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Adsorption of Nicotinic and Isonicotinic Acid Derivatives by Hydroxyapatite from Aqueous Solutions^{1,2)}

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Adsorption of nicotinic and isonicotinic acid derivatives from aqueous solution by hydroxyapatite (HAP), the main component of tooth enamel, was investigated, and the effects of various factors on the adsorption were studied. These nicotinic and isonicotinic acid derivatives are similar in chemical structure to nicotine which is contained in tobacco, and which might be adsorbed by teeth during smoking.

The amounts adsorbed at pH 6.0 and 7.0 at 37°C were determined by measurement of concentration differences (before and after adsorption). Six compounds, that is, nicotinic acid, isonicotinic acid, nicotinamide, isonicotinamide, nicotinic acid hydrazide, and isonicotinic acid hydrazide were used as drugs. All the drugs used were determined by ultraviolet absorption spectrophotometry.

Adsorption isotherms were essentially of almost Langmuir type for every drug. The order of the amount adsorbed was 10^{-6} mol/g, and this value was smaller than that of chlorhexidine adsorption by HAP reported previously. It is suggested that the size of adsorbate molecules affects the adsorbability. The amounts adsorbed of isonicotinic acid derivatives were larger than those of the corresponding nicotinic acid derivatives. The order of the amounts adsorbed for both nicotinic and isonicotinic acid derivatives was as follows: acid > amide > hydrazide. This order was opposite to that of the solubility. The adsorption behavior of HAP was different from that of carbon black, a hydrophobic adsorbent. The amount adsorbed decreased upon addition of sodium chloride and sodium fluoride.

Keywords—adsorption; hydroxyapatite; nicotinic acid derivatives; isonicotinic acid derivatives; molecular weight; solubility; inorganic ions

The effects of various factors on the adsorption of chlorhexidine, which has antibacterial activity against *Streptococcus mutans*, by hydroxyapatite (HAP), the main component of tooth enamel, has already been studied.¹⁾ As part of a series of studies on adsorption by HAP, this study was intended to investigate the effects of various factors such as pH, molecular weight, solubility and inorganic ions on the adsorption of nicotinic and isonicotinic acid derivatives by HAP from aqueous solution. Nicotinic and isonicotinic acid derivatives are similar in chemical structure to nicotine, which is contained in tobacco and are nontoxic compared with nicotine. Although tobacco is believed to be harmful to health because of the inhalation of carcinogenic substances into the lungs, it is possible that some nicotine might be adsorbed by the teeth during smoking, and a fundamental study on this point was considered to be useful.

Experimental

Materials—Very pure hydroxyapatite (HAP) was purchased from Seikagaku Kogyo Ltd. HAP was used after passage through a 200 mesh sieve as described in the previous paper.¹⁾ Other chemicals used were of analytical grade.

Procedures for Determination of the Amount adsorbed—Adsorption procedures were the same as reported in the previous paper.¹⁾ All the drugs were determined by the ultraviolet absorption method using a Hitachi 124 spectrophotometer.

Results and Discussion

Figure 1 shows the adsorption isotherms of nicotinic acid derivatives by HAP at pH 6.0 and 7.0. The adsorption isotherms were of Langmuir type, and there was no large difference between pH 6.0 and 7.0. The amount of nicotinic acid adsorbed was the largest, and the difference between nicotinamide and nicotinic acid hydrazide was small. Figure 2 shows the adsorption isotherms of isonicotinic acid derivatives by HAP at pH 6.0 and 7.0. The amount of isonicotinic acid adsorbed was the largest among the three, but it was small compared with the adsorption of nicotinic acid derivatives. The position of substituent groups may have an effect on the adsorption. The amount of isonicotinic acid hydrazide adsorbed was almost zero at both pHs.

The order of the amount adsorbed was 10^{-6} mol/g, while that of chlorhexidine gluconate was 10^{-7} mol/g.¹⁾ The molecular weight of chlorhexidine is 506, while those of the drugs used in this study are 122–137. Thus, the size of molecules may affect the adsorption.

Fig. 1. Adsorption Isotherms of Nicotinic Acid Derivatives by Hydroxyapatite from 1/30 M Phosphate Buffer Solution (pH 6.0 and 7.0) at 37 °C

○: nicotinic acid, △: nicotinamide, □: nicotinic acid hydrazide.

Fig. 2. Adsorption Isotherms of Isonicotinic Acid Derivatives by Hydroxyapatite from 1/30 M Phosphate Buffer Solution (pH 6.0 and 7.0) at 37 °C

●: isonicotinic acid, ▲: isonicotinamide, ■: isonicotinic acid hydrazide.

Kresak *et al.*³⁾ reported that the order of the amount adsorbed of several amino acids by HAP was 10^{-5} mol/g, and the amount adsorbed was correlated with the largest molecular chain-length, estimated by means of a geometrical model. The molecular weights of nicotinic and isonicotinic acid derivatives are not very different from those of the amino acids, but the amounts adsorbed were quite different. This might be due to the effect of the carboxylic acid groups of the amino acids, or to some difference of HAP used in the two experiments.

TABLE I. The Amount adsorbed on HAP ^{a)} and the Solubility of Nicotinic and Isonicotinic Acid Derivatives at 37 °C

Compounds	<i>a</i> (mol/g) at pH 6.0	<i>a</i> (mol/g) at pH 7.0	Solubility at pH 7.0 (M)
Nicotinic acid	1.59×10^{-5}	1.69×10^{-5}	0.174
Nicotinamide	5.24×10^{-6}	4.48×10^{-6}	4.34
Nicotinic acid hydrazide	3.33×10^{-6}	4.42×10^{-6}	1.64
Isonicotinic acid	1.87×10^{-6}	3.19×10^{-6}	0.0812
Isonicotinamide	1.43×10^{-6}	1.83×10^{-6}	1.57
Isonicotinic acid hydrazide	0	0	1.15

Table I shows the amounts adsorbed at pH 6.0 and 7.0, and also the solubilities at pH 7.0 and at 37°C. The solubilities of nicotinic and isonicotinic acid are small and the amounts adsorbed are large compared with those of the other drugs, as expected.

Further, the adsorption by carbon black (CB) was investigated in the same way as in the previous report.⁴⁾ The contribution of hydrophobic bonding is usually predominant in adsorption by hydrophobic adsorbents such as CB,⁴⁾ and several physical constants were shown to be related to adsorbability by CB.⁵⁾ As shown in Fig. 3, the order of the amount adsorbed was hydrazide > amide > acid for both nicotinic and isonicotinic acid derivatives.

Fig. 3. Adsorption Isotherms of Nicotinic and Isonicotinic Acid Derivatives by Carbon Black from 1/30 M Phosphate Buffer Solution (pH 7.0) at 37 °C

○: nicotinic acid, △: nicotinamide, □: nicotinic acid hydrazide.
●: isonicotinic acid, ▲: isonicotinamide, ■: isonicotinic acid hydrazide.

To investigate the effects of various inorganic ions on the adsorption, sodium chloride and sodium fluoride were added to the adsorption medium. As expected,¹⁾ the amount adsorbed decreased with the addition of these inorganic salts.

As mentioned above, the adsorption properties of nicotinic and isonicotinic acid derivatives were complicated, because the electric charge state of the surface of HAP is very am-

biguous, and also the "hydrophobicity" or organicity/inorganicity value⁶⁾ of the drugs used in this study is not clear. The pK_a values of nicotinic acid and isonicotinic acid are 4.85 and 4.96,⁷⁾ respectively, and more than 91% of these drugs exists in the dissociated state at pH 6.0 and 7.0. However, the pK_a of nicotinamide is 3.35,⁷⁾ and it exists almost entirely in the undissociated state at pH 6.0 and 7.0. The pK_a of nicotine is 6.16.⁸⁾ Further work is necessary on the relation between adsorbability and pK_a .

Nicotine is very poisonous and could not be used in these experiments, but from the results obtained in this study, the amount of nicotine adsorbed by HAP might be small because of its larger molecular weight and solubility in water.

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References and Notes

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Kinetics of Digestive Enzyme Stability in the Solid State. II.¹⁾ Quantitative Prediction of Enzyme Inactivation

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The procedure for predicting digestive enzyme stability in the solid state was further investigated by utilizing the Weibull distribution function, which needs two mathematically meaningful parameters, m and k . These parameters could be estimated by graphic calculation. If the parameter m is independent of temperature, the Arrhenius plots of $(1/m)\ln k$ versus $1/T$ are linear since $k^{1/m}$ is proportional to the inactivation rate constant. The parameter k could be estimated by extrapolating to the desired temperature in the Arrhenius plots and the activation energy could be determined from the slope of this line. These parameters, m and k , made it possible to predict the inactivation ratio of enzymes in the solid state. By comparing the predicted value of the inactivation ratio with the observed value under controlled conditions, it was shown that the proposed method is accurate and useful for studies on enzyme stability in the solid state.

Keywords—Weibull probability paper; digestive enzyme; prediction of stability in solid state; Arrhenius plots; stress test

Many reports on the stability of solid drugs have been published,²⁻⁵⁾ and the degradation ratio of solid drugs during storage could be predicted kinetically by means of the stress test,