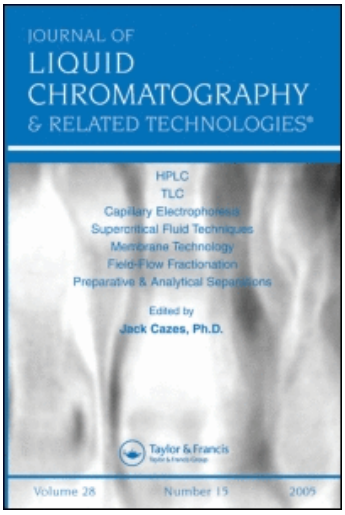

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QUANTITATIVE RETENTION ACTIVITY RELATIONSHIP OF QUINOLONES USING MICELLAR LIQUID CHROMATOGRAPHY

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ABSTRACT

This study investigated the quantitative retention activity relationship of quinolones by using micellar liquid chromatography. Using different types of surfactants, retention behaviors of quinolones were studied. Based on the retention data, obtained from different micellar conditions, a quantitative retention - activity relationship was applied to the representative bacteria*; E. coli, P. aeruginosa, and S. aureus*. In the case of *P. aeruginosa*, a linear relationship was observed for CTAB, and SDS. On the other hand, no relationship was found for any kinds of bacteria, when the retention data, obtained from Brij 35, and SB 3-10 were applied. This study also matches these relationships to the quinolone active site, DNA gyrase, and explains its action mechanism.

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INTRODUCTION

Many applications of the quantitative structure – activity relationship (QSAR) have been used to try to explain drug activity, or design a new drug. In the QSAR, the behavior of biologically active compounds can be viewed on the basis of the physiochemical parameters of those compounds. Hydrophobicity, and electronic and steric properties are usually used to relate bioactivity with structures. However, one of the limitations of the QSAR is its limited applicability to newly – synthesized compounds, for the structural descriptors of these compounds are not known. Especially, the determination of log P has some disadvantages when the traditional shake-flask method is used.

The quantitative retention activity relationship (QRAR) is one recent attempt to overcome these problems. Based on data from the tendency of retention parameters in different RPLC conditions, a simple retention model can be obtained and used to predict bioactivity. Furthermore, it can help provide information about pharmacokinetic properties. Recently, many pieces of research were reported on estimating hydrophobicity, bioactivity, and pharmacokinetic parameters under different RPLC conditions, by emulating the biomembrane or bilipid layer. Of the different types of chromatographic methods, micellar liquid chromatography (MLC) is one of the most useful fields in conducting a QRAR study.

Several advantages of using micellar liquid chromatography have been reported: 1) the retention behavior of compounds has been modeled well, 2) the hydrophobic stationary phase is assumed to be similar to biological membrane, and 3) micelles provide a hydrophobic, electrostatic interaction, making structures more similar to biomembranes . Breyer et. al reported a successful application of MLC to the QRAR.[1] They proved high correlations between the bioactivity of a group of 26 substituted phenols and retention factors in MLC. Gilabert et. al investigated the QSAR and QRAR of local anesthetics by MLC.[2] They found that a linear relationship exists between retention factor and the activity of local anesthetics, and identified the pharmacokinetic parameter.

Yang et.al. studied the influence of surfactant type and mixed micelles on the estimation of hydrophobicity and bioavailability, using micellar electrokinetic chromatography.[3] Important correlations were also observed between the bioactivity of corticosteroids and retention factor. Mallols et. al investigated both the quantitative retention - and structure - activity relationship of ionic and nonionic catecholamines by MLC.[4]

Benito et.al. proposed these relationships for barbiturates with different surfactants.[5] A few groups applied these relationships to quinolone, a kind of antibacterial agent, and tried to explain its action mechanism on representative bacteria. Martorell et. al established the influence of lipophilicity on the antibacterial activity of 22 fluoroquinolones and explained the influence of their electronic, steric, and topological properties on their hydrophobicity.[6] Bazile et.al

correlated the hydrophobicity of 11 fluoroquinolones with antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus*, *and Escherichia coli*.[7] Piddock et. al explained the mechanism of quinolone accumulation by those bacteria, respectively.[8] As far as we know, no report has been made on the study of QRAR on quinolones under different MLC conditions. Also, the quinolone action mechanism has not been explained by relating the character of surfactant with the active site of quinolones. This was the starting point of our study. Basically, we tried to investigate the retention behavior of quinolones in different micellar conditions. These retention data were applied to correlate the bioactivity of quinolones for *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Escherichia coli*. Finally, a quinolone action mechanism was suggested by relating the active site of a DNA gyrase inhibitor with the structure of quinolones.

EXPERIMENTAL

Instrumentation

The HPLC system was composed of a model 600 solvent delivery system (Waters, Milford, U.S.A), a model 600 UV detector (Waters, Milford, U.S.A), and a model 7725 Rheodyne injection valve with a 20 μL sample loop (Rheodyne, Cotati, CA, U.S.A). UV detection was performed at 276nm. The chromatograms were collected using an Autochro-win program (Younglin, Seoul, Korea). Chromatography was performed on a CAP-CELL PAK C_{18} column with dimensions of 250×4.6 mm i. d. (Shiseido, Tokyo, Japan). Column temperature was maintained at 40°C using a column oven (Samsung, Seoul, Korea). The void time was determined for each injection as the first perturbation in the chromatogram.

Chemicals

Micellar mobile phases were prepared by mixing 10% isopropanol with aqueous solutions of cetyltrimethylammonium bromide (CTAB, BDH, Poole, England), sodium dodecyl sulfate (SDS, SIGMA, St. Louis, U.S.A), N-dodecyl-N,N-dimethylammonium-3-propane-1-sulfonic acid (SB3-10, SIGMA, St. Louis, U.S.A), and polyoxoethylene dodecanol (Brij 35, Aldrich, Milwaukee, U.S.A), respectively. A characteristic of these surfactants is presented in Table 1. The pH of the micellar eluent was adjusted to 6.8 with phosphoric acid, so that a similar biological condition would be reproduced. The quinolone samples were obtained from Bayer Co. Their structures are depicted in Figure 1. Stock solutions of each quinolone were prepared by dissolving 1 mg of each compound in 10 mL of each

Table 1. The Surfactants Used in This Study

Surfactant	Type	CMC(M)[a]	Aggregation Number[b]
Cetyltrimethyl ammonium bromide (CTAB)	Cationic	0.0013	78
Sodium dodecyl sulfate (SDS)	Anionic	0.0081	62
Polyoxyethylene dodecanol (Brij 35)	Nonionic	0.0001	40
N-dodecyl-N,N-dimethylammonium 3-propane-1-sulfonic acid (SB 3-10)	Zwitterionic	0.0030	55

[a, b] Values from Ref. 9.
[a] Values for aqueous solution at 25°C.

surfactant used. The standard solutions were prepared by diluting the stock solutions with the same surfactant used in the mobile phase. Water was distilled and deionized in a Milli-Q system (Millipore, Bedford, MA, U.S.A). The mobile phase was filtered through 0.45μ nylon membranes, and degassed with an ultrasonicator for at least 12 hours.

RESULTS AND DISCUSSION

Retention Behavior of Quinolones Under Different Micellar Conditions

The retention behaviors of nine kinds of quinolones were investigated by varying the type and concentration of surfactants. The surfactants used were of a cationic CTAB and anionic type, SDS. Figure 2 shows the plot of k' vs. the concentration of each surfactant. The investigated range of surfactant concentration is over critical micellar concentration (CMC), the CTAB being from 0.005 M to 0.03 M, and the SDS from 0.03 M to 0.07 M. For both the CTAB and SDS, the retention of all solutes decreased as the concentration of each surfactant increased. This trend is typical of the retention behavior of MLC. The overall retention trend of each solute in CTAB and SDS condition is related to the log P and pK_a of each solute. These values are presented in Table 2.

At pH 6.8, acidic quinolones mainly exist in the form of anion, carboxylate at C_3, and substantial proportions of fluoroquinolones exist in the form of zwitterions, carboxylate at C_3 and quaternary ammonium ion at C_7, respectively. When

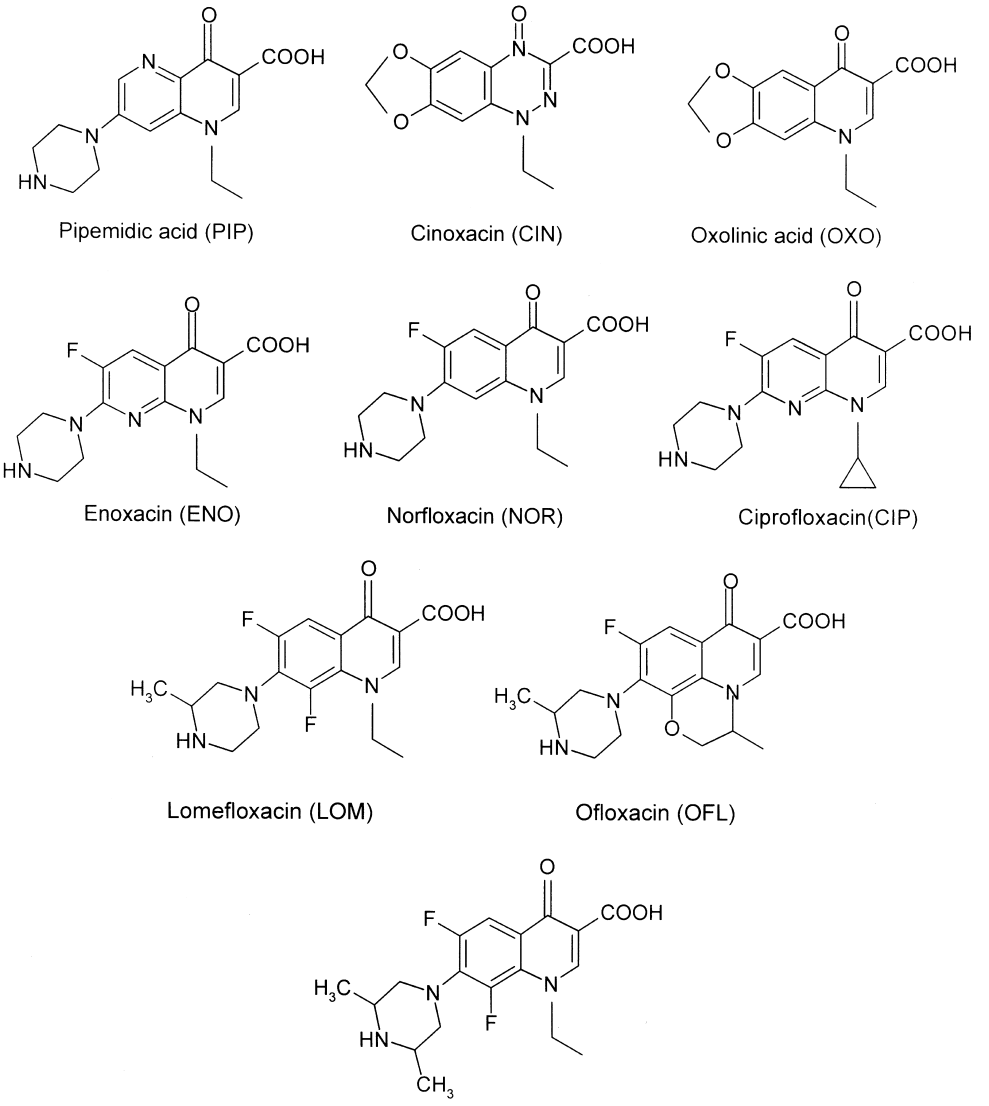

Figure 1. The structures of quinolones used in this study.

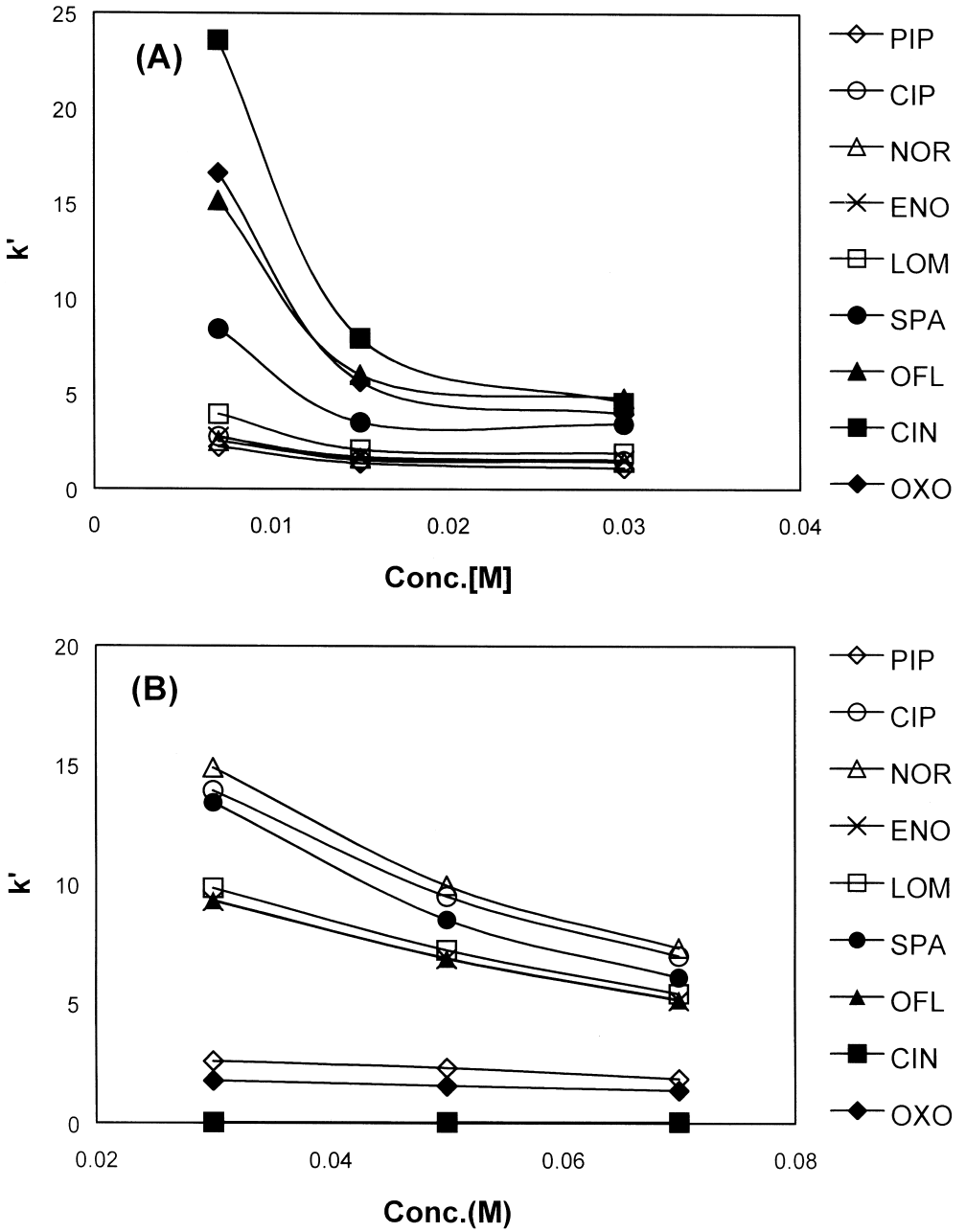

Figure 2. The retention behavior of quinolones, varying the concentration of (A) CTAB, and (B) SDS in the mobile phase.

Table 2. Log Ps and Dissociation Constants of Quinolones, Used in This Study

Compound	Log P_{ow} [a]	pK_{a1} [b]	pK_{a2} [c]
Pipemidic acid (PIP)	0.18	5.55	-
Oxolinic acid (OXO)	0.97	6.91	-
Cinoxacin (CIN)	0.26	4.70	-
Norfloxacin (NOR)	1.64	6.30	8.80
Ciprofloxacin (CIP)	1.46	6.00	8.80
Enoxacin (ENO)	0.77	6.00	8.50
Sparfloxacin (SPA)	1.54	6.20	8.60
Lomefloxacin (LOM)	2.31	5.82	8.30
Ofloxacin (OFL)	2.17	6.05	8.11

[a] Log P_{ow} values from Ref. 10.
[b] pK_{a1} values from Ref. 11.
[c] pK_{a2} values from Ref. 12.

the concentration of CTAB was in the lower range, the acidic quinolones with anionic character would have ionic attractions with the quaternary ammonium group of CTAB monomers, which were adsorbed onto the stationary phase. This phenomenon is observed for cinoxacin and oxolinic acid. However, as the concentration of CTAB was increased, more micelles would be formed in the mobile phase, and the solutes would partition into the micelles rather than the modified stationary phase.

In the case of SDS condition, the retention trend is similar to that of CTAB condition, but the retention order is somewhat different. The retention of acidic quinolones is shorter, compared to that of the other fluoroquinolone derivatives. This can be explained by the ionic repulsion between the anionic quinolones and the sulfate group of SDS monomers, which were adsorbed onto the stationary phase. But more hydrophobic fluoroquinolones easily approach the modified stationary phase, making their retention longer.

Retention-Activity Relationships

Based on the retention data from different micellar conditions, it is possible to predict the activity of quinolones and elucidate its action mechanism at the active sites. In this study, a few types of different characteristic surfactants were selected, including CTAB, SDS, Brij 35, and SB 3-10. Figure 3 shows the relationship between the minimum inhibitory concentration (MIC) of quinolones in *P. aeruginosa*, found in the literature and the retention data, at different CTAB concentrations, in the mobile phase. Regardless of the concentration of CTAB,

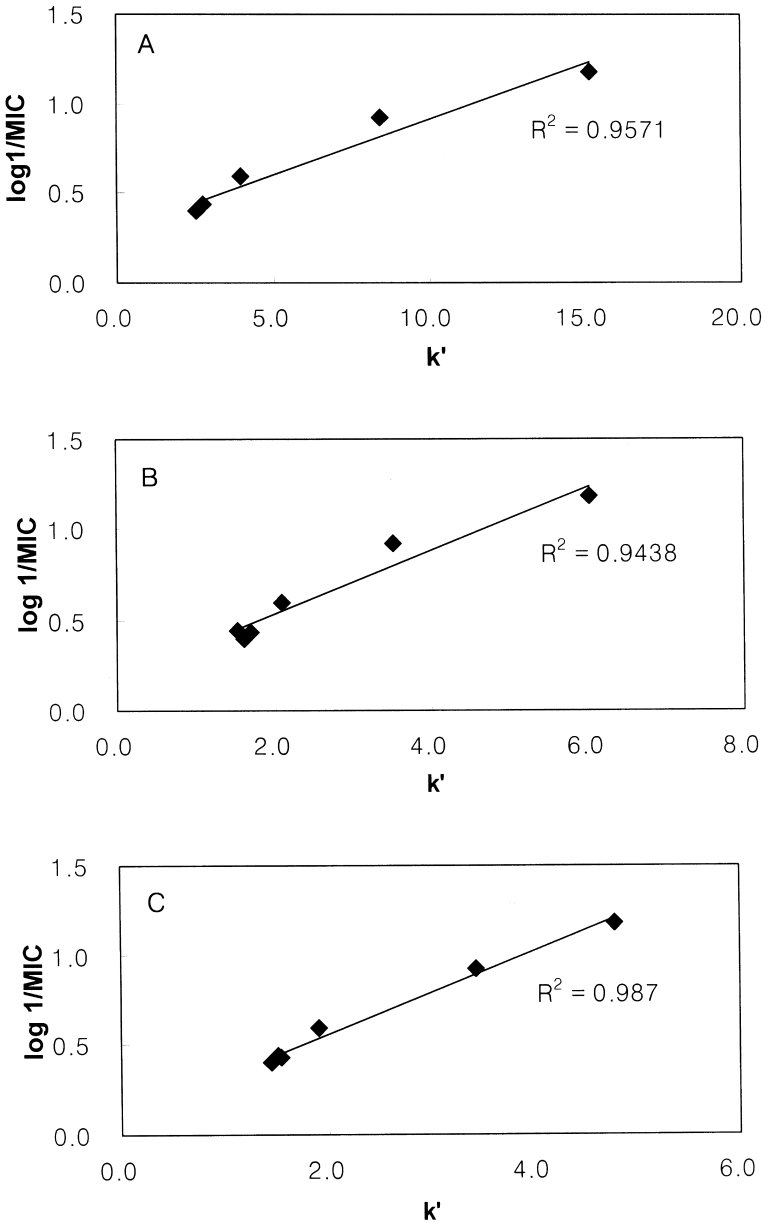

Figure 3. Retention-activity relationships of fluoroquinolones for *P. aeruginosa*, varying the concentration of CTAB in the mobile phase. (A) 0.007M (B) 0.015M (C) 0.030M.

good linearity was observed for fluoroquinolones. This indicates that a CTAB condition is very similar to the environment of a biologically active site.

On the other hand, no special relationship was observed for *E.coli*, or *S.aureus.* This indicates the existence of a different action mechanism from that of *P.aeruginosa*. Figure 4 shows the relationship between bioactivity in *P.aeruginosa*, found in the literature and the retention data, at different SDS concentrations, in the mobile phase. Also, good linearity was observed for *P. aeruginosa* in the investigated concentration range. This indicates that an SDS condition is also very similar to the environment of a biologically active site . On the other hand, for nonionic Brij 35 and zwitterionic SB 3-10, no special retention-activity was observed, as shown in Figure 5 and 6. This tells us that the active site of fluoroquinolones is not close to the nonionic microenvironment. According to Shen's theory, different activity responses of quinolones were found with charged and non-charged C_7 substituents to two different active sites, namely, Asp-426 and Lys-447.[13] These active sites have acidic and basic characters, respectively.

For example, amphoteric quinolones, having a positive charge at the C_7 substituent, such as ciprofloxacin and norfloxacin, were known to be hypersensitive to the mutants which have the negatively charged amino acid, Glu. However, acidic quinolones, such as cinoxacin and oxolinic acid that have no charged substituent at C_7, were equally resistant to two types of mutants which have both the negatively and positively charged amino acid, Glu, Asn. This is the reason why the activities of these acidic quinolones were not correlated with k' in different micellar conditions. In the case of fluoroquinolones, the interaction is possible between the gyrase B, subunit of DNA gyrase, and the positively charged subunit of drugs, such as piperazine, leading to high potency. It is assumed that the sulfate group of SDS may have ionic attractions with the piperazinyl group of quinolones in micellar conditions, and play a role like that of Asp-426, one of the active sites in DNA gyrase.

The CTAB condition would be expected to function as a cationic environment, similar to a DNA base pair, leading to a hydrogen bonding with the CO_2H group of fluoroquinolones at C_3. But, in the case of Brij 35 and SB3-10, only the hydrophobic character increased as the concentration increased. This increment is not directly related with the character of the active site, the DNA gyrase, but is related to the transport of membrane.

As a result, no concrete retention-activity relationship was found for two kinds of surfactants. It is known that the MIC of a quinolone is determined by at least 2 factors: its rate of penetration into the bacterial cell, and its inhibitory activity against the supercoiling reaction, catalysed by its target, the enzyme DNA gyrase. Based on the QRAR plots, the MIC of fluoroquinolones for *P. aeruginosa* is thought to be affected more by their activity on DNA gyrase than on hydrophobic penetration into cells.

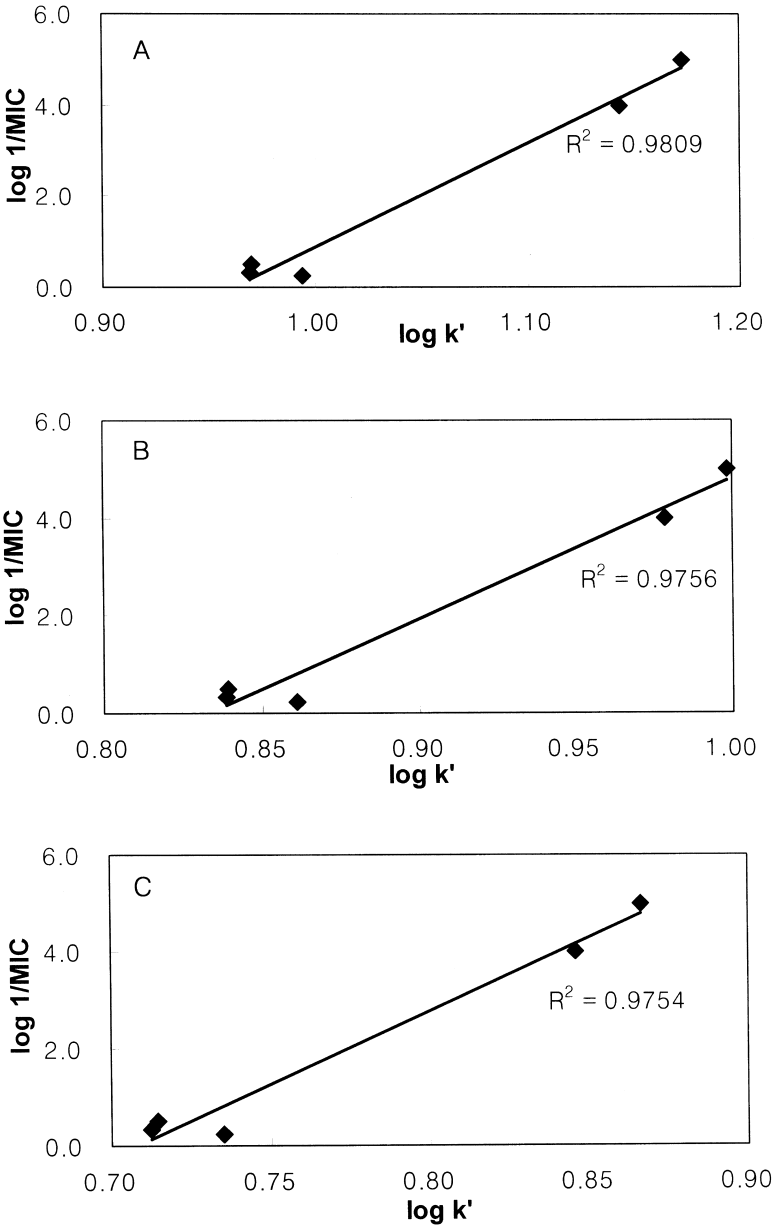

Figure 4. Retention-activity relationships of fluoroquinolones for *P. aeruginosa*, varying the concentration of SDS in the mobile phase. (A) 0.03M (B) 0.05M (C) 0.07M.

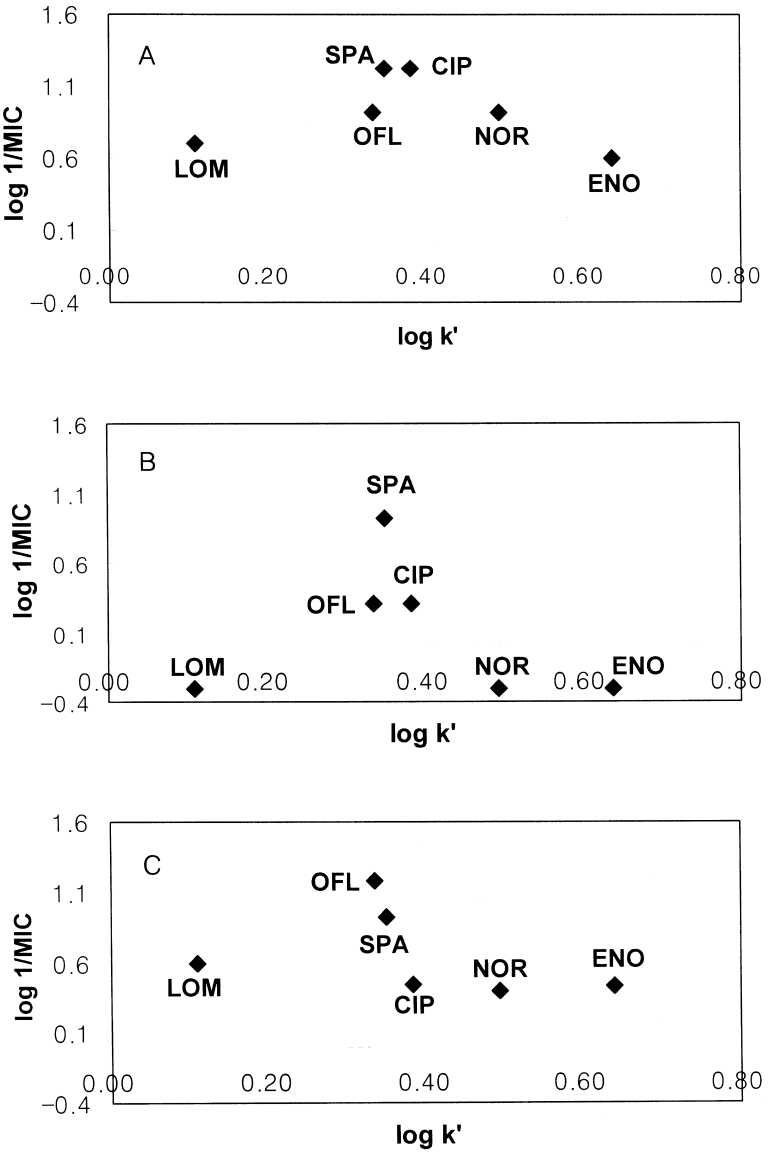

Figure 5. Retention-activity relationships of fluoroquinolones for (A) *E. coli.,* (B) *S. aureus*, and (C) *P. aeruginosa*, using 0.06M Brij 35 as the mobile phase additive.

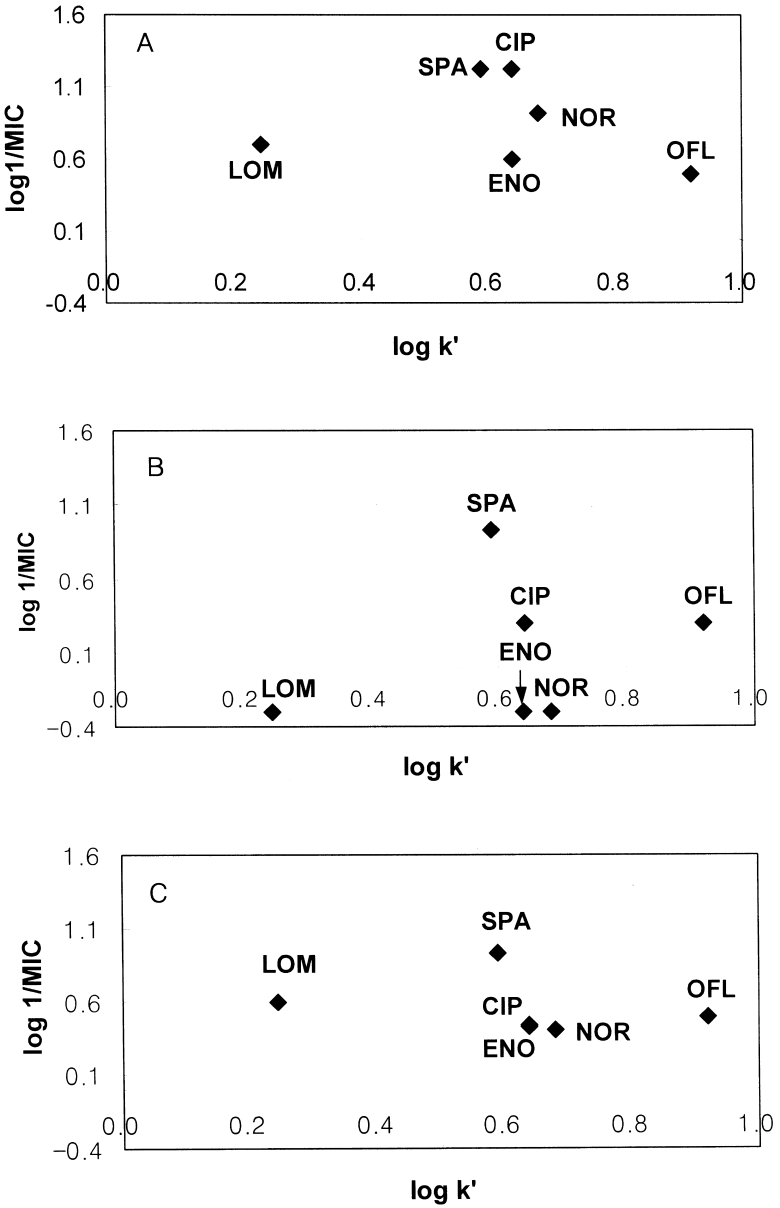

Figure 6. Retention-activity relationships of fluoroquinolones for (A) *E. coli.,* (B) *S. aureus,* and (C) *P. aeruginosa*, using 0.03M SB-3-10 as the mobile phase additive.

CONCLUSIONS

This study investigated the quantitative retention - activity relationship for quinolones by using micellar liquid chromatography. Under CTAB or SDS micellar conditions, a decreasing retention trend was observed as the concentration increased. Also, it was possible to explain their retention trend by hydrophobic or electronic interaction. Based on the retention data obtained from different micellar conditions, a quantitative retention - activity relationship was successfully applied to the representative bacteria, *P.aeruginosa*.

A linear relationship was observed for a CTAB or SDS micellar condition, regardless of their concentration, investigated. On the other hand, no special relationship was found for *E.coli., S.aureus*, and *P.aeruginosa*, by applying the retention data, obtained from Brij 35, SB 3-10 conditions. These phenomena could be interpreted by the similarity to the biological environment, depending on the characteristic of surfactants. In detail, it was assumed that the active site of DNA gyrase or DNA base pair has some interaction with the different structural sites of fluoroquinolones. This information would be helpful in synthesizing new fluoroquinolones with improved activity.

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