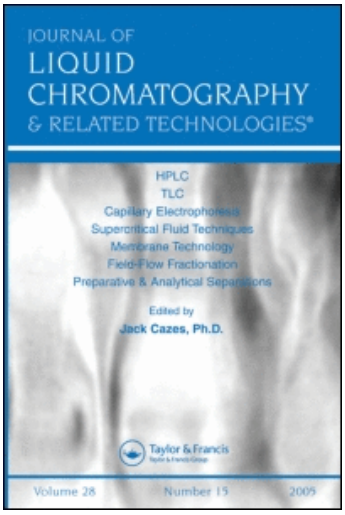

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USE OF RP-TLC AND THEORETICAL COMPUTATIONAL METHODS TO COMPARE THE LIPOPHILICITY OF SALICYLIC ACID AND ITS DERIVATIVES

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USE OF RP-TLC AND THEORETICAL COMPUTATIONAL METHODS TO COMPARE THE LIPOPHILICITY OF SALICYLIC ACID AND ITS DERIVATIVES

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□ *Salicylic acid (SA) and its derivatives, namely acetylsalicylic acid (ASA), salicylanilide (SAND), salicylaldehyde (SALD), salicylamide (SAMD), salicylhydroxamic acid (SHXA), methyl salicylate (MS), phenyl salicylate (PS), 3,5-dinitrosalicylic acid (3,5-DNSA), 2,5-dihydroxybenzoic acid (2,5-DHBA), 3-aminosalicylic acid (3-AMSA), 4-aminosalicylic acid (4-AMSA), and 5-aminosalicylic acid (5-AMSA) were investigated with the use of reversed phase thin layer chromatography on RP8 F_{254}, RP18 F_{254s}, RP18 W, and CN (E. Merck), using methanol-water in different volume compositions as a mobile phase. The chromatographic parameters of lipophilicity ($R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$) of the studied compounds were determined. Lipophilic parameters ($R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$ were compared, both with measured ($\log P_{exp}$), and calculated partition coefficients (Alog Ps, AClog P, AB/log P, COSMOFrag, miLog P, Alog P, mlog P, KOWWIN, xlog P2, and xlog P3). Similarity analysis indicates that the chromatographic parameters of lipophilicity $R_{Mw(RP8)}$, and $R_{Mw(CN)}$ are more appropriate for the experimental n-octanol-water partition coefficient. Comparing all calculation procedures, generally miLog P is more appropriate for the chromatographic parameter of lipophilicities $R_{MW(RP8)}$ and $R_{MW(CN)}$, as well as the experimental n-octanol-water partition coefficient of the studied compounds. The results indicate that the chromatographic parameter of lipophilicity determined on RP8 F_{254s} and CN plates may be used as a measure of lipophilicity of the investigated salicylic acid and its derivatives.*

Keywords densitometry, experimental *n*-octanol-water partition coefficient, lipophilicity parameter R_{MW}, RP-TLC, salicylic acid, theoretical partition coefficient

INTRODUCTION

Salicylic acid and its derivatives have pharmacological and pharmaceutical significances.[1–5] Drugs containing derivatives of salicylic acid, structurally similar to aspirin, have been in medical use since ancient times. Salicylate-rich

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willow bark extract became recognized for its specific effects on fever, pain, and inflammation in the mid-eighteenth century.[5]

Lipophilicity is one of the parameters of chemical substances which influence their biological activities. Lipophilicity is a prime parameter in describing both pharmacodynamic and pharmacokinetic aspects of drug action.[6–11]

Lipophilicity is defined by the partitioning of a compound between a nonaqueous and an aqueous phase. The *n*-octanol-water partition coefficient is generally accepted as a useful parameter in structure activity relationship studies (QSAR) for the prediction of biological or pharmacological activity of compounds. The different partition chromatographic techniques,[7–15] and theoretical methods[6,10,16–23] have been widely used as a reliable alternative to classical determination of log P.

Therefore, the aim of this work was to determine the lipophilicity of salicylic acid and its derivatives by the RP-TLC method on RP8 F_{254s}, and RP18 F_{254s}, RP18 W, and CN plates using a mixture of methanol and water as mobile phases.

The experimental *n*-octanol-water partition coefficient and chromatographic parameters of lipophilicity values were compared with lipophilicity values estimated by computational methods for salicylic acid and its derivatives.

EXPERIMENTAL

Chemicals and Standard Solutions

The following components of the mobile phase: methanol (Merck, Germany; for liquid chromatography), and redistilated water were used for RP-TLC analysis. The commercial samples of SA (Aldrich, lot: S43108-327), ASA (Sigma, lot: 057K0006), SAND (Sigma, lot: 26H0377), SALD (Aldrich, lot: 03523ME), SAMD (Aldrich, lot: S38230–387), SHXA (Aldrich, lot: S44796-447), MS (Sigma-Aldrich, lot: 066K01371), PS (Aldrich, lot: U14124-447), 3,5-DNSA (Sigma, lot: 037K3721), 2,5-DHBA (Aldrich, lot: 05629CE), 3-AMSA (Aldrich, lo: 70626U21384), 4-AMSA (Aldrich, lot: 105H653), and 5-AMSA (Sigma, lot: 106K1589) were used as test solutes. Standard solutions of salicylic acid and its derivatives (1 mg/1 mL) were prepared in absolute ethanol (99.8%, pure for analysis, POCh, Gliwice, Poland).

Application of Reversed–Phase Thin–Layer Chromatography for Determination of Chromatographic Parameters of Lipophilicity

Reversed partition thin–layer chromatography (RP-TLC) was done on TLC RP8 F_{254s} (E. Merck, #1.15424, lot: OB549661), TLC RP18 F_{254s}

(E. Merck, #1.05559, lot: OB687316), HP-TLC RP18 W (E. Merck, #1.14296, lot: OB315589), and HPTLC CN (E. Merck, #1.12571, lot: OB102016) plates. Solutions of the examined compounds were spotted on chromatographic plates in quantities of 5 μg of the compounds in 5 μL of solution. The chromatograms were developed by using the mixture of methanol-water, the content of methanol in mobile phase was gradually varied by 5% (%, v/v) from 20–100 (%, v/v).

Fifty mL of mobile phase was placed into a classical chromatographic chamber (Camag, Switzerland). The chamber was saturated with solvent for 15 min. The chromatograms were developed at the room temperature, e.g., 22(±1)°C. The development distance was 7.5 cm. The plates were dried at the room temperature, e.g., 22(±1)°C. A Camag densitometer was used to obtain R_F values. Densitometric scanning was then performed at the respective absorption maximum (Table 1). The radiation source was a deuterium lamp emitting a continuous spectrum between 190 and 450 nm. The slit dimensions were 8.00 × 0.40 mm, Macro; the optimized optical system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 μm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order. Each track was scanned three times and baseline correction (lowest slope) was used. The R_F values were recalculated on the R_M values.The chromatograms were done in triplicate and mean R_F values were calculated.

The R_M values obtained for the studied compounds on RP8 F_{254}, RP18 F_{254s}, RP18 W, and CN plates, using the methanol-water mobile phases were extrapolated to zero concentration of methanol in eluent

TABLE 1 The Wavelengths of the Fundamental Absorption Band (λ_{max}) of Salicylic Acid and its Derivatives on Particular Chromatographic Sorbents

	λl_{max} [nm] on Particular Chromatographic Sorbent			
Symbol of Compound	RP8	RP18	RP18 W	CN
SA	206	305	204	208
ASA	200	200	200	200
SAND	309	311	206	312
SALD	260	261	254	253
SAMD	204	307	203	206
SHXA	310	306	203	206
MS	206	309	205	207
PS	207	209	206	209
3,5-DNSA	337	345	337	335
2,5-DHBA	211	323	214	338
3-AMSA	211	223	202	312
4-AMSA	313	310	314	311
5-AMSA	206	318	251	203

(R_{MW}), in accordance with Soczewiński- Wachtmeister equation: [10]

$$R_M = R_{Mw} - S \cdot \varphi \quad (1)$$

where: R_M is the R_M value of the examined substance by the content φ of the volume fraction of methanol in mobile phase; R_{MW} is the theoretical value of R_M of the particular compound extrapolated to zero concentration of methanol in mobile phase; S is the slope of the regression curve; φ is the volume fraction of organic modifier in the mobile phase.

Calculation of Theoretical Partition Coefficients

The values of theoretical partition coefficients such as: Alog Ps, AClog P, AB/log P, COSMOFrag, miLogP, Alog P, mlog P, KOWWIN, xlog P2, and xlog P3[17–23] were calculated with the use of the Internet databases.[23]

RESULTS AND DISCUSSION

The lipophilicity of salicylic acid and its derivatives were studied. The theoretical partition coefficients calculated by use of different methods and for experimental n-octanol-water partition coefficients for investigated compounds are presented in Table 2. The scientific literature does not publish the experimental n-octanol-water partition coefficients for salicylhydroxamic acid (SHXA), phenyl salicylate (PS), 3-aminosalicylic acid (3-AMSA), and 5-aminosalicylic acid (5-AMSA).[23]

Salicylic acid and its derivatives were investigated with the use of reversed phase thin layer chromatography on $RP8F_{254}$, $RP18F_{254s}$, RP18W, and CN plates, using methanol-water in different volume compositions as a mobile phase. The R_M values obtained for the studied compounds were extrapolated to zero concentration of methanol in mobile phase, in accordance with Soczewiński- Wachtmeister Equation (1). The terms of the regression equations (Eqs. (2)–(52)) describing the dependence of the R_M values of the salicylic acid and its derivatives on the methanol content (φ) of the mobile phase are listed in Tables 3, 4, 5, and 6 for analysis performed on $RP8F_{254s}$ ($R_M = R_{Mw(RP8)} - S\varphi$), on $RP18F_{254s}$ ($R_M = R_{Mw(RP18)} - S\varphi$), on RP18W ($R_M = R_{Mw(RPW)} - S\varphi$), and on CN ($R_M = R_{Mw(CN)} - S\varphi$) plates, respectively.

The high correlation coefficients (r), significance levels (p), the values of the Fisher test (F), and small values of the standard errors of the estimates (s) indicate that all the equations were highly significant.

It was found that the lipophilicity values $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$ obtained by the use of RP-TLC depend linearly on the slope of the regression curve *S* with Eq. (1). The regression

TABLE 2 The Numerical Values of Experimental and Theoretical *n*-Octanol-Water Partition Coefficients.[23]

Symbol of Compound	log P_{exp}	Alog P_s	AClog P	AB/log P	COSMOFrag	miLogP	Alog P	mlog P	KOWWIN	xlog P2	xlog P3
SA	2.26	1.96	1.20	2.04	1.55	1.87	1.17	1.64	2.24	2.43	2.26
ASA	1.19	1.43	1.43	1.22	0.92	1.43	1.20	1.70	1.13	1.42	1.19
SAND	3.27	2.65	2.77	2.49	2.93	3.32	2.35	3.08	3.30	3.02	3.27
SALD	1.81	1.22	1.37	1.52	0.82	1.67	1.32	1.67	2.01	1.74	1.81
SAMD	1.28	0.74	0.66	0.76	0.25	1.25	0.56	1.24	1.03	0.93	1.28
SHXA	-	0.44	1.44	1.36	1.03	0.82	1.30	1.14	−0.02	1.90	2.52
MS	2.55	2.07	1.66	1.94	2.55	2.13	1.42	1.98	2.60	2.54	2.34
PS	–	3.58	3.09	3.21	4.11	3.83	2.99	3.48	3.82	4.10	3.83
3,5-DNSA	1.71	1.80	1.21	2.51	1.10	1.49	0.96	1.76	2.46	2.64	1.30
2,5-DHBA	1.74	1.23	0.90	1.24	0.66	1.37	0.90	1.10	1.76	1.60	1.63
3-AMSA	–	0.84	0.48	0.54	0.86	1.10	0.42	1.10	1.33	1.83	0.87
4-AMSA	0.89	0.62	0.48	1.18	0.81	0.92	0.42	0.00	0.98	1.61	1.32
15-AMSA	–	0.75	0.48	0.34	1.17	0.92	0.42	1.10	0.98	1.19	1.32

TABLE 3 Parameters of the Linear Regression (±SE) Relating the R_M Values of Salicylic Acid and its Derivatives to the Methanol Content (φ) of Methanol – Water Mobile Phase (According to Eq. (1): $R_M = R_{Mw(RP8)} - S \cdot \varphi$) for Analysis Performed on RP8 F_{254s} Plates

Symbol of Compound	$R_{Mw(RP8)}$ (±SE)	S (±SE)	n	r	SEE	F	Range of the Volume Fraction of Methanol (φ)	Eq. No.
SA	1.040 (±0.038)	2.00 (±0.05)	12	0.996	0.030	1551	1.00 ÷ 0.45	(2)
ASA	0.618 (±0.045)	1.72 (±0.06)	15	0.990	0.055	679	1.00 ÷ 0.30	(3)
SAND	2.871 (±0.044)	3.68 (±0.06)	15	0.998	0.054	2305	1.00 ÷ 0.30	(4)
SALD	1.541 (±0.027)	2.23 (±0.04)	15	0.997	0.033	2121	1.00 ÷ 0.30	(5)
SAMD	1.323 (±0.036)	2.26 (±0.05)	15	0.996	0.044	1753	1.00 ÷ 0.30	(6)
SHXA	0.832 (±0.023)	1.74 (±0.03)	15	0.997	0.029	2502	1.00 ÷ 0.30	(7)
MS	2.399 (±0.070)	3.04 (±0.10)	15	0.992	0.086	882	1.00 ÷ 0.30	(8)
PS	3.583 (±0.086)	4.23 (±0.12)	13	0.996	0.080	1273	1.00 ÷ 0.40	(9)
3,5-DNSA	1.174 (±0.059)	2.67 (±0.08)	13	0.995	0.055	1088	1.00 ÷ 0.40	(10)
2,5-DHBA	1.008 (±0.041)	2.48 (±0.06)	15	0.996	0.050	1644	1.00 ÷ 0.30	(11)
3-AMSA	0.600 (±0.039)	2.28 (±0.05)	14	0.996	0.041	1734	1.00 ÷ 0.35	(12)
4-AMSA	0.496 (±0.039)	1.74 (±0.06)	14	0.993	0.042	981	1.00 ÷ 0.35	(13)
5-AMSA	0.120 (±0.022)	1.17 (±0.03)	15	0.995	0.027	1349	1.00 ÷ 0.30	(14)

Where: SE – standard error; n – number of points to drive the particular regression equation; r – correlation coefficient; SEE – standard error of the estimation; F - the values of the Fisher test; for all regression equation the significance level (p) is <0.0001.

equations Eqs. (53), (54), (55), and (56) describe these linear relationships with high correlation coefficients:

$$R_{Mw(RP18W)} = -1.037(\pm 0.134) + 1.122(\pm 0.075)S \quad n = 13; r = 0.9763; F = 224; p < 0.0001; s = 0.259 \tag{53}$$

$$R_{Mw(RP8)} = -1.382(\pm 0.288) + 1.139(\pm 0.113)S \quad n = 13; r = 0.9495; F = 100; p < 0.0001; s = 0.332 \tag{54}$$

$$R_{Mw(RP18)} = -1.756(\pm 0.129) + 1.382(\pm 0.060)S \quad n = 13; r = 0.9897; F = 525; p < 0.0001; s = 0.208 \tag{55}$$

$$R_{Mw(CN)} = -0.769(\pm 0.105) + 1.043(\pm 0.060)S \quad n = 13; r = 0.9823; F = 304; p < 0.0001; s = 0.154 \tag{56}$$

Equations (53), (54), (55), and (56) confirm the fact that the studied compounds comply with Soczewiński-Wachtmeister Equation (1).

Obtained by the use of methanol + water mobile phase, the chromatographic parameters of lipophilicity $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$ indicate that salicylanilide, metyl salicylate, and phenyl salicylate have the highest lipophilicities. The remaining investigated compounds have smaller lipophilic properties.

TABLE 4 Parameters of the Linear Regression (±SE) Relating the R_M Values of Salicylic Acid and its Derivatives to the Methanol Content (φ) of Methanol – Water Mobile Phase (According to Eq. (1): $R_M = R_{Mw(RP18)} - S \cdot \varphi$) for Analysis Performed on $RP18F_{254s}$ Plates

Symbol of Compound	$R_{Mw(RP18)}$ (±SE)	S (±SE)	n	r	SEE	F	Range of the Volume Fraction of Methanol (φ)	Eq. No.
SA	−0.084 (±0.034)	1.38 (±0.05)	17	0.989	0.053	691	1.00 ÷ 0.20	(15)
ASA	−0.218 (±0.022)	0.84 (±0.03)	14	0.990	0.026	599	0.90 ÷ 0.30	(16)
SAND	2.818 (±0.070)	3.52 (±0.10)	15	0.994	0.085	1194	1.00 ÷ 0.30	(17)
SALD	1.909 (±0.038)	2.59 (±0.06)	15	0.996	0.047	2144	1.00 ÷ 0.30	(18)
SAMD	1.396 (±0.039)	2.21 (±0.06)	17	0.994	0.060	1368	1.00 ÷ 0.20	(19)
SHXA	0.905 (±0.030)	1.84 (±0.05)	17	0.995	0.047	1603	1.00 ÷ 0.20	(20)
MS	2.698 (±0.060)	3.14 (±0.09)	15	0.995	0.074	1262	1.00 ÷ 0.30	(21)
PS	3.291 (±0.078)	3.58 (±0.11)	14	0.994	0.083	1053	1.00 ÷ 0.35	(22)
3,5-DNSA	0.510 (±0.025)	1.51 (±0.04)	17	0.995	0.039	155	1.00 ÷ 0.20	(23)
2,5-DHBA	−0.288 (±0.036)	1.27 (±0.05)	14	0.989	0.041	554	0.90 ÷ 0.30	(24)
3-AMSA	−0.271 (±0.038)	1.11 (±0.05)	13	0.988	0.035	459	1.00 ÷ 0.40	(25)
4-AMSA	−0.624 (±0.030)	0.90 (±0.05)	17	0.980	0.048	367	1.00 ÷ 0.20	(26)
5-AMSA	−0.463 (±0.028)	1.01 (±0.04)	17	0.986	0.044	536	1.00 ÷ 0.20	(27)

Where: SE – standard error; n – number of points to drive the particular regression equation; r – correlation coefficient; SEE – standard error of the estimation; F – the values of the Fisher test; for all regression equation the significance level (p) is <0.0001.

TABLE 5 Parameters of the Linear Regression (±SE) Relating the R_M Values of Salicylic Acid and its Derivatives to the Methanol Content (φ) of Methanol – Water Mobile Phase (According to Eq. (1): $R_M = R_{Mw(RP18W)} - S \cdot \varphi$) for Analysis Performed on RP18 W Plates

Symbol of Compound	$R_{Mw(RP18W)}$ (±SE)	S (±SE)	n	r	SEE	F	Range of the Volume Fraction of Methanol (φ)	Eq. No.
SA	0.150 (±0.033)	0.90 (±0.04)	13	0.986	0.031	384	1.00 ÷ 0.40	(28)
ASA	−0.241 (±0.012)	0.65 (±0.02)	15	0.995	0.015	1329	1.00 ÷ 0.30	(29)
SAND	2.103 (±0.063)	2.73 (±0.09)	13	0.994	0.058	996	1.00 ÷ 0.40	(30)
SALD	−0.224 (±0.017)	0.63 (±0.02)	15	0.990	0.021	636	1.00 ÷ 0.30	(31)
SAMD	1.130 (±0.032)	1.75 (±0.05)	15	0.996	0.038	1448	1.00 ÷ 0.30	(32)
SHXA	0.430 (±0.047)	1.37 (±0.07)	15	0.984	0.057	403	1.00 ÷ 0.30	(33)
MS	2.317 (±0.043)	2.99 (±0.13)	13	0.998	0.040	2546	1.00 ÷ 0.40	(34)
PS	3.001 (±0.053)	3.70 (±0.08)	14	0.998	0.057	2396	1.00 ÷ 0.35	(35)
3,5-DNSA	0.334 (±0.013)	1.20 (±0.02)	15	0.998	0.016	4104	1.00 ÷ 0.30	(36)
2,5-DHBA	−0.089 (±0.024)	0.81 (±0.03)	13	0.991	0.022	616	1.00 ÷ 0.40	(37)
3-AMSA	0.178 (±0.030)	0.92 (±0.04)	13	0.989	0.028	479	1.00 ÷ 0.40	(38)
4-AMSA	0.100 (±0.019)	0.91 (±0.03)	15	0.994	0.023	1109	1.00 ÷ 0.30	(39)
5-AMSA	0.049 (±0.042)	0.98 (±0.06)	15	0.975	0.051	254	1.00 ÷ 0.30	(40)

Where: SE – standard error; n – number of points to drive the particular regression equation; r – correlation coefficient; SEE – standard error of the estimation; F – the values of the Fisher test; for all regression equation the significance level (p) is <0.0001.

TABLE 6 Parameters of the Linear Regression (±SE) Relating the R_M Values of Salicylic Acid and its Derivatives to the Methanol Content (φ) of Methanol – Water Mobile Phase (According to Eq. (1): $R_M = R_{Mw(CN)} - S \cdot \varphi$) for Analysis Performed on CN Plates

Symbol of Compound	$R_{Mw(CN)}$ (±SE)	S (±SE)	n	r	SEE	F	Range of the Volume Fraction of Methanol (φ)	Eq. No.
SA	0.490 (±0.038)	1.40 (±0.05)	11	0.994	0.026	797	1.00 ÷ 0.50	(41)
ASA	0.568 (±0.067)	1.53 (±0.09)	11	0.985	0.046	303	1.00 ÷ 0.50	(42)
SAND	2.368 (±0.101)	3.05 (±0.14)	13	0.989	0.094	476	1.00 ÷ 0.40	(43)
SALD	0.887 (±0.052)	1.40 (±0.07)	11	0.989	0.036	421	1.00 ÷ 0.50	(44)
SAMD	0.878 (±0.041)	1.64 (±0.06)	12	0.994	0.035	786	0.90 ÷ 0.40	(44)
SHXA	0.620 (±0.053)	1.41 (±0.07)	13	0.986	0.049	372	1.00 ÷ 0.40	(45)
MS	1.622 (±0.099)	2.14 (±0.13)	11	0.984	0.068	275	1.00 ÷ 0.50	(46)
PS	2.454 (±0.088)	2.96 (±0.12)	13	0.991	0.082	596	1.00 ÷ 0.40	(47)
3,5-DNSA	0.303 (±0.030)	0.90 (±0.04)	11	0.991	0.021	519	1.00 ÷ 0.50	(48)
2,5-DHBA	1.005 (±0.060)	1.83 (±0.07)	9	0.994	0.028	618	1.00 ÷ 0.60	(49)
3-AMSA	0.130 (±0.034)	0.84 (±0.04)	9	0.991	0.016	391	1.00 ÷ 0.60	(50)
4-AMSA	0.251 (±0.020)	0.83 (±0.02)	9	0.997	0.010	1136	1.00 ÷ 0.60	(51)
5-AMSA	0.062 (±0.038)	0.82 (±0.05)	13	0.979	0.035	249	1.00 ÷ 0.40	(52)

where: SE – standard error; n – number of points to drive the particular regression equation; r – correlation coefficient; SEE – standard error of the estimation; F – the values of the Fisher test; for all regression equation the significance level (p) is <0.0001.

We compared the values of $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$ lipophilicity parameters with the experimental and theoretical *n*-octanol-water partition coefficients for the studied compounds. Obtained values of $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$ for ASA, 5-AMSA, SA, 4-AMSA, 3-AMSA, and 3,5-DNSA are lower in relation to their values of experimental and theoretical partition coefficients. The $R_{Mw(RP18W)}$ value for SHXA and the $R_{MW(RP8)}$ value for SALD show the best agreement with the experimental *n*-octanol-water partition coefficients. However, the remaining chromatographic parameters of lipophilicity for SHXA and for SALD are lower in relation to their values of experimental and theoretical partition coefficients. The $R_{Mw(RP18W)}$, $R_{Mw(RP8)}$, $R_{Mw(RP18)}$ and $R_{Mw(CN)}$ values for SAMD show the best agreement with the experimental and theoretical *n*-octanol-water partition coefficients. The $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, and $R_{Mw(RP18W)}$ values for PS show the best agreement with the experimental and theoretical *n*-octanol-water partition coefficients.

All determined chromatographic parameters of lipophilicity for 2,5-DHBA are lower in relation to its value of the experimental theoretical partition coefficients. The $R_{Mw(RP8)}$ and $R_{Mw(CN)}$ for 2,5-DHBA show the best agreement with the theoretical *n*-octanol-water partition coefficients. Remaining chromatographic parameters of lipophilicity for 2,5-DHBA are lower in relation to their values of theoretical partition coefficients.

Similarity analysis was also used for comparison of experimental partition coefficients ($\log P_{exp}$) with chromatographic lipophilicity ($R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, and $R_{Mw(CN)}$) of the salicylic acid and its derivatives. The results (Euclidean distance, single linkage) are presented in Figure 1. It was apparent that the lipophilicity $R_{Mw(RP8)}$ was most similar to $R_{Mw(CN)}$; however, $R_{Mw(RP8)}$ and $R_{Mw(CN)}$ values were most similar to the experimental partition coefficient. Good correlation was obtained between lipophilicity $R_{Mw(RP8)}$ and $R_{Mw(CN)}$:

$$R_{MW(RP8)} = 0.260(\pm 0.132) + 1.222(\pm 0.112)R_{MW(CN)}$$
$$n = 13; r = 0.9568.\ s = 0.307.\ F = 119.\ p < 0.0001 \tag{57}$$

The correlation coefficients for the linear relationships between chromatographic lipophilicity and experimental, as well as theoretical partition coefficients are listed in Table 7 for all the compounds.

The experimental partition coefficient correlated best with miLog P, xLog P3, $R_{Mw(RP8)}$ and $R_{Mw(CN)}$:

$$\log P_{exp} = 0.12(\pm 0.23) + 1.01(\pm 0.12) \cdot \text{miLog P}$$
$$n = 9; r = 0.9508;\ F = 65;\ p < 0.0005;\ s = 0.24 \tag{58}$$

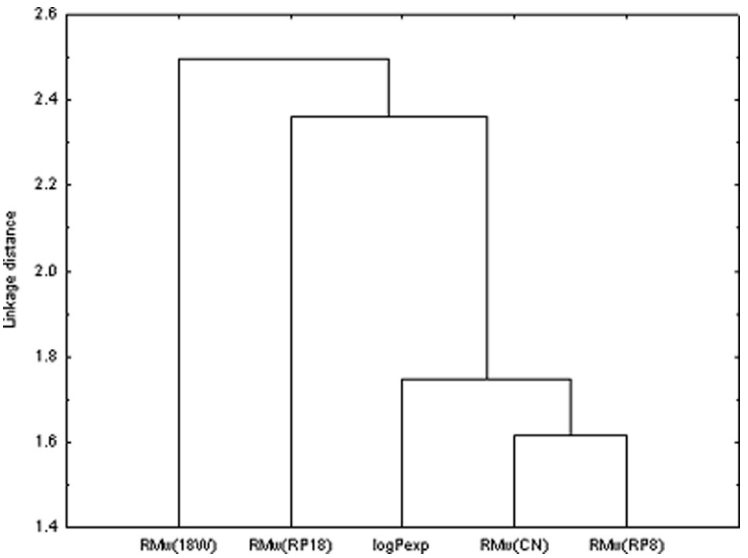

FIGURE 1 Similarity analysis of the chromatographic parameters of lipophilicity $R_{Mw(RP8)}$, $R_{Mw(RP18)}$, $R_{Mw(RP18W)}$, $R_{Mw(CN)}$ and the experimental n-octanol-water partition coefficients ($\log P_{exp}$) for salicylic acid and its derivatives (Euclidean distance, single linkage).

TABLE 7 The Values of Correlation Coefficients of Linear Relationships between Theoretical and Experimental Partition Coefficients as Well as Chromatographic Parameters of Lipophilicity (n = 13)

	$\log P_{exp}$[a]	$R_{Mw(RP8)}$	$R_{Mw(RP18)}$	$R_{Mw(RP\ 18W)}$	$R_{Mw(CN)}$
Alog P_s	0.910	0.872	0.692	0.720	0.809
AClog P	0.863	0.898	0.822	0.744	0.899
AB/log P	0.744	0.810	0.666	0.626	0.708
COSMOFrag	0.889	0.865	0.742	0.852	0.828
miLog P	0.951	0.940	0.78	0.787	0.913
Alog P	0.887	0.899	0.811	0.725	0.896
mlog P	0.847	0.878	0.805	0.739	0.843
KOWWIN	0.938	0.848	0.676	0.648	0.759
xlog P2	0.818	0.826	0.664	0.723	0.719
xlog P3	0.954	0.850	0.781	0.748	0.862

[a] n = 9 for $\log P_{exp}$.

$$\log P_{exp} = -0.01(\pm 0.24) + 1.02(\pm 0.12) \cdot \text{xLog P3}$$
$$n = 9; r = 0.9537;\ F = 70;\ p < 0.0005;\ s = 0.24 \tag{59}$$

$$R_{MW(RP8)} = -0.364(\pm 0.366) + 0.943(\pm 0.185) \cdot \log P_{exp}$$
$$n = 9; r = 0.889;\ F = 26;\ s = 0.387; p < 0.0014 \tag{60}$$

$$R_{MW(CN)} = -0.485(\pm 0.389) + 0.763(\pm 0.196) \cdot \log P_{exp}$$
$$n = 9; r = 0.827;\ F = 15;\ s = 0.411; p < 0.01 \tag{61}$$

The lipophilicity values $R_{Mw(RP8)}$ and $R_{Mw(CN)}$ correlated best with the theoretical n-octanol-water partition coefficient miLogP:

$$R_{MW(RP8)} = -0.406(\pm 0.217) + 1.034(\pm 0.113)\,\text{miLog P}$$
$$n = 13; r = 0.9402;\ F = 83;\ s = 0.360.\ p < 0.0001 \tag{62}$$

$$R_{MW(CN)} = -0.443(\pm 0.203) + 0.787(\pm 0.106)\,\text{miLog P}$$
$$n = 13; r = 0.9133\ s = 0.337.\ F = 55.\ p < 0.0001 \tag{63}$$

CONCLUSIONS

It was stated that the $RP8F_{254s}$ as well as CN plates and methanol-water mobile phase are suitable for the estimation of lipophilicity of examined salicylic acid and its derivatives. The chromatographic parameters of lipophilicity $R_{Mw(RP8)}$, and $R_{Mw(CN)}$, and theoretical n-octanol-water partition coefficient miLog P may be the alternative methods of lipophilicity determination of examined salicylic acid and its derivatives.

These methods of determining lipophilicity on the basis of theoretical calculation of log P and chromatographic methods complement other well established methods and applications, i.e., methods of normal measurement with the *n*-octanol-water system. Because of experimental difficulties, including solubility limits, chemical instability, formation of emulsions, or impure compounds, evaluation of log P values by the analytical methods described in this paper is justified. The methodology described in this paper can be used for the study and comparison of the lipophilic properties of other organic compounds of biological significance.

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