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# THERMODYNAMIC MODEL FOR REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH MULTISOLVENT MOBILE PHASES

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## SUMMARY

A basic reversed-phase liquid chromatographic model is presented. It is based on the thermodynamic treatment of adsorption, dissolution and mixing of binary solvents. It has been applied to the prediction of the capacity ratio of five antiepileptic drugs in a multisolvent mobile phase. Correlation coefficients between the observed and the calculated values of the logarithm of the capacity ratio were *ca.* 0.95–0.98.

## INTRODUCTION

Various attempts have been made to predict retention characteristics of multisolvent high-performance liquid chromatography (HPLC), both reversed phase (RP) and normal phase (NP), especially for the optimization of sample separation. It is possible to treat retention in RPLC in a very similar way to retention in NPLC[1]. Snyder[2] introduced the well-known empirical parameter, solvent strength, to predict the capacity ratio in the mobile phase of an organic–water mixture for NPLC. Glajch *et al.*[3] extended his work to predict the optimum mobile phase statistically by using the solvent selectivity triangle for NPLC and RPLC. Soczewinski[4] proposed an empirical equation for the prediction of capacity ratio for NPLC, and Hara *et al.*[5] systematically investigated its validity. Jandera and Churáček[6] and Tijssen *et al.*[7] predicted the capacity ratio on the basis of solubility parameters. Recently, Colin *et al.*[8] proposed an improved equation for RPLC by considering the polar interactions between two solvent molecules and between the sample solutes and the solvent molecules, and found a good agreement. However, they did not include the entropic contribution to the retention characteristics. Almost all these authors have based their reports on the linear relationship between the logarithm of the capacity ratio and the mole fraction of the organic solvent, and have added some terms or modified the shape.

This paper presents a basic RPLC model, based on the full thermodynamic treatment of adsorption, dissolution and mixing of solvents. It is applied to the prediction of the capacity ratio in a multisolvent mobile phase, and to obtain the resolution functions for the separation of five antiepileptic drugs.

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## THEORETICAL

Sample molecules that are in equilibrium between the two-component mobile phase and the adsorbent are described in Fig. 1A. Here, S and B denote the sample molecule and the adsorbent, respectively. Both $\alpha$ and $\beta$ denote each group of solvent molecules which solvate to the same area of adsorption site surface of sample molecule and adsorbent, respectively (Surface Site Model; SSM). Solvent molecules, which usually solvate on the sample molecule or on the adsorbent bed, regardless of the sample adsorption or desorption from the adsorbent, can be neglected for simplicity as illustrated in Fig. 1B. We need to deal only with species that definitely adsorb or desorb in the equilibrium process for a rough evaluation of the change in Gibbs free energy $\Delta G$. So the equilibrium process is described as the following equation:

$$\mathrm{SB} + \alpha\beta = \mathrm{S}\alpha + \mathrm{B}\beta + \Delta G \tag{1}$$

This equation is the sum of the following four equations:

$$\mathrm{SB} = \mathrm{S} + \mathrm{B} - \Delta G_0 \tag{2}$$
$$\mathrm{S} + \alpha = \mathrm{S}\alpha + \Delta G_\alpha \tag{3}$$
$$\mathrm{B} + \beta = \mathrm{B}\beta + \Delta G_\beta \tag{4}$$
$$\alpha\beta = \alpha + \beta - \Delta G_\phi \tag{5}$$

Here, the values which are symbolized with $\Delta$ are corresponding Gibbs free energy changes. In case of RPLC, solvent molecules are far more polar than the adsorbent. The ratio of values of small non-polar affinity of the two solvent molecules to the adsorbent bed might be large, whereas there will not be such a difference between these two absolute values compared with the $RT$ value. Therefore, as a first approximation, we assume that the ratio of binary solvent molecules on the adsorption site surface is equal to that in the mobile phase.

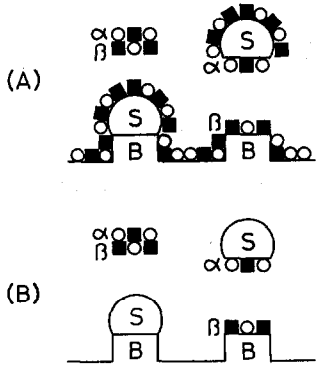


Fig. 1. Visualization of the dissolution–adsorption equilibrium in binary-solvent liquid chromatography. (A) Whole illustration as it is; S = sample molecule, B = adsorbent; open circles and filled squares show two kinds of solvent molecule; (B) unchanged solvent molecules can be neglected; see text for further discussion.

The $\Delta G_0$ is described as the following equation:

$$\Delta G_0 = \Delta H_{50} - RT X_S \ln(X_S) \tag{6}$$

Here, $\Delta H_{50}$ is the corresponding enthalpy change of eqn. 2, and the second term corresponds to the entropy change of the same equation; $X_S$ denotes the mole fraction of the sample. $\Delta G_\alpha$ is defined by the following equation:

$$\begin{aligned}\Delta G_\alpha = {} & W_{HS}N_{HS} + W_{IS}N_{IS} + W_{SHH}M_{SHH} + W_{SII}M_{SII} + W_{SHI}M_{SHI} \\ & + RT[X_H \ln(X_H) + X_I \ln(X_I) - X_H(1 - X_S y) \ln\{X_H(1 - X_S y)\} - \\ & X_I(1 - X_S y) \ln\{X_I(1 - X_S y)\}]\end{aligned} \tag{7}$$

Here, H and I denote each solvent molecule in the binary solvent mobile phase; S denotes the sample molecule; $W_{HS}$ and $W_{IS}$ denote the adsorption energies of solvent molecules, H and I, to the adsorption site surface of the sample molecule, respectively; $N_{HS}$ and $N_{IS}$ denote the number of solvent H molecules and solvent I molecules that solvate to the adsorption site surface of the sample molecule; $W_{SHH}$, $W_{SII}$ and $W_{SHI}$ denote the interaction energy between each pair of two molecules HH, II and HI on the adsorption site, respectively, $M_{SHH}$, $M_{SII}$ and $M_{SHI}$ denote the number of corresponding pair interactions of the nearest neighbours on the adsorption site surface; $X_H$ and $X_I$ denote the mole fraction of each solvent; the factor $y$ denotes the area of the adsorption site surface in units of solvent molecule cross-section; the final term corresponds to the entropy change of eqn. 3.

$\Delta G_\beta$ differs from $\Delta G_\alpha$ only in that S is replaced by B; $X_B$ is equal to $X_S$ because the number of sample molecules equals the number of adsorption sites that will be adsorbed perfectly.

$\Delta G_\phi$ is defined by the following equation:

$$\Delta G_\phi = W_{HH}(y n_S N)X_H^2 + W_{HI}(y n_S N)2X_H X_I + W_{II}(y n_S N)X_I^2 - RT X_S \ln(X_S) \tag{8}$$

Here, $W_{HH}$, $W_{HI}$ and $W_{II}$ denote the interaction energies of each pair of solvent molecules; $N$ and $n_S$ denote the Avogadro constant and the mole number of the sample in the system. The value in parentheses describes the number of all solvent molecules that are assigned to group $\alpha$ or $\beta$.

Therefore, $\Delta G$ can be written as the following equation:

$$\begin{aligned}\Delta G = {} & -\Delta H_0 + W_\alpha + W_\beta - (W_{HH}X_H^2 + 2W_{HI}X_H X_I + W_{II}X_I^2) y n_S N \\ & + 2RT X_S \ln(X_S) + 2RT\{X_H(1 - X_S y) \ln(1 - X_S y) + \\ & + X_I(1 - X_S y) \ln(1 - X_S y) - X_S y X_H \ln(X_H) - X_S y X_I \ln(X_I)\}\end{aligned} \tag{9}$$

where

$$W_\alpha = W_{HS}N_{HS} + W_{IS}N_{IS} + W_{SHH}M_{SHH} + W_{SII}M_{SII} + W_{SHI}M_{SHI} \tag{10}$$

$$W_\beta = W_{HB}N_{HB} + W_{IB}N_{IB} + W_{BHH}M_{BHH} + W_{BII}M_{BII} + W_{BHI}M_{BHI} \tag{11}$$

$$N_{HS} = N_{HB} = (y n_S N)X_H \tag{12}$$

$$N_{IS} = N_{IB} = (yn_S N)X_I \quad (13)$$

$$M_{SHH} = M_{BHH} = (yn_S N)X_H^2 \quad (14)$$

$$M_{SII} = M_{BII} = (yn_S N)X_I^2 \quad (15)$$

$$M_{SHI} = M_{BHI} = (yn_S N)2X_H X_I \quad (16)$$

Fig. 2 shows the various interactions between solvent molecules, sample molecules and adsorbent. Rearrangement of eqn. 9 gives the following equation:

$$\Delta G - X_H \Delta G_H - X_I \Delta G_I = - yn_S[2\,RT\,\{X_H \ln(X_H) + X_I \ln(X_I)\} + N X_H X_I(\Delta g - \Delta g_S - \Delta g_B)] \quad (17)$$

where

$$\Delta g = W_{HH} + W_{II} - 2W_{HI} \quad (18)$$

$$\Delta g_S = W_{SHH} + W_{SII} - 2W_{SHI} \quad (19)$$

$$\Delta g_B = W_{BHH} + W_{WII} - 2W_{BHI} \quad (20)$$

Here, $\Delta G_H$ and $\Delta G_I$ are the free energy changes of the adsorption processes in the case of pure solvent mobile phases. The Gibbs free energy change is related to the capacity ratio $k'$ by the equation:-

$$\log k' = \log \varphi - \Delta G/(2.303RT) \quad (21)$$

where $\varphi$ is the phase ratio of the HPLC column. Substitution of eqn. 21 into eqn. 17 gives the following equation:

$$\log k' = X_H \log k'_H + X_I \log k'_I + y\{2(X_H \log X_H + X_I \log X_I) + N X_H X_I(\Delta g - \Delta g_S - \Delta g_B)/(2.303RT)\} \quad (22)$$

Approximating $\Delta g_S$ and $\Delta g_B$ as equal to $\Delta g$, we can obtain the following equation:

$$\log k' = X_H \log k'_H + X_I \log k'_I + y\{2(X_H \log X_H + X_I \log X_I) - N X_H X_I \Delta g/(2.303RT)\} \quad (23)$$

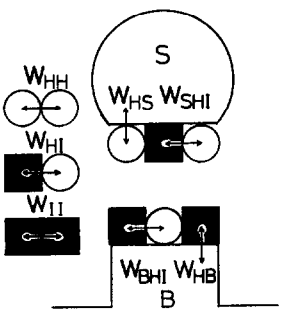


Fig. 2. Visualization of the solvation interaction.

which can also be expressed as:

$$\log k' = a(y)\, X_H^2 + b(y)\, X_H + c(y) + 2y\{X_H \log X_H + (1 - X_H)\log(1 - X_H)\} \quad (24)$$

Here, $a(y)$, $b(y)$ and $c(y)$ denote the coefficients as a function of $y$. The fourth term on the right-hand side is an additional term of the well-known quadratic equation.

Next, consider the separation factor or selectivity factor, $\alpha$, defined as

$$\alpha = k'_1/k'_2 \quad (25)$$

Here, the subscripts 1 and 2 denote the different sample peaks. The logarithm of $\alpha$ is defined as follows:

$$\log \alpha = X_H(\log k'_{H1} - \log k'_{H2}) + X_I(\log k'_{I1} - \log k'_{I2}) + (y_1 - y_2)2(X_H \log X_H + X_I \log X_I) - N\Delta g(y_1 - y_2)X_H X_I/(2.303RT) \quad (26)$$

Here, the subscript of $y$ and the second subscript of $k'$ denote the peak number, whereas $\Delta g$ has no subscript because it is independent of the sample molecules. The overall shape of eqn. 26 is about the same as that of eqn. 23.

Next, consider the resolution function $R_S$, defined as follows[9]:

$$R_S = \frac{1}{4}(\alpha - 1)\sqrt{N_{TP}}\frac{k'_1}{k'_1 + 1} = \frac{\sqrt{N_{TP}}}{2}\frac{k'_2 - k'_1}{k'_1 + k'_2 + 2} \quad (27)$$

where $N_{TP}$ is the number of theoretical plates of the column. Substituting eqns. 23 and 26 into eqn. 27, we can obtain $R_S$ values for any desired pair of peaks in the various mobile phase compositions.

Further, the SSM can be extended to multisolvent mobile phase systems in order to optimize the composition. Eqn. 23 can be extended to $n$ solvents, as follows:

$$\log k'_1 = \sum_{i=1}^{n} X_i \log k'_{i1} + y\left\{2\sum_{i=1}^{n} X_i \log X_i - \frac{N}{2.3RT}\sum_{i \neq j}^{n} X_i X_j \Delta g_{ij}\right\} \quad (28)$$

where $\sum_{i=1}^{n} X_i = 1$ and $\Delta g_{ij}$ denote the exchange energy of solvent molecules $i$ and $j$. If it is desired to estimate the overall value of $k'$ or $R_S$ from experiments in the $n$-solvents system, it is necessary to evaluate only following number, $Q$, of unknown parameters in eqn. 28 from at least the same number of experiments:

$$Q = n + 1 + \frac{n(n-1)}{2} = \frac{n^2 + n + 2}{2} \quad (29)$$

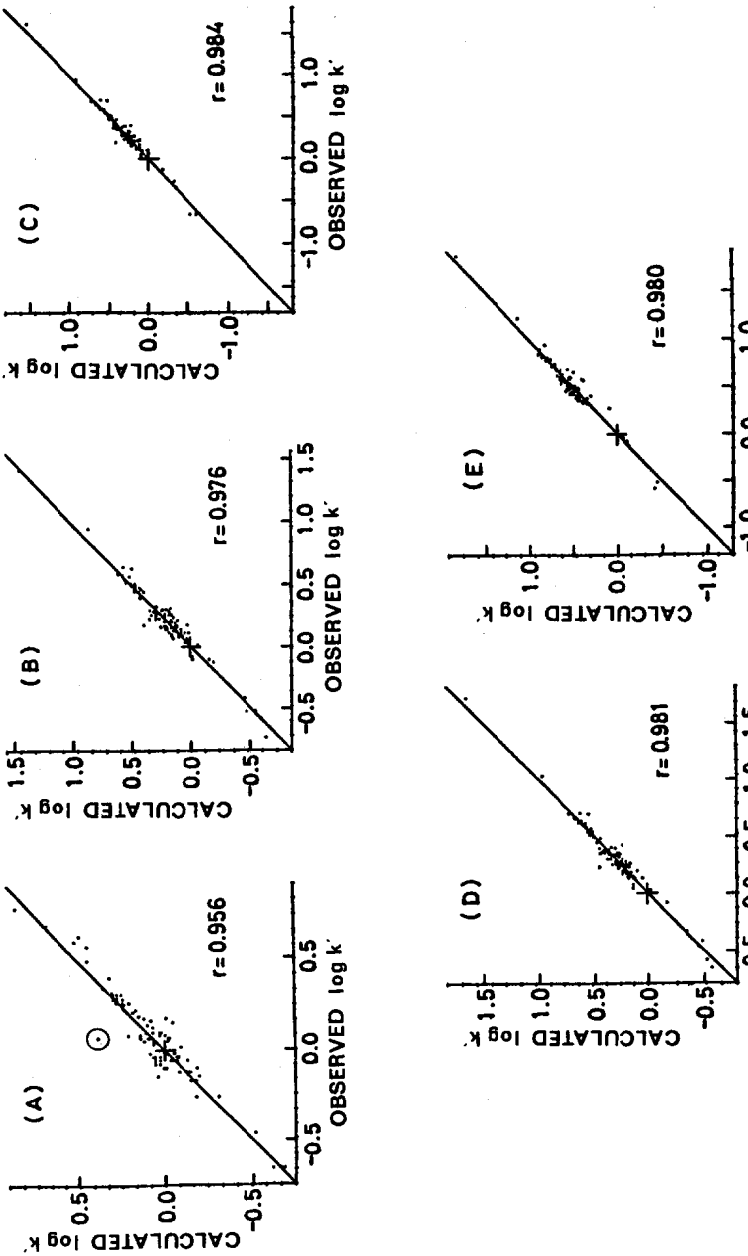


Fig. 3. Correlations between calculated and observed values of the logarithm of the capacity ratio for each drug by fitting eleven parameters. (A) Phenobarbital; (B) phenytoin; (C) nitrazepam; (D) clonazepam; (E) diazepam; the crosses indicate where $k' = 1$; $r$ = correlation coefficient.

## EXPERIMENTAL

### *Samples and columns*

Antiepileptic drugs, phenobarbital, phenytoin, nitrazepam, clonazepam, and diazepam, were purchased from Fujinaga Pharmaceutical (Tokyo, Japan), Dainippon Pharmaceutical (Osaka, Japan), Sankyo (Tokyo, Japan), Sumitomo Chemical (Osaka, Japan), and Yamanouchi Pharmaceutical (Tokyo, Japan), respectively. ODS columns, μBondapak $C_{18}$ (Waters Assoc., Milford, MA, U.S.A.) were used.

### *Chromatography*

A constametric metering pump Model 3 (LDC/Milton Roy, Riviera Beach, FL, U.S.A.) and a Model KLS-3T injector (Kyowa Seimitsu, Tokyo, Japan) were linked to a Model UVIDEC 100 UV detector (Nihon Bunko, Tokyo, Japan). HPLC-grade solvents, acetonitrile, tetrahydrofuran, and methanol, were purchased from Wako (Osaka, Japan), Kanto Chemical (Tokyo, Japan), and Tokyo Kasei (Tokyo Japan), respectively. The flow-rate was 1.0 ml/min. Each sample was dissolved in methanol or ethanol. A volume of 10 μl of sample solution was injected into the column. The hold-up volume was measured by using water as a sample. The results were obtained at ambient temperature (22 ± 4°C).

## RESULTS AND DISCUSSION

Five antiepileptic drugs were eluted by 114 arbitrary compositions of mobile phases, and the corresponding capacity ratios for each drug were evaluated. The ranges of the eluting solvents were 0–100% for tetrahydrofuran, 0–100% for acetonitrile, 0–100% for methanol and 0–80% for water. Eqn. 28 was fitted to the experimental points. Fig. 3 shows the correlations between the observed values and fitted (calculated) values of the logarithm of the capacity ratio. A good correlation was obtained with eleven parameters for quaternary solvent mobile phase HPLC. The circled point in Fig. 3A corresponds to an elution with 39% tetrahydrofuran and 61% water. Correlation coefficients between observed and calculated values of the logarithm of the capacity ratio were *ca.* 0.95–0.98. Better correlations can of course be obtained with few and completely empirical parameters.

Our attention is now focused on the values of these parameters rather than the correlation itself. All the parameters obtained are shown in Table I. It can be seen that the number of solvent molecules that adsorb on the sample is less than 1. Parameters that indicate the exchange energy of specific pairs of solvent molecules have the same sign and show similar tendencies. Next, we tried to predict the capacity ratios by using these exchange energy parameters, $\Delta g_{ij}$. These six parameters obtained from the fitting procedures for all drugs other than phenobarbital were averaged at each parameter and were used to predict the capacity ratios of phenobarbital. These $\Delta g_{ij}$ values were substituted into eqn. 28, and the resulting equation was fitted to the experimental points to obtain the $\log k'_{i1}$ and $y$ values specific to phenobarbital.

Fig. 4 shows the correlations between observed and calculated values of the logarithm of the capacity ratio for each drug from the fitted equation. Correlation coefficients between observed values and calculated values were *ca.* 0.94 – 0.97. This means that if we obtain $\Delta g_{ij}$ values we can accurately predict the capacity ratio of

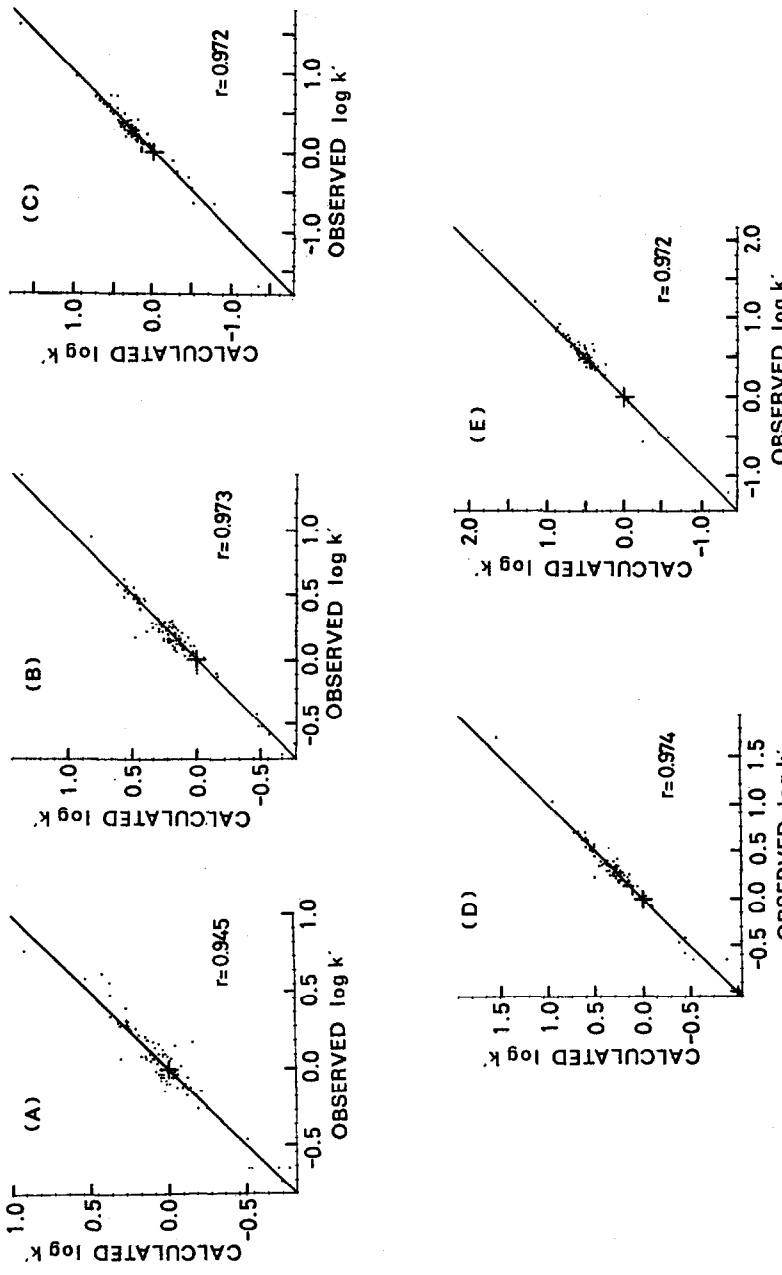


Fig. 4. Correlations between calculated and observed values of the logarithm of the capacity ratio for each drug by fitting five parameters. (A) Phenobarbital; (B) phenytoin; (C) nitrazepam; (D) clonazepam; (E) diazepam; the crosses indicate where $k' = 1$; $r$ = correlation coefficient.

TABLE I

PARAMETERS OBTAINED FROM $k'$ FITTING ON THE EXPERIMENTAL POINTS

$\alpha$ = Tetrahydrofuran; $\beta$ = acetonitrile; $\gamma$ = methanol; $\delta$ = water.

| *Parameter* | *Antiepileptic drugs* | | | | |
|---|---|---|---|---|---|
| | *Phenobarbital* | *Phenytoin* | *Nitrazepam* | *Clonazepam* | *Diazepam* |
| $\log k'_\alpha$ | −0.66 | −0.66 | −1.69 | −0.65 | −1.21 |
| $\log k'_\beta$ | −0.67 | −0.54 | −0.35 | −0.45 | −0.11 |
| $\log k'_\gamma$ | −0.61 | −0.63 | −0.53 | −0.57 | −0.43 |
| $\log k'_\delta$ | 1.83 | 2.85 | 2.95 | 3.08 | 3.16 |
| $y$ | 0.42 | 0.38 | 0.79 | 0.55 | 0.68 |
| $\frac{N}{2.3RT}\Delta g_{\alpha\beta}$ | −2.94 | − 3.84 | − 4.50 | − 2.61 | −3.59 |
| $\frac{N}{2.3RT}\Delta g_{\alpha\gamma}$ | −12.44 | −14.39 | −10.19 | −11.06 | −8.66 |
| $\frac{N}{2.3RT}\Delta g_{\alpha\delta}$ | 2.34 | 9.49 | 1.39 | 6.52 | 3.63 |
| $\frac{N}{2.3RT}\Delta g_{\beta\gamma}$ | − 7.35 | − 9.05 | − 6.97 | − 8.04 | −7.77 |
| $\frac{N}{2.3RT}\Delta g_{\beta\delta}$ | 4.40 | 9.17 | 3.42 | 5.68 | 3.57 |
| $\frac{N}{2.3RT}\Delta g_{\gamma\delta}$ | 3.09 | 5.73 | 1.19 | 3.11 | 0.64 |

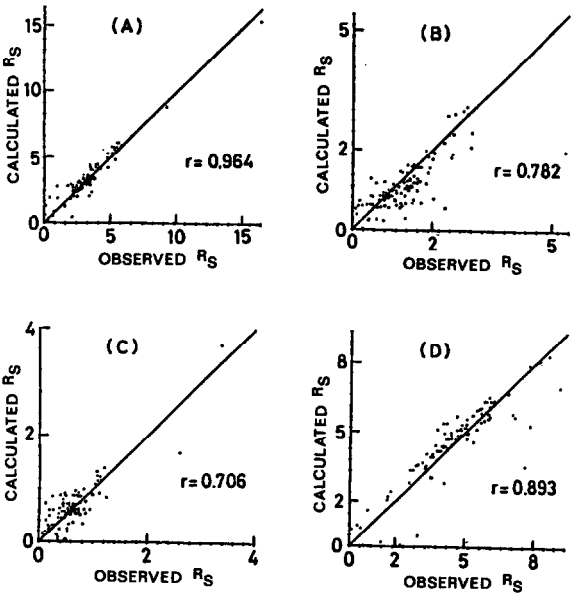


Fig. 5. Correlations between calculated and observed values of $R_S$ for the nearest pairs of the drugs. (A) Phenobarbital–phenytoin; (B) phenytoin–nitrazepam; (C) nitrazepam–clonazepam; (D) clonazepam–diazepam; $r$ = correlation coefficient.

each drug by using eqn. 28 and at least five capacity ratio data (the number specific values, log $k'_{i1}$ and $y$, is five). Reliability of the prediction, of course, depends on the number of the capacity ratio data used.

Next, the $R_S$ values were evaluated from the parameters obtained. Fig. 5 shows the correlation of observed and calculated $R_S$ values: correlation coefficients were *ca.* 0.70–0.96, relatively low compared with those of the capacity ratios. This shows that the sensitive $R_S$ parameter cannot be easily reproduced by the $k'$ fitting equation, whereas the overall feature of $k'$ itself can be reproduced well.

We may state that capacity ratios can be reproduced well by our thermodynamic model with several parameters. They seem to depend mainly on the exchange energies of the solvent molecules. It remains to be seen whether all these energy values can be related to the chemical thermodynamic values of binary liquids that have not yet been determined. It is still difficult to evaluate $R_S$ values because of their sensitivity caused by the difference between two independent substances.

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