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Introduction of *In Silico* Chromatography

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Abstract

Molecular interaction (MI) is fundamental phenomena in nature. Chromatography is a tool to quantitatively measure the degree of molecular interactions. The qualitative explanation has been performed using solubility factors [1]. The quantitative explanation is achieved using computational chemical calculation (*In silico*). The molecular interaction forces are combination of solubility factors, and can be obtained as van der Waals, hydrogen bonding and electrostatic energy values after molecular mechanics (MM2) calculations. Simple study can be done using small molecules. The model analyses should help to quantitatively understand the chromatographic retention mechanisms. When one small molecule is replaced to a macro molecule, (chromatography model phase), we can quantitatively analyze chromatography retention time with the retention times of standard compounds. Furthermore, when the macro molecule is a protein, we can study affinity level of proteins. In addition, the apc calculated using MOPAC program indicates the enzyme reactivity. Prediction of boiling point, dissociation constant, and albumin-drug binding affinity were demonstrated as practical applications of *in silico* chromatography [2].

Keywords: Chromatography; *In silico*; Molecular interactions; Moleules

Molecular Interaction *In Silico*

The final structure energy (FS) for a pair of identical molecules was less than two times from the FS of a single alcohol, indicating that the energy values calculated using a molecular mechanics (MM2) program can explain the degree of molecular interactions. The final structure energy is a combination of hydrogen bonding (HB), electrostatic (ES), van der Waals (VW), bond stretch, bond angle dihedral angle, and improper torsion energies. The electrostatic, hydrogen bonding and van der Waals energies are measured as ion-ion interaction level, hydrogen bonding contribution, and hydrophobic interaction, respectively. Further, steric hindrance is contributed by other energies.

Molecular interaction (MI) energy value of final (optimized) structure (MIFS), hydrogen bonding (MIHB), electrostatic (MIES), and van der Waals (MIVW) can be calculated using following equations:

MIFS=FS (molecule A)+FS (molecule B)–FS (molecule A and molecule B complex),

MIHB=HB (molecule A)+HB (molecule B)–HB (molecule A and molecule B complex),

MIES=ES (molecule A)+ES (molecule B)–ES (molecule A and molecule B complex),

MIVW=VW (molecule A)+VW (molecule B)–VW (molecule A and molecule B complex). The relative MIHB, MIES, and MIVW values indicate the contribution level.

When these equations are applied, one of molecules is model phase and target molecules are analytes. Only molecular size is different for analytes and model phases, even model phases can be replaced to proteins for analyzing affinity level.

In addition, when two molecules contact together, localization of electron is observed. The phenomena can be extracted as difference of atomic partial charge (apc) even the weak hydrophobic interaction is occurred. The apc is calculated using MOPAC program. The difference increases stronger the contact like π–π interaction<hydrogen bonding<ion-ion interaction. These typical examples are described using simple model compounds [2].

Basic Concepts of *In Silico* Chromatography

Hydrophobic interaction (van der waals energy contribution)

n-Hexane was constructed as a model compound to study hydrophobic interaction. The structure was optimized using MM2 calculation. Furthermore, the structure was optimized using MOPACPM5 to obtain apc. The FS (optimized) energy value was 3.472 kcal mol^{-1}, and VW energy value was 2.615 kcal mol^{-1} among the final energy value. That is, alkanes are completely saturated molecules having no specific physico-chemical property except van der Waals volume. The most contributed property of n-hexane is VW energy. Two n-hexane molecules were docked as a complex. The initial conformations and complex forms are shown in Figure 1 with their calculated energy values. For conformation A, one n-hexane was turned 180°, and located above of another n-hexane, then these two n-hexane molecules were optimized using MM2 calculation, the FS energy value was 1.627 kcal mol^{-1}. For complex B, a copied n-hexane was moved to upper location; then the conformation was optimized using MM2 calculation. The FS energy value was 2.433 kcal mol^{-1}. These two n-hexane molecules were tightly contacted. The difference of tightness was obtained as calculated energy value difference. They interact together using van der Waals force. The MIFS of complex A was 5.316 kcal mol^{-1}, and that of complex B was 4.511. The MIVW of complex A was 5.376 kcal mol^{-1}, and that of complex B was 4.514 kcal/mol. The complex A is more stable than the complex B. It is about 0.8 kcal mol^{-1} stable. The final (optimized) structure energy values are smaller than the sum of individual energy values. The balance is considered as the molecular interaction energy values. These pairs demonstrated only van der Waals energy value change. That is, these molecules interact with hydrophobicity.

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Then, the complex structure was further optimized using MOPACPM5 to obtain atomic partial charge (apc) to analyze the electron localization level. The calculated values are summarized in Figure 2 with their complex forms. The apc value of center hydrogen of n-hexane was 0.094 au (atomic unit). The value increased to 0.096 at the contact site. Even the apc change is very small, but it indicated localization of electron by complex formation.

π–π interaction (electrostatic energy contribution)

Diethylether was selected to demonstrate π–π interaction. The molecular size is similar to n-hexane, and no possible substitute is included to form hydrogen bonding and ionic interactions. The FS energy value was 1.904 kcal mol^{-1}, the main contributed energy was VW energy, 1.979 kcal mol^{-1}. However, when two molecules docked together, contribution of electrostatic energy became clear. The FS energy values of complex A and B were -1.674 and 1.706 kcal mol^{-1}, respectively. Complex A was formed with a 180° turned molecule, and complex B was formed with parallel molecules. These conformations are shown in Figure 3 with calculated molecular properties. Complex A is more stable and the MIFS and MIES energy values were 5.483 and 1.521 kcal mol^{-1}. Those of complex B were 2.103 and -0.901 kcal mol^{-1}, respectively. Contribution of MIVW was less than MIFS, and the values were 4.234 and 2.976 kcal mol^{-1} for complexes A and B, respectively.

The apc of ethylether oxygen was -0.391, and that of complex was -0.423. The value decreased 0.032 au after complex formation. The conformation and the molecular properties are shown in Figure 4. The electro localization due to π–π interaction is clearer than that in hydrophobic interaction.

Hydrogen bonding (hydrogen bonding energy contribution)

The example of hydrogen bonding contribution in molecular interactions was demonstrated by complex formation of pentanol. When two pentanol molecules were located like complex A of n-hexane or ethylether, and their complex conformation was optimized using MM2 calculation. The complex conformation is shown in Figure 5. FS and VW energy values of pentanol were 2.779 and 2.036 kcal mol^{-1}. The HB and ES values were zero. After complex formation these values were changed. The FS, VW, HB, and ES values were -1.821, 0.665, -5.047, -0.486 kcal mol^{-1}, respectively. These MIFS, MIVW, MIHB, and MIES values were 7.379, 3.407, 5.047, and 0.486, respectively. The contribution of hydrogen bonding energy value was clear, and contributed about 70% of MIFS energy value. The electro localization by formation of hydrogen bonding was also observed as apc value change (Δapc) of 0.007 au of hydrogen and oxygen as shown in Figure 6.

Ion-ion interaction by coulombic force

Before ion-exchange, two molecules form complex by either ion-ion or ion-hydrogen interactions due to the dissociation form at certain pH solution. Therefore, the molecular interaction analysis was carried out at the condition where one molecule was ionic form and another molecule was either molecular or ionic form. Two type of ion-exchanges, cation and anion, were studied. A simplified model experiment *in silico* demonstrated that carboxyl groups interact with ionized amine by Coulombic force and with molecular form of amine by Lewis acid-base interaction mainly hydrogen bonding.

Interaction of both molecular forms butyric acid and pentylamine: Molecular form acid forms a complex with molecular form amine based on hydrogen bonding, especially in non-aqueous system (normal-phase liquid chromatography). The conformation of butyric acid, pentylamine and the complex were optimized by

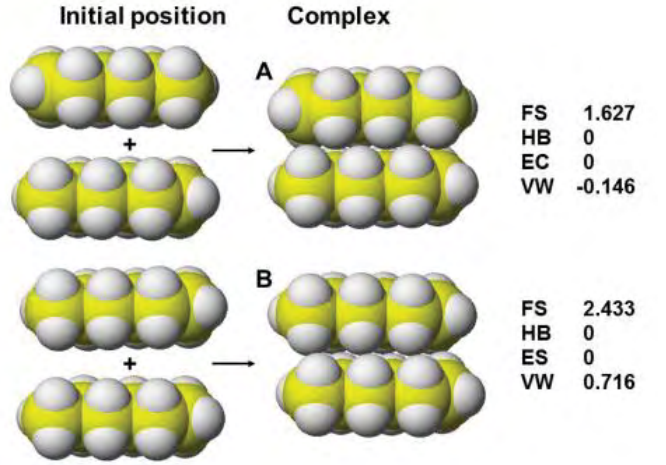


Figure 1: Complex conformation and their optimized energy values of n-hexane (unit: kcal mol-1). Large and small white balls: carbon and hydrogen.

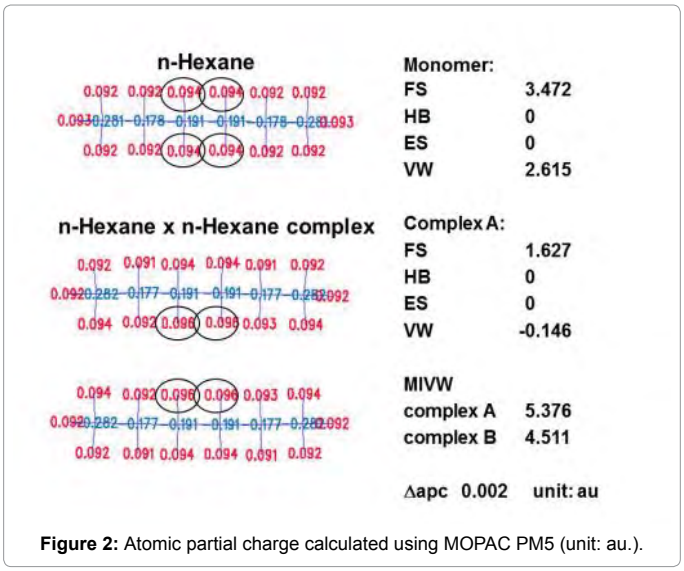


Figure 2: Atomic partial charge calculated using MOPAC PM5 (unit: au.).

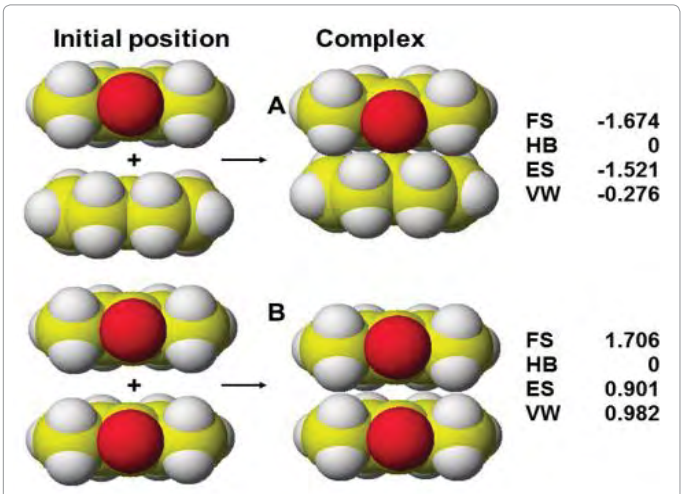


Figure 3: Complex conformation and their optimized energy values of ethylether (unit: kcal mol-1). Small and large white, and black balls: hydrogen, carbon and oxygen.

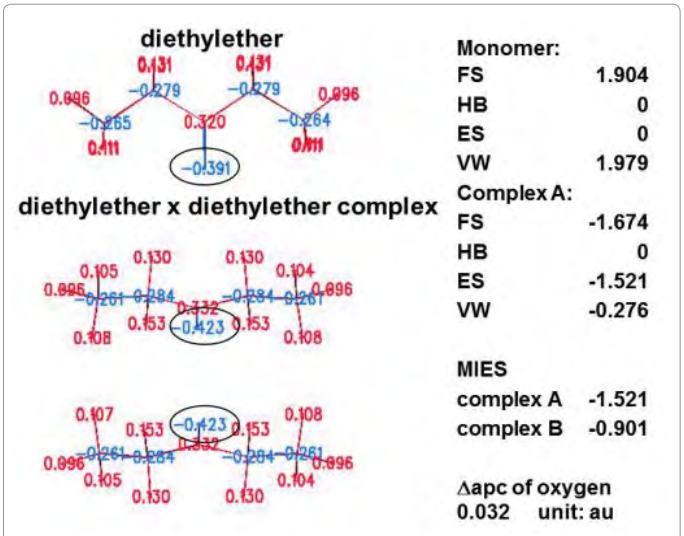


Figure 4: Atomic partial charge calculated using MOPAC PM5 (unit: au).

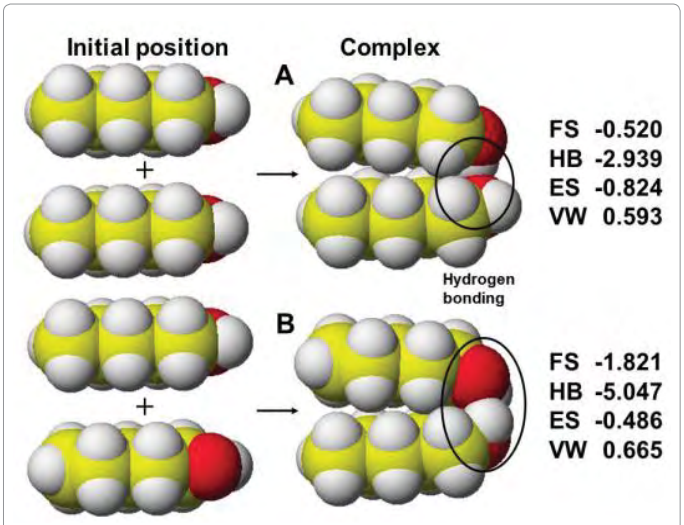


Figure 5: Complex conformation and their optimized energy values of pentyl alcohol (unit: kcal mol^{-1}). Small and large white, and black balls: hydrogen, carbon and oxygen.

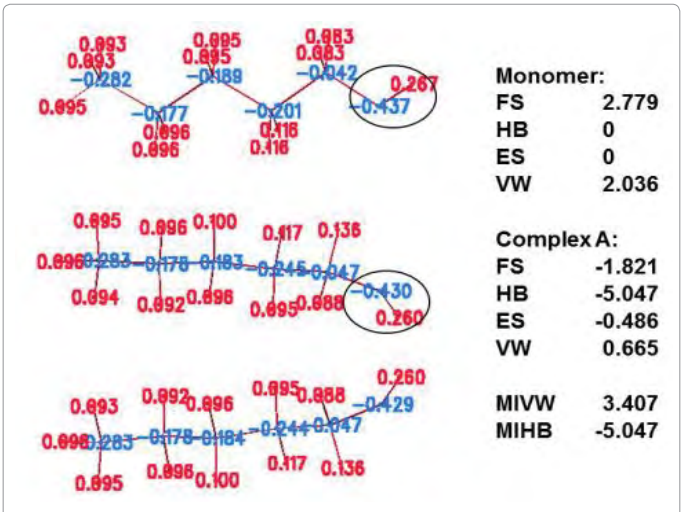


Figure 6: Atomic partial charge calculated using MOPAC PM5 (unit: au).

using MM2 calculation, and the apc values were obtained by using MOPACPM5 calculation as shown in Figures 7 and 8. The MIFS energy value was 8.976 kcal mol^{-1}, and the MIHB was 5.440 kcal mol^{-1}. Hydrogen bonding energy mainly contributed. In addition, Δapc of amino hydrogen was 0.060 au, and that of nitrogen was 0.134 au. Δapc of butyric acid oxygen and hydrogen was a little.

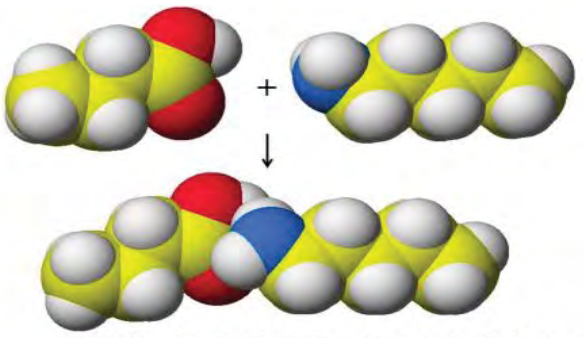
Molecular form butyric acid and ionic form pentylamine at low pH: At low pH, carboxyl group is molecular form and amino group is ionized. The MIFS energy value of molecular form butyric acid and ionic form amine complex was 37.822 kcal mol^{-1}. The MIHB, MIES, and MIVW energy values were 28.704, 17.389, and 3.442 kcal mol^{-1}, respectively. This difference clearly indicated the contribution of hydrogen bonding in the ion-exchange system in Figure 9. Coulombic force should be the main molecular interaction force, but hydrogen bonding does contribute in the ion-exchange system, depending on the molecular structure of the ion-exchangers. The difference can be examined based on the apc of the targeted atoms. The Δapc of butyric acid oxygen and hydrogen was 0.065 and 0.035 au, respectively. That of amine nitrogen and hydrogen was 0.029 as shown in Figure 10.

Both ionic form butyric acid and pentylamine at neutral pH: When both acid and amine are ionized at middle neutral pH, the true ion-ion interaction can be studied. The optimized structure is shown in Figures 11 and 12. The MIFS energy value of ionic form butyric acid and pentylamine complex was 133.803 kcal mol^{-1}. The MIES and MIHB energy value was 120.902 and 30.486 kcal mol^{-1}, respectively. These values clearly indicated the contribution of Coulombic force in the ion-exchange system as shown in Figure 11. Hydrogen bonding does contribute a little in the ion-exchange system, depending on the dissociation of the ion-exchangers. The difference can be examined based on apc of the targeted atoms. The Δapc of butyric acid oxygen was 0.044 au, and that of amino group nitrogen was about 0.036 au as shown in Figure 12.

Ionic butyric acid and molecular from pentylamine at high pH: The MIFS energy value of molecular form pentylamine and ionic form butyric acid complex was 8.692 kcal mol^{-1}. The MIHB, MIES, and MIVW energy values were 0.008, 3.634, and 2.614 kcal mol^{-1}, respectively. This difference clearly indicated the contribution of Coulombic force in the ion-exchange system; however, contribution of van der Waals force was not neglect as shown in Figure 13. Van der Waals force is not the main molecular interaction force for ionized acids, but does contribute in the ion-exchange system, depending on the molecular structure of the ion-exchangers. The difference can be examined based on the apc of the targeted atoms. An example is shown in Figure 14. The Δapc of butyric acid oxygen was 0.026 au.

Propylguanidine and molecular form butyric acid at low pH: The MIFS energy value of propylguanidine and molecular form butyric acid was 27.978 kcal mol^{-1}, and MIHB energy value was 28.676 kcal mol^{-1}. These values clearly indicated the contribution of hydrogen bonding in the ion-exchange system as shown in Figure 15. The MIES energy value was 1.648 kcal mol^{-1}, and the MIVW energy value was 3.413 kcal mol^{-1}. Hydrogen bonding does contribute in the ion-exchange system, depending on the molecular structure of the ion-exchangers. The difference can be examined based on apc of the targeted atoms. The Δapc of hydroxyl hydrogen of butyric acid was 0.034 au, and that of guanidyl group hydrogen was about 0.010 au as shown in Figure 16.

Propylguanidine and ionic form butyric acid at neutral pH: The MIFS energy value of propylguanidine and ionic form butyric acid complex was 85.010 kcal mol^{-1}. The MIHB and MIES energy values were 24.789 and 33.938 kcal mol^{-1}, respectively. This difference clearly



	Butyric acid	Pentylamine	Complex	Balance
FS	-9.672	3.288	-15.361	8.976
HB	-5.689	0	-11.129	5.440
ES	-5.937	0	-6.234	0.297
VW	2.353	1.937	3.365	0.925

Figure 7: Complex conformation and their optimized energy values of molecular form butyric acid and pentylamine (unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

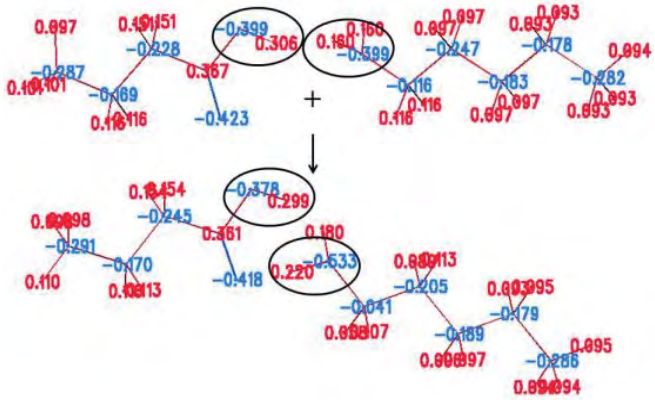
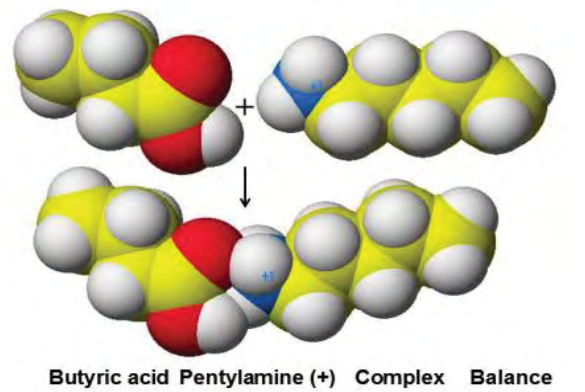


Figure 8: Atomic partial charge calculated using MOPAC PM5 (unit: au).



	Butyric acid	Pentylamine (+)	Complex	Balance
FS	-9.672	3.475	-50.969	37.822
HB	-5.689	0	-34.393	28.704
ES	-5.937	0	-23.326	17.389
VW	2.353	2.237	8.032	3.442

Figure 9: Complex conformation and their optimized energy values of molecular form butyric acid and ionized pentylamine (unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

indicated the contribution of Coulombic force in the ion-exchange system; however, contribution of hydrogen bonding could not be neglected as shown in Figure 17. Hydrogen bonding is not the main molecular interaction force for ionized acids, but does contribute in the ion-exchange system, depending on the molecular structure of the ion-exchangers. The difference can be examined based on the apc of the targeted atoms. An example is shown in Figures 18. The Δapc of butyric acid oxygen was about 0.03 au.

The above analyses were carried out without solvent and pH control components. The solvent and pH control components, however, contribute to the replacement of an analyte for elution from the column, and the initial molecular interaction must occur directly between the analyte and the molecular recognition phase.

Steric Hindrance for Chiral Recognition

Steric hindrance cannot be directly calculated, but a lower MI energy value indicates lower steric hindrance in a complex. This phenomenon can be observed in chiral recognition and protein-substrate interactions, with the latter also being known as affinity. Such phenomenon can be studied via amino acid enantiomer separation. The major interaction force for enantiomer separation in normal-phase liquid chromatography is the Lewis acid-base interaction including hydrogen bonding, and steric hindrance affects stereo-selectivity.

Naturally, amino acid and saccharide are enantioselective compounds. Therefore, enantioselective phases are synthesized from amino acid and saccharide. 4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) derivatized amino acids are commonly used for trace amount analysis of amino acids as highly sensitive fluorescence compounds. The enantiomers have been separated either amino acid based enantioselective (Pirkle type) or modified polysaccharide phases. The enantiomer separation of NBD-alanine is demonstrated using model phases.

Separation using a pirkle type phase

One of Pirkle type phase, Sumichiral OA-2500S was constructed and optimized using MM2 calculation. The complex forms with NBD-S-alanine and NBD-R-alanine are shown in Figure 19. MIFS energy values for S-alanine and R-alanine were 28.109 and 27.617 kcal mol^{-1}. Contribution of MIHB was significant, MIHB for S-alanine and R-alanine were 17.865 and 14.352 kcal mol^{-1}, respectively. Contribution of MIVW was not neglected, and MIVW values for NBD-s-alanine and NBD-R-alanine were 9.840 and 10.332 kcal mol^{-1}, respectively.

Separation sing a derivatized polysaccharides phase

NBD-amino acid methyl ester enantiomers were also separated using a derivatized polysaccharide phase (Chiralpak 1A). Therefore, an oligomer of six methoxyglucoses was constructed and used to study chiral recognition of NBD-alanine enantiomers. The complex conformations with NBD-S-alanine or NBD-R-alanine are shown in Figure 20. The MIFS energy values for NBD-S-alanine and NBD-R-alanine were 24.126 and 22.240 kcal mol^{-1}, respectively. MIVW was main contributed energy due to the molecular recognition. MIVW energy values for s-alanine and R-alanine were 16.068 and 14.708 kcal mol^{-1}, respectively. The contribution of MIHB was not neglected, and the MIHB values for NBD-s-alanine and NBD-R-alanine were 7.167 and 4.350 kcal mol^{-1}, respectively. These model analyses demonstrated the feasibility of *in silico* analysis of enantiomer recognition.

Prediction of boiling point

Boiling point is sometimes related to retention time measured on a non-polar phase in gas chromatography. The capacity ratios of 48 compounds measured using two methylsilicone capillary columns under isocratic conditions correlated well to the boiling point. The correlations between the boiling points and log *k* values at 240°C were 0.992 and 0.988 (n=48) for DB1 and CPSil5 columns, respectively.

In general, the boiling point is not available. Computational chemical methods, however, can be used to calculate a variety of molecular properties. The calculated molecular interaction energy, the analyte's van der Waals volume, enthalpy, and optimized energy values can be used to further quantitative study.

The molecular interaction energy values of alkanes were used as the standard, similar to Kovats' retention index method. The energy value change after complex formation is considered the molecular interaction energy value and comprises the final (optimized) structure (MIFS), hydrogen bonding (MIHB), and electrostatic (MIES), and van der Waals (MIVW) forces. The MIHB and MIVW indicate the contribution of the hydrogen bonding and molecular size effects, respectively. The calculated molecular interaction energy values were correlated with the log *k* values of these compounds. For example, to predict retention time using MIFS=a × log *k*+b, the slope a and constant b of all groups should be the same for an ideal system.

The retention (molecular interaction) force on methylsilicone phases should be van der Waals force on non-polar methylsilicone phases; therefore, MIVW was used for the quantitative analysis of retention time on methylsilicone phases. The van der Waals energy values (MIVW) are near equal to the final structure energy values (MIFS) on model methylsilicone phases. The balance (ΔMIVW) between alkanes and the selected PAH, benzene, naphthalene, and anthracene was considered to be the vaporization energy from the methylsilicone phase.

The desorption energy (Δdes) was related to the stability of a pair of analytes which was considered to be the simplest cluster model. That is, the addition of necessity energy to the cluster should disperse the individual molecules. The final structure energy value of a pair of analytes (fs*p*) was used to obtain Δdes, but the Δdes of alkanols was small. Generally, cutting a hydrogen bond requires more energy, then the van der Waals force. The boiling point of molecules with hydrogen donor substitutes was relatively high by comparison with the van der Waals volume. The typical example is water with two hydrogens that has a high boiling point. The boiling point of molecules with a hydrogen acceptor is a little high. The contribution of hydrogen bonding and electrostatic energy values were therefore considered to increase Δdes. The Δdes were obtained as a combination of fs*p*, 200x hb*p*, and es*p* as Δvap, instead of fs*p*. The sum of MIVW and Δdes correlated with measured log *k* values.

This new method demonstrated an excellent correlation with the measured log *k* values. The correlation coefficient was 0.99, for all temperatures on both non-polar CPSil5 and DB1 methylsilicone phases. That is, boiling point is related to retention time measured on a non-polar phase. The capacity ratio measured on a non-polar phase can be predicted using computational chemical calculations. Thus, boiling point can be predicted using *in silico* analysis [2].

Prediction of dissociation constant (p*K*a)

Dissociation constants can be predicted using Hammett's equations p*K*a=A+BΣσ where A and B are constant and σ is Hammett's σ constant. A is the p*K*a value of non-substituted compounds and B has to be selected for each series of compounds. There were no standard equations for A and B values. The reference values of dissociation constants were used to predict dissociation constants from atom partial charges (apc) calculated by the MM/AM1 program.

The correlation between p*K*a values measured by liquid chromatography and apc of non-substituted aromatic acids including phenol was:

p*K*a=-210.485 × (apc)+55.797, r^2=0.994, (n=8), where the reference compounds used for this calculation were benzoic acid, phenylacetic acid, 3-phenylpropionic acid, mandelic acid, trans-cinnamic acid, indole-3-acetic acid, indole-3-butyric acid and phenol. The constant A of Hammett's equation can be calculated from this equation.

BΣσ of Hammett's equation was:

BΣσ=(-9.356 × p*K*a-116.314) × Δ(apc of substitute)

where B is (-9.356 × p*K*a-116.314) and Σσ is Δ (apc of substituent). The ortho-effect of p*K*a values of ortho-substituted benzoic acids were 0.6177 and 1.3558 unit for a methyl group and a halogen atom, respectively. Therefore, p*K*a of benzoic acid derivatives can be predicted from above equations and ortho-effects. The correlation between predicted and reference p*K*a values for benzoic acid derivatives was as follows:

p*K*a (pred)=0.991 × p*K*a (ref) -0.017, r^2=0.982, (n=22).

Dissociation constant of acidic compounds can be predicted using apc instead of Hammett's σ constant. The predicted p*K*a values were used for the prediction of retention times of partly ionized acidic compounds a given pH-controlled eluent [2].

Prediction of human serum albumin-drug binding affinity (log n*K*)

The discovery of new drugs has been accelerated by combinatorial chemistry, and fast screening of drug candidates is very important, and the octanol-water partition coefficient (log *P*) and dissociation constant (p*K*a) are easily measured. Human serum albumin (HSA) is a 66500 Da protein and the most common and abundant plasma protein and is considered as a multifunctional plasma transport protein. It constitutes approximately 60% of total serum protein and is a small globular protein with a high electrophoretic mobility; it acts to maintain homeostasis in the body providing a protein reserve. HSA displays the property of conformational adaptability which allows the binding of ligands including bilirubin, fatty acid, tryptophan, and many drugs, and binding to HSA can prolong the *in vivo* half-life of a drug. The physiological function of HSA does not depend on specific interactions but on the broad non-specific physicochemical character of the protein. The binding of lead compounds for HSA represents a major challenge in drug discovery while a degree of albumin binding may be desirable in helping to solubilize compounds. An excessively high affinity for a protein (>95% bound) requires correspondingly high doses to achieve an effective concentration *in vivo*. Thus, the binding of drugs to HSA is one of the most important factors determining their pharmacokinetics.

The main binding forces are hydrophobic interactions and ion-ion interactions; therefore, albumin-drug binding affinity could be measured from the *k* of drugs measured by reversed-phase and ion-exchange liquid chromatography for standard drugs using a pentyl-bonded silica gel column for measuring hydrophobic interaction and a guanidine-bonded and carboxyl-bonded silica gel columns for

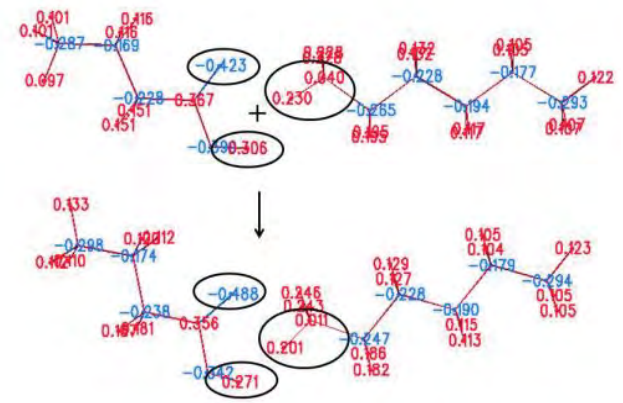
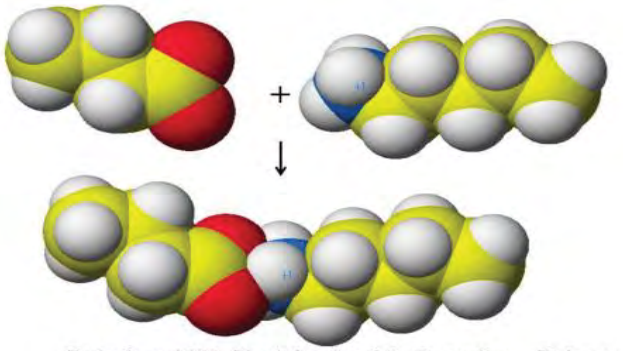


Figure 10: Atomic partial charge calculated using MOPAC PM5 (unit: au).



	Butyric acid(-)	Pentylamine (+)	Complex	Balance
FS	-4.483	3.475	-134.811	133.802
HB	0	0	-30.486	30.486
ES	0	0	-120.902	120.902
VW	2.175	2.237	12.549	8.137

Figure 11: Complex conformation and their optimized energy values of ionized butyric acid and pentylamine (Unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

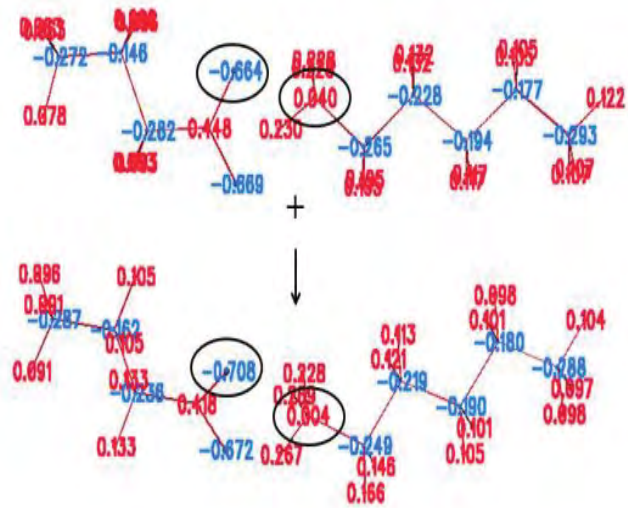
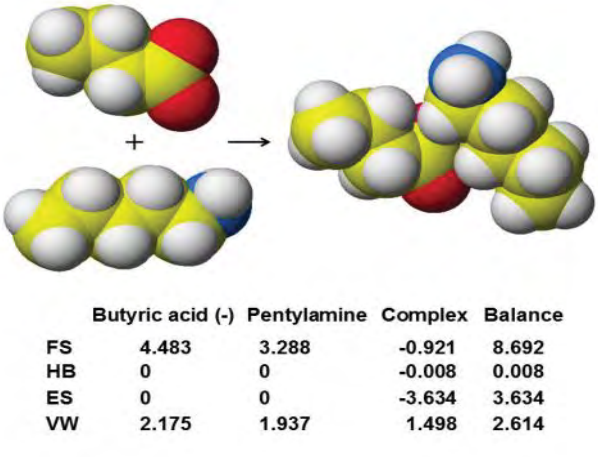


Figure 12: Atomic partial charge calculated using MOPAC PM5 (unit: au).



	Butyric acid (-)	Pentylamine	Complex	Balance
FS	4.483	3.288	-0.921	8.692
HB	0	0	-0.008	0.008
ES	0	0	-3.634	3.634
VW	2.175	1.937	1.498	2.614

Figure 13: Complex conformation and their optimized energy values of ionized butyric acid and molecular form pentylamine (unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

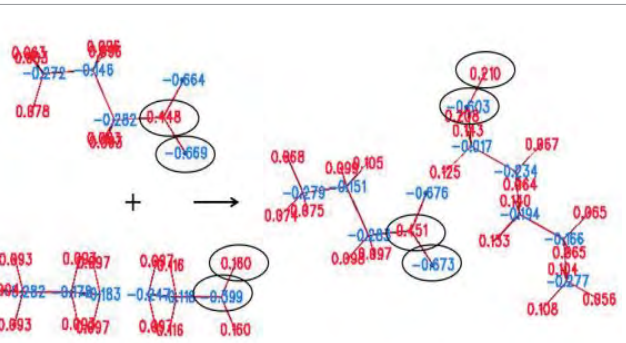
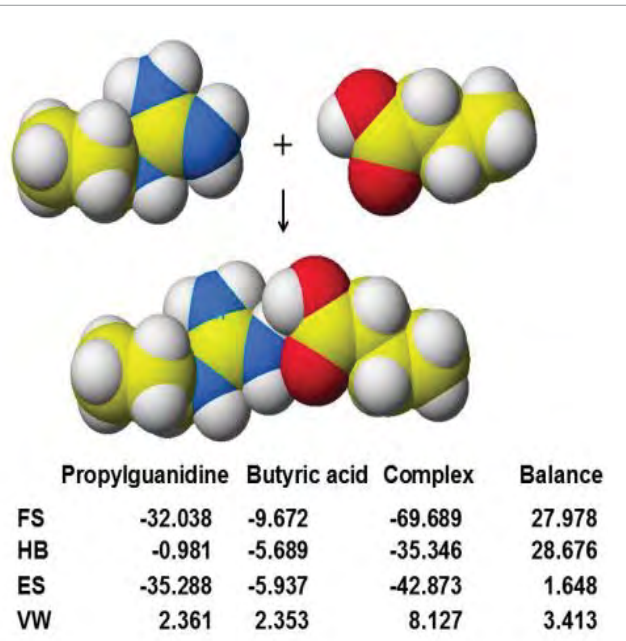


Figure 14: Atomic partial charge calculated using MOPAC PM5 (unit: au).



	Propylguanidine	Butyric acid	Complex	Balance
FS	-32.038	-9.672	-69.689	27.978
HB	-0.981	-5.689	-35.346	28.676
ES	-35.288	-5.937	-42.873	1.648
VW	2.361	2.353	8.127	3.413

Figure 15: Complex conformation and their optimized energy values of propylguanidine and molecular form butyric acid (unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

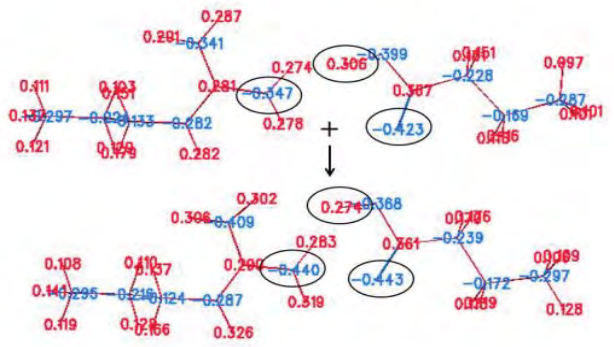
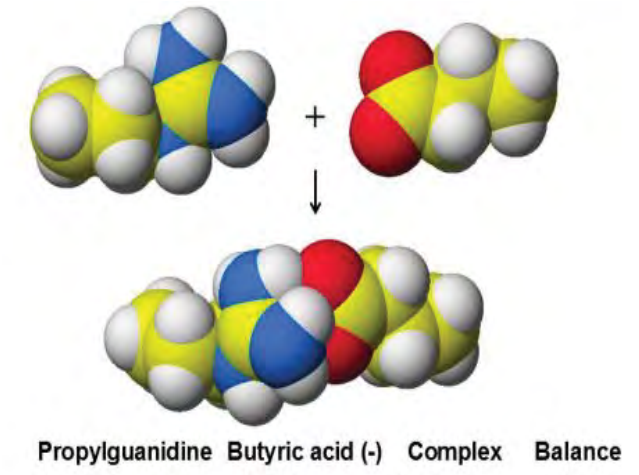


Figure 16: Atomic partial charge calculated using MOPAC PM5 (unit: au).



	Propylguanidine	Butyric acid (-)	Complex	Balance
FS	-32.038	4.483	-112.565	85.010
HB	-0.981	0	-26.751	24.789
ES	-35.288	0	-104.514	33.938
VW	2.361	2.175	8.378	3.842

Figure 17: Complex conformation and their optimized energy values of propylguanidine and ionized butyric acid (unit: kcal mol^{-1}). Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen.

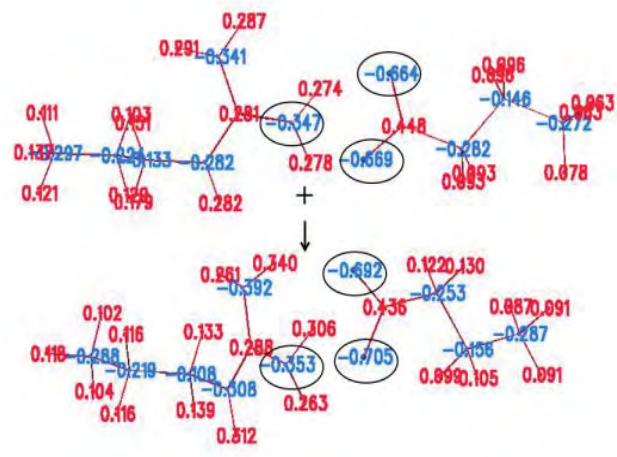


Figure 18: Atomic partial charge calculated using MOPAC PM5 (unit: au).

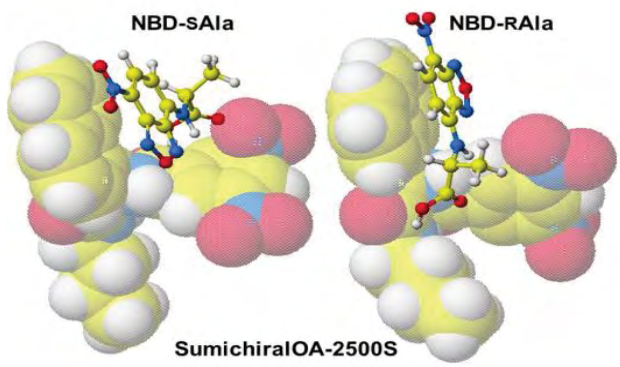


Figure 19: NBD-alanine enantiomer recognition of a Pirkle type phase; Small and large white, black, and dark gray balls: hydrogen, carbon, oxygen, and nitrogen; molecular size of NBD-alanine is 20% of chiral phase.

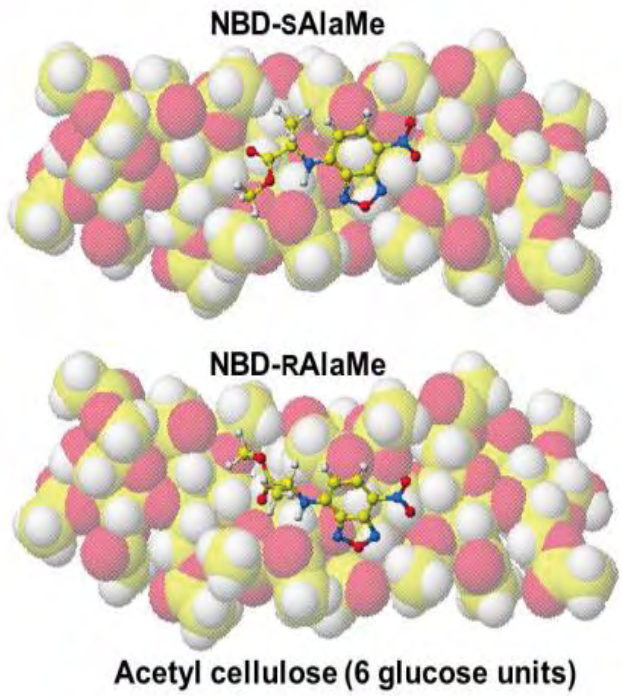


Figure 20: NBD-alanine enantiomer recognition of acetylglucose (six units of acetylglucose).

measuring ion-ion interaction. Standard acidic drugs were furosemide, naproxen, phenylbutazone, salicylic acid, sulfamethoxazole, tolbutamide and warfarin, and those of basic drugs were scopolamine, lidocaine, quinine, dextromethorphan and imipramine.

The *ks* of acidic and basic drugs measured by the above systems were correlated to their binding affinity log n*K* values. The log n*K* values of acidic drugs were measured by the modified Hummer-Dreyer method. The calculated results were as follows.

log nK=2.614(log k(R)+0.453log k(I))+3.120, r=0.974, n=7, for acidic drugs,

log nK=0.708(log k(R)+0.365log k(I))+3.211, r=0.991, n=5, for basic drugs,

where log k(R) is log k measured on reversed-phase and log k(I)

was that determined on ion-exchange liquid chromatography. Such simple liquid chromatography may be useful to measure the albumin-drug binding affinity without albumin.

Furthermore, a faster analytical method is required. The retention time of acidic drugs was measured using a guanidino-phase with pH-controlled eluent to determine their molecular form in pH 7.4 eluent. Their log n*K* values were investigated with a computational chemical analysis using a molecular mechanics calculation program (MM2).

The albumin-acidic drug binding affinity was measured at pH 7.40. The log *k* values measured at pH 7.40 correlated with the predicted log n*K* values from reference. The following relation was obtained, log n*K*=1.597 (log *k*)+5.808, r=0.887, n=13. The log n*K* values may be derived from log *k* values measured on a guanidino phase at pH 7.40. Therefore, the addition of *k* measured by reversed-phase liquid chromatography improved the correlation as previously described.

MIFS (pH 7.40)=4.702 (log n*K*)+14.314, r=0.932, n=16,

where these energy values were calculated from the following equation. MI energy=(MI enrgy$_i$+MI energy$_m$ × [H^+]/[*K*a])/(1+[H^+]/[*K*a]), where MI energy$_i$ is MI energy value of ionic form analyte, MI energy$_m$ is MI energy value of molecular form analyte and H^+ is the hydrogen ion concentration at pH 7.4.

This simple model carboxyl-phase was used to investigate basic drug-carboxyl phase interactions. The MIFS was calculated using MIFSm+hbm of the molecular form, MIFSi+hbi of the ionized form, and p*K*a from references. The correlation between MIFS and the new log n*K* is given as the following equations:

MIFS (pH 7.50)=18.432 × log n*K*–38.996, r=0.928, n=17.

Prediction of retention times in both reversed-phase and ion-exchage liquid chromatography is feasible using *in silico* chromatography. On the other hand, albumin-drug binding affinity was analyzed as a combination of capacity ratio measured using reversed-phase and ion-exchange liquid chromatography. That is, albumin-drug binding affinity was predicted using *in silico* chromatography [2].

Summary

As a complementary approach to these technological advances, computational chemical analysis is a promising technique with the potential to analyze the mechanisms of molecular interaction between analytes and solid phases, especially given the feasibility of modeling three dimension structures of biological macromolecules, such as proteins. Importantly, this technology can be easily used to study the retention mechanisms in chromatography for a variety of model phases. Furthermore, theoretical calculations can provide significant insights into organic reaction mechanisms, which can be applied to study highly sensitive detection in chromatography, such as bromate and chemiluminescence detections [2]. As a consequence, combining chromatography and computational chemistry offers new possibilities in developing a quantitative description of molecule interactions relevant to analytical chemistry. Prediction of boiling point, dissociation constant, and albumin-drug binding affinity were demonstrated as the practical applications of *in silico* chromatography [2]. Furthermore, a combination of the quantitative molecular recognition analysis and electron transfer study permits the quantitative analysis of enzyme reaction mechanisms [3].

The details of above demonstrations are summarized in a book [2]. It contains following chapters; Basic Concept of Molecular Interaction Energy Values, Design Model-phases in Chromatography, Retention in Gas Chromatography, Retention in Normal-phase Liquid Chromatography, Retention in Reversed-phase Liquid Chromatography, Retention in Ion-exchange Liquid Chromatography, Enantiomer Recognition, Human Serum Albumin-Drug Binding Affinity Based on Liquid Chromatography, Protein Affinity Chromatography, and Mechanism of Highly Sensitive Detection (Bromate Detection in Ion Liquid Chromatography, Chemiluminescence Detection, and Derivative Reagents for Highly Sensitive Analysis of Amino Acids). Furthermore, the retention mechanisms of reversed-phase ion-pair liquid chromatography was described [4].

References

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2. Hanai T (1999) HPLC: A Practical Guide. Royal Society of Chemistry, Cambridge, UK, p: 134.
3. Hanai T (2015) Basic properties of a molecular mechanics program and the generaton of unknown stereo structures of proteins for quantitative analysis of enzyme reactions. In: Watkins P (ed.), Molecular Mechanics and Modeling. Nova Science, New York, USA, pp: 25-48.
4. Hanai T (2016) Quantitative in silico analysis of retention of phenylthiohydantoin-amino acids in reversed-phase ion-pair liquid chromatography. J Chromatogr Sci 54: 604-609.