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Relationship between the Lipophilicity and Specific Hydrophobic Surface Area of Non-Homologous Series of Synthetic Dyes

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The lipophilicity and specific hydrophobic surface area of 43 synthetic dyes were determined on reversed-phase alumina layers using water-methanol mixtures as eluents. Carminic acid and hematoxylin remained on the start in each eluent system. The majority of dyes demonstrated regular retention behaviour, their retention decreased monotonously with increasing concentration of the organic modifier in the mobile phase. The retention of Rubin C increased with increasing concentration of methanol in the eluent (anomalous retention behaviour). Significant linear relationship was found between the lipophilicity and specific hydrophobic surface area of dyes indicating that, from the chromatographic point of view, they behave as a homologous series of compounds; however, their chemical structures are markedly different.

Key words: lipophilicity, specific hydrophobic surface area, synthetic dyes

INTRODUCTION

During the last decades, quantitative structure-activity relationship (QSAR) studies have been extensively used for the rational design of pharmaceuticals[1,2] and pesticides.[3] QSAR methods have been employed not only

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in the design of new bioactive compounds but also for elucidation of the biochemical[4] and biophysical[5] processes underlying biological activity. Lipophilicity, as one on the most important molecular parameters, has been frequently used in QSAR studies.[6,7] Lipophilicity modifies the penetration of bioactive molecules through the apolar cell mebranes and their uptake by target organs or organisms. Many methods have been developed for the determination of molecular lipophilicity. The traditional partition method between water and *n*-octanol is time-consuming, and the bioactive molecule has to be very pure because impurities may markedly influence the partition.[8] Various chromatographic methods, such as reversed-phase thin-layer (RP-TLC),[9] reversed-phase high-performance liquid chromatography,[10] micellar electrokinetic chromatography,[11] and gas-liquid chromatography[12] have been tested as rapid procedures for the determination of molecular lipophilicity. The chromatographic determination of lipophilicity offers some advantages: it is rapid and relatively simple, it does not require pure solutes because the impurities are separated during the chromatographic procces, and a very low quantity of a compound is needed.

Many RP-TLC systems were employed for the determination of lipophilicity, generally using silica as support material. The hydrophobic ligand can be bound to the support surface either by adsorption forces or by covalent bonding. When the ligand is bonded by adsorption forces, the silica is impregnated with paraffin[13] or silicone oils[14] dissolved in an appropriate solvent (*n*-hexane, diethyl ether, chloroform, *etc.*). The concentration of the oils in the solvent is generally 5% (*V*/*V*) but the application of lower (1% (*V*/*V*)) and higher (15% (*V*/*V*)) concentrations has also been reported.[15] Silica supports with covalently bonded hydrophobic ligands (silanized silica[16] and octyl-[17] or octadecyl-bonded silica[18]) have also been successfully applied for the determination of lipophilicity. Other inorganic and organic supports, such as alumina and cellulose, have not been frequently used for the study of the lipophilic character of bioactive compounds.[19]

As the majority of compounds show negligible mobility in water as the mobile phase, the solvent strength of the mobile phase has to be increased by adding an organic modifier miscible with water. In order to increase the reliability of lipophilicity determination, the R_M values [$R_M = \log(1/R_f - 1)$] characterizing lipophilicity in RP-TLC have been extrapolated to zero concentration of organic modifier.[20] The slope value of the relationship between the lipophilicity and the concentration of the organic modifier in the mobile phase was considered as a characteristic of the specific hydrophobic surface area of the compound.[21] It was supposed that, in the case of homogeneous series of solutes, the lipophilicity and specific hydrophobic surface area are intercorrelated.[22]

The objectives of the study were to determine the lipophilicity and specific hydrophobic surface area of synthetic dyes for further QSAR studies and to elucidate the relationship between these two parameters.

EXPERIMENTAL PART

DC-aluminiumoxide F_{254} plates (Merck, Darmstadt, Germany) were impregnated by an overnight predevelopment in *n*-hexane-paraffin oil 95:5 (*V*/*V*), as previously described.[23] The common and IUPAC names of synthetic dyes are compiled in Table I. The dyes were separately dissolved in methanol at a concentration of 3 mg/mL and 2 μL of the solutions were spotted on the plates. Mobile phase consisted of water-methanol mixtures. The methanol

TABLE I

Common and IUPAC names of synthetic dyes

No. of dyes	Common name	IUPAC name
1.	Acridine O	*N*,*N*,*N*',*N*'-Tetramethyl-3,6-acridinediamine monohydrochloride
2.	Amidoblack	4-Amino-5-hydroxy-3-[4-nitrophenyl)azo]-6-(phenylazo)-2,7-naphtalenedisulfonic acid disodium salt
3.	Aniline Blue	Aminomethyl[[4-[sulfophenyl)-amino]-phenyl][4-[(sulfophenyl)imino]-2,5-cyclohexadien-1-ylidene]methyl]-benzenesulfonic acid disodium salt
4.	Azobenzene	1,2-Diphenyldiazene
5.	Bengal Rose	3,4,5,7-Tetrachloro-3',6'-dihydroxy*spiro*-[isobenzofuran-1(3*H*),9'-[9*H*]xanthen]-3-one] disodium salt
6.	Brilliant Green	*N*-[4-[[-(Diethylamino)phenyl]phenylmethylethylene]-2,5-cyclohexadien-1-ylidene]-*N*-ethylethanaminium sulfate
7.	Bromthymol Blue	4,4'-(3*H*-2,1-Benzoxathiol-3-ylidene)-bis[2-bromo-3-methyl-6-(1-methylethyl)phenol]*S*,*S*-dioxide
8.	Carminic Acid	7-α-D-Glucopyranosyl-9,10-dihydro--3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracene-carboxylic acid

TABLE I (continued)

No. of dyes	Common name	IUPAC name
9.	Congo Red	3,3'-[[1,1'-Biphenyl]-4,4'-diylbis(azo)]-bis[4-amino-1-naphtalenesulfonic acid] disodium salt
10.	Coumassie R (R-250)	*N*-[4-[[4-[(4-Ethoxyphenyl)amino]phenyl]-[4-[ethyl[(3-sulfophenyl)methyl]amino]-phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-*N*-ethyl-3-sulfobenzene-methanaminium monosodium salt
11.	Coumassie R (G-250)	*N*-[4-[[4-[(4-Ethoxyphenyl)amino]phenyl]-[4-[ethyl[(3-sulfophenyl)methyl]amino]-2-methylphenyl]methylene]-3-methyl-2,5-cyclohexadien-1-ylidene]-*N*-ethyl-3-sulfobenzenemethanaminium monoso-dium salt
12.	Crystal Violet Gentian Violet	*N*-[4-[Bis[4-dimethylamino)-phenyl]-methylene]-2,5-cyclohexadien-1-ylidine]-*N*-methyl-methanaminium chloride
13.	Eosin Yellowish	2',4',5',7'-Tetrabromo-3',6'-dihydroxy-*spiro*[isobenzofuran-1(3*H*),9'-[9*H*]xan-then-3-one disodium salt
14.	Evan's Blue	6,6'-[3,3'-Dimethyl[1,1'-biphenyl]-4,4'-diyl)bis(azo)bis[4-amino-5-hydroxy-1,3-naphtalelenedisulfonic acid] tetra-sodium salt
15.	Hematoxylin	7,11b-Dihydroxybenz[*b*]indeno[1,2-*d*]-pyran-3,4,6a,9,10(6*H*)-pentol
16.	Janus Green B	3-(Diethylamino)-7-[[4-dimethyl-amino)phenyl[azo]-5-phenyl-phenazinium chloride
17.	Litmus	Natural dye mixture
18.	Malachite Green	*N*-[4-[[4-(Dimethylamino)phenyl]phenyl-methylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride
19.	Methylene Blue	2,2'-Methylenebis[3,4,5-trihydroxy-benzoic acid]
20.	Methyl Green	4-[[4-(Dimethylamino)phenyl][4-(di-methylimino)-2,5-cyclohexadien-1-ylidene]methyl]-*N*-ethyl-*N*,*N*-dimethyl-benzeneaminium bromide chloride

TABLE I (continued)

No. of dyes	Common name	IUPAC name
21.	Methyl Violet	*N*-[4-[Bis[4-dimethylamino)-phenyl]-methylene]-2,5-cyclohexadien-1-ylidine]-methanaminium chloride
22.	Neutral Red	N^8,N^8,3-Trimethyl-2,8-phenazinediamine monohydrochloride
23.	Nile Blue	5-Amino-4-(diethylamino)benzo[*a*]-phenazoxonium hydrogen sulfate
24.	Orange GS	4-[[4-(phenylamino)phenyl]azo]-benzenesulfonic acid monosodium salt
25.	Orcein	Oxidation product of orcein
26.	*p*-Methoxyazobenzene	
27.	*p*-Dimethylaminoazobenzene	*N*,*N*-Dimethyl-4-(phenylazo)benzenamine
28.	Pararosaniline	4-((4-Aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)mehtyl)benzenamine monohydrochloride
29.	Yellow AB	1-Phenylazo-2-naphtalenamine
30.	Phloxime B	2',4',5',7'-Tetrabromo-4,5,6,7-tetra-chloro-3',6'-dihydroxy-*spiro*[isobenzofuran--1(3*H*),9'-[9*H*]xanthen-3-one-sodium salt
31.	Pyronine G Pyronine Y	*N*-[6-(Dimethylamino)-3*H*-xanthene-3-ylidene]-*N*-methylmethanaminium chloride
32.	Rubine C	2-Amino-5-[(4-amino-3-sulfophenyl)(4--imino-3-sulfo-2,5-cyclohexadien-1-ylidene) acid disodium salt
33.	Safranine O	3,7-Diamino-2,8-dimethylphenyl phenazinium chloride
34.	Sudan Black B	2,3-Dihydro-2,2-dimethyl-6-[[4-(phenylazo-1*H*-perimidine
35.	Sudan III	1-[[4-(Phenylazo)phenyl]azo]-2-naphthalenol
36.	Sudan IV Scarlet Red	1-[[2-Methyl-4-[(2-methylphenyl)-azo]-phenyl]azo-2-naphtalenol
37.	Sudan Red	2,3-Dihydro-2,2-dimethyl-6-[[4-(phenylazo)-1-naphtyl]azo]-1*H*-perimidine
38.	Thionine	3,7-Diaminophenothiazin-5-ium chloride

TABLE I (continued)

No. of dyes	Common name	IUPAC name
39.	Trypan Blue	3,3'-[(3,3'-Dimethyl[1,1'-biphenyl]-4,4'--diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphtalenedisulfonic acid] tetrasodium salt
40.	Trypan Red	4,4'-[(3-Sulfo[1,1'-biphenyl]-4,4'-diyl)bis(azo)[3-amino-2,7-naphtalene-disulfonic acid] pentasodium salt
41.	Rhodamine B	N-[9-(2-Carboxyphenyl)-6-diethylamino)-3*H*-xanthen-3-ylidene]-*N*-ethylethanaminium chloride
42.	2,6-Dichloroindophenol Sodium	2,6-Dichloro-4-[(4-hydroxyphenyl)-imino]-2,5-cyclohexadien-1-one sodium
43.	Methyl Red	2-[[4-(Dimethylamino)phenyl]azo]-benzoic acid

concentration varied between 25 – 95% (V/V) in steps of 5% (V/V). The application of this wide concentration range was motivated by the very different lipophilicity of synthetic dyes. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, and the development distance was about 16 cm. After development, the plates were dried at 105 °C and the spots of dyes were revealed by their visible spectra. In the case of natural or synthetic mixtures, the position of the main fraction was determined. Each experiment was run in quadruplicate. The R_M values were calculated for each dye in each eluent. When the relative standard deviation of parallel determinations was higher than 5%, the R_M value was omitted from the following calculations. To increase the reliability of the determination of chromatographic parameters, the R_M values were extrapolated to zero concentration of organic modifier:

$$R_M = R_{M0} + b \times C \quad (1)$$

where R_M is the R_M value for a dye actually determined at a given concentration of organic modifier; R_{M0} is the R_M value extrapolated to zero concentration of organic modifier (best estimate of the lipophilicity); b reflects the decrease in the R_M value caused by 1% (V/V) concentration change of organic modifier in the eluent (this parameter is related to the specific hydrophobic surface area of synthetic dyes).

In order to elucidate the relationship between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b) of dyes, linear correlation between these two hydrophobicity parameters was calculated:

$$R_{M0} = A + B \times b \quad (2)$$

where A and B are the intercept and slope values of the correlation.

RESULTS AND DISCUSSION

Dyes carminic acid and hematoxylin (compounds 8 and 15 in Table I) remained on the start even at the highest concentration of methanol. It means that these compounds are bonded very strongly to the stationary phase. Hence, their lipophilicity and specific hydrophobic surface area cannot be determined under these experimental conditions. The strong binding of these dyes can be tentatively explained by the supposition that paraffin oil do not entirely cover the adsorption centers on the alumina surface. The alkaline adsorption centers are available for the acidic substructures of the dyes resulting in strong electrostatic interactions between them. These interactions account for the immobilization of the dyes at the start.

The majority of synthetic dyes showed regular retention behaviour in each RP-TLC system. Their retention decreased monotonously with increasing the concentration of the organic modifier in the mobile phase (Figure 1). This fact indicates that Eq. (1) can be successfully applied to the calculation

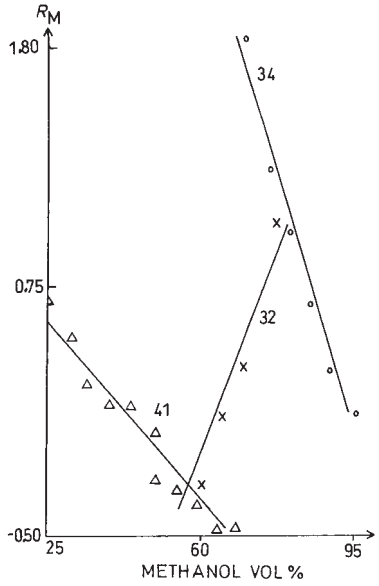

Figure 1. Dependence of the R_{M0} value of some synthetic dyes on methanol concentration in the mobile phase. Numbers refer to dyes in Table I.

of the hydrophobicity parameters of this class of solutes. Surprisingly, the Rubin C dye demonstrated anomalous retention behaviour. Its retention increased with an increasing concentration of methanol in the eluent (Figure 1). It can be assumed that higher concentrations of methanol suppress the dissociation of the polar groups of the molecule. The undissociated or less dissociated forms show a higher affinity to the apolar paraffin layer, resulting in enhanced retention.

The parameters of Eq. (1) are compiled in Table II. Eq. (1) describes well the experimental data. The significance level was over 95% in each instance. The ratio of variance explained was high in each instance, indicating good reproducibility of the method. The parameters of Eq. (1) show marked differ-

TABLE II

Parameters of the linear relationships between the R_{M0} values of synthetic dyes and the concentation of methanol in the eluent (C vol.%)

$$R_M = R_{M0} + b \times C$$

No of dyes	Common name	R_{M0}	$-b \times 10^2$	$r_{calc.}$
1.	Acridine O	2.68	2.95	0.9861
2.	Amidoblack	4.15	5.48	0.9955
3.	Aniline Blue	1.43	2.50	0.9952
4.	Azobenzene	3.88	4.84	0.9913
5.	Bengal Rose	4.43	5.61	0.9768
6.	Brilliant Green	5.32	6.53	0.9716
7.	Bromthymol Blue	3.41	4.93	0.9908
8.	Carminic Acid		remains on the start	
9.	Congo Red	5.90	8.70	0.9968
10.	Coumassie G Red	5.99	8.25	0.9905
11.	Coumassie R	6.02	8.31	0.9912
12.	Crystal Violet	3.15	4.15	0.9912
13.	Eosin Yellowish	3.43	4.64	0.9989
14.	Evan's Blue	5.55	7.04	0.9944
15.	Hematoxylin		remains on the start	
16.	Janus Green B	4.58	5.26	0.9908
17.	Litmus	5.45	6.77	0.9718
18.	Malachite Green	3.87	5.23	0.9936

TABLE II (continued)

No of dyes	Common name	R_{M0}	$-b \times 10^2$	$r_{calc.}$
19.	Methylene Blue	2.04	2.85	0.9649
20.	Methyl Green	6.37	8.02	0.9951
21.	Methyl Violet	3.58	4.66	0.9918
22.	Neutral Red	2.41	4.10	0.9618
23.	Nile Blue	4.97	5.62	0.9799
24.	Orange GS	4.18	4.46	0.9419
25.	Orcein	3.39	4.77	0.9876
26.	*p*-Methoxyazobenzene	3.96	4.98	0.9883
27.	*p*-Dimethylaminoazo-benzene	3.74	4.71	0.9917
28.	Pararosaniline	1.99	2.32	0.9728
29.	Yellow AB	3.97	5.02	0.9899
30.	Phloxine B	4.55	5.86	0.9882
31.	Pyronine G	2.72	3.60	0.9913
32.	Rubine C	–3.25	–5.27	0.9443
33.	Safranine O	2.61	3.21	0.9792
34.	Sudan Black B	6.29	6.64	0.9902
35.	Sudan III	6.85	7.60	0.9971
36.	Sudan IV	6.12	6.04	0.9983
37.	Sudan Red	6.21	6.17	0.9894
38.	Thionine	2.15	1.66	0.9765
39.	Trypan Blue	4.15	5.30	0.9740
40.	Trypan Red	3.99	5.47	0.9987
41.	Rhodamine B	1.04	2.25	0.9576
42.	2,6-Dichloroindophenol Sodium	2.75	3.98	0.9841
43.	Methyl Red	2.50	3.72	0.8600

ences between the synthetic dyes, suggesting that RP-TLC systems employing surface modified alumina can be used for their separation. The results can be further used for rational design of the reversed-phase high-performance liquid chromatographic separation of these class of dyes.

Highly significant relationship was found between the lipophilicity and specific hydrophobic surface area of dyes (significance level over 99.9%) (Figure 2). This finding indicates that, from the chromatographic point of view, these compounds behave as a homologous series of solutes despite the fact that, their chemical structures are considerably different.

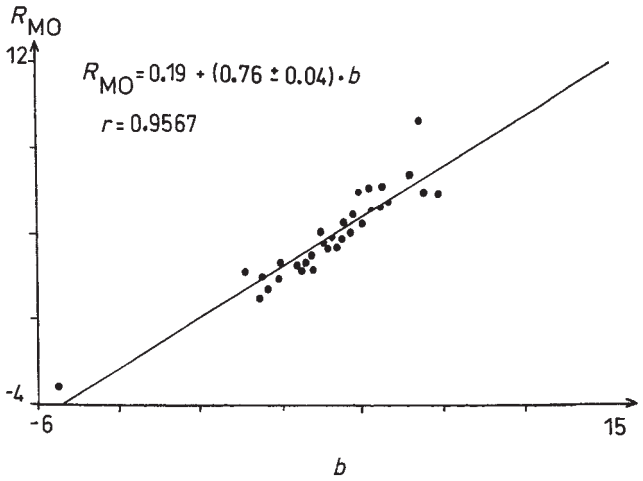

Figure 2. Relationship between the lipophilicity (R_{M0}) and specific hydrophobic surface area (b) of synthetic dyes (n = 41).

It can be concluded from the data that RP-TLC carried out on impregnated alumina support can be successfully used for determination of the hydrophobicity parameters of synthetic dyes and these parameters can be applied in future QSAR studies.

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REFERENCES

1. E. D. Moret, M. De Boer, H. W. Hilbers, J. P. Tollenaere, L. H. M. Janssen, J. J. M. Holthuis, R. J. Driebergen, W. Verboom, and D. N. Reinhardt, *J. Med. Chem.* **39** (1996) 720–728.
2. V. Nguyen-Cong and B. M. Rode, *J. Chem. Inf. Comput. Sci.* **36** (1996) 114–117.
3. M. W. Goosey, *Pestic. Sci.* **34** (1992) 313–320.
4. A. Elass, G. Vergoten, D. Legrand, J. Mazurier, E. Elass-Rochard, and G. Spik, *Quant. Struct.-Act. Relat.* **15** (1996) 94–101.
5. F. Yoshi and S. Hirono, *Chem. Senses* **21** (1996) 201–210.

6. C. E. Lipinski, E. F. Fiese, and R. J. Korst, *Quant. Struct. Act. Relat.* **10** (1991) 109–117.
7. U. Norinder and T. Högberg, *Quant. Struct. Act. Relat.* **10** (1991) 1–5.
8. C. Steinborn, C. Taube, and H. J. Mest, *Pharmazie* **45** (1990) 370–371.
9. G. L. Biagi, A. M. Barbaro, and M. C. Guerra, *J. Chromatogr.* **41** (1969) 371–379.
10. R. Kaliszan, A. Nasal, and M. Turowski, *J. Chromatogr. A* **722** (1996) 25–32.
11. S. Yang, J. G. Bumgartner, L. F. R. Kruk, and M. G. Khaledi, *J. Chromatogr. A* **721** (1996) 323–335.
12. D. R. Clifford and D. A. M. Watkins, *Pestic. Sci.* **2** (1971) 41–47.
13. T. Cserháti, E. Forgács, and G. Hajós, *J. Planar Chromatogr.* **11** (1998) 64–69.
14. N. Houngbedji and K. Waisser, *J. Chromatogr.* **509** (1990) 400–405.
15. A. Pyka, *J. Planar Chromatogr.* **9** (1996) 215–217.
16. K. P. Dross, K. Mannhold, and R. F. Rekker, *Quant. Struct. Act. Relat.* **11** (1992) 36–44.
17. R. M. Kleyle, D. Nurok, A. D. Kossoy, and S. C. Burris, *J. Chromatogr. A* **749** (1996) 211–217.
18. A. Podgórny and T. Kowalska, *J. Planar Chromatogr.* **7** (1994) 291–293.
19. T. Cserháti and Z. Illés, *Chromatographia* **31** (1991) 152–156.
20. Y. Darwish, T. Cserháti, and E. Forgács, *J. Planar Chromatogr.* **6** (1993) 458–462.
21. C. Horvath, W. Melander, and I. Molnar, *J. Chromatogr.* **125** (1976) 129–156.
22. K. Valkó, *J. Liq. Chromatogr.* **7** (1984) 1405–1424.
23. T. Cserháti, B. Bordás, E. Fenyvesi, and J. Szejtli, *J. Chromatogr.* **259** (1983) 107–110.

SAŽETAK

Veza između lipofilnosti i specifične hidrofobne površine nehomolognog niza sintetskih boja

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Određena je lipofilnost i specifična hidrofobna površina niza od 43 sintetske boje na slojevima glinice upotrijebivši kao eluent smjese vode i metanola. Bez obzira na upotrijebljen eluentni sustav, karminska kiselina i hematoksilin nisu se micale sa starta. Većina ostalih boja pokazala je očekivane retencije, koje su opadale jednolično s povećanjem udjela metanola u pokretnoj fazi. Rubin C je pokazao nepravilno retencijsko ponašanje, jer je njegova retencija rasla s porastom koncentracije metanola u eluensu. Nađena je signifikantna linearna veza između lipofilnosti i specifične hidrofobne površine proučavanih boja. Taj rezultat pokazuje da se s kromatografske točke gledanja proučavane boje ponašaju kao homologni niz spojeva, premda su njihove kemijske strukture izrazito različite.