Correlation between the Adsorption Tendency of Cationic Disinfectant onto a Synthetic Multibilayer and Its Minimum Inhibitory Concentration

Masahiro MAKINO,*[,a] Shin OHTA,[b] and Hiroshi ZENDA[b]

Department of Research and Development, Nicca Chemical Co., Ltd.,[a] 4–23–1 Bunkyo, Fukui, Fukui 910, Japan and Department of Pharmacy, Shinshu University Hospital,[b] 3–1–1 Asahi, Matsumoto, Nagano 390, Japan.
Received August 31, 1994; accepted December 9, 1994

Minimum inhibitory concentration (the lowest concentration able to inhibit growth of microorganisms after 48 h contact) could be useful as a measure of bactericidal activity of cationic disinfectants. The relation between the minimum inhibitory concentration (μg/ml) of cationic disinfectants against gram-negative microorganisms and the adsorption tendency of these compounds onto a lipid bilayer was therefore studied using a quartz crystal microbalance (QCM) coated with synthetic multibilayer film. The adsorbed amounts of these compounds were obtained from the frequency decrease of the QCM in aqueous solutions. The calculated partition coefficients showed a good correlation with the minimum inhibitory concentrations of cationic disinfectants against *E. coli* and *K. pneumoniae*, supporting the validity of this method for predicting minimum inhibitory concentration. The results suggest that effective antibacterial agents are only slightly absorbed, and may mostly penetrate through the lipid bilayer.

Key words minimum inhibitory concentration; bilayer; lipid; quartz crystal microbalance; adsorption; disinfectant

In a previous paper,[1] we have shown that the relationship between the minimum inhibitory concentration (*MIC*) of cationic disinfectants against *E. coli* and the ζ-potential of *E. coli* in disinfectant solutions is linear with a correlation coefficient of $r=0.989$. This supported the idea that synthetic cationic surfactants (**1, 2, 3**) with antibacterial activity penetrate the cell membrane.

The frequency of a quartz crystal microbalance (QCM) is known to decrease linearly with increase in the amount adsorbed on the electrode. Sauerbrey[2] has proposed a relationship between the amount adsorbed on the QCM electrode and the frequency decrease of the QCM, which leads to the following Eq. 1:

$$\Delta F=\frac{-2F_0^2}{A\sqrt{(\rho_q\mu_q)}}\Delta m \qquad (1)$$

ΔF: measured frequency shift (Hz)
F_0: parent frequency of the QCM (9×10^6 Hz)
Δm: mass change (g)
A: electrode area ($0.196\,cm^2$)
ρ_q: density of quartz ($2.65\,g\,cm^{-3}$)
μ_q: shear modulus (2.95×10^{11} dyne cm^{-2})

Okahata and coworkers[3-6] found a good linear correlation ($r=0.84$) between the Draize score (irritation of the rabbit cornea) of a surfactant and the partition coefficient to synthetic multibilayer films on the QCM. They concluded that hydrophobic surfactants are easily adsorbed on a lipid bilayer and thus cause severe eye irritation, and they suggested this method as an alternative to the Draize test.

If we can confirm a linear relationship between amount adsorbed on a lipid bilayer in the QCM test and bactericidal activity of disinfectants, QCM could be used as a safe, easy and economical screening method for effective disinfectants. Furthermore, on the basis of the relationship between the increase or decrease of amount of disinfectant adsorbed on a lipid bilayer and the increase of bactericidal activity, we should be able to establish whether a disinfectant with strong bactericidal activity penetrates through the lipid bilayer or is adsorbed on it.

For these reasons, we examined the adsorption tendency of a cationic disinfectant using a $2C_{18}N^+2C_1/PSS^-$ multibilayer-coated QCM sensor as described by Okahata *et al.* (Fig. 1).[3-6] Calibration of the QCM used in our experiments showed that a frequency change of 1 Hz corresponded to a mass increase of 1.05 ± 0.01 ng on the electrode of the QCM. The following Eq. 2 was obtained for the AT-cut shear of the QCM.

$$\Delta m=(1.05\pm0.01)\times10^{-9}\Delta F \qquad (2)$$

When a disinfectant was added to an aqueous solution into which the QCM had been placed (Fig. 1), the amount adsorbed on the multibilayer film-coated QCM could be determined easily from the measured frequency shift. However, the amount adsorbed on the multibilayer film-coated QCM changes depending on the concentration of disinfectant added or the mass of the multibilayer film on the QCM. Therefore, it is preferable to estimate the adsorption tendency in terms of the partition coefficient. The partition coefficient (P) is given by the following equation.

$$P=\frac{C_1}{C_0}$$

C_1: amount adsorbed on the multibilayer film (g)
C_0: mass in the aqueous solution (g)

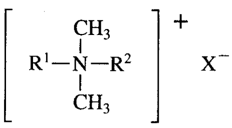
We report here the correlation between the partition coefficients obtained from the adsorption of cationic disinfectants (Table 1) on synthetic multibilayer film and the *MIC* values of these disinfectants against *E. coli* and *K. pneumoniae* (Table 2).

Results and Discussion

Adsorption of Cationic Disinfectant onto a Multibilayer Film We studied the adsorption of benzalkonium chloride (BAC), as a standard cationic disinfectant, and

* To whom correspondence should be addressed.

Table 1. Compounds Tested



Compound	R^1	R^2	X	Mole ratio
1a	C_8H_{17}	C_2H_4OH	$BuO-P(=O)(O^-)-O^-$ / $BuO-P(=O)(BuO)-O^-$	Monoester/diester = 49/51
1b	$C_{10}H_{21}$	C_2H_4OH		
1c	$C_{12}H_{25}$	C_2H_4OH		
1d	$C_{14}H_{29}$	C_2H_4OH		
1e	$C_{16}H_{33}$	C_2H_4OH		
1f	$C_{18}H_{37}$	C_2H_4OH		
2c	$C_{12}H_{25}$	$CH_2CH(OH)CH_2O-C_6H_5$	$BuO-P(=O)(O^-)-O^-$ / $BuO-P(=O)(BuO)-O^-$	Monoester/diester = 49/51
2d	$C_{14}H_{29}$	$CH_2CH(OH)CH_2O-C_6H_5$		
2e	$C_{16}H_{33}$	$CH_2CH(OH)CH_2O-C_6H_5$		
3c	$C_{12}H_{25}$	$CH_2CH(OH)CH_2O-C_6H_5$	Cl^-	
3d	$C_{14}H_{29}$	$CH_2CH(OH)CH_2O-C_6H_5$		
3e	$C_{16}H_{33}$	$CH_2CH(OH)CH_2O-C_6H_5$		

Table 2. *MIC* of Cationic Disinfectants against Two Organisms

Organism	No.[a]	*MIC* (μg/ml)												
		BAC	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**2c**	**2d**	**2e**	**3c**	**3d**	**3e**
E. coli	92411	200	>1600	800	200	400	800	>1600	400	800	1600	400	800	1600
K. pneumoniae	92511	200	>1600	1600	800	400	800	>1600	400	1600	≧1600	200	400	≧1600

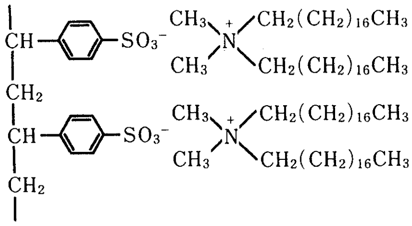
The *MIC* is the lowest concentration that inhibits visible growth after 48 h of incubation at 37 °C. The *MIC* was determined by the agar dilution method using Muller-Hinton Agar (Nissui Seiyaku Co., Ltd.). BAC, benzalkonium chloride. *a*) The classification number of the organism is that assigned by Shinshu University Hospital.

twelve synthetic compounds (**1a**—**f**, **2c**—**e**, and **3c**—**e**).[7,8] Figure 2 shows a typical frequency response of the multibilayer film-coated QCM to the addition of cationic disinfectant in aqueous solution at 45 °C. After the cationic disinfectant is injected into distilled water, the frequency decreases immediately, and a few minutes later, the adsorption reaches equilibrium. The adsorbed amounts of these cationic disinfectants and *P* in the multibilayer film on the QCM were obtained. The values of the logarithm of the partition coefficients (log *P*) were 3.20 (**1b**), 2.90 (**1c**), 3.11 (**1d**), 3.22 (**1e**), 3.55 (**1f**), 3.28 (**2c**), 3.73 (**2d**), 3.79 (**2e**), 3.07 (**3c**), 3.40 (**3d**), 3.45 (**3e**), and 2.84 (BAC).

The adsorption tendency of these cationic disinfectants into the multibilayer films increased linearly in the order **1c**, **1d**, **1e**, and **1f**, with the exception of **1b**, in the homologues of **1**; in the order **2c**, **2d**, and **2e** in the homologues of **2**; and in the order **3c**, **3d**, and **3e** in the homologues of **3**. These results showed that longer alkyl chain groups and/or larger hydrophobic compounds were easily adsorbed by the lipid layer. The reason why **1b**, which has a shorter alkyl chain, was strongly adsorbed is unknown. There was no correlation between the adsorbed amount (C_1) and the *MIC*.

Correlation between log *MIC* and log *P* of Disinfectants Figures 3 and 4 show plots of the logarithm of *MIC* against *E. coli vs.* log *P*. The relationship for the homologues of **1** was essentially linear with a correlation coefficient of $r = 0.963$ at the 1% level of significance. Compounds **1**, **2**, and **3**, including the BAC type, had a good correlation ($r = 0.815$) at the 1% level of significance. Similarly, a plot of the logarithm of the *MIC* against *K. pneumoniae vs.* log *P* for the homologues of **1** gave a correlation coefficient of $r = 0.744$ at the 5% level of significance. For compounds **1**, **2**, and **3**, including the BAC type, the correlation coefficient was $r = 0.626$ at the 5% level of significance. These four correlation coefficients, taken together, showed a statistically significant linear relationship at the 5% level of significance.

These results showed that larger hydrophobic compounds are adsorbed onto a lipid bilayer to a greater ex-



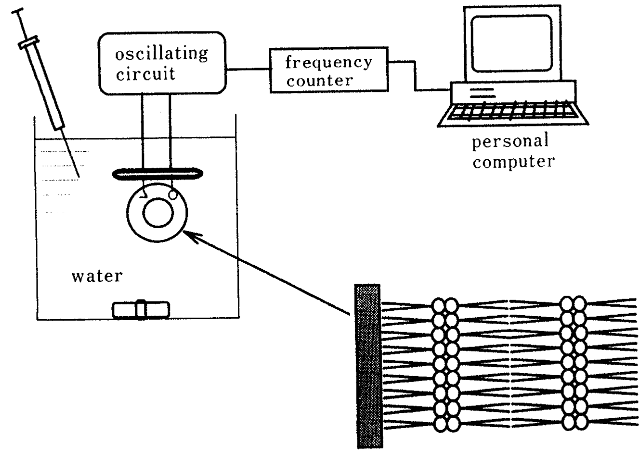


Fig. 1. Apparatus for Frequency Measurements of Multibilayer Film-Coated QCM

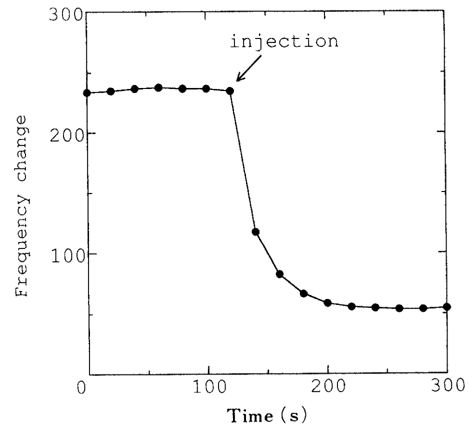


Fig. 2. Typical Frequency Response of the $2C_{18}N^{+}2C_{1}/PSS^{-}$ Coated QCM to the Injection of a Cationic Disinfectant in Water at 45°C

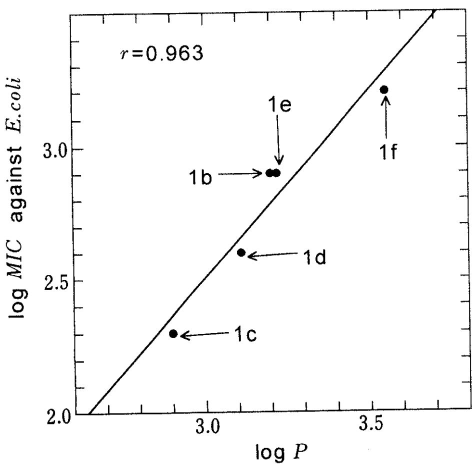


Fig. 3. Relationship between log *P* and log *MIC* against *E. coli*

Here, *P* is the partition coefficient in the liquid matrix measured on QCM at 45°C. *r*, correlation coefficient.

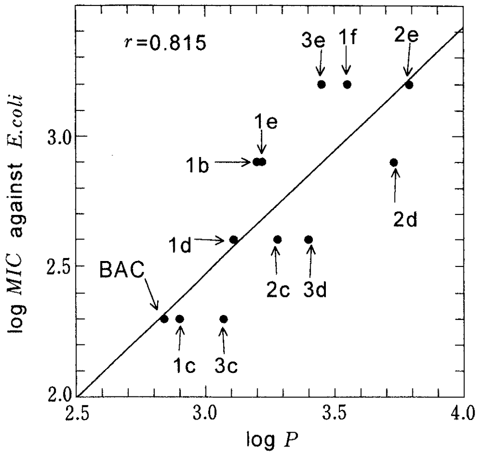


Fig. 4. Relationship between log *P* and log *MIC* against *E. coli*

Here, *P* is the partition coefficient in the liquid matrix measured on QCM at 45°C. *r*, correlation coefficient.

tent than hydrophilic compounds, and hydrophilic compounds have greater bactericidal activity than hydrophobic compounds. This idea suggested that the compounds with greater bactericidal activity were accepted by receptor sites on cell membranes without a lipid bilayer, or penetrated through the lipid bilayer and diffused intracellularly.

Conclusion

We investigated the relationship between *MIC* of cationic disinfectants against microorganisms and the adsorption tendency of cationic disinfectants onto a lipid bilayer-coated QCM. We found a statistically significant linear relationship at the 5% level of significance between the logarithm of the *MIC* (log *MIC*) and the logarithm *P* of partition coefficient (log *P*). This result suggested that QCM would be a useful screening method of cationic bactericidal agents.

It appears that effective antibacterial agents (BAC, **1c**, **2c**, and **3c**) are only slightly adsorbed by and mostly penetrate through the lipid bilayer, then they diffuse intracellularly, or are bound at receptor sites within the cell membrane. Conversely, ineffective antibacterial agents (**1f**, **2e**, and **3e**) are mainly adsorbed onto the lipid layer, and thus can not penetrate through the lipid bilayer or be accepted at receptor sites in the cell membrane.

Experimental

Chemicals The compounds (**1**, **2** and **3**) were synthesized as described in the previous study.[1] The alkyl groups were octyl (C_8), decyl (C_{10}), dodecyl (C_{12}), tetradecyl (C_{14}), hexadecyl (C_{16}), and octadecyl (C_{18}). Benzalkonium chloride (BAC) (long alkyl chain components, C_{12}: 83% and C_{14}: 17%) was used as a standard for comparison.

Antibacterial Assay *Escherichia coli* and *Klebsiella pneumoniae* obtained from a patient at Shinshu University hospital were used for an antibacterial assay. The *MIC* values against *E. coli* and *K. pneumoniae* were measured by agar dilution, using nutrient agar (Nissui) plates.

Apparatus As described by Okahata *et al.*,[3-6] the apparatus consisted of a 9 MHz, AT-cut quartz crystal plate (0.568 cm^2), on each side of which a 0.196 cm^2 silver electrode had been deposited, with a custom-made oscillator designed to drive the quartz at its resonance frequency. The QCM was driven at 5V d.c. and the frequency of the vibrating quartz was measured with an Iwatsu frequency counter (SC7201 model) attached to a microcomputer system (NEC model PC9801).

Multibilayer Film-Coated Quartz Crystal Microbalance Sensor A polyion complex-type multibilayer-immobilized film, $2C_{18}N^+2C_1/PSS^-$, was used. This film was prepared by mixing an aqueous dispersion of dioctadecyldimethylammonium bromide ($2C_{18}N^+2C_1Br^-$) and an aqueous solution of sodium poly(styrenesulfonate) (PPS^-Na^+) at 70 °C. The precipitate, $2C_{18}N^+2C_1/PSS^-$, was collected by filtration and dried. This complex was dissolved in chloroform, and coated on the electrodes of both sides of a QCM, which was then aged in hot water at 60 °C for 20 h. The coating mass was calculated from the decrease in frequency.

Frequency Measurements The QCM coated with $2C_{18}N^+2C_1/PSS^-$ multibilayer film was dipped in 60 ml of distilled water at 45 °C; 0.25 ml of 1.0% aqueous solution of a cationic disinfectant was injected into the water, with stirring. The frequency decrease that occurred with increasing mass adsorbed on the multibilayer film was followed by using a frequency counter connected to the microcomputer.

Partition Coefficient The partition coefficient (P) of the multibilayer film from the aqueous phase was calculated by dividing the amount adsorbed on the bilayer film (g) by the concentration in the aqueous phase (g).

References

1) Makino M., Ohta S., Zenda H., *Chem. Pharm. Bull.*, **42**, 2472 (1994).
2) Sauerbrey G. Z., *Z. Phys.*, **155**, 206 (1959).
3) Ebato H., Okahata Y., *Fragrance J.*, **8**, 92 (1990).
4) Okahata Y., Ebato H., Taguchi K., *J. Chem. Soc., Chem. Commun.*, **1987**, 1363.
5) Okahata Y., Ebato H., Taguchi K., *J. Chem. Soc., Chem. Commun.*, **1987**, 1365.
6) Okahata Y., Ye X., Shimizu A., Ebato H., *Thin Solid Films*, **180**, 51 (1989).
7) Makino M., Ohta S., Zenda H., *Yakugaku Zasshi*, **112**, 712 (1992).
8) Makino M., Ohta S., Zenda H., *Yakugaku Zasshi*, **114**, 73 (1992).