Studies on The Elution Behavior of Ni(II)-α-isonitroso-β-diketone Imine Chelates in Reversed-Phase Liquid Chromatography

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Abstract : The retention mechanism of Ni(II)-α-isonitroso-β -diketone imine chelates in reversed-phase HPLC has been studied by examining the effect of temperature, mobile phase composition in acetonitrile-water mixture, and molecular structure on retention.

The empirical retention equation was investigated to evaluate the properties of S (hydrophilic index). The value of the S index of the Ni(II) chelates decrease with the increasing column temperature and a linear relationship between S and log k_w' has been found. The results showed that the S index is influenced by the interaction between Ni(II) chelates and mobile phase.

Molecular properties, van der Waals molar volume, polarizability and dipole moment, of the Ni(II) chelates were calculated by Cerius 2 program and the calculations were performed at Universal Force Field (UFF) model. The S value and log k_w' increase with decreasing the dipole moment of Ni(II) chelates.

Keywords : RPLC, Ni(II) Chelate, Molecular Structure

1. Introduction

Reversed-phase high performance liquid chromatography (RPLC) has been the most widely used liquid chromatographic method in recent years, yet the retention mechanism in reversed-phase HPLC remains unclear. Many studies have been conducted that examine the retention mechanism in RPLC, and two main theories have evolved.

First, solvophobic theory applied to RPLC by Horvath and co-workers proposed that retention is primarily related to hydrophobic interactions between the mobile phase and solutes[1,2]. Vtacil and co-workers have reported that retention mechanism of metal chelates is largely affected by the solvophobic interactions[3]. Lee and co-workers[4,5] have also been reported that elution behavior of diethyldithiocarbamate (DDTC) and isonitroso-acetylacetone (HIAA) metal chelates in RPLC is affected by the solvophobic interactions.

Second, thermodynamic investigations were made by Knox and Vasvari[6]. Knox plotted ln k' vs. $1/T$. The resultant van't Hoff plots gave absolute enthalpies and relative entropies of transfer of the solutes between the mobile phase and the stationary phase. Since this early work, Guiochon and Horvath have been instrumental in adavancing understanding of separation mechanism through various thermo-dynamic consideration[7,8]. Numerous studies on the effect of temperature and mobile phase composition on RPLC retention have been reportd by Melander, Horvath and Guiochon and co-workers[9~11]. They found that the standard enthalpies of transfer of a nonpolar solute from the mobile phase to the stationary phase become to decrease and standard entropies of transfer become to increase with increasingly water percentage in mobile

phase[9~11]. It means that retention is related to solvophobic interactions between the mobile phase and solutes.

The stoichiometric displacement model (SDM), which was developed by Geng and Regnier[12] is also important in the development of reversed-phase retention theory. They proposed a double logarithmic plot to represent the dependence of retention on solvent composition. According to the solubility parameter concept[13], a quadratic dependence of the retention on mobile phase composition has been derived and predicted theoretically. This general equation was found to describe precisely the retention over a wide range of concentrations of the mobile phase.

The S index (hydrophilic index) in this empirical retention equation, log k' = log k_w' − $S\phi$, in RPLC and effect of moleculor structure on the S index was investigated by Chen and co-workers[14,15]. Recently solvato-chromic comparison method have been used. Solvatochromism is a well-established tool of physical organic chemistry that has recently been used to study and correlate factors involved in solvation of ground states, activated complexes, and equilibrium constants of reactions. Sadek and co-workers[16] first reported the correlation of HPLC retention properties with fundermental dipolarity/polarizability and hydrogen bonding properities of the solutes and mobile phases. They used solvatochromic parameters and the methodolo-gy associated with the linear solvation energy relationships (LSERs) to evaluate the multiple interaction effects that influence HPLC capacity factors[17]. Also Cheong and co-workers[18] showed upon examination of the activity coefficients of solute in mobile phase of various compositions that changes in solute retention in RPLC with mobile phase

composition is due mainly to solute-mobile phase interactions. Many studies of retention process in RPLC by the use of the solvatochromic comparison method have been reported continuously[19,20]. Most of these studies are on the elution mechanism of organic compound such as benzene derivatives, and studies of retention mechanism of metal chelates have a little.

The study of the separation of β-diketone metal chelates by HPLC was first reported by Huber and co-workers[21]. Separation of β-diketone chelates by RPLC was also reported by Risby and Tollinche[22]. And Gurira and Carr have been reported that β-diketone chelates can be separated quite conveniently by taking advantage of the hydrophobic nature of complexes[23]. The attempt for separation of metal chelates of α-isonitroso-β-diketone derivatives such as isonitrosoacetylacetone (HIAA), isonitrosobenzoylacetone (HIBA) was reported by Lee and co-workers[5]. Lee and co-workers[24,25] also reported that the liquid chromatographic behavior of Ni(II) and Ni(II) isonitrosoethylacetoacetate Imine (IEAA-NR, where R=H, CH_3, C_2H_5, n-C_3H_7, n-C_4H_9, C_6H_5-CH_2) chelates was largely affected by the hydrophobic effect based both on examination of relationship between capacity factors (k′) and mobile phase compositions and distribution ratio, and on thermodynamic study.

In this work, we studied the effect of temperature, the composition of acetonitrile-water mixed mobile phase, and molecular structure on retention of Ni(II)-α-isonitroso-β-diketone imine chelates in RPLC was studied. The empirical retention equation was utilized to evalutate the properties of the S. The S values for Ni(II) chelates were related to van der Waals volume, polarizability and dipole moments calculated by Cerius 2 program.

2. Experimental

Instrumentation

Varian VISTA 5500 liuid chromatograph equipped with a variable wavelength UV absorbance detector was used for chromatographic measurements on a Novapak C_{18} (3.9mm I.D. × 15cm L., particle size 4μm) column. The colume was thermostated using universal column heater. Young-in D520A Integrator was used to record chromatograms. Sample injection was performed using 10μℓ micro syringe (Hamilton).

Materials

The α-isonitroso-β-diketone imine used are listed in *Table 1*. The α-isonitroso-β-diketone imine and their Ni(II) chelates were prepared by using reported methods[25,26]. HPLC-grade methanol and acetonitrile (J. T. Bakers) were filtered with Millipore membrane filter (type FH, pore size 0.45μm), mixed with appropriate volumes of water, then degassed before use. Water was obtained from Nano Pure Deionization system, and was used after filtration with Millipore membrane filter (type HA, pore size 0.45μm), Degassing was accomplished by placing the moblie phase vessel in an ultrasonic bath and applying a vacuum for a few minutes. Metal chelate solutions were filtered by 5μm SEP-PAK (Waters) syringe filter.

Procedure

The optimum conditions for the separation of Ni(II) chelates were examined with respect to the effect of the flow rate, the type of the sample solvents and the mobile phase composition. The results are summarized in *Table 2*. The capacity factor (k′) was evaluated from the t_o value and the retention

Table 1. Abbrevition of α-isonitroso-β-diketone Imine Ligands

$$R \underset{\underset{\text{C}}{\overset{\overset{\text{NH}}{\|}}{}}{} \underset{\underset{\text{C}}{\overset{\overset{\text{NOH}}{\|}}{}}{} \underset{\underset{\text{C}}{\overset{\overset{\text{O}}{\|}}{}}{} R'$$

Abbreviation	R	R'
H-IMAA-NH	CH_3	OCH_3
H-IAA-NH	CH_3	CH_3
H-IBA-NH	CH_3	C_6H_5
H-IDBM-NH	C_6H_5	C_6H_5

Table Ⅱ. The Optimum Conditions for HPLC Operation

Descriptions	Conditions
Column	Novapak C_{18} (3.9mm I.D. × 25cm L., particle size 10μm)
Mobile Phase	Acetonitrile/Water = 47/53
Flow Rate	0.7 ml/min.
Solvent of Sample	Methanol
Detection Wavelength	254 nm
AUFS	0.02

time of the sample, t_R, using the following equation

$$k' = (t_R - t_o) / t_o$$

where t_o is dead time which is the retention time of sample solvent.

Retention of Ni(Ⅱ) chelates were measured as a function of temperature by varying temperature from 18℃ to 65℃ at 5 or 10℃ interval and as a function of mobile phase composition by varying the volume percentage of acetonitrile from 42% to 57% in mobile phase at 2~3 % interval.

Calculation of the Molecular Descriptors of the Ni(Ⅱ) Chelates

In order to calculate van der Waals molar volumes, polarizabilities and dipole moments of Ni(Ⅱ) chelates, the bond length, bond angle and dihedral angle of each metal chelates were first calculated through simulration of 3-dimensions structure of chelates using Chem Draft software. The molecular parameters of Ni(Ⅱ) chelates were calculated by Cerius 2 program. The calculations were performed at Universal Force Field (UFF) model. The all calculations were performed by the Super-computer of Cornell university (USA).

3. Result and Discussion

3.1 The Retention Mechanism of Ni(II) chelates on the Empirical Retention Equation

Generally, studies on the retention mechanism in RPLC was accomplished by using the solvophobic theory and the extra-thermodynamic approach method. However, the factors that have influenced on the retention behavior are not simple, various and very complicated. With these theories only, retention mechanism of Ni(Ⅱ) chelates can't illustrated accurately, quantitatively and systematically. Accordingly, in this work, we studied each Ni(Ⅱ) chelates on retention behavior by using empirical retention equation in RPLC.

A linear approximation of the retention equation to describe the effect of organic solvent composition on log k' has been widely accepted in practical RPLC and no significant errors in retention prediction have been found. It is expressed as[27,28]

$$\log k' = \log k_w' - S \phi \qquad (1)$$

where k' is capacity factor, ϕ is the volume fraction of strong solvent in a binary mobile phase, log k_w' and S are constants for a given

column system, log k_w' is the capacity factor obtained by extrapolation of retention data from binary eluents to 100% water. The log k_w' value plays an important role in understanding the hydrophobic interactions of the solute and stationary phase. The log k_w' or the "hydrophobic index" is an intrinsic property of solute. The S index or the "hydrophilic index" is defined as the slope of log k' versus volume fraction (ϕ). The S value is characterizes the solvent strength[27, 29] and plays an important role in understanding the interactions in binary mobile phases and in computer simulations of RPLC[1,30]. A thermodynamic interpretation of S can be obtained if the free energy of interaction between the solute and solvent molecules in the eluent is taken as a linear function of eluent composition[1,14]. We have used a thermodynamic method in combination with an empirical relationship to derive the retention equation. The parameters log k_w' and S can be expressed as

$$\log k_w' = \log \varphi + (\Delta G^{\circ}_{A,C} - \Delta G^{\circ}_{A,L})/RT \quad (2)$$

$$S = (\Delta G^{\circ}_{A,C} - \Delta G^{\circ}_{A,B})/RT \quad (3)$$

where R is the gas costant, T is the column absolute temperature, φ is the phase ratio, the subscipts A, B, C and L refer to solute, strong solvent, weak solvent and stationary phase, $\Delta G^{\circ}_{A,B}$, $\Delta G^{\circ}_{A,C}$ and $\Delta G^{\circ}_{A,L}$ are the free energy change for solute-strong solvent, solute-weak solvent and solute-stationary phase interactions. As can be seen, S is determined mainly by the interactions in the mobile phase and the S index characterizes the properties of the mobile phase.

In order to determine the S index for Ni(II) chelates log k' values were plotted vs. volume fraction of acetonitrile in Fig. 1. Log k_w' and
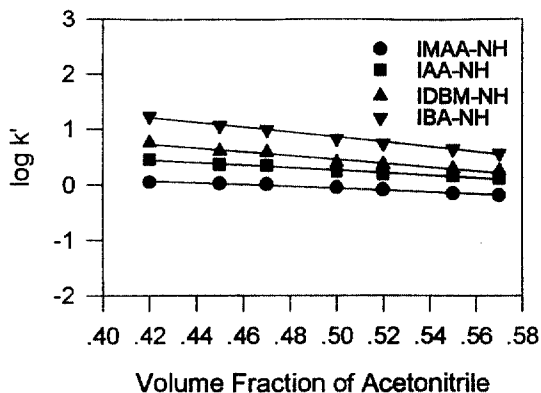

Fig. I. Dependence of log k' of Ni(II)-α-isonitroso-β-diketone imine chelates on the volume fraction of acetonitrile in acetonitrile/water mixed eluent.

S values are shown in Table 3. When a solute is very polar or ionizable in an aqueous mobile phase, a nonlinearity of the log k' vs. ϕ plot are observed[31]. This means that the retention mechanism is different according to mobile phase composition due to specific interaction between the solute and the stationary phase (silanophilic interaction) and/or between the solute and the mobile phase (ionic interaction). Fig. 1 showed good linear relationships with negative slope. This indicated that rentention of the Ni(II) chealtes is determined maimly by nonspecific interactions between the solute and organic solvent in mobile phase and that retention mechanism for the metal chelates is independent of the mobile phase composition.

The value of the S index for each of the Ni(II) chelates is a constant, characterizing the solvent. However, Snyder and co-workers[32, 33] have been found that S value is variable, tending to increase with increasing log k' for a solute, and that there is a general trend of increasing the S value and retention as the molecular size of the solute increases. This illustrate that S value is a property of the solvent and approximately relate to the solute

Won Lee, In-Whan Kim, Mi-Kyoung Kim, Yong-Jun Kim, Hae-Rim Jung, Kyoung-Tai No and Su-Yeon Kim

Table III. Comparison of S-values for Ni(II)-α-isonitroso-β-diketone Imine Chelates at 42~57% Acetonitrile

Ligands	log k_w' [a]	S^b	r^2	S.D.^{c}
IMAA-NH	0.761	0.016	0.976	0.083
IAA-NH	1.391	0.022	0.982	0.113
IDBM-NH	2.151	0.034	0.964	0.173
IBA-NH	3.050	0.044	0.991	0.220

a. The extrapolated value for the capacity factor in pure water, b. Hydrophilic Index, c. Standard Deviation

Fig. II. Relationship between S and log k_w' for Ni(II)-α-isonitroso-β-diketone Imine Chelates

size and molecular structure. The S values and log k_w' for Ni(II) chelates are shown in *Table 3*. The S values generally increase as retention (log k′) for the Ni(II) chelates increase. The S values also tend to increase with increasing the molecular size of the solutes. It means that the S index is not only related to the characteristics of the solvent but to the molecular structure of the solute.

Schoenmakers and co-workers[34~36] reported that log k_w' value plays an important role in understanding the hydrophobic interactions of the solute with stationary phase on particular column. The log k_w' is an intrinsic property of the solute: log k_w', which is a function of the molecular structure parameter of solute, has been found to be linearly related with S. *Fig. 2* shows that there are linear relationships between log k_w' and S for the Ni(II) chelates : (Ni[(IMAA-NH)(IMAA-NH)′], r^2=0.989 ; Ni[(IAA-NH)(IAA-NH)′], r^2=0.800 ; Ni[(IDBM-NH)(IDBM-NH)′], r^2=0.892 ; Ni[(IBA-NH)(IBA-NH)′], r^2=0.970). Linear relationships between log k_w' and S support that the S index is a function of the characteristic of both the solvent and solute.

Temperature plays an important role in RPLC as GC[13,29,37]. The difference between the solute-weak solvent and solute-strong
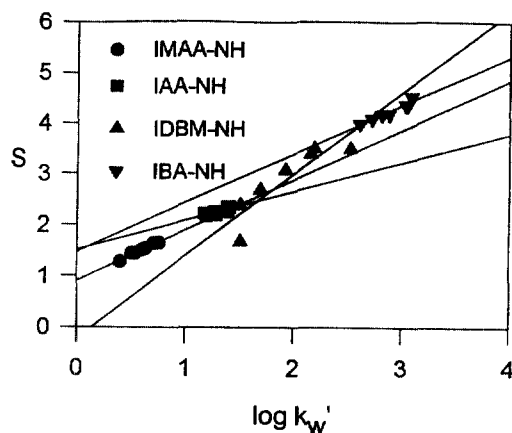
solvent interaction free energy change characterizes the solte interactions in the mobile phase. As values of this difference for a particular solute are approximately constant at different column temperatures, the value of ST approaches a constant for non-ionic compounds as is demonstrated by the following equation[15].

$$\Delta G^\circ_{A,C} - \Delta G^\circ_{A,B} = S_1 T_1 = S_2 T_2 = S_3 T_3 \quad (4)$$

where S_1, S_2 and S_3 are S values at temperatures T_1, T_2 and T_3, respectively. Therefore. the S values decrease with increasing column temperature in order for ST to remain almost constant for a particular solute. *Table 4* illustrates the effect of temperature on the S index for Ni(II) chelates in acetonitrile-water mobile phase. In the temperature range investigated (18 ~ 65℃), the S values is observed to decrease with increasing column temperature (from 271K to 338K) and the value of ST are nearly constant. It means that there is a larger solute-water than solute-acetonitrile free energy change and relatively well explain by eqn. 4. Therfore it found that the S index is a characteristic

Table IV. Effect of Column Temperature S index for Ni(II)-α-isonitroso-β-diketone Imine Chelates

| T(℃)	Ligands	log k_w'	S	ST	r^2	S.D.
18	IMAA-NH	0.761	0.016	4.656	0.976	0.083
	IAA-NH	1.391	0.022	6.402	0.982	0.113
	IDBM-NH	2.151	0.034	10.185	0.964	0.173
	IBA-NH	3.050	0.044	12.804	0.991	0.220
25	IMAA-NH	0.708	0.016	5.662	0.969	0.083
	IAA-NH	1.410	0.023	6.854	0.994	0.118
	IDBM-NH	2.183	0.035	10.430	0.981	0.177
	IBA-NH	3.036	0.044	13.112	0.991	0.220
30	IMAA-NH	0.635	0.015	4.545	0.982	0.077
	IAA-NH	1.393	0.023	6.969	0.987	0.118
	IDBM-NH	2.517	0.035	10.605	0.978	0.177
	IBA-NH	3.091	0.045	13.635	0.992	0.228
35	IMAA-NH	0.605	0.015	4.620	0.996	0.075
	IAA-NH	1.283	0.022	7.084	0.958	0.120
	IDBM-NH	1.920	0.031	9.240	0.979	0.155
	IBA-NH	2.877	0.042	12.936	0.984	0.212
45	IMAA-NH	0.537	0.014	4.452	0.992	0.072
	IAA-NH	1.271	0.022	6.996	0.982	0.113
	IDBM-NH	1.691	0.027	8.586	0.966	0.137
	IBA-NH	2.807	0.042	13.356	0.992	0.210
55	IMAA-NH	0.501	0.014	4.592	0.971	0.073
	IAA-NH	1.194	0.022	7.216	0.989	0.109
	IDBM-NH	1.510	0.024	7.872	0.943	0.123
	IBA-NH	2.716	0.041	13.448	0.993	0.206
65	IMAA-NH	0.395	0.013	4.394	0.917	0.067
	IAA-NH	1.177	0.022	7.436	0.968	0.113
	IDBM-NH	1.510	0.017	5.746	0.965	0.085
	IBA-NH	2.603	0.040	13.520	0.986	0.202
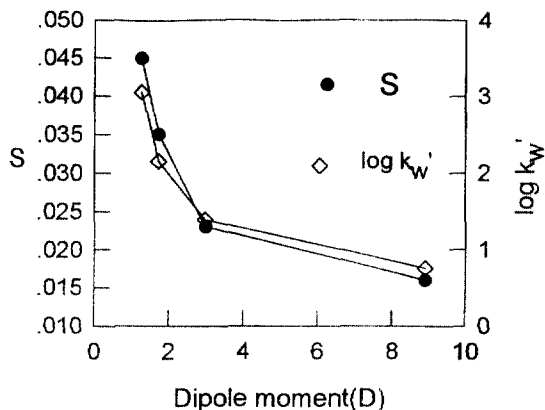
Fig. III. Plot of S and log k_w' vs. dipole moment for Ni(II)-α-isonitroso-β-diketone imine chelates in acetonitrile/water. = 47/53

Table V. The S index, log k_w' and molecular parameters for Ni(II)-α-isonitroso-β-diketone imine chelates

Ligands	S	log k_w'	Molecular parameter		
			Dipole moment (D)	Polarizability	van der Waals volume
IMAA-NH	0.016	0.761	8.910	8.83×10^3	207.691
IAA-NH	0.023	1.391	2.981	9.57×10^3	221.551
IDBM-NH	0.035	2.151	1.728	2.14×10^4	395.041
IBA-NH	0.045	3.051	1.278	1.51×10^4	301.050

constant of solute defference between solute-weak solvent (water) and solute-strong solvent (acetonitrile) free energy changes. However it should be born in mind that the S values are also affeced by the solute characteristics represented by the solute size, as shown earlier.

3.2 The effect of the Structure of Ni(II) Chelates on retention mechanism

Chen, Snyder and Lu and co-workers[14,38 ~41] have reported that the S index is correlated with some physical property which is related to the molecular structure of the solute. This is in accord with the results of the this work, as described in previous section. These authers illustrated that there is a tendency for the more polar compounds to exhibit smaller values of S and that the S index quantitatively describes the difference in the free energy change of the solute in a weak solvent and strong solvent. In order to understand the retention mechanism of the Ni(II) chelates in RPLC, we examined the relationship between retention and three molecular parameters of the chelates; van der

Waals volume, polarizability and dipole moment. The calculated values of van der Waals volume, dipole moment and polarizability for Ni(II) chelates are listed in *Table 5* along with the S and log k_w' values. *Table 5* shows that S index increases with decreasing the dipole moment. Chen and co-workers[15] found that S becomes increases as the solute becomes increasingly hydrophobic, S will become increasingly positive; in contrast, as the solute becomes more hydrophilic, S will decease. *Fig. 3* shows that the S values for the Ni(II) chelates increases with decreasing their dipole moment. This is in agreement with the observation by Chen and co-workers[15] and indicates values of S increase with solute hydrophilicity.

The relationship between dipole moment and log k_w' (*Fig. 3*) and linear relationships between S and log k_w' (*Fig. 2*) for Ni(II) chelates together indicate that the S index and retention of the Ni(II) chelates increase with decreasing dipole moment. This seems to indicate that the contribution of dipole moment to retention of the Ni(II) chelates in RPLC was much larger than the contribution of van der Waals volume and polarizability.

Acknowledgement

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