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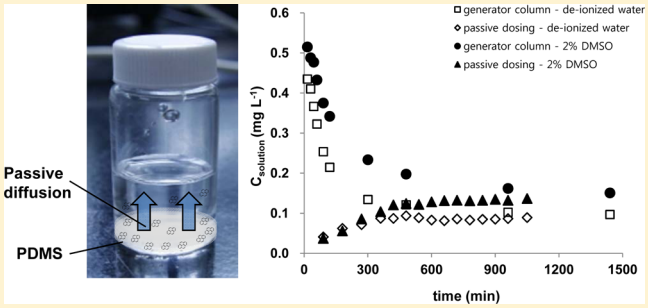
# Measuring Aqueous Solubility in the Presence of Small Cosolvent Volume Fractions by Passive Dosing

Hyuck-Chul Kwon and Jung-Hwan Kwon*

Department of Environmental Engineering, Ajou University, Woncheondong, Yeongtonggu, Suwon 443-749, Republic of Korea

Supporting Information

**ABSTRACT:** A new passive dosing method was developed to determine aqueous solubility of hydrophobic chemicals. In the passive dosing method, chemical crystals were loaded to a polydimethylsiloxane (PDMS) phase to maintain the maximum chemical activity and to prevent direct contact of chemical crystals with the aqueous solution. Eight polycyclic aromatic hydrocarbons (PAHs) were chosen covering their literature aqueous solubility from 0.001 to 30 mg $L^{-1}$. Values of the aqueous solubility for less hydrophobic PAHs (naphthalene, 2-methylnaphthalene, and fluorene) were measured by an Organization for Economic Co-ordination and Development (OECD)-recommended generator column method and those for more hydrophobic PAHs (anthracene, chrysene, and benzo(*a*)pyrene) were measured by the passive dosing method. For phenanthrene and pyrene, both methods were used for comparison. The results obtained by the passive dosing method were very close to those obtained by the generator column method. Aqueous solubilities in deionized water for more hydrophobic PAHs obtained using the passive dosing method agreed very well with values reported in the literature, suggesting the utility of the passive dosing method for the determination of aqueous solubility for highly hydrophobic chemicals. Because hydrophobic chemicals are often introduced in aqueous solutions by using a cosolvent, the solubility enhancement for PAHs at low cosolvent volume fractions was also evaluated. Three cosolvents (dimethyl sulfoxide, ethanol, and acetone) were chosen and their volume fractions in water were between 0.2% and 2%. The enhancement of the aqueous solubility could be well explained quantitatively by using a log−linear cosolvency model. The measured values of cosolvency power at the range of volume fraction investigated ($\sigma_{0.02}$) correlated very well with log $K_{ow}$ of the PAHs. The combination of the log−linear model and semiempirical relationships between $\sigma_{0.02}$ and log $K_{ow}$ would be useful for the prediction of the solubility enhancement of hydrophobic chemicals.




## INTRODUCTION

Aqueous solubility is one of the most important chemical properties for assessing the aquatic toxicity of chemicals as well as for environmental fate modeling. Although the aqueous solubilities of many hydrophobic chemicals are very low, the freely dissolved fraction is very important because it is believed that only this fraction can permeate cell membranes and exhibit bioavailability.[1] In spite of its critical importance, determination of aqueous solubility for highly hydrophobic chemicals is still challenging because of the difficulties with accurate measurement of aqueous concentration.

Passive dosing is an emerging technique for maintaining the aqueous activity or freely dissolved concentration of hydrophobic chemicals by delivering them via thermodynamic partitioning from a condensed phase, such as a silicone polymer, to the test medium.[2−9] Because the distribution coefficient between the dosing phase and the medium is high for hydrophobic chemicals and the volume of the medium is small compared to that of the dosing phase, the mass of the hydrophobic chemical in the dosing phase remains almost constant during an experiment. This technique has been applied to maintain constant exposure conditions in both in vivo[2,3] and in vitro[4−8] bioassays and to determine chemical speciation in water.[9] Although the working principle of passive dosing is similar to that of standard and modified generator column methods,[10−12] the passive dosing device is much simpler. Thus, this method would also be useful for determining the aqueous solubility if the aqueous concentration of a solute can be measured with the limited volume used in passive dosing.

In (eco)toxicity tests, hydrophobic chemicals are often introduced in the test medium by using a cosolvent because of the difficulty of dissolving them directly in the test medium.[13] However, the nominal concentration in the medium often exceeds the limit of the aqueous solubility of a compound.[14−17] Although the presence of a cosolvent and disssolved organic phases should increase the solubility of the compound in the test medium, it is very difficult to be sure that the nominal concentration dosed above the solubility in pure

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water provides only dissolved solutes unless the fraction of freely dissolved species is known.[18] In many cases, chemicals dosed with cosolvent are considered dissolved because they look dissolved when spiked with cosolvent. However, the administration of hydrophobic solutes using cosolvents may result in inhomogeneous distribution of the chemicals in the test medium.[19] Thus, it would be useful to know the limits of the solubility enhancement of a hydrophobic chemical in the presence of cosolvents used as carriers of hydrophobic chemicals in order to provide an accurate estimate of the solubility in the test medium.

Earlier studies investigated the effects of cosolvents to enhance the solubility of hydrophobic organic compounds that are sparingly soluble in water, which has significance in many areas including chemical engineering and pharmaceutical and environmental sciences.[20−31] To explain the changes in solubility in binary mixtures, the range of cosolvents used in the majority of previous studies covered from 0 to 100%. Only a small number of studies have focused on small cosolvent volume fractions. Although the existing cosolvency models[20,23,27,32] could be used to estimate the solubility enhancement at low volume fraction of cosolvents, the predictability of models could be improved with experimentally determined cosolvency effects on highly hydrophobic chemicals at low cosolvent volume fraction.

Consequently, we carefully measured the solubility of hydrophobic chemicals in deionized water and aqueous solution containing a small volume fraction of a cosolvent (≤2%) using both the generator column method[10] and the newly developed passive dosing method. The two major objectives were (1) to develop a passive dosing method for the determination of the aqueous solubility of hydrophobic chemicals, which is easier and potentially more accurate than the recommended generator column method, and (2) to quantify the degree of solubility enhancement at small volume fractions of cosolvents typically used in bioassays. Eight polycyclic aromatic hydrocarbons (PAHs), with log $K_{ow}$ ranging between 3.3 and 6.3, were chosen as model compounds, and dimethylsulfoxide (DMSO), ethanol, and acetone were chosen as model cosolvents. Aqueous solubility values were measured in deionized water and for 0.2, 0.5, 1.0, and 2.0% (v/v) of cosolvents. A log−linear cosolvency model was used to obtain the "cosolvency power" of the selected cosolvents for each PAH at low volume fractions. Quantitative relationships between the cosolvency power and log $K_{ow}$ were obtained to evaluate the effects of hydrophobicity on the solubility enhancement, and practical model equations were proposed to estimate the solubilities of hydrophobic chemicals in the presence of cosolvents.

**Theory.** Many models have been developed to explain the solubility in water/cosolvent systems. Among them, the log−linear cosolvency model is the simplest, assuming a linear relationship between the logarithmic solubility and the volume fraction of a cosolvent.[24,32] Because the range of cosolvent volume fractions of interest in this study did not exceed 2% (v/v), the increase in the logarithmic solubility was assumed to be linear with increasing volume fraction of a cosolvent.

The detailed theoretical bases of log−linear cosolvency model are found in the literature[24,27,32] and briefly described in the Supporting Information. In short, logarithmic solubility in a mixed solvent at low volume fraction of a cosolvent (log $S_m$) relative to that in pure water (log $S_w$) could be represented by a product of cosolvency power and volume fraction of a cosolvent (eq 1).

$$\log S_m = \log S_w + f\sigma_{0.02} \quad (1)$$

where $f$ is the volume fraction of a cosolvent and $\sigma_{0.02}$ is cosolvency power up to 2% volume fraction. Assuming that the log activity coefficient in a cosolvent/water mixture is proportional to that in octanol, $\sigma_{0.02}$ can be correlated linearly with log $K_{ow}$ (eq 2).

$$\sigma_{0.02} = a\log K_{ow} + b \quad (2)$$

where $a$ and $b$ are empirical constants dependent on the cosolvent. Empirical relationships between the cosolvency power and log $K_{ow}$ have been reported in the literature for the range of cosolvent volume fractions from 0 to 0.5 or 1.0 for various cosolvents.[32,33]

## EXPERIMENTAL SECTION

**Materials and Chemicals.** Eight PAHs (naphthalene, 2-methylnaphthtalene, phenanthrene, fluorene, anthracene, pyrene, chrysene, and benzo[$a$]pyrene) were chosen as model hydrophobic solutes. All were of high purity (at least 97%) and purchased from Sigma-Aldrich (St. Louis, MO, USA). The reported purities of three cosolvents—DMSO (Sigma-Aldrich),

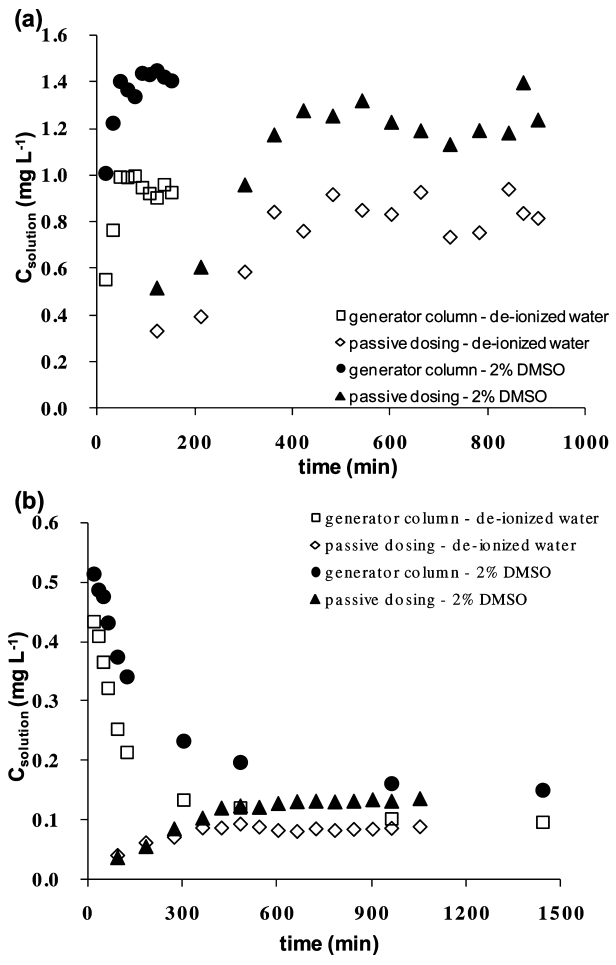


**Figure 1.** Comparison of the time-course changes in the aqueous concentration of (a) phenanthrene and (b) pyrene in deionized water and 2% DMSO solution using the generator column method and the passive dosing method.


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Table 1. Solubility of Selected PAHs in Water Measured in This Study and Values Reported in the Literature

| | | this study ($mg \cdot L^{-1}$)[b] | | | |
|---|---|---|---|---|---|
| | log $K_{ow}$[a] | generator column | passive dosing | literature ($mg \cdot L^{-1}$)[c] | ref |
| naphthalene | 3.35 | 30.6 ± 1.8 | | 30.6−34.8 | 37, 38, 40−44 |
| 2-methylnaphthalene | 4.00 | 21.5 ± 0.1 | | 27.3 | 44 |
| fluorine | 4.18 | 1.57 ± 0.03 | | 1.62−2.23 | 38, 40−44 |
| phenanthrene | 4.52 | 0.94 ± 0.02 | 0.82 ± 0.08 | 0.82−1.29 | 37−45 |
| anthracene | 4.50 | | 0.044 ± 0.003 | 0.0434−0.446 | 38, 39 |
| pyrene | 5.00 | 0.103 ± 0.009 | 0.086 ± 0.002 | 0.107−0.136 | 11, 38, 40−44 |
| chrysene | 5.86 | | 0.00070 ± 0.00006 | 0.00102−0.00327 | 37, 38, 42−44 |
| benzo[a]pyrene | 6.35 | | 0.00147 ± 0.00027 | 0.0012−0.00182 | 37, 40, 43, 45−47 |

[a]Suggested values by Sangster Research Laboratory.[46] [b]Mean ± standard deviation ($n$ = 5). [c]Range of experimental literature values obtained using the generator column method.

ethanol (Sigma-Aldrich), and acetone (Daejung Chemicals, Siheung, Korea)—were higher than 99.5%. Deionized water (≥ 18 MΩ cm) was prepared by reverse osmosis. Silicone elastomer (Sylgard 184A) and a curing agent (Sylgard 194B) were purchased from Sewang Hitech (Kimpo, Korea). Glass beads (0.2−0.3 mm) were purchased from Sigmund Linder (Warmensteinach, Germany).

**Determination of Aqueous Solubility.** Changes in the aqueous solubility of the eight PAHs were measured in deionized water and 0.2, 0.5, 1.0, and 2.0% (v/v) cosolvent fractions. DMSO, ethanol, and acetone were chosen as three model cosolvents as recommended by the Organization for Economic Co-ordination and Development (OECD).[13,34] For the less hydrophobic chemicals (naphthalene, 2-methylnaphthalene, fluorene, and phenanthrene), solubilities were obtained using a generator column method. For more hydrophobic chemicals with lower aqueous solubilities (anthracene, pyrene, chrysene, and benzo[a]pyrene), solubility values were obtained using a passive dosing method. To compare the performance of the two methods, the solubilities of phenanthrene and pyrene in deionized water and 2% (v/v) DMSO were determined using both methods.

**Generator Column Method.** A schematic diagram of the custom-made generator column apparatus used in this study is shown in Figure S1a in the Supporting Information. The aqueous solubility was measured according to the OECD test guidelines.[10] Briefly, glass beads (1.1−1.3 g) coated by a chemical species were packed in the column. The amount of chemical coated on the surface of the glass beads was sufficiently greater than the calculated mass required for the measurement of solubility by a factor of at least 10. Deionized water or the aqueous solution containing the desired volume fraction of a cosolvent was circulated by an HPLC pump. The volumetric flow rate of the solution was approximately 1.2−1.5 $mL \cdot min^{-1}$. The temperature of the solution was maintained at 25 ± 0.2 °C by circulating water via a peristaltic pump in the water jacket. Aqueous solution (0.3 mL) was carefully taken using a glass syringe from the Erlenmeyer flask and subjected to HPLC quantification. Due to high aqueous solubility, samples containing naphthalene, 2-methylnaphthalene, fluorene, and phenanthrene were diluted with methanol prior to HPLC analysis. For pyrene, aqueous samples obtained in the generator column test were promptly injected into the HPLC system in order to minimize any experimental artifacts associated with storage. Experiments were terminated after the measured concentrations of at least five successive samples did not differ by more than ±30% as suggested by OECD Test Guideline.[10] Then, the solubility was determined as the average of the last five samples.

**Passive Dosing.** For more hydrophobic PAHs, solubilities were measured using the passive dosing method, in which chemical crystals were embedded in the PDMS phases. The amount of chemical required for the passive dosing method was calculated such that a sufficient amount of PAH crystals was guaranteed to remain after equilibration was reached. Acetone/methanol (1:10) solution containing a solute was transferred to a 20-mL glass vial and evaporated at 45 °C in a fume hood until dry, and the evaporation resulted in the precipitation of a thin layer of crystals. Polydimethylsiloxane (PDMS) elastomer and the curing agent were mixed at 10:1 mass ratio, and the liquid mixture (0.5−0.7 g) was carefully poured in the glass vial containing the precipitated PAH crystals. First, curing was carried out at 55 °C for 6 hours. Deionized water (10 mL) was added to rinse the surface and remove any remaining crystalline phases on the surface, and water was removed using lint-free tissue. This curing and rinsing procedure was repeated three times in order to prevent direct contact between the solute crystals and the solution.

After the solute was embedded in the PDMS phase (Figure 1b, Supporting Information) by curing, 12 mL of deionized water or solution containing the desired volume fraction of a cosolvent was added to the vial. The vial was gently agitated in a shaking incubator at 25 °C and 80 rpm. Sample aliquots (400 μL) were taken at hourly intervals and promptly subjected to HPLC analysis. Values of the solubility were determined as for the generator column method.

**Instrumental Analyses.** The concentration of PAHs was quantified using an HPLC system equipped with a Waters 600E pump (Milford, MA, USA), an autosampler (Waters 717+), and a fluorescence detector (Waters 2475) or a diode-array detector (Waters 2996). Acetonitrile was used as the mobile phase in an isocratic mode with a flow rate of 1 $mL \cdot min^{-1}$. PAHs were separated on a Thermo C18 column (4.6 × 150 mm, 5 μm particle size; Thermo Scientific) at 35 °C. Naphthalene and 2-methylnaphthalene were detected using the diode-array detector at 219.5 and 220 nm, respectively. Phenanthrene, fluorene, pyrene, anthracene, chrysene, and benzo[a]pyrene were detected using the fluorescence detector, with excitation ($\lambda_{ex}$) and emission wavelengths ($\lambda_{em}$) of 260 and 350 nm for phenanthrene, 270 and 310 nm for fluorene, 270 and 390 nm for pyrene, 244 and 400 nm for anthracene, 266 and 382 nm for chrysene, and 380 and 404 nm for benzo[a]pyrene.

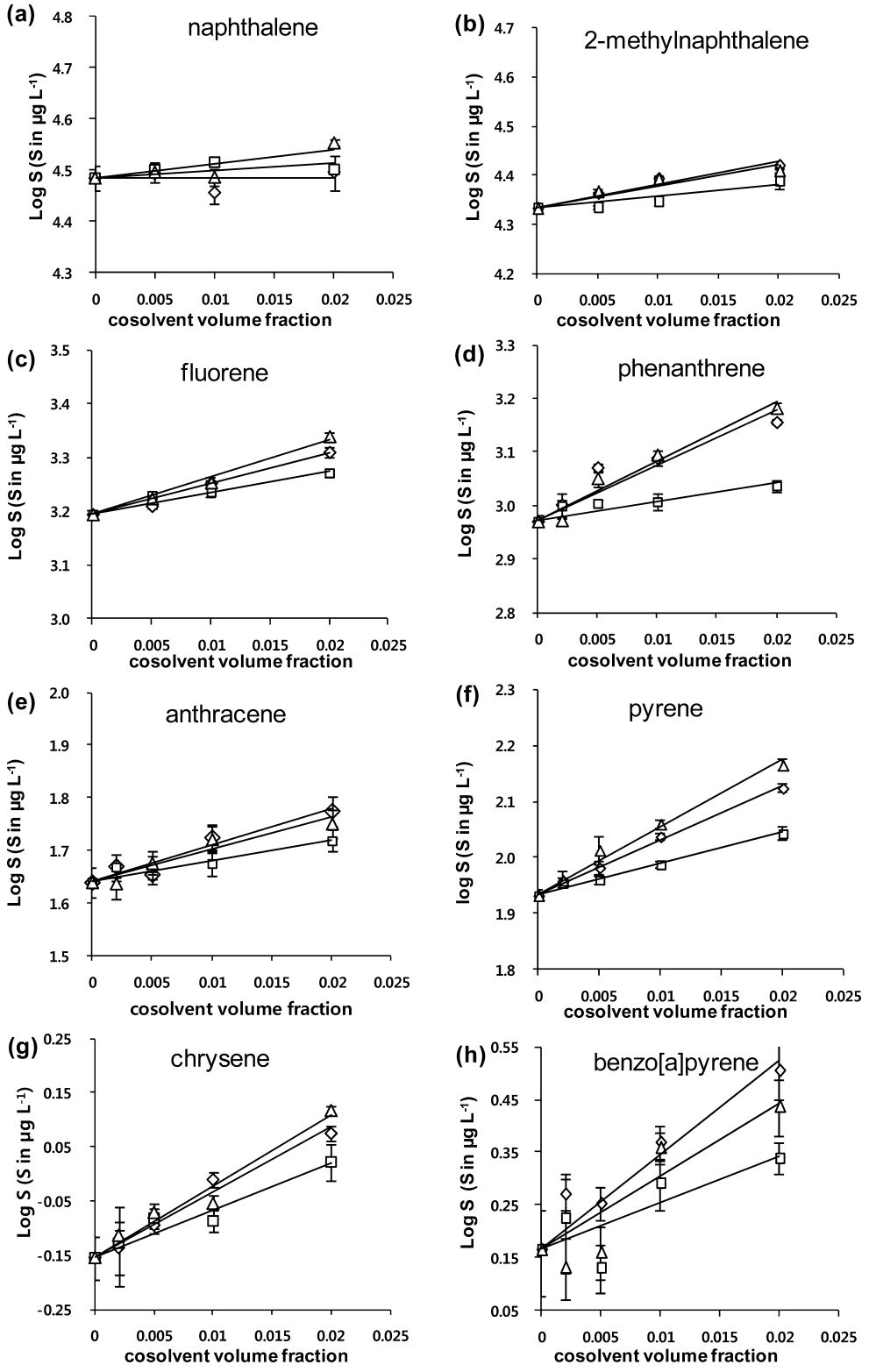


**Figure 2.** Relationships between log solubility (log $S_m$) and the volume fraction of cosolvents ($f$). Diamonds, squares, and triangles represent DMSO, ethanol, and acetone, respectively. Error bars denote the standard deviations, and linear lines were obtained using linear regression to derive cosolvency power, $\sigma_{0.02}$.

## RESULTS AND DISCUSSION

**Comparison of the Two Methods for Determining Aqueous Solubility.** Figure 1 shows the concentration changes in deionized water and 2% (v/v) DMSO with experimental time for (a) phenanthrene and (b) pyrene for the comparison of the generator column method and the passive dosing method. For phenanthrene, the aqueous concentration increased with the experimental time and reached a steady-state value regardless of solution in both the generator column and the passive dosing methods. However,


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**Table 2. Values of Cosolvency Power ($\sigma_{0.02}$) Obtained in This Study at Low Cosolvent Volume Fractions with Literature Values**

| | $\sigma_{0.02}$ (this study)[a] | | | $\sigma$ (literature) | | |
|---|---|---|---|---|---|---|
| chemical | DMSO | ethanol | acetone | DMSO | ethanol | acetone |
| naphthalene | 0.02 (0.80) | 1.39 (0.57) | 2.78 (0.74) | 3.31[b] | 1.85[c], 3.48[d] | 5.05[e], 4.17[d] |
| 2-methyl naphthalene | 4.77 (0.42) | 2.43 (0.41) | 4.38 (0.58) | | | |
| fluorene | 5.66 (0.34) | 4.05 (0.39) | 6.93 (0.33) | | | |
| phenanthrene | 10.30 (1.23) | 3.62 (0.60) | 11.10 (0.79) | | 4.49[f], 4.50[g], 4.83[h] | |
| anthracene | 6.99 (0.66) | 4.01 (0.49) | 6.08 (0.61) | | 2.72[c], 4.31[f], 4.42[g], 4.74[h] | 5.36[d] |
| pyrene | 9.77 (0.22) | 5.56 (0.24) | 12.06 (0.51) | | 3.14[c], 4.29[d] | 5.22[d] |
| chrysene | 12.02 (0.58) | 8.75 (0.79) | 13.10 (0.86) | | | 6.11[d] |
| benzo[*a*]pyrene | 17.88 (1.65) | 8.80 (2.11) | 13.74 (2.41) | | 5.80[g], 6.08[h] | |

[a]Values in parentheses are standard errors of regression. [b]Volume fraction 0–50%.[47] [c]Volume fraction 0–20%.[28] [d]Volume fraction 0–100%.[24] [e]Volume fraction 0–100%.[30] [f]Volume fraction 0–100%.[27] [g]Volume fraction 0–100%.[31] [h]Volume fraction 0–50%.[31]

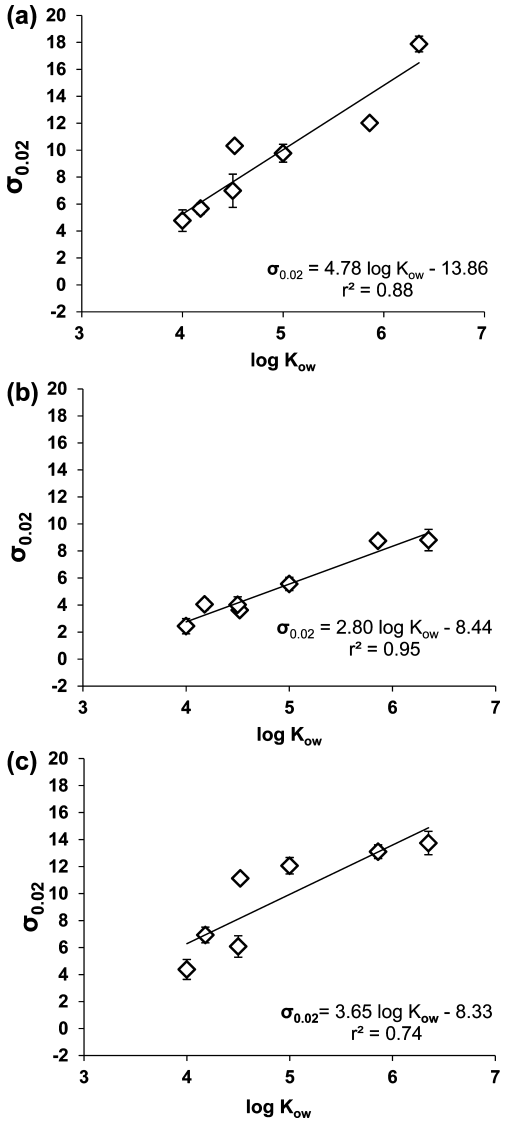


**Figure 3.** Relationships between cosolvency power ($\sigma_{0.02}$) and log $K_{ow}$ for (a) DMSO, (b) ethanol, and (c) acetone. Error bars denote the 95% confidence intervals of $\sigma$ and lines represent the best fit obtained using linear regression.

pyrene concentrations in both deionized water and 2% (v/v) DMSO solution showed abnormally high initial values in the generator column method. It decreased to a steady-state value with the passage of time, and the concentration approached those obtained using the passive dosing method after 10 and 24 h in deionized water and 2% DMSO solution, respectively. A similar observation was also documented by de Maagd et al.[35] This is likely to be due to the initial detachment of crystals from the coating at the surfaces of the glass beads, which caused the measured values of the aqueous concentration to be above the solubility and the continuous filtration of the crystals by circulating the solution through the HPLC pump. In contrast, passive dosing resulted in an initial increase in the aqueous concentration followed by a plateau, as was the case with phenanthrene and other relatively hydrophilic solutes in the generator column method (Figure 1). This indicates that the passive dosing method would be robust in determining the aqueous solubility of a highly hydrophobic solute because it prevented the overshooting of the solubility observed in the generator column method.

Table 1 shows the aqueous solubility values of the PAHs obtained in this study using the generator column and passive dosing methods with those reported in the literature using the generator column method.[11,35−45] As shown, the experimental values in this study agree well with literature values. The aqueous solubilities of the more hydrophobic PAHs were close to the lower ends of the ranges of suggested literature values. For example, the aqueous solubility of chrysene measured in this study was slightly lower than the lowest value in the literature[41] by a factor of 1.5. This indicates that the passive dosing method is very promising for the generation of reliable solubility values for hydrophobic chemicals, although its usefulness should be evaluated with a larger data set.

**Enhancement of Aqueous Solubility by Cosolvents.** Figure 2 shows the increase in the logarithmic solubility (log $S_m$) of all PAHs tested with increasing volume fractions of the three cosolvents. A noticeable increase in aqueous solubility was observed when the volume fractions of the three cosolvents were less than 2%. The best-fit lines using eq 1 were obtained by fixing the intercept at the mean aqueous solubility of the compound. Values of the solubilities for all solutes are presented in Table S1. As shown in Figure 2 and Table S1, the increase in the solubility was well explained by the log–linear cosolvency model. Values of the regression coefficients ($r^2$) were above 0.9, except for naphthalene with the smallest $\sigma_{0.02}$ values and the most hydrophobic benzo[*a*]pyrene. For benzo[*a*]pyrene, relatively large error bars were obtained in deionized water and at lower volume fractions of cosolvents. However, the degree of increase in log $S_m$ was the highest within the range of cosolvent volume fractions.

The slopes in Figures 2 tend to increase with increasing hydrophobicity of the PAHs. A similar trend was also observed

in earlier literature using DMSO, ethanol, or acetone as model cosolvent.[24,28,31,32] Table 2 presents all values of cosolvency power ($\sigma_{0.02}$) obtained from the linear regression in this study as well as values reported in the literature. The $\sigma_{0.02}$ values for ethanol obtained in this study were in good agreement with literature values, although the range of the cosolvent volume fractions was different.[24,27,28,30] However, the $\sigma_{0.02}$ values for acetone showed significant differences. The values obtained in this study increased with the increasing hydrophobicity of a solute, whereas Morris et al.[24] reported $\sigma$ values of 4.17, 5.36, 5.22, and 6.11 for naphthalene, anthracene, pyrene, and chrysene, respectively, at the cosolvent volume fraction between 0 and 100%. The cosolvency power depends on the activity coefficients both in water and in cosolvents and it is believed that the activity coefficient in water increased with increasing hydrophobicity whereas that in cosolvent is relatively invariant. Thus, it is expected that $\sigma$ increases with log $K_{ow}$ of a solute.[28,31−33] The insensitivity of $\sigma$ to log $K_{ow}$ in earlier studies might be due to difficulties in determining accurate aqueous solubility for more hydrophobic solutes and the difference in the range of cosolvents investigated. According to more sophisticated cosolvent models, such as the Margules equation, the solubility of a solute in a binary mixture often resulted in a concave curve.[27] Therefore, the estimated $\sigma$ value at a lower volume fraction is likely to be higher than that obtained using data covering the entire range of the solvent mixture.

**Effects of the Hydrophobicity of PAHs on Solubility Enhancement.** Figure 3 presents the linear relationships (eq 2) between $\sigma_{0.02}$ and log $K_{ow}$ for (a) DMSO, (b) ethanol, and (c) acetone as model cosolvents. Because $\sigma_{0.02}$ for naphthalene was not significantly different from zero, values for naphthalene were excluded in the regression. The highest slope was found for DMSO, followed by acetone and ethanol. As discussed previously, the relatively higher slopes found in this study may be due to the overestimated water solubility for more hydrophobic chemicals in earlier studies at lower cosolvent volume fractions.

Although the hydrophobic solutes tested in this study were limited to only eight PAHs, the results shown in Figure 3 provide a useful estimation of the solubility enhancement of hydrophobic chemicals within the range of cosolvent volume fractions typically used in (eco)toxicity tests. Because the values of log $K_{ow}$ for the hydrophobic chemicals introduced with a cosolvent are mostly between 3 and 7, the range of log $K_{ow}$ values investigated in this study encompasses the hydrophobicity of many chemicals of interest. Many publications reported that the effective concentrations of hydrophobic chemicals such as PAHs[14−16] and phthalates[17] in in vivo and in vitro tests are much greater than their aqueous solubilities even if the potential solubility enhancement by adding a cosolvent is considered. Because the addition of 2% (v/v) cosolvent enhanced the solubility of benzo[*a*]pyrene, the most hydrophobic chemical tested in this study, by a factor of approximately 2, the usage of the reported dose−response relationships above the aqueous solubility in those studies is only limited. A simple combination of the linear relationships shown in Figure 3 with the log−linear cosolvency model (eq 1) would be useful because it provides at least a crude estimation of the expected aqueous solubility in the presence of low volume fractions of cosolvents, which cannot be exceeded to consider the reliable exposure conditions.

## ■ ASSOCIATED CONTENT

### Supporting Information

Detailed derivation of linear cosolvency model, the schematic diagrams of the generator column, and the passive dosing method (Figure S1); determination of all solubility values (Figure S2); and all values of solubilities (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION

### Corresponding Author

*Phone: +82 31 219 1942; fax: +82 31 215 5145; e-mail: jhkwon@ajou.ac.kr.

### Notes

The authors declare no competing financial interest.

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