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LIQUID CHROMATOGRAPHIC RETENTION BEHAVIOR OF ORGANO-METALLIC COMPOUNDS AND LIGANDS WITH AMINE-, OCTADECYL-SILICA- AND β-CYCLODEXTRIN-BONDED PHASE COLUMNS

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SUMMARY

   Effects of solvent composition and ligand variation on the retention of organometallic compounds have been studied using an amino, an octadecylsilica (ODS) and a β-cyclodextrin (β-CD) bonded phase column in either a normal-phase or a reversed-phase mode. The retention behavior for the organometallic compounds with the amino column can be rationalized using the displacement model. The "apparent" molecular areas are greater for compounds capable of strong hydrogen bonding. The retention in the ODS column roughly follows an argument based on the expected solubility behavior while mixed retention mechanisms are involved for the β-CD column, *i.e.* both inclusion process and solubility or solvophobic interactions are possibly operative.

INTRODUCTION

   The application of high-performance liquid chromatographic (HPLC) techniques for the separation of organometallic compounds has been very fruitful. Many separations have been reported since the first separation of some arene tricarbonyl-chromium(0) complexes[1], and both normal-phase and reversed-phase modes have been used. For example, Uden and his co-workers[2,3] have reported separation of titanium, zirconium, and hafnium metallocene dichlorides and homologous titanaindene complexes using various polar and non-polar adsorbents[2,3]. An updated review of HPLC separations on organometallic and metal coordination compounds has also appeared[4]. Despite such activity, a systematic study on their retention mechanisms has not been reported.

   We are interested in the retention mechanisms and effects of solvent composition on normal-phase HPLC separations. It has been reported that using an amine-bonded phase column for the normal-phase separation of anilines, the

solute–stationary phase interaction mode changes gradually with the change of mobile phase composition[5]. On the other hand, this phenomenon is not observed when a silica column is used, nor is it found when phenols are separated[5,6]. Thus, several different types of intermolecular interactions between substrates and surface–surface bonded reagents which are tuned by the mobile phase composition have emerged. A detailed understanding of all the interactions during the retention process will certainly be invaluable in terms of future development. Because of the similarity of structures and properties, organometallic compounds and their ligands can serve as good probes for the study of retention mechanisms during the separation.

In this paper, we wish to report an initial study on the liquid chromatographic retention behavior of several arene tricarbonylchromium(0) compounds together with the arene ligands. Emphasis is placed on the effects of solvent composition on the separation using an amino, an octadecyl silica (ODS) and a β-cyclodextrin (β-CD) bonded phase columns.

EXPERIMENTAL

Apparatus

A Beckman 332 liquid chromatograph with two Altex 110 pumps and a microprocessor control unit together with a Micromeritics 786 variable-wavelength (200–600 nm) detector with a deuterium lamp was used. A Micromeritics Model 7500 liquid chromatography system was also used. This latter system was equipped with a Model 750 solvent delivery system, a Model 752 ternary solvent mixer, and a Model 731 column compartment with a universal injector and a variable-temperature controller from ambient to 150°C.

Pressure-Lok series C-160, 10-μl and 25-μl syringes were used. Chromatograms were recorded with a Linear Model 555 single-channel recorder.

Materials

Amino bonded phase, Rosil-NH$_2$, was purchased from Alltech Associates. The column (25 cm × 4.6 mm I.D.) was packed using an up-flow method with a Micromeritics Model 705 stirred-slurry column packer. Carbon tetrachloride was used as the solvent for column packing. Ultrasphere ODS, 5 μm, 25 cm × 4.6 mm I.D. column from Altex (Beckman) was used for reversed-phase separation. β-CD column, 25 cm × 4.6 mm I.D., was obtained from Advanced Separations Technology.

HPLC-grade methanol, 2-propanol, and *n*-heptane were obtained from Fisher Scientific. HPLC water (18 MΩ) was obtained by passing boiled deionized water through a Milli-Q Type I reagent grade water system. Tricarbonylchromium complexes were prepared by published techniques and/or purchased from Strem Chemical. All other chemicals were reagent grade and obtained from various sources.

Chromatographic procedures

The general procedures to study the effects of solvent composition for various columns were described in a previous publication with minor modifications[5]. Mobile phase pre-equilibration was always achieved before any separation occurred. A flowrate of 1 ml/min was used. A back pressure less than 1000 p.s.i. was usually observed for the normal-phase separations and 3000 p.s.i. for reversed-phase separations.

All data points were collected by averaging more than three reproducible injections. Column void volumes were determined according to published methods[5,7].

RESULTS AND DISCUSSION

A total of nine arene ligands and their corresponding tricarbonylchromium(0) organometallic compounds were studied. They can be classified into two groups. Group I consists of mesitylene, toluene, benzene, methylbenzoate, and anisole which are either non-polar or less polar and incapable of strong hydrogen bonding. Group II consists of four aniline derivatives, aniline, N-methylaniline, o-toluidine, and m-toluidine, all of which are polar and capable of hydrogen bonding. Some representative chromatograms using three different columns at various conditions are shown in Fig. 1.

Amino-bonded phase column in a normal-phase mode
    Under the normal-phase mode using an amino-bonded phase column, it is observed that group I ligands always elute faster than group II ligands with all 2-propanol–n-heptane solvent compositions tested. A similar observation was found for the organometallic compounds. N-methylaniline is a borderline case, it always has a relatively smaller capacity factor than the other three members in group II but a substantially larger capacity factor than those of group I. Tables I and II list capacity factors of all solutes at all mobile phase compositions studied.
    As the solvent eluotropic strength increases, *i.e.* as the concentration of 2-propanol in *n*-heptane increases, the capacity factor decreases as expected. However, the extent of decrease of capacity factor is greater for group II compounds. Snyder and co-workers[8,9] have derived an equation to account for the mobile phase effect on liquid–solid chromatography

$$\log k'_2 = \log k'_1 - (A_s/n_b) \log X_s \qquad (1)$$

where X_s is the mole fraction of strong solvent in a binary mobile phase, A_s is the molecular area of the solute, n_b is the molecular area of the strong solvent, k'_2 is the capacity factor for a solute eluted with the binary mobile phase, and k'_1 is the capacity factor of the solute eluted with just the strong solvent. A recent study has been reported using another form of the equation to rationalize the mobile phase effects on normal-phase separations of some less polar compounds using an amino-bonded phase column[10]. In Table III are listed the A_s/n_b values which were obtained from the values of slopes of the plots of $\log k'_2$ *vs.* $\log X_s$. An example of the plot is shown in Fig. 2.
    In general, the molecular area ratio, *i.e.* A_s/n_b, is in the following order: group II orgmetallic > group II ligands > group I orgmetallic $\approx$ group I ligands. Thus, larger "apparent" molecular areas are observed for group II ligands and organometallic compounds which may be due to their capability of strong hydrogen bonding and some competition interactions against solvent at the amino-bonded phase surface[9]. The tricarbonylchromium(0) moiety has been said to be similar to that of a *p*-NO$_2$ substituent which makes the coordinated anilines less basic than the free anilines because of its electron withdrawing ability[11]. The capacity factors of group
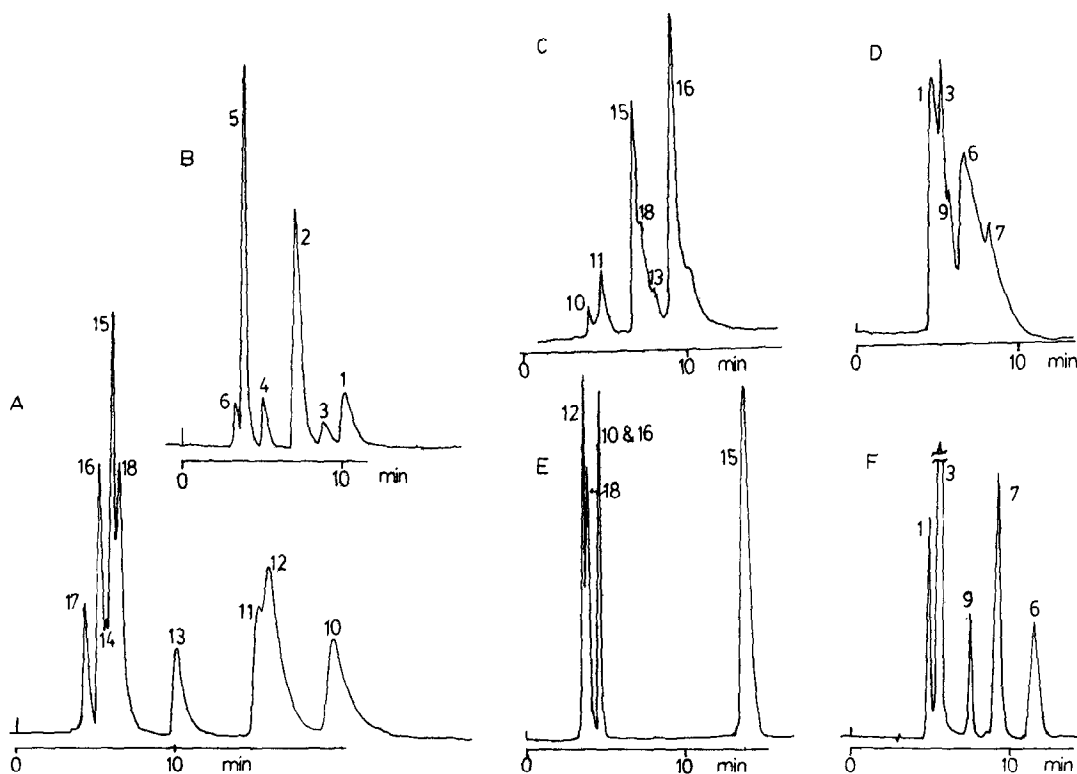
Fig. 1. Chromatograms of arene and arene tricarbonylchromium compounds using three different columns at various conditions. Flow-rate = 1 ml/min, 254 nm UV *detection.* (A) Amine-bonded phase, 2-propanol–*n*-heptane (15:85), arene $Cr(CO)_3$; (B) amine-bonded phase, 2-propanol–*n*-heptane (4:96), arene; (C) ODS, water–methanol (40:60), arene $Cr(CO)_3$; (D) ODS, water–methanol (30:70), arene; (E) β-CD, water–methanol (40:60), arene $Cr(CO)_3$; (F) β-CD, water–methanol (60:40), arene. Peak identification: 1, Aniline; 2, *o*-toluidine; 3, *m*-toluidine; 4, N-methylaniline; 5, methylbenzoate; 6, benzene; 7, toluene; 8, mesitylene; 9, anisole; 10, aniline $Cr(CO)_3$; 11, *o*-toluidine $Cr(CO)_3$; 12, *m*-toluidine $Cr(CO)_3$; 13, N-methylaniline $Cr(CO)_3$; 14, methylbenzoate $Cr(CO)_3$; 15, benzene $Cr(CO)_3$; 16, toluene $Cr(CO)_3$; 17, mesitylene $Cr(CO)_3$; 18, anisole $Cr(CO)_3$.

II organometallic compounds are much longer than their corresponding ligands, consistent with the facts that nitroanilines are much longer retained than chloroanilines and toluidines[5].

The data for the A_s/n_b values for the group I ligands show large errors. This is presumably due to their relatively short retention times. However, it is seen that the "apparent" molecular areas of group I ligands are not significantly different from those of group I organometallic compounds, indicating the effects of the "*p*-NO₂" group are relatively less pronounced. Summarizing all facts, it is reasonable to state that hydrogen bonding is important and fundamental to the chromatographic process. This is because hydrogen bonding is directional and specific which is different from the less-directional, less-specific dipole–dipole and London dispersion interactions.

TABLE I

CAPACITY FACTORS OF ARENE LIGANDS USING AN AMINO-BONDED PHASE COLUMN

The relative errors are within ±1%.

Arene	2-Propanol–n-heptane (%)							
	1:99	2:98	3:97	4:96	5:95	10:90	25:75	30:70
Aniline	26.05	4.31	2.98	2.29	1.94	1.28	0.64	0.57
m-Toluidine	23.80	3.69	2.48	1.94	1.57	1.07	0.54	0.45
o-Toluidine	22.39	2.92	1.61	1.30	1.07	0.82	0.41	0.37
N-Methylaniline	–	1.16	0.89	0.71	0.59	0.52	0.27	0.24
Anisole	0.68	0.20	0.17	0.17	0.16	–	–	–
Benzene	0.18	0.11	0.08	0.09	0.07	–	–	–
Methylbenzoate	1.91	0.37	0.28	0.30	0.28	–	–	–
Toluene	0.23	0.09	0.07	0.08	0.07	–	–	–
Mesitylene	0.12	0.06	0.05	0.06	0.04	–	–	–

TABLE II

CAPACITY FACTORS OF ARENE TRICARBONYLCHROMIUM(0) COMPOUNDS USING AN AMINO-BONDED PHASE COLUMN

The relative errors are within 1%.

Arene tricarbonyl-chromium	2-Propanol–n-heptane (%)							
	3:97	4:96	5:95	10:90	15:85	20:80	25:75	30:70
Aniline	–	–	38.30	13.30	6.75	5.14	2.82	2.10
m-Toluidine	–	–	26.70	10.50	5.38	–	2.41	1.75
o-Toluidine	–	–	22.70	9.02	4.83	3.53	2.18	1.67
N-Methylaniline	–	–	10.79	4.64	2.72	1.93	1.38	1.04
Anisole	3.07	2.40	2.11	1.38	1.06	0.88	0.69	0.64
Benzene	2.06	1.67	1.46	1.00	0.82	0.69	0.54	0.48
Methylbenzoate	2.17	1.70	1.42	1.00	0.74	0.63	0.49	0.43
Toluene	1.40	1.17	1.07	0.72	0.62	0.53	0.42	0.37
Mesitylene	0.66	0.54	0.49	0.39	0.32	0.27	0.23	0.22

TABLE III

VALUES OF A_s/n_b FOR SOME ARENE–ARENE TRICARBONYLCHROMIUM COMPOUNDS USING AN AMINO-BONDED PHASE COLUMN

Values in parenthesis are corresponding correlation coefficients of the linear fits between log k'_2 and log X_s.

|	Organic compound	Tricarbonylchromium derivative
Mesitylene	0.64 ± 0.13 (0.941)	0.50 ± 0.02 (0.994)
Toluene	0.76 ± 0.19 (0.913)	0.61 ± 0.03 (0.993)
Benzene	0.58 ± 0.08 (0.970)	0.67 ± 0.03 (0.995)
Anisole	0.90 ± 0.23 (0.915)	0.74 ± 0.02 (0.998)
Methylbenzoate	1.20 ± 0.34 (0.897)	0.74 ± 0.03 (0.995)
N-Methylaniline	0.52 ± 0.05 (0.969)	1.44 ± 0.05 (0.998)
o-Toluidine	1.09 ± 0.18 (0.924)	1.63 ± 0.08 (0.996)
m-Toluidine	1.08 ± 0.14 (0.951)	1.72 ± 0.07 (0.998)
Aniline	1.04 ± 0.14 (0.951)	1.78 ± 0.09 (0.994)
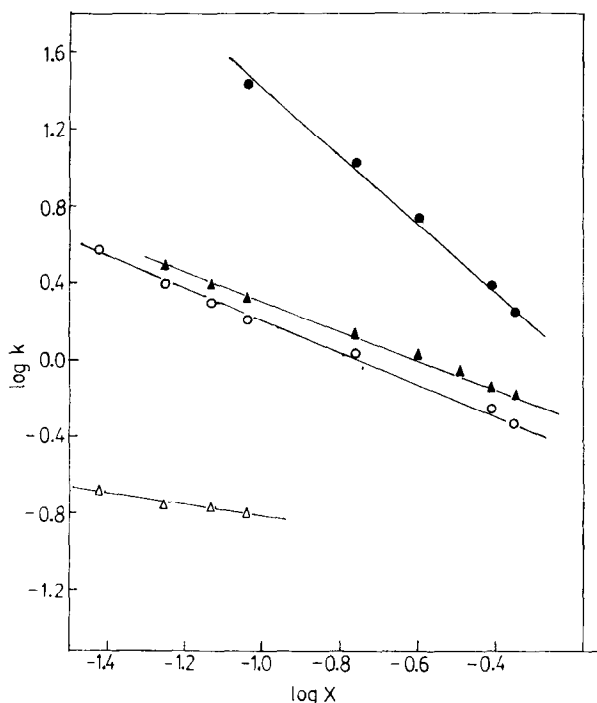
Fig. 2. Plots of log k'_2 vs. log X_s for m-toluidine and anisole and their tricarbonylchromium(0) derivatives at various mole fractions of 2-propanol–n-heptane using an amino-bonded phase column ○, m-toluidine; ●, m-toluidine Cr(CO)$_3$; △, anisole, ▲, anisole Cr(CO)$_3$.

The data obtained for group II organometallic compounds can be rationalized using the displacement model. This is presumably because the hydrogen bonding site, *i.e.* –NHR group, of group II organometallic compounds has a relatively smaller area as compared to the total molecular areas which reduces its probability to interact with the surface. On the other hand, the rest of the less polar area is subjected to lipophilic interaction. Thus, the relative effect of hydrogen bonding is diminished proportionally and dispersion and dipole interactions become significant. This is similar to several cases concerning normal-phase separation of steroids[12,13].

ODS column in a reversed-phase mode
    The capacity factors of compounds of both group I and group II are listed in Table IV and V using an ODS column with various water–methanol mixtures as mobile phases. In general, the capacity factor increases with increasing water content in the mobile phase as expected. Group I compounds always elute slower than group II compounds, for both ligands and their organometallic derivatives. Furthermore, within each group, the elution order seems to follow their expected solubility behavior. Thus, for group I ligands, the elution order is: mesitylene > toluene > methylbenzoate > anisole > benzene. The order is slightly changed for organometallic compounds: mesitylene > toluene > benzene > anisole > methylbenzoate. On the other hand, for group II compounds, the retention time follows the following se-

TABLE IV

CAPACITY FACTORS OF ARENE TRICARBONYLCHROMIUM(0) COMPOUNDS USING AN ODS AND A β-CD COLUMN

The relative errors are within 1%.

Arene tricarbonyl-chromium	Water–methanol (%)									
	20:80		30:70		40:60		50:50		60:40	
	β-CD	ODS	β-CD	ODS	β-CD	ODS	β-CD	ODS	β-CD	ODS
Mesitylene	0.15	1.41	0.36	—	0.93	—	3.15	—	12.21	—
Toluene	0.25	0.84	0.48	1.78	1.00	4.74	2.48	—	6.06	—
Benzene	1.64	0.59	2.85	1.24	5.46	2.95	12.24	8.16	23.68	—
Anisole	0.14	0.57	0.26	1.32	0.52	3.38	1.31	10.30	3.28	—
Methylbenzoate	0.13	0.64	0.20	1.46	0.42	3.90	1.12	11.92	2.98	—
N-Methylaniline	0.15	0.40	0.25	0.79	0.46	2.00	1.13	6.65	2.75	—
o-Toluidine	0.16	0.28	0.22	0.54	0.40	1.28	0.94	3.47	1.93	—
m-Toluidine	0.15	0.31	0.22	0.60	0.44	1.51	1.09	4.23	2.48	—
Aniline	0.31	0.23	0.49	0.39	0.88	0.90	1.84	2.39	3.95	—

TABLE V

CAPACITY FACTORS OF SOME ARENES USING AN ODS AND A β-CD COLUMN

Te relative errors are within 1%.

	Water–methanol (%)									
	20:80		30:70		40:60		50:50		60:40	
	β-CD	ODS	β-CD	ODS	β-CD	ODS	β-CD	ODS	β-CD	ODS
Mesitylene	0.02	5.21	0.08	—	0.21	—	0.49	—	1.12	—
Toluene	0.19	1.77	0.38	4.86	0.76	6.10	1.35	—	2.48	—
Benzene	0.46	1.16	0.85	2.35	1.33	3.14	2.18	7.35	3.52	—
Anisole	0.19	1.11	0.32	1.89	0.58	2.95	1.02	6.97	1.82	—
Methylbenzoate	0.18	0.95	0.33	1.04	0.64	2.57	1.21	7.92	2.38	—
N-Methylaniline	—	0.66	0.23	0.92	0.39	1.62	0.64	3.16	1.06	—
o-Toluidine	0.13	0.50	0.17	0.70	0.29	0.89	0.43	2.05	0.66	—
m-Toluidine	0.14	0.47	0.20	0.56	0.29	0.92	0.50	2.23	0.81	—
Aniline	0.19	0.35	0.26	0.43	0.37	0.59	0.53	1.16	0.76	—

quence: N-methylaniline > m-toludine > o-toluidine > aniline. This order is observed for both ligands and organometallic derivatives except that at low water content, m-toluidine elutes faster than o-toluidine.

Fig. 3 shows an example of the plot of log k (capacity factor) vs. % water in the mobile phase. It is seen that for m-toluidine, the organometallic compound elutes faster than the corresponding ligand at low water content. However, the order is reversed at high water content. Similar phenomena are also observed for aniline, N-methylaniline, o-toluidine, anisole, methylbenzoate, and benzene. The cross-over
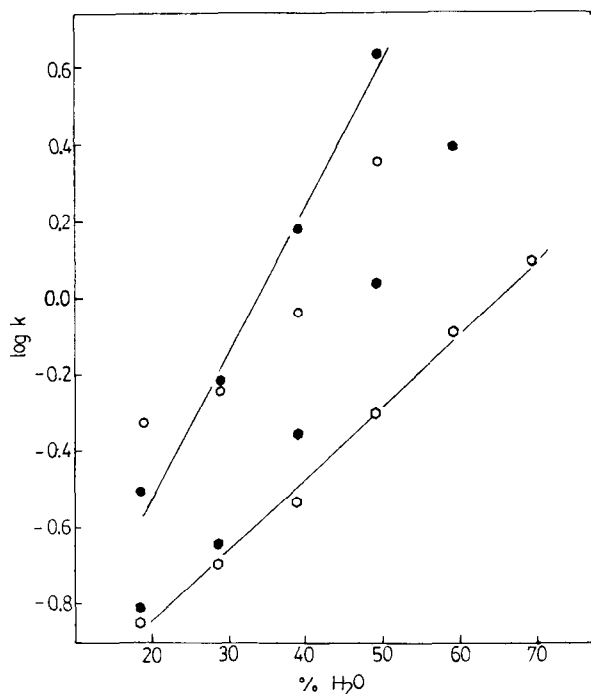
Fig. 3. Plots of log k (capacity factor) *vs.* % water for the retention of *m*-toluidine using an ODS and a β-CD column. ODS: $\bigcirc$, *m*-toluidine; $\bullet$, *m*-toluidine $Cr(CO)_3$. β-CD: $\bigcirc$; *m*-toluidine; $\bullet$, *m*-toluidine $Cr(CO)_3$.

point, *i.e.* at where ligand and organometallic compound co-elute, is somewhere be-
tween 20% and 40% of water in methanol. Our tentative rationalization of this fact
is that the solubility of organometallic derivatives changes much more with a change
of the mobile phase[14]. The organometallic compounds are much more hydrophobic
than the simple ligands therefore at high water concentration in methanol, they elute
slower than the ligands.

β-CD-bonded phase column in a reversed-phase mode

β-CD-bonded phase columns have been shown to be useful in separating en-
antiomers, diastereomers, and structural isomers[15-17]. Separation of metallocene en-
antiomers is also described[18]. It is noticed that chiral recognition using cyclodextrin-
bonded phase columns occurs only in the reversed-phase mode. An inclusion mech-
anism is proposed to rationalize these separations. Thus, it has been suggested that
cyclodextrin columns might prove useful as an alternative to reversed-phase columns
in a variety of more conventional separations.

In Table IV and V are also listed capacity factors of compounds of both group
I and group II using a β-CD-bonded phase column under a reversed-phase mode. It
is observed that the elution order is completely different from that using an ODS
column, indicating that the retention mechanisms are not the same. For example,
among group I compounds, both the benzene ligand and its tricarbonylchromium(0)

derivative elute later than their corresponding members throughout the solvent composition range tested. On the other hand, aniline in group II ligands elutes the slowest when water concentration is low but faster than N-methylaniline and *m*-toluidine when water content is high. However, the organometallic derivative of aniline always elutes later than other group II members. It is also observed that group I compounds do not have very long retention times as compared to those of group II compounds.

Detailed rationalization of the retention mechanisms involved in β-cyclodextrin column at the molecular level is difficult. However, several factors should be considered. First, if an inclusion process is indeed present, the configuration of inclusion for the free ligand may be different from that of the organometallic compounds. The ligand can get into the cyclodextrin cavity with a vertical configuration but the organometallic compounds may have to be in a flat one. (Fig. 4) This flat configuration is consistent with the one proposed when metallocene enantiomers are resolved[18]. The elution order is benzene > toluene > and mesitylene (fast) which corresponds very well with the stabilities of the inclusion complex using model systems at all solvent compositions tested[19]. On the other hand, the organometallic compounds do not follow the same trend. Particularly at high water content, the elution order is benzene (slow) > mesitylene > toluene. Thus, a blend of at least two mechanisms, *i.e.* both inclusion process and solubility or solvophobic type interaction are required to interpret the retention. Further studies are currently in progress to clarify this point.
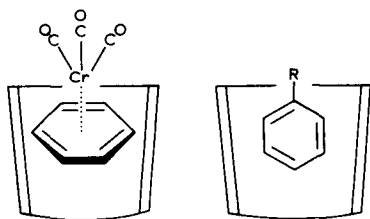

Fig. 4. Configurations of the inclusion complexes of an arene and an arene tricarbonylchromium(0).

CONCLUSION

The applications of HPLC in organometallic chemistry have been extended to monitoring the course of reactions, identifying intermediates, determination of reaction rates and the composition of equilibrium mixtures, and preparative purification. Little work has been done using HPLC techniques to study their physical properties such as solubilities and polarities. Our results reported in this paper have demonstrated the utilities of both normal- and reversed-phase HPLC for such purposes other than its applications on separation. Of particular interest is that the cyclodextrin-bonded phase has proven to be very selective toward certain group of compounds, *i.e.* benzene structural analogues. More specific applications of organometallic separation are expected in the near future.

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