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Acid Modified Diatomaceous Earth-A Sorbent Material for Thin Layer Chromatography

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Abstract

Natural diatomaceous earth (DE) is modified by flux calcination and refluxing with acid. To characterize natural DE, modified DE's [flux calcinated (FC)DE and FCDE-I] and silica gel 60GF$_{254}$ (Si-60GF$_{254}$) are analyzed microscopically, physically, and chemically by various techniques. FCDE-I and Si-60GF$_{254}$ are investigated for their usefulness in the stationary phase of thin layer chromatography (TLC) both individually and in composition. Sodium diethyldithiocarbamate (DEDTC) and ammonium pyrrolidinedithiocarbamate (PyDTC) are prepared as Co or Cu (M) complexes [M(DEDTC)$_2$ and M(PyDTC)$_2$, respectively]. These complexes and their mixtures are run on thin layers of Si-60GF$_{254}$ and FCDE-I individually, and on various FCDE-I and Si-60GF$_{254}$ mixtures. Pure toluene and various toluene–cyclohexane mixtures (3:1, 1:1, 1:2, 1:3, v/v) are used as mobile phases for the running the complexes. The best analytical separations of both M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes are obtained when using pure toluene and toluene–cyclohexane (3:1, 1:1, v/v) as mobile phases on FCDE-I–Si-60GF$_{254}$ (1:3, 1:1, w/w) layers as stationary phases. This study shows that it is possible to qualitatively analyze and to satisfactorily separate a mixture Cu^{2+} and Co^{2+} cations on cited chromatographic systems.

Introduction

A number of minerals have previously been used as thin layer chromatography (TLC) adsorbents, including activated bentonite (1), kaolinite (2), china clay (3), activated bleaching earth (4), modified perlite (5), and modified diatomaceous earth (6). Natural diatomaceous earth (DE) is a biogenic sedimentary mineral and originates from the deposition of hard frustules of siliceous algaes (diatoms), which lived in fresh or seawater in the Miocene and Pliocene periods. It is composed of amorphous SiO$_2$, a variety of inorganic compounds based on metals (such as iron, aluminium, alkaline metals, and earth alkaline metals), and a number of organic compounds (6,7). To characterize DE, it is necessary to analyze it microscopically, physically, and chemically (8,9).

Chromatography is one of the most important analytical techniques used to separate components of mixtures. Thin layer chromatography (TLC) is a quick, easy, and simple separation method extensively used for organic species but rarely used for inorganic cations. While TLC is not common for inorganic cations, in the literature, some researchers have recently revealed that its utility is also valid for inorganic samples (9–24). Additionally, the chromatographic behaviors of M(DEDTC)$_2$ and M(PyDTC)$_2$ (M: Cu or Co) complexes on activated and non-activated thin layers of silica gel 60GF$_{254}$ (Si-60GF$_{254}$) using two different mobile phases are discussed in the context of the variation of stationary phase activation, mobile phase polarity, separation mechanisms, and the nature of the metal, ligand, and complexes (25).

Effective separation by TLC depends on the properties of the sample, mobile phase, and stationary phase. The best representation of the interrelationship between properties of the sample, mobile phase, and stationary phase is given by Stahl's diagram in Figure 1 (6,26). In the diagram, the angles of the triangle correspond to the properties of the sample, stationary phase, and mobile phase. The appropriate conditions for a good separation are determined by rotating the triangle (6,26). The outstanding characteristics of the stationary phase are physical parameters such as particle size and distribution, particle shape, pore size

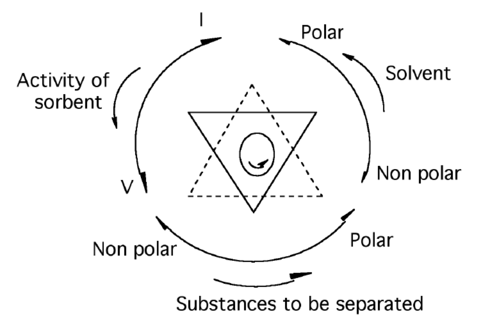

Figure 1. Stahl's diagram for choosing experimental conditions for TLC.

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and distribution, specific surface area (a_s), and chemical parameters such as surface hydroxyl group density [$\alpha_{OH(s)}$] (5,6,9). The chromatographic behaviors of components of commercial ink samples were investigated to give a comprehensive understanding of the systematic effects of stationary phase properties such as a_s and $\alpha_{OH(s)}$, and also those of the mobile phase such as polarity and acidity, on R_f values and separability of the components (6).

In practice, the optimal separation conditions are investigated by changing the properties of the mobile phase. Stationary phase properties are not investigated because sorbents with varying activity are not available. In this context, the chromatographic behaviors of commercial red and blue ink components, which are soluble in water (6), were investigated, but the chromatographic behaviors of $M(DEDTC)_2$ and $M(PyDTC)_2$ complexes, which are not soluble in water were not investigated. Therefore, in this study, the utility of DE was investigated by modifying it through flux calcinations (FC) and refluxing with acid processes. Acid modified FCDE (FCDE-I) was used to systematically change stationary phase activity by mixing with Si-60GF$_{254}$. The retention factors (R_f), theoretical plate number (N) values, and separability of complex mixtures were examined and are discussed in the context of the variation of the stationary and mobile phase properties, retention mechanism, and the nature of the metal, ligand, and complexes.

Experimental

Chemicals, reagents and materials

DE was sourced from the Afyon-Tinaztepe district in Anatolia (Afyon, Turkey). Toluene, cyclohexane, Si-60GF$_{254}$, NaDEDTC, NH_4PyDTC, $Cu(NO_3)_2$, $Co(NO_3)_2$, Na_2SO_4, HCl, $CHCl_3$, CH_3COOH, and CH_3COONa were purchased from Merck (Darmstadt, Germany).

$Cu(DEDTC)_2$, $Co(DEDTC)_2$, $Cu(PyDTC)_2$, and $Co(PyDTC)_2$ complexes were prepared by the reactions of NaDEDTC and NH_4PyDTC with $Cu(NO_3)_2$ and $Co(NO_3)_2$. Pure toluene and toluene–cyclohexane mixtures (3:1, 1:1, 1:2, 1:3 v/v) were used as the mobile phases.

Si-60GF$_{254}$ and FCDE-I individually, and three FCDE-I–Si-60GF$_{254}$ (1:3, 1:1, 3:1, w/w) mixtures were used as the stationary phases. The plates were prepared using a Loughborough, Griffin & George, TLC Unikit (Leicestershire, U.K.). All chemicals were of analytical grade.

Modification of natural DE

DE was ground, sized with a 50 µm sieve, and calcinated with Na_2CO_3 as the flux reagent at 900–1000°C. The product was named flux calcinated (FC)DE. FCDE (100 g, < 50 µm particle size) was then refluxed with 500 mL of 3 mol/L HCl at 100–110°C for 3 h. After cooling to room temperature, the mixture was filtered and washed until the filtrate gave a negative reaction for Cl^-. The product was dried at 110°C for 24 h and then sized with a 50 µm sieve. The product, acid modified FCDE, was named FCDE-I. FCDE-I was used as the principal stationary phase component for TLC applications.

Scanning electron microscopy

DE, FCDE, FCDE-I, and Si-60GF$_{254}$ were characterized by using a scanning electron microscope (JEOL-JMS840, Instruments, Tokyo, Japan). The micrographs are shown in Figure 2.

Infrared spectroscopy

The IR spectra of DE, FCDE, FCDE-I, and Si-60GF$_{254}$ were investigated with a BX-II model Fourier-transform (FT) IR spectrometer from Perkin Elmer (Beaconsfield-Buckinghamshire, U.K.). The DE, FCDE, FCDE-I, and Si-60GF$_{254}$ were first dried at 110°C overnight. They were then accurately weighed and 3.5 mg of dried sample was mixed with 350 mg of KBr, ground in an agate mortar, and pelletted under vacuum with an applied pressure of 10 tons/m^2. The transmittance spectra of samples in the region of 4000–400 cm^{-1} are given in Figure 3.

Particle size analysis

Particle size was analyzed with a Series 2600 particle size analyzer from Malvern Instruments (Worcestershire, U.K.), in combination with a computer. The particle size analysis data for FCDE-I and Si-60GF$_{254}$ are shown in Table I, and their particle size distributions are shown in Figures 4 and 5, respectively.

In Table I, span is the measurement of the range of the particle

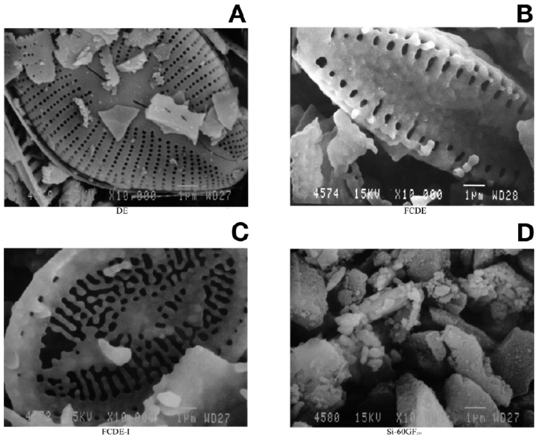

Figure 2. Scanning electron micrographs of DE (A), FCDE (B), FCDE-I (C), and Si-60GF$_{254}$ (D) with 10.000× magnification.

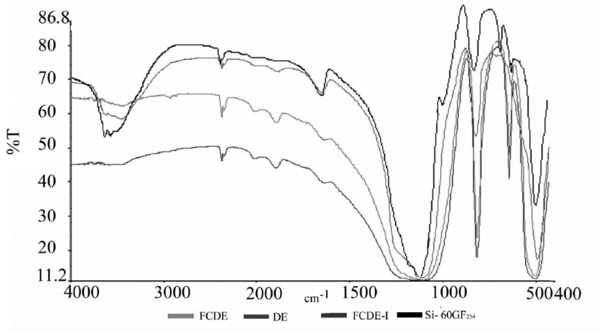

Figure 3. Transmittance spectra of DE, FCDE, FCDE-I, and Si-60GF$_{254}$ in the region 4000–400 cm^{-1}.

size distribution. It was calculated using the following equation:

$$\text{Span} = \frac{D[v,0.9] - D[v, 0.1]}{D[v,0.5]} \quad \text{Eq 1}$$

Span is a dimensionless number, which indicates whether the distribution is narrow or wide; 90% of the distribution is below the value D [v, 0.9]; 10% of the distribution is below the value D [v, 0.1]; and 50% of the distribution is above and 50% below the value D [v, 0.5]. The volume median diameter, D [v, 0.5], should not be confused with the diameter, D [4, 3]. It divides the distribution exactly in half. In Table I, the parameter D [4, 3] refers to the arithmetically derived volume mean diameter. It is the diameter of a sphere having the same values as the real particle. D [3, 2] refers to the surface area mean diameter. It is the diameter of a sphere having the same surface area as the real particle (5,6). Table I shows that the average particle diameters for FCDE-I and Si-60GF$_{254}$ are 11.95 and 24.24 µm, respectively.

Determination of specific surface area

The specific surface areas of FCDE-I and Si-60GF$_{254}$ were determined by using a Brunauer-Emmet-Teller (B.E.T.) analyzer from Quantachrome (Syosset, NY), combined with a computer at TUBITAK in Turkey. Specific surface areas of FCDE-I and Si-60GF$_{254}$ were 1.39 m^2/g and 306 m^2/g, respectively. The reproducibility of specific surface area values was ± 5%.

Table I. Particle Size Analysis Data of FCDE-I and Si-60GF$_{254}$

Adsorbent	D[4,3]	D[3,2]	D[v,0.9]	D[v,0.1]	D[v,0.5]	Span
FCDE-I	11.95	5.10	22.32	3.15	10.86	1.80
Si-60GF$_{254}$	24.24	8.03	63.81	3.59	21.47	2.00

Table II. Physical and Chemical Parameters of FCDE, FCDE-I, and Si-60GF$_{254}$

Adsorbent	Specific surface area (α_s)	Surface hydroxyl group density ($\alpha_{OH(s)}$)
FCDE	1.55	0.00
FCDE-I	1.39	0.00
Si-60GF$_{254}$	306.0	4.84

Table III. Pore Diameter and Volume of FCDE-I and Si-60GF$_{254}$

Adsorbent	Pore diameter (µm)	Pore volume (mL/g)
FCDE-I	6.9432	2.7859
Si-60GF$_{254}$	0.0066	1.2999

Surface hydroxyl group density

The method applied by Chertov et al. (27) for the determination of surface hydroxyl group density [$\alpha_{OH(s)}$], based on the ion exchange of surface hydrogen for Ca^{2+} in Ca(OH)$_2$ solution, was used. The $\alpha_{OH(s)}$ values for FCDE-I and Si-60GF$_{254}$ were 0.00 and 4.84 µmol/m^2, respectively. Experimental data for specific surface area and surface hydroxyl group density parameters of FCDE-I and Si-60GF$_{254}$ are given in Table II.

Determination of pore size

The pore size analyses of FCDE-I and Si-60GF$_{254}$ were carried out using an Autopore 9220 mercury porosimeter from Micromeritics Instrument Corp. (Norcross, GA). Pore diameter and pore volume data are given in Table III.

Preparation of thin layer plates

Slurries of Si-60GF$_{254}$ in water (1:2, w/v) were spread with the spreader kit on clean glass plates measuring 7.5 × 15 cm, with a thickness of 250 µm. Non-activated plates were obtained by storing the plates at room temperature for 12 h. They were then activated by heating in an oven at 110°C for 2 h. For TLC applications, activated plates were used.

Other activated layers were prepared using different FCDE-I and FCDE-I–Si-60GF$_{254}$ (1:3, 1:1, 3:1, w/w) mixtures. The water–FCDE-I–Si-60GF$_{254}$ ratio required to prepare slurries was approximately 2:1 (v/w) and that of water–FCDE-I was approximately 3:1 (v/w).

Synthesis of M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes

Prepared were 0.1 mol/L solutions of metal nitrates [Cu(NO$_3$)$_2$, and Co(NO$_3$)$_2$] at pH 5.5–6.0 (adjusted by acetic acid-sodium acetate buffer). From these solutions, a 1.0-mL aliquot was poured into a beaker and 1.0 mL of 0.1 mol/L NaDEDTC (or NH$_4$PyDTC) solution was added to it and then was shaken. Four milileters of pure chloroform was added to the beaker and was shaken for 1 min. This mixture was tranfered into a separatory funnel and shaken. The phases were allowed to separate for 5 min. The aqueous phase was separated from the chloroform phase and discarded. Subsequently, the chloroform phase containing the complex was dried by treating with anhydrous Na$_2$SO$_4$. The dried phase was used as sample for TLC applications.

TLC applications

Two microliter aliquots from each of the complex solutions and their mixtures were spotted with micropipettes on the starting line, which was 2 cm from the bottom of the five activated Si-60GF$_{254}$ plates. The original spots on layers were dried at room temperature for 3 min. A pencil line was marked 5.5 cm above the starting line of each plate. Five developing chambers with 10 × 50 × 20 cm dimensions were used for running. Sixty milliliters of pure toluene and toluene–cyclohexane mixtures (3:1, 1:1, 1:2, 1:3, v/v) were poured into each developing chamber individually. The lids of the chambers were closed, and the chambers were allowed to stand for 15 min to ensure that saturation of the air in each chamber with solvent vapors occurred. The plates containing the spotted samples were then carefully immersed in the developing chambers. When the solvent fronts reached 5.5 cm above the starting line of each plate, the plates were removed and dried. The migration distances of the solvent (Z_f) and of each spot (Z_x), as well as their width (W), were measured. R_f (from $R_f = Z_x/Z_f$) and N [from $N = 16(Z_x/W_x)^2$] were calculated (22,25). The same procedure was also applied to FCDE-I individually, and three FCDE-I–Si-60GF$_{254}$ (1:3, 1:1, 3:1, w/w) layers.

Results and Discussion

Samples from different natural and synthetic sources contain various compounds as major and minor components. Many of these compounds may have very similar physical and chemical properties. In such cases, sample components generate mutual interference spectra in qualitative, quantitative, and structural analyses. Therefore, in analytical operations, interfering compounds with similar properties have to be well separated.

Successful TLC separation depends on the properties of the sample and also those of the mobile and stationary phases. Finding a suitable resolution for a TLC application usually involves changing the properties of the mobile phase only. It does not involve changing the properties of stationary phase, although it is possible that a sorbent exists to change the properties of the stationary phase (6,9). Activated layers with low activity are usually not investigated because of the assumption that the components cannot be successfully separated because of low activity. In this study, the chromatographic behaviors of M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes were investigated to give a comprehensive understanding of the systematic effects of stationary phase properties such as a_s and $\alpha_{OH(s)}$, and also those of the mobile phase such as polarity on R_f values, N values, and separability of these complexes.

TLC applications for M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes were carried out on various combinations of stationary and mobile phases. These complexes are colored and can be easily visualized in the chromatograms. In addition, the spots of the complexes and their mixtures were developed without any tailing or decomposition and were successfully separated into components. DE was modified by FC and refluxing with acid and FCDE-I was used to systematically change the stationary phase properties for normal phase TLC applications. Si-60GF$_{254}$, FCDE-I, and their mixtures were used as stationary phases. Because M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes are soluble in toluene and toluene–cyclohexane mixture, pure toluene and toluene–cyclohexane mixtures were used as mobile phases.

To prevent interference, the organic compounds in DE were ignited by the FC process and, thus, removed volatile compounds such as CO_2 and H_2O. Reacting and interfering inorganic compounds were converted to soluble salts and removed from DE by successive FC and refluxing with acid processes, but the SiO_2 remained unchanged. Through the modification process, the color of the DE changed from dirty white to white, and the color of the HCl solution during successive refluxing with acid changed from colorless to yellow-green. These qualitative indicators suggested that some interfering organic and inorganic compounds had been successfully removed.

Diatomic frustules were among the first objects examined in early electron microscopic studies. They are divided into two main categories (8,9): centric (discoid) and pennate (elongated), shown in Figures 2A–2C. As shown in the figures, the pennate structure of diatomic frustules was not changed by FC and refluxing with acid processes, whereas the texture of frustules was partially broken down because of heating and mixing during the modification processes. Likewise, the structure was also found to have decreased specific surface area and increased diameter of its pores. Data concerned with the latter are shown in Tables II and III. The increased pore diameter and volume was because of the removal of organic and inorganic compounds from voids within the SiO_2 framework. Because most chemicals, except HF and concentrated NaOH solution, do not react with amorphous SiO_2 (6,9), it appears that HCl did not react with the SiO_2 of DE.

Because DE is principally amorphous silica (SiO_2 nH_2O) with free surface silanol (Si-OH) and siloxane (Si-O-Si) groups, it was expected that FCDE and FCDE-I would also have these groups, as well as some kristobolite phase. This expectation was confirmed by observation. The DE, FCDE, and FCDE-I in Figure 3 show spectral bands appearing at 3695–3400, 1101, 1031 and 912 cm^{-1}. The bands between 3695 and 3400 cm^{-1} are because of the free surface silanol group (Si-OH), the bands at 1101 and 1031 cm^{-1} are mainly because of siloxane (Si-O-Si) stretching, and the band at 912 cm^{-1} is due to (Si-O) stretching of the silanol group (6,9). The intensity of the band between 3695 and 3400

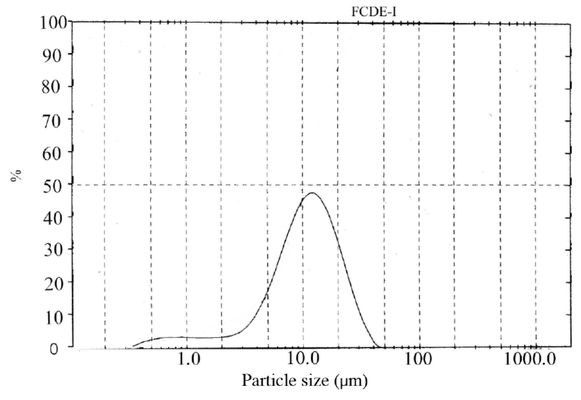

Figure 4. Particle size distribution of FCDE-I.

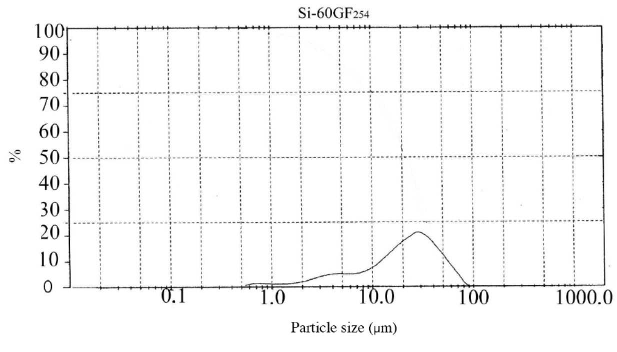

Figure 5. Particle size distribution of Si-60GF$_{254}$.

cm^{-1} was significantly decreased because of the removal of the surface silanol groups by modification of DE, while the band intensities at 1031 cm^{-1} and 912 cm^{-1} were significantly increased. The IR spectra and all other indicators suggest that DE was successfully modified. When the O-H band intensities of Si-60GF$_{254}$ and FCDE-I between 3695 and 3400 cm^{-1} were compared, the O-H band intensity of Si-60GF$_{254}$ was higher. Thus, it can be said that Si-60GF$_{254}$ has more surface silanol groups than FCDE-I. Also, $\alpha_{OH(s)}$ values in Table II support this view.

For adsorbents to have optimal chromatographic properties, they must exhibit a particle size distribution as narrow as possible for a given particle size. A D[v,0.9]/D[v,0.1] ratio between 1.5 and 2.0 is preferred, or the span should be > 1.0 (28). In Figures 4 and 5, the particle size distributions of FCDE-I and Si-60GF$_{254}$ exhibit a Gaussian curve. The spans for FCDE-I and Si-60GF$_{254}$ are 1.80 and 2.00, respectively, as shown in Table I. The D [v, 0.1] values of FCDE-I and Si-60GF$_{254}$ are 3.15 and 3.59 µm, respectively, indicating rather fine particles. This reveals that FCDE-I and Si-60GF$_{254}$ and their mixtures are appropriate for TLC applications, with regard to particle size and size distribution.

Experimental a_s and $\alpha_{OH(s)}$ values of adsorbents in Table II show that Si-60GF$_{254}$ has an a_s of 306 m^2/g and an $\alpha_{OH(s)}$ of 4.84 µmol/m^2. FCDE-I has an a_s of 1.39 m^2/g and an $\alpha_{OH(s)}$ of 0.00 µmol/m^2. Compared with FCDE-I, Si-60GF$_{254}$ has much higher a_s and $\alpha_{OH(s)}$ values. Thus, the activity of Si-60GF$_{254}$ layers was expected to be higher than the activity of FCDE-I layers in normal phase TLC applications. Accordingly, as the ratio of FCDE-I in the stationary phase increased, the a_s and $\alpha_{OH(s)}$ values of layers prepared from Si-60GF$_{254}$–FCDE-I mixtures decreased. Consequently, the polarities or activities of any prepared TLC layers are also lowered. It is, therefore, easy to understand the systematic effects of stationary phase properties such as a_s and $\alpha_{OH(s)}$ on R_f, N values, and separability of the complexes. For the best separation, it may be necessary to prepare the layers from Si-60GF$_{254}$–FCDE-I mixtures systematically.

On the other hand, Stahl's diagram suggests that the Si-60GF$_{254}$ layer has the highest polarity (corresponding to activity V), but the FCDE-I layer has the lowest polarity (corresponding to activity I), and the layers of various ratios have intermediate polarities (corresponding to activities II–IV).

In this study, to investigate the chromatographic behaviors of complexes, M(DEDTC)$_2$, M(PyDTC)$_2$, and their mixtures were run on the selected stationary phase with toluene and toluene–cyclohexane mixtures. In all Si-60GF$_{254}$ and Si-60GF$_{254}$–FCDE-I layers, the complex mixtures were successfully separated into components when solvent front values were 5.5 cm. The red and blue in components, which are soluble in water, were separated successfully on the layers of Si-60GF$_{254}$–FCDE-I mixtures (6). This study indicates that M(DEDTC)$_2$, M(PyDTC)$_2$, and their mixtures can be also separated successfully on the layers of Si-60GF$_{254}$–FCDE-I mixtures. The R_f and N values for these complexes are given in Table IV and V, respectively.

As seen from Table IV, the R_f values of these complexes increase when Si-60GF$_{254}$ is replaced by FCDE-I–Si-60GF$_{254}$ (3:1, w/w), when using the same mobile phase. An FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer has lower a_s and $\alpha_{OH(s)}$ values than an Si-60GF$_{254}$ layer because the corresponding values of FCDE-I are lower than for Si-60GF$_{254}$. Hence, the activity of an FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer is also lower. In this context, it follows that the increase of R_f values stems from weakening of the interactions responsible for retention of complex components because of the decrease in activity of the layer. When a similar comparision was done for other mobile phases, the same result was seen. In summary, the chromatographic behavior of complex components is susceptible to changing a_s and $\alpha_{OH(s)}$ parameters in the stationary phase.

R_f values of complex components on the FCDE-I layers in all

Table IV. R_f Values of M(DEDTC)$_2$ and M(PyDTC)$_2$ (M: Cu and Co) Complexes*

Stationary phase	Mobile phase	Complex			
FCDE-ISi–60GF$_{254}$ Ratios	Toluene–cyclohexane ratios	Cu(DEDTC)$_2$ (R_f ± SD)	Co(DEDTC)$_2$ (R_f ± SD)	Cu(PyDTC)$_2$ (R_f ± SD)	Co(PyDTC)$_2$ (R_f ± SD)
(0:1, w/w)	(1:0, v/v)	0.53±0.03	0.19 ± 0.01	0.42 ± 0.02	0.13 ± 0.01
	(3:1, v/v)	0.45 ± 0.02	0.15 ± 0.01	0.32 ± 0.02	0.08 ± 0.01
	(1:1, v/v)	0.33 ± 0.02	0.07 ± 0.01	0.22 ± 0.01	0.00
	(1:2, v/v)	0.11 ± 0.01	0.00	0.00	0.00
	(1:3, v/v)	0.05 ± 0.01	0.00	0.00	0.00
(1:3, w/w)	(1:0, v/v)	0.65 ± 0.03	0.26 ± 0.01	0.51 ± 0.03	0.16 ± 0.01
	(3:1, v/v)	0.56 ± 0.03	0.18 ± 0.01	0.38 ± 0.02	0.09 ± 0.01
	(1:1, v/v)	0.42 ± 0.02	0.09 ± 0.01	0.25 ± 0.01	0.04 ± 0.01
	(1:2, v/v)	0.15 ± 0.01	0.00	0.00	0.00
	(1:3, v/v)	0.07 ± 0.01	0.00	0.00	0.00
(1:1, w/w)	(1:0, v/v)	0.75 ± 0.04	0.36 ± 0.02	0.65 ± 0.03	0.27 ± 0.01
	(3:1, v/v)	0.64 ± 0.03	0.24 ± 0.01	0.50 ± 0.03	0.16 ± 0.01
	(1:1, v/v)	0.48 ± 0.02	0.07 ± 0.01	0.20 ± 0.01	0.02 ± 0.01
	(1:2, v/v)	0.34 ± 0.02	0.00	0.00	0.00
	(1:3, v/v)	0.21 ± 0.01	0.00	0.00	0.00
(3:1, w/w)	(1:0, v/v)	0.83 ± 0.04	0.46 ± 0.02	0.73 ± 0.04	0.31 ± 0.02
	(3:1, v/v)	0.76 ± 0.04	0.29 ± 0.02	0.64 ± 0.03	0.22 ± 0.01
	(1:1, v/v)	0.54 ± 0.03	0.18 ± 0.01	0.36 ± 0.02	0.11 ± 0.01
	(1:2, v/v)	0.15 ± 0.01	0.00	0.00	0.00
	(1:3, v/v)	0.12 ± 0.01	0.00	0.00	0.00
(1:0, w/w)	(1:0, v/v)	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05
	(3:1, v/v)	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05
	(1:1, v/v)	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05
	(1:2, v/v)	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05
	(1:3, v/v)	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05	1.00 ± 0.05

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* Number of repeated runs: 3.

of mobile phases were 1.00 ± 0.05, as shown in Tables IV. The complex components on these TLC layers moved simultaneously, with these mobile phases showing no separation. This result is not surprising because the activity of FCDE-I is poor because of its low a_s and $\alpha_{OH(s)}$ values. This result can be explained by Stahl's diagram in Figure 1. According to this diagram, the separability of polar substances by a polar solvent on an FCDE-I layer with poor activity should not be high. In fact, FCDE-I is not a good sorbent for normal-phase TLC, at least for separating complex mixtures. However, layers of FCDE-I and Si-60GF$_{254}$ mixtures were quite suitable for separating complex mixtures, as well as the Si-60GF$_{254}$ by itself. Although it is not common practice to change the polarity of the stationary phase by adding other sorbents in normal phase TLC applications, this study indicates that activity of the stationary phase can be changed systematically by adding FCDE-I to Si-60GF$_{254}$. As a result, FCDE-I can be used to optimize the stationary phase properties for chromatographic purposes.

As seen from Table V, the *N* values of M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes increase when the Si-60GF$_{254}$ layer is replaced by FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer, when using the same mobile phase. Although the activity of an FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer is also lower than that of a Si-60GF$_{254}$ layer, an FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer has higher N values of complexes than a Si-60GF$_{254}$ layer. When a similar comparison was done for other mobile phases, the same result was seen. An FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer has lower a_s and $\alpha_{OH(s)}$ values than a Si-60GF$_{254}$ layer because the corresponding values of FCDE-I are lower than for Si-60GF$_{254}$. Hence, the activity of an FCDE-I–Si-60GF$_{254}$ (3:1, w/w) layer is also lower. In summary, the chromatographic behavior of complex components was susceptible to the changing of the activity of the stationary phase, and the FCDE-I–Si-60GF$_{254}$ layers were more successful in separation than the Si-60GF$_{254}$ layers because of the higher N values. The original spots of the mixtures on the FCDE-I–Si-60GF$_{254}$ layers were separated better than on the Si-60GF$_{254}$ layers because of the lower activity of the FCDE-I–Si-60GF$_{254}$ layers and poor polarity of the complexes. Therefore, the spots expand less on the FCDE-I–Si-60GF$_{254}$ layers. This means that the overall effects of Eddy diffusion, longitudinal diffusion, and mass transfer on the FCDE-I–Si-60GF$_{254}$ layers were smaller than on the Si-60GF$_{254}$ layers.

Table V. *N* Values of M(DEDTC)$_2$ and M(PyDTC)$_2$ (M: Cu and Co) Complexes*

Stationary phase	Mobile phase	Complex			
FCDE-1–Si-60GF$_{254}$ Ratios	Toluene–cyclohexane ratios	Cu(DEDTC)$_2$ (*N* ± SD)	Co(DEDTC)$_2$ (*N* ± SD)	Cu(PyDTC)$_2$ (*N* ± SD)	Co(PyDTC)$_2$ (*N* ± SD)
(0:1, w/w)	(1:0, v/v)	1061 ± 53	256 ± 13	529 ± 27	87 ± 4
	(3:1, v/v)	752 ± 38	114 ± 6	306 ± 15	52 ± 3
	(1:1, v/v)	324 ± 16	21 ± 1	51 ± 2.55	0
	(1:2, v/v)	64 ± 3	0	0	0
	(1:3, v/v)	16 ± 1	0	0	0
(1:3, w/w)	(1:0, v/v)	2178 ± 109	502 ± 25	1394 ± 70	324 ± 16
	(3:1, v/v)	1708 ± 86	256 ± 13	784 ± 39	100 ± 5
	(1:1, v/v)	529 ± 27	44 ± 2	348 ± 18	16 ± 1
	(1:2, v/v)	114 ± 6	0	0	0
	(1:3, v/v)	28 ± 2	0	0	0
(1:1, w/w)	(1:0, v/v)	1681 ± 84	711 ± 36	1296 ± 65	900 ± 45
	(3:1, v/v)	2178 ± 109	300 ± 15	784 ± 39	324 ± 16
	(1:1, v/v)	697 ± 35	28 ± 2	215 ± 11	4 ± 0.2
	(1:2, v/v)	500 ± 25	0	0	0
	(1:3, v/v)	260 ± 13	0	0	0
(3:1, w/w)	(1:0, v/v)	1296 ± 65	625 ± 31	1600 ± 80	514 ± 26
	(3:1, v/v)	1129 ± 57	256 ± 13	1225 ± 61	256 ± 13
	(1:1, v/v)	576 ± 29	178 ± 9	711 ± 36	144 ± 7
	(1:2, v/v)	64 ± 3	0	0	0
	(1:3, v/v)	87 ± 4	0	0	0
(1:0, w/w)	(1:0, v/v)	–	–	–	–
	(3:1, v/v)	–	–	–	–
	(1:1, v/v)	–	–	–	–
	(1:2, v/v)	–	–	–	–
	(1:3, v/v)	–	–	–	–

* Number of repeated runs: 3.

As seen in Tables IV and V, the R_f and N values of the M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes decreased when pure toluene was replaced by the toluene–cyclohexane mixture (3:1, v/v), using the same stationary phase. In this context, it follows that the decreases in the R_f values stem from decreasing the mobile phase polarity. This can be explained in the following way: the polarity of toluene is higher than that of cyclohexane because of its π-electron system. Consequently, when the percentage of toluene decreases, the polarity of the solvent system, the interaction of the complex molecules with the mobile phase, and R_f also decreases. On the other hand, pure toluene as the mobile phase was more successful than the toluene–cyclohexane mixture (3:1, v/v) because of the higher N values of the complexes. In addition, the best analytical separations of both M(DEDTC)$_2$ and M(PyDTC)$_2$ complexes were obtained when using pure toluene and toluene–cyclohexane (3:1, 1:1, v/v) as mobile phases on FCDE-I–Si-60GF$_{254}$ (1:3, 1:1, w/w) layers as stationary phases.

In a chromatographic application, the retention mechanism depends on the liquid preadsorbed on the layer's surface, the nature of the mobile phase, and the properties of the sample components (25). In this context, the surfaces of the activated Si-60GF$_{254}$ and other layers were not covered by water or another solvent and adsorption equilibriums were established between the stationary and mobile phases, as in solid–liquid chromatography (SLC). Therefore, adsorption equilibriums are established, as in SLC, on the basis of the retention mechanisms of $M(DEDTC)_2$ and $M(PyDTC)_2$ complexes on all layers of Si-60GF_{254} and Si-60GF_{254} mixtures.

As seen in Table IV, the R_f values of either group complexes show significant difference when the ligands and the mobile and stationary phases are the same. This results from the difference in the electronic structures of the metal atoms. For example, the difference in the R_f values of the $Cu(DEDTC)_2$ and $Cu(PyDTC)_2$ complexes on the activated Si-60GF_{254} layer using pure toluene was 0.11, whereas it was 0.34 for $Cu(DEDTC)_2$ and $Co(DEDTC)_2$. This shows that the metal in the complex had a greater effect than the ligand on the R_f value. Although the charges, radii, and charge densities of Cu^{2+} and Co^{2+} in aqueous solutions were very similar, their d^7 and d^9 electronic configurations lead to the different physical and chemical properties of the complexes. The wavelength values for maximum reflections of the $Cu(DEDTC)_2$ and $Co(DEDTC)_2$ spots were 273 nm and 325 nm, respectively (23). Thus, it followed that because of these d electron distributions in the metal complexes with the same ligand, the extra stabilization energies decreased from Co^{2+} to Cu^{2+}. Hence, the interactions of the corresponding complexes decreased with the stationary phase, though the interactions with the mobile phase increased in the same order. This relationship was also valid for these metals and their complexes in all the other chromatographic systems with layers of Si-60GF_{254}–FCDE-I mixtures as the stationary phase.

Conclusions

This work was carried out on mixtures of $M(DEDTC)_2$ and $M(PyDTC)_2$ complexes in order to better understand the systematic effects of mobile phase polarity, stationary phase properties, such as a_s and $\alpha_{OH(s)}$, and retention mechanisms, as well as the effects of the metal, ligand and complexes on the chromatographic parameters (e.g. R_f and N), and to determine the separability of cation mixtures.

As a first step, FCDE-I was prepared by flux calcination and refluxing with acid processes. As a second step, the a_s and $\alpha_{OH(s)}$ values of adsorbents were determined. As a third step, TLC layers were prepared with Si-60GF_{254}, FCDE-I, and various mixtures of these. As a fourth step, TLC applications were performed on various combinations of stationary and mobile phases. In light of these studies, conclusions are as follows:

(*i*) Scanning electron micrographs and FTIR spectra showed that modification has a physical basis.

(*ii*) As the ratio of FCDE-I in the stationary phase increased, a_s and $\alpha_{OH(s)}$ values of the layers prepared from Si-60GF_{254}–FCDE-I mixtures decreased. Thus, to understand the systematic effects of stationary phase properties such as a_s and $\alpha_{OH(s)}$ on R_f values, N values and the separability of complex components, it is necessary to prepare layers from Si-60GF_{254} and FCDE-I mixtures at systematically altered ratios.

(*iii*) R_f values of the complex components increased when the Si-60GF_{254} layer was replaced with FCDE-I–Si-60GF_{254} layers, while the mobile phase was constant. When the percentage of FCDE-I in the layer increased, R_f values increased because the interactions responsible for retention of complex components weakened because of the decrease in activity of the layers.

(*iv*) According to the diagram in Figure 1, the separability of polar substances by a polar solvent on an FCDE-I layer with poor activity is not high. Thus, FCDE-I was not a good sorbent for normal-phase TLC, but layers of FCDE-I and Si-60GF_{254} mixtures were quite suitable for separating the complex components. The activity of the stationary phase can be changed systematically by adding FCDE-I to Si-60GF_{254}. Thus, the chromatographic properties of the adsorbent can be optimized by FCDE-I for the separation $M(DEDTC)_2$ and $M(PyDTC)_2$ complex mixtures.

(*v*) Si-60GF_{254}–FCDE-I layers were used for separation of commercial red and blue ink components which are soluble in water (6). In this study, these layers were also used for the separation of complex components which are not soluble in water. Thus, these layers can be used for separation of polar and non-polar components.

(vi) The R_f and N values of the complexes decrease when pure toluene is replaced with toluene–cyclohexane mixture (3:1, 1:1, 1:2, 1:3, v/v) as the mobile phase, using the same stationary phase. It follows that the decrease of the R_f values stems from the decrease of the mobile phase polarity.

(vii) The separation of $M(DEDTC)_2$ and $M(PyDTC)_2$ molecules on the activated Si-60GF_{254} and Si-60GF_{254}–FCDE-I layers was carried out via adsorption equilibriums, as in SLC, because there was no adsorbed liquid which was not miscible with pure toluene or toluene–cyclohexane mixture.

(viii) This study showed that it is possible to qualitatively analyze and satisfactorily separate a mixture of Cu^{2+} and Co^{2+} cations using TLC following complexing of the cations with DEDTC or PyDTC ligands. In addition, the best analytical separations of both $M(DEDTC)_2$ and $M(PyDTC)_2$ complexes were obtained when using pure toluene and toluene-cyclohexane (3:1, 1:1, v/v) as mobile phases on FCDE-I–Si-60GF_{254} (1:3, 1:1, w/w) layers as stationary phases.

(ix) Although the ligand and the mobile and stationary phases were the same, the significant difference in the R_f values of the two group complexes resulted from the difference in the electronic structure of the metal atoms. The metal in the complex had a greater effect on the R_f value than the ligand. Although the charges, radii, and charge densities of Cu^{2+} and Co^{2+} in aqueous solutions are close to each other, their d^9 and d^7 electronic configurations lead to the different physical and chemical properties of the complexes. Thus, when the extra stabilization energies of the metal complexes with the same ligand decrease from Co^{2+} to Cu^{2+}, the interactions of the corresponding complexes with the stationary phase decrease, though the interactions with the mobile phase increase in this order.

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