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Note

Influence of stationary phase modifications on lipophilicity measurements of benzophenones using reversed-phase thin-layer chromatography

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In a previous publication on reversed-phase thin-layer chromatography (RPTLC), Kakoulidou and Rekker¹ reported data obtained from a series of benzophenones. The experiments were performed on silica gel plates impregnated with paraffin oil and elution was done with acetone-water, preferably a 65:35 (v/v) mixture. The R_M values could be perfectly correlated in the following equation:

$$\Sigma f = 4.247 (\pm 0.116)R_M - 0.312 (\pm 0.027)kn + 4.482 (\pm 0.034) \quad (1)$$

$n = 17, r = 0.998, s = 0.077, F = 2052$

where Σf is the sum of the hydrophobic fragmental values² of the constituent parts of the benzophenone, including one c_M factor (= 0.289) to account for cross-conjugation in molecules that are not too hindered sterically (occupation of both *ortho*-positions in one of the two phenyl moieties by methyl groups will fully prevent cross-conjugation).

Parameter kn in eqn. 1 is the coefficient of the constant c_M factor (= 0.289). It has a corrective role in the final fit and is connected with resonance, steric and systemic peculiarities of the structure under consideration³. Although the relationship between the three indicated factors is not yet completely understood, they are useful in improving relationships such as eqn. 1 to a statistical level comparable to that attained for alkyl benzenes⁴.

The results obtained so far have encouraged further study. The present investigations are mainly concerned with a comparison of a number of reversed-phase thin-layer conditions, which seem to influence the moving pattern of the benzophenones over the thin-layer plate. We take into account:

(a) replacement of silica gel by a silica gel-Kieselguhr mixture; (b) application of methanol-water instead of acetone-water elution. (Grünbauer *et al.*⁵, in their RPTLC study on *n*-alkylphenyl ketones, observed a distinct non-linearity between R_M and the acetone-water ratio of high acetone concentrations. These deviations can be ascribed to acetone-induced perturbations of the stationary phase and are not observed in the corresponding methanol-water elutions.); (c) replacement of paraffin

oil by silicone oil, *i.e.*, modification of experimental conditions as frequently applied by Biagi *et al.*⁶.

Some of the benzophenones investigated are identical to the Kakoulidou-Reker series, but others have not been considered in RPTLC experiments before.

MATERIALS AND METHODS

The benzophenones investigated were of several origins, from laboratory stock. They were all of sufficient purity.

Experiments were performed on DC Fertigplatten Kieselgel 60 F254 (Merck) and on DC Fertigplatten Kieselgel 60-Kieselguhr F254 (Merck) impregnated with liquid paraffin oil (Baker) or with silicone oil (Merck). Impregnation was carried out by a standard method as described by Grünbauer *et al.*⁵. The plates were eluted with methanol-water [65:35 (v/v), Baker, double-distilled water] in closed tanks at 21°C. The plates were dried at 75°C and the spots were located under UV light.

The R_F values given are the averages of at least ten determinations. The R_M values were calculated from R_F values using the following equation:

$$R_M = \log (1/R_F - 1)$$

Only R_M values derived from R_F values of between 0.2 and 0.8 were used in the calculations.

Part of the investigation was carried out by nine students during a short QSAR laboratory course.

RESULTS AND DISCUSSION

Tables I, II and III summarize both R_F and R_M values and include the calculated standard deviations.

TABLE I

RPTLC DATA [METHANOL-WATER (65:35)] FOR BENZOPHENONES USING SILICA GEL PLATES COATED WITH PARAFFIN OIL

Substituent	R_F	R_M	Σf^*	kn	Σf_{est}
None	0.522 (± 0.017)	-0.039 (± 0.030)	3.193	0	3.217
2-Methyl	0.402 (± 0.030)	0.172 (± 0.055)	3.712	0	3.782
4-Methyl	0.430 (± 0.019)	0.123 (± 0.034)	3.712	0	3.648
2-Ethyl	0.328 (± 0.026)	0.312 (± 0.052)	4.231	0	4.159
4-Trichloromethyl	0.184 (± 0.011)	0.647 (± 0.031)	5.071	0	5.074
4-Methoxy	0.514 (± 0.022)	-0.024 (± 0.038)	3.273	0	3.271
4-Fluoro	0.507 (± 0.030)	-0.012 (± 0.053)	3.402	0	3.298
4-Chloro	0.361 (± 0.028)	0.248 (± 0.052)	3.935	0	3.998
4-Bromo	0.336 (± 0.020)	0.295 (± 0.038)	4.127	0	4.132
4-Nitro	0.507 (± 0.029)	-0.012 (± 0.051)	2.958	1	3.020
2-Trifluoromethyl	0.517 (± 0.033)	-0.029 (± 0.058)	4.339	-4	4.355

* Including one kn to account for cross-conjugation.

TABLE II

RPTLC DATA [METHANOL-WATER 65:35] FOR BENZOPHENONES USING SILICA GEL-KIESELGUHR PLATES COATED WITH PARAFFIN OIL

Substituent	R_F	R_M	Σf^*	kn	Σf_{est}
None	0.583 (± 0.018)	-0.146 (± 0.033)	3.193	0	3.276
2-Methyl	0.447 (± 0.023)	0.093 (± 0.040)	3.712	0	3.814
4-Methyl	0.490 (± 0.029)	0.017 (± 0.050)	3.712	0	3.657
2-Ethyl	0.360 (± 0.017)	0.250 (± 0.032)	4.231	0	4.172
4-Trichloromethyl	0.193 (± 0.010)	0.621 (± 0.027)	5.071	0	5.001
4-Methoxy	0.598 (± 0.023)	-0.173 (± 0.042)	3.273	0	3.231
4-Fluoro	0.577 (± 0.029)	-0.135 (± 0.052)	3.402	0	3.298
4-Chloro	0.390 (± 0.007)	0.194 (± 0.013)	3.935	0	4.038
4-Bromo	0.359 (± 0.024)	0.252 (± 0.046)	4.127	0	4.194
4-Nitro	0.603 (± 0.036)	-0.183 (± 0.065)	2.958	1	2.939
2-Trifluoromethyl	0.591 (± 0.031)	-0.160 (± 0.055)	4.339	-4	4.334

* Including one kn for cross-conjugation.

TABLE III

RPTLC DATA [METHANOL-WATER (65:35)] FOR BENZOPHENONES USING SILICA GEL-KIESELGUHR PLATES COATED WITH SILICONE OIL

Substituent	R_F	R_M	Σf^*	kn	Σf_{est}
None	0.685 (± 0.035)	-0.339 (± 0.071)	3.193	0	3.284
2-Methyl	0.544 (± 0.030)	-0.077 (± 0.052)	3.712	1	3.741
4-Methyl	0.606 (± 0.045)	-0.188 (± 0.083)	3.712	0	3.713
2-Ethyl	0.474 (± 0.050)	0.046 (± 0.087)	4.231	1	4.112
4-Trichloromethyl	0.323 (± 0.018)	0.322 (± 0.036)	5.071	0	5.170
4-Methoxy	0.699 (± 0.026)	-0.367 (± 0.052)	3.273	0	3.198
4-Fluoro	0.649 (± 0.039)	-0.269 (± 0.075)	3.402	0	3.484
4-Chloro	0.563 (± 0.051)	-0.111 (± 0.090)	3.935	0	3.941
4-Bromo	0.553 (± 0.052)	-0.093 (± 0.092)	4.127	0	3.998
4-Nitro	0.687 (± 0.021)	-0.341 (± 0.042)	2.958	1	2.998
2-Trifluoromethyl	0.600 (± 0.050)	-0.178 (± 0.091)	4.339	-2	4.314

* Including one kn for cross-conjugation.

The three sets of experiments are coded as follows: *gelpar*, elutions on silica gel plates coated with paraffin oil; *gelgurpar*, elutions on silica gel-kieselguhr plates coated with paraffin oil; *gelgursil*, elutions on silica gel-kieselguhr plates coated with silicone oil. The DC Fertigplatten Kieselgel 60 F254 coated with silicone oil could not be eluted with a methanol-water mixture.

Eqs. 2, 3 and 4 allow a first comparison of the three modes of separation

$$R_{M(\text{gelpar})} = 0.835 (\pm 0.054)R_{M(\text{gelgurpar})} + 0.106 (\pm 0.014) \quad (2)$$

$$n = 11, r = 0.996, s = 0.020, F = 1180$$

$$R_{M(\text{gelpar})} = 1.133 (\pm 0.302)R_{M(\text{gelgursil})} + 0.325 (\pm 0.069) \quad (3)$$

$$n = 11, r = 0.941, s = 0.076, F = 69.8$$

$$R_{M(\text{gelgurpar})} = 1.362 (\pm 0.338)R_{M(\text{gelsursil})} + 0.263 (\pm 0.079) \quad (4)$$

$n = 11, r = 0.948, s = 0.085, F = 80.6$

The discriminative power of gelgurpar (the spread that is effected in the eluted spots) is slightly better than that of gelpar and gelsursil. The mutual ratios can be expressed as *ca.* 1.4:1.1:1.0, in the order gelgurpar > gelpar > gelsursil. The decreased statistical quality of eqns. 3 and 4 is mainly due to the behaviour of 2-trifluoromethylbenzophenone. This compound has too high a lipophilicity (R_F value too low) in the gelsursil system.

The R_M values from Table I, II and III were correlated with Σf values. These Σf values were composed from the constituent fragments of the structure, applying one c_M factor to account for cross-conjugations.

$$\Sigma f = 2.345 (\pm 1.215)R_{M(\text{gelpar})} + 3.454 (\pm 0.310) \quad (5)$$

$n = 11, r = 0.820, s = 0.369, F = 18.5$

$$\Sigma f = 1.978 (\pm 1.005)R_{M(\text{gelgurpar})} + 3.701 (\pm 0.251) \quad (6)$$

$n = 11, r = 0.826, s = 0.364, F = 19.2$

$$\Sigma f = 2.763 (\pm 0.920)R_{M(\text{gelsursil})} + 4.216 (\pm 0.222) \quad (7)$$

$n = 11, r = 0.913, s = 0.264, F = 44.8$

Eqns. 5–7 are all of unacceptable quality, especially eqns. 5 and 6. Notorious outliers in all three equations are 4-nitrobenzophenone and 2-trifluoromethylbenzophenone:

	4-NO ₂	2-CF ₃
eqn. 5	$\Delta = -0.47$	0.96
eqn. 6	$\Delta = -0.39$	0.95
eqn. 7	$\Delta = -0.32$	0.62

Optimal fits of the compounds, including both 4-NO₂ and 2-CF₃ derivatives, could be achieved by assigning a proper kn parametrization:

$$\Sigma f = 2.691 (\pm 0.222)R_{M(\text{gelpar})} - 0.278 (\pm 0.037)kn + 3.325 (\pm 0.058) \quad (8)$$

$n = 11, r = 0.995, s = 0.069, F = 440$

$$\Sigma f = 2.240 (\pm 0.240)R_{M(\text{gelgurpar})} - 0.270 (\pm 0.048)kn + 3.612 (\pm 0.061) \quad (9)$$

$n = 11, r = 0.992, s = 0.089, F = 261$

$$\Sigma f = 2.858 (\pm 0.318)R_{M(\text{gelsursil})} - 0.286 (\pm 0.077)kn + 4.256 (\pm 0.077) \quad (10)$$

$n = 11, r = 0.990, s = 0.694, F = 230$

The observed regressor values of kn are well within the range of the expected value of 0.289. The kn parameterizations used in the three equations (see Tables I–III) emphasize the similarities between gelpar and gelgurpar experiments, as evident from eqn. 2. They also confirm the unusual behaviour of gelsursil found in eqns. 3 and 4. Table IV gives five representative items.

TABLE IV

COMPARISON OF ESTIMATED LIPOPHILICITIES OF FIVE REPRESENTATIVE BENZOPHENONES

Substituent	Σf_{est}^*			$\Sigma f_{(est.av.)}$	$\Sigma f_{(calc.)}$
	gelpar	gelgurpar	gelgursil		
None	3.22	3.28	3.29	3.26	3.19
2-Methyl	3.78	3.81	$4.04 - 1c_M$	3.78	3.71
2-Ethyl	4.16	4.17	$4.39 - 1c_M$	4.15	4.23
4-NO ₂	$3.29 - 1c_M$	$3.21 - 1c_M$	$3.28 - 1c_M$	2.98	2.96
2-CF ₃	$3.24 + 4c_M$	$3.25 + 4c_M$	$3.74 + 2c_M$	4.34	4.34

* $c_{M(av.)} = 0.278$.

The 2-methyl- and 2-ethyl-benzophenones on the paraffin-oil-coated plates fully agree with previous experiments by Kakoulidou and Rekker¹. The steric effects of both methyl and ethyl groups can be neglected. On a gelgursil plate, however, the steric effect has to be considered in order to obtain an acceptable fit (*i.e.*, a correlation coefficient of at least 0.99 as attained in previous studies^{1,4}). Assuming a comparable steric effect for the 2-CF₃ in our gelgursil experiments, this would mean that the kn parameter, given as -2 for the 2-CF₃ group, is actually the sum of two terms -3 and $+1$, respectively. We believe that the value of -3 (as well as the values of -4 in Tables I and II) is connected with systemic factors. The comparison between an octanol-water partition system and an RPTLC system with a less soluble organic phase needs a down-correction for halogen lipophilicity³. The value of $+1$ is connected with steric effects.

The 4-NO₂ group with $kn = 1$, independent of the applied plate-type, has to be considered a resonance-decreasing group. Its negative mesomeric effect counteracts the similar negative mesomeric effect of C=O, resulting in decreased lipophilicity.

Not all factors that govern the transport of benzophenones over a thin-layer plate are understood as yet. We intend to perform more experiments with some structures that have not been investigated before.

The modifications of the stationary phase envisaged in the present study show that the replacement of paraffin oil by silicone oil may cause significant changes in retention behaviour of particular functional groups. However, these changes might make it difficult to explain the investigated structures in terms of lipophilicity. The addition of kieselguhr to the silica gel coating of the thin-layer plate seems to be important for silicone-oil-coated plates but does not influence the paraffin-oil-coated plates.

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