preventive and therapeutic agent in the dental field.

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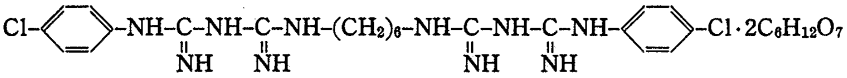

(mol. wt. = 897.8; Without gluconate, mol. wt. = 505.5)

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