

## Reverse Phase Thin Layer Chromatography of Aminoalkanethiosulfuric Acids, Mercaptoalkanamines and Aminoalkyl Disulfides

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**Abstract:** The experimental  $R_m$  values for a series of aminoalkanethiosulfuric acids, mercaptoalkanamines and aminoalkyl disulfides were determined by reverse phase thin layer chromatography. The  $R_m$  values were determined for various concentrations of methanol:water and the correlation obtained was extrapolated to 100% water. These values permitted the calculation of the log P values for each substance. The log P values obtained by this method were compared with those obtained using the shake-flask method and by theoretical calculations utilizing fragment constants.

**Keywords:** aminoalkanethiosulfates, mercaptoalkanamines, aminoalkyl disulfides, thin layer chromatography, log P.

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### Introduction

Chromatographic methods have frequently been applied in the determination of relative partition coefficients (P) for use in quantitative relationships between chemical structure and biological activity (QSAR) [1]. Reverse phase thin layer chromatography and high performance liquid chromatography are used to evaluate the lipophilicity of a series of organic compounds. These correlations are based on

the studies of Dearden [2], Consdem [3] and Martin and Syngé [4] on the relationship between chromatographic distribution and the partition coefficient. The correlation which involves  $R_m$  is defined by equation 1 [5]:

$$R_m = \log (1/R_f - 1) \quad (1)$$

They demonstrated experimentally that the relationship predicted by Martin and Syngé [4] was indicated for a number of flavones, anthocyanines and some reference compounds. However, because of the nature of the substituents studied (e.g. hydroxyl groups), the data necessarily were restricted to a narrow range of compounds. Therefore, they analyzed the effect of substituents on the  $R_f$  value of an unsubstituted compound.

According to Tomlinson [6], the partition coefficient can be defined as an equilibrium constant similar to equation 2.

$$P = K_a / K_b \quad (2)$$

and  $\Delta R_m$  is similar to  $\pi$ . Many times the term  $\Delta R_m$  is ignored and  $\pi$  is expressed as:

$$\pi = \log \frac{(1/R_{f_X}) - 1}{(1/R_{f_H}) - 1} \quad (3)$$

In the study of the structural characteristics of compounds active against the fungus *Podosphaera leucotricha* using regression analysis, they examined the relationship between the fungicidal activities of a series of alkyldinitrophenols and the  $\Delta R_m$  values of the substituents. The  $R_m$  values were measured in cellulose layers impregnated with 10% ethyl oleate using 60% aqueous ethanol as the mobile phase. A quadratic relationship was found for a series of 4-(1-cyclopentyl-n-alkyl)-2,6-dinitrophenols [5] (Equation 4).

$$\log Br = 7,583 - 11,815 \Delta R_m + 6,434 (\Delta R_m)^2 \quad (4)$$

Later, Draber *et al.* [7] studied the herbicidal activity of some triazinones. They demonstrated that the  $\Delta R_m$  constants of the substituents, obtained from measured  $R_m$  values in a reverse phase thin layer chromatography (RPTLC) system with the chromatographic solvent pair — paraffin oil and water/dioxane — together with its  $\pi$  values, correlate satisfactorily with its ability to inhibit electron transport in isolated chloroplasts.

Tomlinson [6] determined the  $R_m$  values for acids and bases. In this case,  $R_m$  was described by the following expression, stipulating that the degree of association in the organic phase can be ignored:

$$R_m = \log (1/R_f - 1) + \log \frac{K_a + [H^+]}{[H^+]} \quad (5)$$

where  $K_a$  is the dissociation constant of the solute and  $[H^+]$  it is the hydrogen ion concentration in the mobile phase. Iwasa *et al.* [8] suggested the usefulness of the chromatographic data for QSAR. According to them, the relationship between the partition coefficient and  $R_m$  values constitutes an extension of the Collander equation [9, 10], that correlates the  $\log P$  values in octanol/water with those obtained in other apolar/polar solvent pairs. Thus, the following equation was formulated:

$$\log P = bR_m + a \quad (6)$$

where  $a$  and  $b$  are constants. Also, Hulshoff and Perrim [11, 12] emphasized the study of the classification and of the system model, through which the values of  $R_m$  can be measured.

In measuring of the  $R_m$  values by reverse phase chromatographic methods, the systems are generally constituted of silica gel impregnated with a lipophilic phase (liquid paraffin, mineral oil, *n*-octanol) or chemically bound (C-8, C18) and an aqueous mobile phase of variable composition and polarity. Due to the nature of some of the solutes studied, an increase or decrease in the polarity of the mobile phase is generally necessary to obtain a reasonable migration of the solute and to obtain measurable values of  $R_f$  [13].

Boyce and Milborrow [14] have also demonstrated linear relationships between the  $R_m$  values and the number of methylene groups in the alkyl chain in a series of *N*-alkyltriethylamines in an aqueous-acetone system. This fact was related to the work of Irsherwood [15] which correlated the water content of the mobile phase with the  $R_m$  values of oligosaccharides. Such evidence showed that the composition of the mobile phase can significantly affect  $R_m$  and  $\Delta R_m$  data measured in similar. Biagi and coworkers [16] mention  $R_m$  data for values found by extrapolation of the curve obtained for the percent of acetone in an aqueous acetone mixture versus  $R_m$ . These values of  $R_m$  corresponding to a system containing 100% water can then be used for any QSAR model. Such an extrapolation is generally valid when  $R_m$  varies linearly with the fraction of volume of the organic solvent in the aqueous mobile phase, as represented by the equation of Soczewinski – Wachtmeister [17].

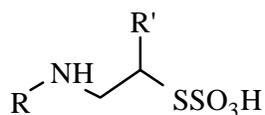
$$R_m = R_{m_w} + bc \quad (7)$$

where  $R_{m_w}$  is the value of  $R_m$  for an mobile phase containing pure water,  $b$  is a constant and  $c$  represents the concentration of the organic modifier in the mobile phase.

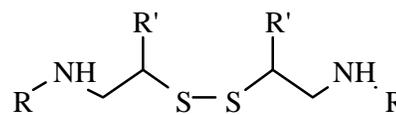
To measure  $\log P$  by the conventional Shake-flask method is difficult and time consuming. It is difficult to determine  $\log P$  for substances that are not very soluble in water or that cannot be detected by conventional methods. Instead of measuring the  $\log P$  values by equilibrium methods, partition chromatography data can be used. The main advantages of the chromatographic methods over the direct partition methods for determining the hydrophobicity index consists of the fact that the former are

simpler to use, are faster and less tedious [6]. In addition, less material is required and these methods can be used to determine the hydrophobicity of drug molecules with very high or very low log P values (such solutes require long equilibration periods in the normal Shake-flask methods), the material doesn't need to be ultra pure; there is no need to make a specific quantitative analysis of the solute and more satisfactory results are obtained.

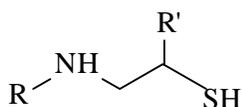
Based on these facts, the objective of the present study was the determination of the chromatographic parameters of three series of sulfur containing alkylamines — aminoalkanethiosulfuric acids (**1**), aminoalkyl disulfides (**2**) and mercaptoalkanamines (**3**) — using a reverse phase system to find satisfactory correlations for the lipophilicity by extrapolation of the values of  $R_m$ . These data are compared with those obtained by the shake flask technique which is used in the determination of the partition coefficient.

Aminoalkanethiosulfuric Acids (**1a-j**)

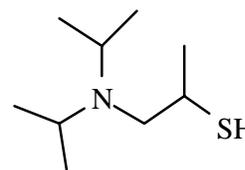
Comp.	R	R'	Comp.	R	R'
<b>1a-</b>	isopropyl	hexyl	<b>1f-</b>	isobutyl	hexyl
<b>1b-</b>	<i>sec</i> -butyl	hexyl	<b>1g-</b>	butyl	pentyl
<b>1c-</b>	butyl	hexyl	<b>1h-</b>	isobutyl	pentyl
<b>1d-</b>	propyl	hexyl	<b>1i-</b>	butyl	ethyl
<b>1e-</b>	cyclohexyl	hexyl	<b>1j-</b>	<i>sec</i> -butyl	ethyl

N-alkyl-2,2'-dithiobis(1-alkanamines) (**2a-c**)

Comp.	R	R'
<b>2a-</b>	cyclohexyl	methyl
<b>2b-</b>	butyl	methyl
<b>2c-</b>	propyl	methyl

N-alkyl-2-mercapto-1-alkanamines (**3a-g**)

Comp.	R	R'	Comp.	R	R'
<b>3a-</b>	butyl	methyl	<b>3e-</b>	cyclohexyl	methyl
<b>3b-</b>	isopropyl	methyl	<b>3f-</b>	butyl	H
<b>3c-</b>	propyl	methyl	<b>3g-</b>	cyclohexyl	H
<b>3d-</b>	butyl	ethyl			

N,N-Diisopropyl-2-mercapto-1-propanamine (**4**)

## Materials and Methods

The compounds studied were synthesized in this laboratory by previously established methods [18-21] and their structures were established by physical and spectroscopic techniques (infrared, nuclear magnetic resonance and mass spectrometry).

### Determination of the Chromatographic Parameter $R_{m_{wol}}$ (the $R_m$ value for pure water as the eluent)

Glass plates (10 x 10 cm) were coated with a 0.25 mm layer of silica gel DGF-254 (Merck). The plates were activated for 5 h at 110 °C, and, after cooling in a desiccator, were placed in a closed chromatography chamber in contact with a solution 5% of octanol in chloroform for 18 h to impregnate them with the reverse phase [13].

Individual solutions containing 0.3 mg/ml of each of the 21 derivatives in methanol were applied to the chromatographic plates. The mobile phase was composed of binary mixtures of 0.2 M ammonium acetate buffer (pH 6,0) and methanol in the following proportions 50:50; 60:40; 70:30; 80:20; 90:10. The plates were developed at 25 °C. After the mobile phase had migrated for a distance of 8.5 cm, the plates were removed from the chambers and the solvent was evaporated. The spots were visualized with iodine vapor and the  $R_f$  values were determined. From the average of the results from three plates. From the  $R_f$  values, the  $R_m$  values could be calculated from the following equation:

$$R_m = \log (1/R_f - 1) \quad (8)$$

## Results and Discussion

To obtain the  $R_{m_{wol}}$  values, the chromatographic parameter for the pure aqueous eluent the linear correlation of the  $R_m$  values versus the percent of organic solvent in the eluent was extrapolated to the 0% point. To guarantee the experimental efficiency in the sense of assuring the reproducibility of the results the  $R_f$  values obtained in the absence and in the presence of octanol were compared. These values are shown in Table 1.

The  $R_{m_{wol}}$  values obtained with varying proportions of methanol in the eluent can be observed in Table 2. The correlation coefficient shown in Table 2 indicates that this is indeed a linear correlation, thus assuring that extrapolation will furnish the  $R_{m_{wol}}$  value for the 100% aqueous eluent (Equation 9).

$$R_{m_{wol}} = a (\% \text{ methanol}) + b \quad (9)$$

Table 1. Comparison of  $R_f$  values of 2-(N-alkylamino)-1-hexanethiosulfuric acids ( $R' = \text{hexyl}$ ) obtained on plates coated with silica gel with and without being impregnated with octanol. (Eluent: methanol: ammonium acetate buffer = 40:60, pH = 6.0).

Substance	R	Without Octanol	With Octanol
<b>1a</b>	isopropyl	0.655	0.555
<b>1b</b>	sec-butyl	0.614	0.477
<b>1c</b>	butyl	0.659	0.411
<b>1d</b>	propyl	0.683	0.422
<b>1e</b>	cicloexyl	0.603	0.242
<b>1f</b>	isobutyl	0.694	0.320

The results obtained from reverse phase thin layer chromatography showed that the tested compounds did not migrate when the mobile phase was only composed of ammonium acetate buffer. The addition of the methanol was necessary to obtain longer migrations and, therefore, more reliable  $R_m$  values. The comparison of the  $R_m$  values versus the composition of the mobile phase showed a linear relationship. An increase of 10% of methanol in the eluent resulted in a decrease of 2.6 in the  $R_m$  values for substance **1a**. The experimental data for the mobile phase containing 5% methanol were not used because, at this concentration, several compounds showed very small migrations, presenting, therefore,  $R_m$  values outside the limits of maximum precision. The  $R_{mWOL}$  values are shown in Tables 3 and 4, together with the log P values in the series of the compounds discussed above.

Table 2.  $R_m$  values with varying proportions of ammonium acetate buffer (pH = 6.0) and methanol

Substance	Proportions of ammonium acetate buffer:methanol					$r^*$
	50:50	60:40	70:30	80:20	90:10	
<b>1a</b>	-0.158	-0.096	0.159	0.301	0.720	-0.932
<b>1b</b>	0.000	0.161	0.493	0.673	1.043	-0.990
<b>1c</b>	-0.036	0.156	0.505	0.624	0.861	-0.905
<b>1d</b>	-0.099	0.136	0.549	0.623	1.194	-0.952
<b>1e</b>	0.133	0.364	0.547	0.719	1.130	-0.980
<b>1f</b>	0.234	0.327	0.405	0.550	0.720	-0.986
<b>1g</b>	-0.434	-0.154	0.020	0.235	0.347	-0.980
<b>1h</b>	-0.292	-0.076	-0.076	0.039	0.239	-0.888
<b>1i</b>	-0.299	-0.286	-0.286	0.091	0.188	-0.805
<b>1j</b>	-0.117	0.012	0.010	0.029	-0.124	-0.914
<b>2a</b>	0.610	0.632	0.726	0.733	0.784	-0.937
<b>2b</b>	0.659	0.710	0.833	0.869	0.939	-0.937
<b>2c</b>	0.739	0.743	0.954	1.026	1.091	-0.925
<b>3a</b>	0.282	0.474	0.796	0.969	1.150	-0.987
<b>3b</b>	-0.110	0.034	0.582	0.869	1.007	-0.980
<b>3c</b>	0.120	0.513	0.733	0.921	1.179	-0.962
<b>3d</b>	0.726	0.845	1.020	1.136	1.143	-0.936
<b>3e</b>	0.094	0.133	0.206	0.383	0.598	-0.960
<b>3f</b>	0.573	0.580	0.784	0.903	1.015	-0.953
<b>3g</b>	0.781	0.788	0.821	0.916	0.949	-0.912
<b>4</b>	-0.578	-0.534	-0.412	-0.278	-0.141	-0.974

Table 3.  $R_{mWOL}$ ,  $\log P_{Ap}^*$  and  $\log P_{calc}^{**}$  values for the alkylaminoalkanethiosulfuric acids [22-24].

Substance	$R_{mWOL}$	$\log P_{Ap}$	$\log P_{Calc}$	
			Hansch e Leo	Nys e Rekker
<b>1a</b>	1.31	-0.30	-0.44	-0.44
<b>1b</b>	0.83	-0.38	-0.58	-0.47
<b>1c</b>	1.18	-0.34	0.03	0.08
<b>1d</b>	0.80	-0.46	0.04	0.04
<b>1e</b>	1.25	-0.38	-0.18	0.04
<b>1f</b>	1.27	-0.15	0.35	0.77
<b>1g</b>	0.58	-0.72	-0.51	-0.44
<b>1h</b>	0.31	-0.54	-0.58	-0.04
<b>1i</b>	0.28	-1.85	-2.06	-2.02
<b>1j</b>	0.16	-2.00	-2.27	-2.05
<b>4</b>	-0.04	-2.10	-2.15	-2.28

\*Determined by the shake-flask method.

\*\*Calculated from fragmental constants by the methods of Hansch and Leo and of Nys and Rekker

Table 4.  $R_{mWOL}$ ,  $\log P_{Ap}^*$  and  $\log P_{calc}^{**}$  values for the mercaptoalkanamines and aminoalkyl disulfides [22-24].

Substance	$R_{mWOL}$	$\log P_{Ap}$	$\log P_{cal}$	
			Hansch e Leo	Nys e Rekker
<b>2a</b>	1.20	-1.10	-1.01	2.12
<b>2b</b>	1.10	-0.26	-0.07	3.75
<b>2c</b>	0.83	-0.14	-0.11	4.47
<b>3a</b>	1.44	-1.64	-0.16	2.03
<b>3b</b>	1.39	-1.76	-0.30	2.01
<b>3c</b>	1.13	-1.57	-0.09	2.08
<b>3d</b>	1.40	0.14	0.31	2.57
<b>3e</b>	1.31	-0.39	-0.26	3.09
<b>3f</b>	0.99	-1.65	-0.17	2.67
<b>3g</b>	0.66	-0.52	0.23	3.25

\*Determined by the shake-flask method.

\*\*Calculated from fragmental constants by the methods of Hansch and Leo and of Nys and Rekker

The analysis of the quantitative structure-activity relationship of the series of aminoalkanethiosulfuric acids, mercaptoalkanamines and aminoalkyl disulfides lead to the following equations (calculated and observed values) [23].

## Conclusions

The chromatographic values of the three series of sulfur-containing amines – aminoalkanethiosulfuric acids, mercaptoalkanamines and aminoalkyl disulfides were determined by reverse phase thin layer

chromatography. A satisfactory correlation with other methods was found when the extrapolated values of  $R_m$  were used. Thus, this technique can be used in place of more tedious methods for these classes of compounds.

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