

# Modified Diatomaceous Earth as a Principal Stationary Phase Component in TLC

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## Abstract

Modified natural diatomaceous earth (DE) is a principal component of the stationary phase in normal thin-layer chromatography (TLC) applications and is mixed with commercial silica gel 60GF<sub>254</sub> (Si-60GF<sub>254</sub>). Modification is carried out by flux calcination and refluxing with acid. Natural DE, modified DEs [flux calcinated (FC)DE and FCDE-I], and Si-60GF<sub>254</sub> are characterized by scanning electron microscopy and Fourier-transform-IR spectroscopy. Particle size, specific surface area, pore distribution, pore volume, and surface hydroxyl group density parameters of materials are determined by various techniques. FCDE-I and Si-60GF<sub>254</sub> are investigated for their usefulness in the stationary phase of TLC both individually and in composition. Commercially available red and blue ink samples are run on layers of Si-60GF<sub>254</sub> and FCDE-I individually, and on various FCDE-I and Si-60GF<sub>254</sub> mixtures. Butanol-ethanol-2M ammonia (3:1:1, v/v) and butanol-acetic acid-water (12:3:5, v/v) mixtures are used as mobile phases. The polarities of stationary phases decrease, and the retention factor ( $R_f$ ) values of ink components increase when the FCDE-I content of the stationary phase increases. The properties of the stationary phase can be optimized by adding FCDE-I to Si-60GF<sub>254</sub>. This study may be useful in understanding both the systematic effects of stationary phase properties [e.g., specific surface area and surface hydroxyl group density,  $\alpha_{\text{OH(s)}}$ ] and those of the mobile phase (e.g., polarity and acidity) on  $R_f$  values and the separability of components.

## 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), and modified perlite (5). Natural diatomaceous earth (DE) is a biogenic sedimentary mineral and originates from the deposition of hard frustules of siliceous algae (diatoms), which lived in fresh or seawater in the Miocene and Pliocene periods. It is composed of amorphous SiO<sub>2</sub>, 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). To characterize DE, it is necessary to analyse it microscopically, physically, and chemically (7,8).

Chromatography is one of the most important analytical techniques used to separate components of mixtures. In chromatographic separation, TLC is often used because it is a quick, easy, and simple method. There are numerous applications of TLC concerned with the qualitative and quantitative analysis of components of natural and artificial mixtures referred to in the literature (9–14).

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 (15). 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 (15). The outstanding characteristics of the stationary phase are physical parameters, such as particle size and distribution, particle shape, pore size and distribution, specific surface area ( $a_s$ ), and chemical parameters such as surface hydroxyl group density [ $\alpha_{\text{OH(s)}}$ ] (7).

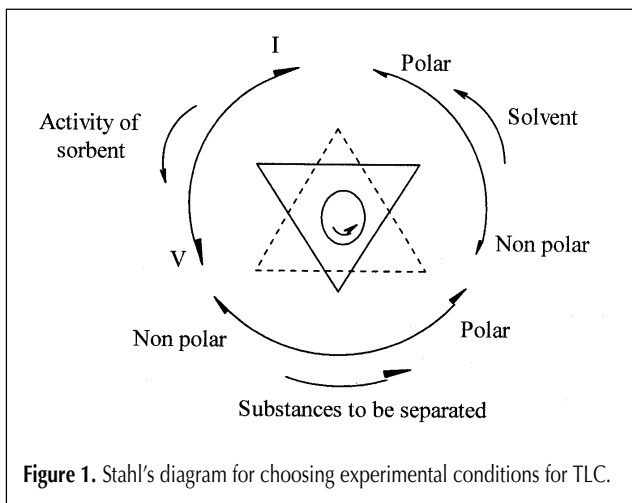


Figure 1. Stahl's diagram for choosing experimental conditions for TLC.

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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. Therefore, in this study, the utility of DE was investigated by modifying it through flux calcination (FC) and refluxing with acid processes. Modified DE (FCDE-I) was used to systematically change stationary phase activity by mixing with Si-60GF<sub>254</sub>. The retention factors ( $R_f$ ) and separability of commercial ink components were examined and are discussed in the context of the variation of the stationary and mobile phases.

## Experimental

### Chemicals, reagents, and materials

DE was sourced from the Afyon-Tinaztepe district in Anatolia (Afyon, Turkey). Si-60GF<sub>254</sub>, *n*-butanol, ammonia, and acetic acid were purchased from Merck (Darmstadt, Germany). Pelikan 4001 red and blue inks were used as the samples for TLC applications (Hanover, Germany).

Ammonia solution (2 mol/L) was prepared by adding distilled water to 150.4 mL of ammonia ( $d = 0.904\text{ g/mL}$ , 25%) in a 1.0-L volumetric flask. Butanol–ethanol–2M ammonia (3:1:1, v/v) and butanol–acetic acid–water (12:3:5, v/v) mixtures were used as the mobile phases. Si-60GF<sub>254</sub> and FCDE-I individually, and five FCDE-I–Si-60GF<sub>254</sub> (1:1, 1:2, 1:3, 2:1, 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- $\mu\text{m}$  sieve, and calcinated with  $\text{Na}_2\text{CO}_3$  as the flux reagent at 900–1000°C. The product was named “FCDE”. FCDE (100 g, < 50- $\mu\text{m}$  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  $\text{Cl}^-$ . The product was oven dried at 110°C for 24 h and then sized with a 50- $\mu\text{m}$  sieve. The product, acid modified FCDE, was named FCDE-I.

### Scanning electron microscopy

DE, FCDE, FCDE-I, and Si-60GF<sub>254</sub> were characterized by using a scanning electron microscope (JEO-JMS840, Instruments, Tokyo, Japan). The micrographs are shown in Figure 2.

### IR spectroscopy

The IR spectra of DE, FCDE, FCDE-I, and Si-60GF<sub>254</sub> were investigated with a BX-II model Fourier-transform (FT) IR spectrometer from PerkinElmer (Beaconsfield-Buckinghamshire, U.K.). The DE, FCDE, FCDE-I, and Si-60GF<sub>254</sub> 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 pelleted under vacuum with an applied pressure of 10 tons/ $\text{m}^2$ . The transmittance

spectra of samples in the region of 4000–400  $\text{cm}^{-1}$  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<sub>254</sub> 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 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,  $D[v, 0.5]$ , must 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 the sphere having the same surface area as the real particle (5). Table I shows that the average particle diameters for FCDE-I and Si-60GF<sub>254</sub> are 11.95 and 24.24  $\mu\text{m}$ , respectively.

### Determination of specific surface area

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

### Surface hydroxyl group density

The method applied by Chertov et al. (16) for the determination of surface hydroxyl group density [ $\alpha_{\text{OH(s)}}$ ], based on the ion exchange of surface hydrogen for  $\text{Ca}^{2+}$  in  $\text{Ca}(\text{OH})_2$  solution, was used. Values of  $\alpha_{\text{OH(s)}}$  for FCDE-I and Si-60GF<sub>254</sub> were 4.84 and 0.00  $\mu\text{mol}/\text{m}^2$ , respectively. Experimental data for specific surface area and surface hydroxyl group density parameters of FCDE-I and Si-60GF<sub>254</sub> are given in Table II.

### Determination of pore size

The pore size analyses of FCDE-I and Si-60GF<sub>254</sub> 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<sub>254</sub> in water (1:2, w/v) were spread with the spreader kit on clean glass plates measuring  $7.5 \times 15\text{ cm}$ ,

with a thickness of 250  $\mu\text{m}$ . Nonactivated 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<sub>254</sub> (1:1, 1:2, 1:3, 2:1, 3:1, w/w) mixtures. The water-FCDE-I-Si-60GF<sub>254</sub> ratio required to prepare slurries was approximately 2:1 (v/w) and that of water-FCDE-I was approximately 3:1 (v/w).

### TLC applications

The red and blue ink samples and a mixture of both (1:1, v/v) were spotted with micropipettes on the starting line, which was 2 cm from the bottom of the plate. The original spots on the layers were dried at room temperature for 5 min. A pencil line was marked 8 cm above the starting line of each plate. Two developing chambers measuring 10  $\times$  50  $\times$  20 cm were used. Sixty milliliters of butanol-ethanol-2M ammonia (3:1:1, v/v) was poured into one chamber and 60 mL of butanol-acetic acid-water (12:3:5, v/v) into the other. The lids of the chamber were closed, and the chambers were allowed to stand for 25 min to ensure saturation of the air in each chamber with solvent vapors. Then, the Si-60GF<sub>254</sub> plates with their ink-samples were carefully immersed in the chambers. The plates, which were taken out of the chambers when the solvent fronts reached 8.0 cm above the starting line of each plate, were dried. The migration distances of the solvent ( $Z_f$ ) and of each spot ( $Z_x$ ) were then measured. The  $R_f$  of the ink components were calculated from  $R_f = Z_x/Z_f$  (7). The same procedure was also applied to FCDE-I and FCDE-I-Si-60GF<sub>254</sub> layers. From the chromatograms, the  $R_f$  values of ink components are given in Tables IV and V.

## 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. TLC is extensively used for organic compounds, but its use for inorganic cation separations is relatively uncommon (17,18).

TLC applications were carried out on various combinations of stationary and mobile phases. Successful TLC separation depends on the properties of the sample and also those of the mobile and stationary phases. Finding suitable resolution for a TLC application usually involves changing the properties of the mobile phase only. It does not involve changing the properties of the stationary phase, although it is a possibility that sorbent is present to change the properties of the stationary phase. In this study, 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  $\alpha_{\text{OH(s)}}$ , and also

those of the mobile phase such as polarity and acidity, on  $R_f$  values and separability of the components. Commercial red and blue ink samples were chosen because of their low cost and availability. In addition, the developed spots can be determined easily with the naked eye.

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<sub>254</sub>, FCDE-I, and their mixtures were used as stationary phases. Because the ink components are acidic, basic, or neutral and also soluble in water, basic butanol-ethanol-2M ammonia (3:1:1, v/v) and acidic butanol-acetic acid-water (12:3:5, v/v) mixtures were used as mobile phases.

To prevent interference, the organic compounds in DE were ignited by the FC process and thus removed as volatile compounds such as CO<sub>2</sub> and H<sub>2</sub>O. 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<sub>2</sub> 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.

Diatom frustules were among the first objects examined in early electron microscopic studies. They are divided into two main categories (7,8): centric (discoid) and pennate (elongated), shown in Figures 2A-C. 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 the diameter of the 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<sub>2</sub> framework. Because most chemicals, except HF and concentrated NaOH solution, do not react with amorphous SiO<sub>2</sub> (7), it appears that HCl did not react with the SiO<sub>2</sub> of DE.

Because DE is principally amorphous silica (SiO<sub>2</sub>  $n\text{H}_2\text{O}$ ) 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  $\text{cm}^{-1}$ . The bands between 3695 and 3400  $\text{cm}^{-1}$  are because of the free surface silanol group (Si-OH), the bands at 1101 and 1031  $\text{cm}^{-1}$  are mainly because of siloxane (Si-O-Si) stretching, though the band at 912  $\text{cm}^{-1}$  is because of (Si-O) stretching of the silanol group (7). The intensity of the band between 3695 and 3400  $\text{cm}^{-1}$  was significantly decreased because of the removal of the surface silanol groups by modification of DE, while the band intensities at 1031 and 912  $\text{cm}^{-1}$  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<sub>254</sub> and FCDE-I between 3695 and 3400  $\text{cm}^{-1}$  were compared, the O-H band intensity of Si-

60GF<sub>254</sub> was higher. Thus, it can be said that Si-60GF<sub>254</sub> has more surface silanol groups than FCDE-I. Also,  $\alpha_{\text{OH(s)}}$  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  $> 1.0$  (19). In Figures 4 and 5, the particle size distributions of FCDE-I and Si-60GF<sub>254</sub> exhibit a Gaussian curve. The spans for FCDE-I and Si-60GF<sub>254</sub> are 1.80 and 2.00, respectively, as shown in Table I. The  $D[v, 0.1]$  values of FCDE-I and Si-60GF<sub>254</sub> are 3.15 and 3.59  $\mu\text{m}$ , respectively, indicating rather fine particles. This reveals that FCDE-I and Si-60GF<sub>254</sub> and their mixtures are appropriate for TLC applications, with regard to particle size and size distribution.

Experimental  $a_s$  and  $\alpha_{\text{OH(s)}}$  values of adsorbents in Table II show that Si-60GF<sub>254</sub> has an  $a_s$  of 306  $\text{m}^2/\text{g}$  and an  $\alpha_{\text{OH(s)}}$  of 4.84  $\mu\text{mol}/\text{m}^2$ . FCDE-I has an  $a_s$  of 1.39  $\text{m}^2/\text{g}$  and an  $\alpha_{\text{OH(s)}}$  of 0.00  $\mu\text{mol}/\text{m}^2$ . Compared with FCDE-I, Si-60GF<sub>254</sub> has much higher  $a_s$  and  $\alpha_{\text{OH(s)}}$  values. Thus, the activity of Si-60GF<sub>254</sub> 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  $\alpha_{\text{OH(s)}}$  values of layers prepared from Si-60GF<sub>254</sub>-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 effect of stationary phase properties such as  $a_s$  and  $\alpha_{\text{OH(s)}}$  on  $R_f$  values and the separability of the ink components. For the best separation, it may be necessary to prepare the layers from Si-60GF<sub>254</sub>-FCDE-I mixtures systematically.

On the other hand, Stahl's diagram suggests that the Si-60GF<sub>254</sub> 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 behavior of components, commercial ink samples were run on the selected stationary phases with acidic and basic mobile phases. In all Si-60GF<sub>254</sub> and Si-60GF<sub>254</sub>-FCDE-I layers, the red ink was separated into yellow and pink spots when the solvent front values were 2.0 cm for both acidic and basic mobile phases.

The blue ink separated into two main spots (dark blue and green) on running with the acidic mobile phase when the solvent front was 8.0 cm. However, in the basic mobile phase application, blue ink components did not separate even when the solvent front was 8.0 cm. On the FCDE-I layers, ink components moved together in both acidic and basic mobile phases. In relation to varia-

tion of stationary phases,  $R_f$  values obtained for TLC applications with the acidic and basic mobile phases are given in Tables IV and V, respectively.

In Table IV, by substituting Si-60GF<sub>254</sub> with FCDE-I-Si-60GF<sub>254</sub> (3:1, w/w), the  $R_f$  value change from  $0.38 \pm 0.02$  to  $0.62 \pm 0.03$  for the yellow component, from  $0.91 \pm 0.05$  to  $0.98 \pm 0.05$  for the pink, from  $0.49 \pm 0.03$  to  $0.78 \pm 0.04$  for the dark blue, and from  $0.56 \pm 0.03$  to  $0.82 \pm 0.04$  for the green component. In Table V, by substituting Si-60GF<sub>254</sub> with FCDE-I-Si-60GF<sub>254</sub> (3:1, w/w),  $R_f$  values changed from  $0.40 \pm 0.02$  to  $0.68 \pm 0.03$  for the yellow component, from  $0.71 \pm 0.04$  to  $0.94 \pm 0.05$  for the pink, and from  $0.56 \pm 0.03$  to  $0.81 \pm 0.04$  for the dark blue component. Based on these results, the  $R_f$  values of ink components increase when Si-60GF<sub>254</sub> is replaced with FCDE-I-Si-60GF<sub>254</sub> (3:1, w/w), when using the same mobile phase. An FCDE-I-Si-60GF<sub>254</sub> (3:1, w/w) layer has lower  $a_s$  and  $\alpha_{\text{OH(s)}}$  values than an Si-60GF<sub>254</sub> layer because the corresponding values of FCDE-I are lower than for Si-60GF<sub>254</sub>. Hence, the activity of an FCDE-I-Si-60GF<sub>254</sub> (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 ink components because of the decrease in activity of the layer. In summary, the chromatographic behavior of ink components is susceptible to changing  $a_s$  and  $\alpha_{\text{OH(s)}}$  parameters in the stationary phase.

$R_f$  values of ink components on the FCDE-I layers in both acidic and basic mobile phases were  $1.00 \pm 0.05$ , as shown in Tables IV and V. The ink 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  $\alpha_{\text{OH(s)}}$  values. This

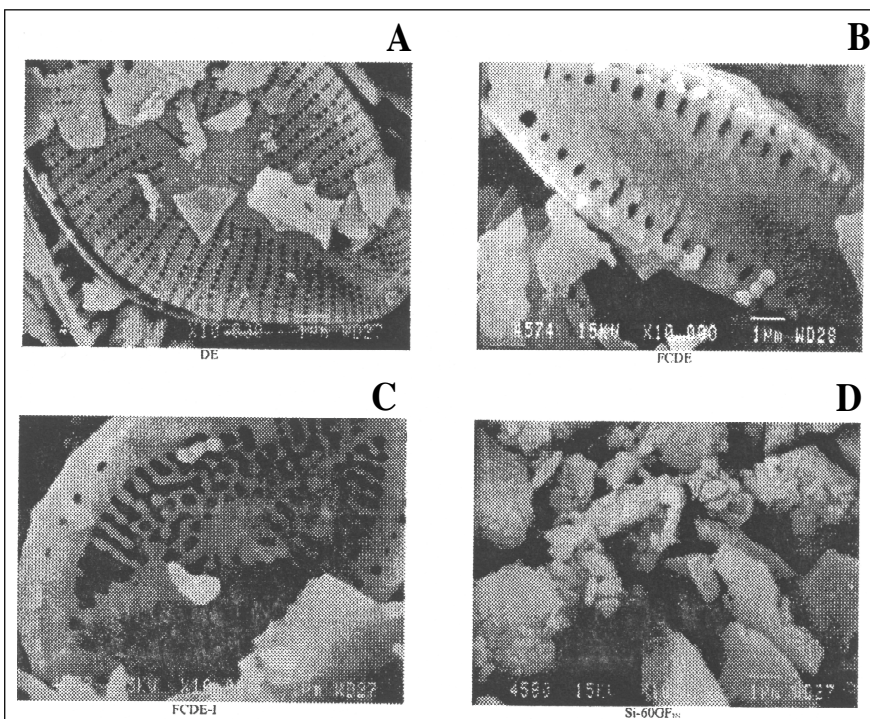


Figure 2. Scanning electron micrographs of DE (A), FCDE (B), FCDE-I (C), and Si-60GF<sub>254</sub> (D) with 10,000  $\times$  magnification.

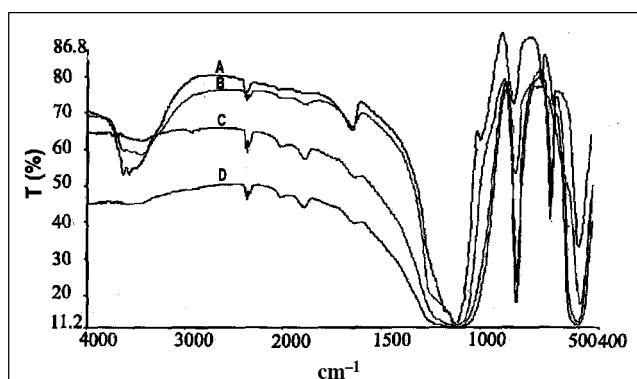


Figure 3. Transmittance spectra of DE, FCDE, FCDE-I, and Si-60GF<sub>254</sub> in the region 4000–400 cm<sup>-1</sup>. Si-60GF<sub>254</sub> (A), DE (B), FCDE (C), and FCDE-I (D).

Table I. Particle Size Analysis Data of FCDE-I and Si-60GF<sub>254</sub>

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 <sub>254</sub>	24.24	8.03	63.81	3.59	21.47	2.00

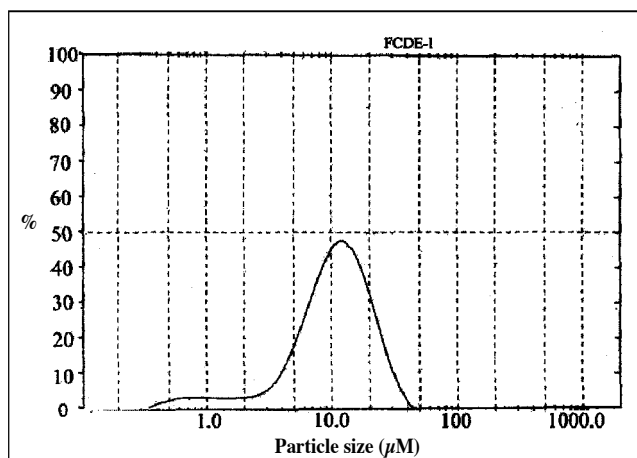


Figure 4. Particle size distribution of FCDE-I.

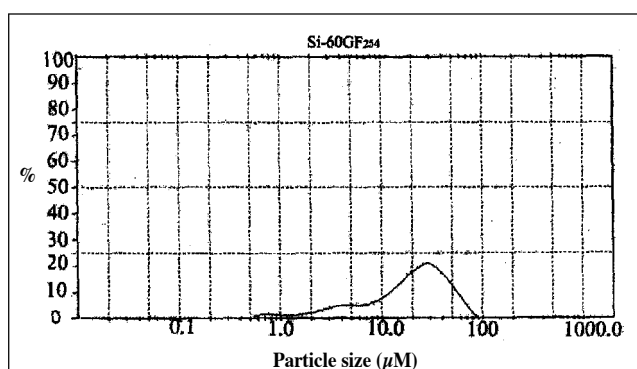


Figure 5. Particle size distribution of Si-60GF<sub>254</sub>.

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 ink samples. However, layers of FCDE-I and Si-60GF<sub>254</sub> mixtures were quite suitable for separating ink components, as well as the Si-60GF<sub>254</sub> 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<sub>254</sub>. As a result, FCDE-I can be used to optimize the stationary phase properties for chromatographic purposes.

According to Tables IV and V, for Si-60GF<sub>254</sub> layers, when the acidic mobile phase was replaced by the basic mobile phase,  $R_f$  values changed from  $0.91 \pm 0.05$  to  $0.71 \pm 0.04$  for the pink, from  $0.38 \pm 0.02$  to  $0.40 \pm 0.02$  for the yellow, from  $0.49 \pm 0.03$  to  $0.56 \pm 0.03$  for the dark blue, and from  $0.56 \pm 0.03$  to  $0.56 \pm 0.03$  for the green component. Replacing the acidic mobile phase with the basic phase changed the  $R_f$  value significantly for the pink component only.  $R_f$  values for the yellow, dark blue, and green components did not change. When a similar comparison was done for other layers, the same result was seen. On the basis of the experimental data and their changing patterns, the polarity of the mobile phase was more effective in contributing to the chromatographic behavior of all the dye components. Considering all components are water soluble dyes, it is possible to attribute this behavior to the acid–base properties of components. In this study, the pH was 3.00 for the acidic mobile phase and 11.00 for the basic mobile phase (7). The  $R_f$  value for the pink component in the acidic mobile phase was higher than that of the basic mobile phase. This means that the pink component was favored by the mobile phase because of its basic chemical nature. Because the  $R_f$  values of the other ink components were affected in both acidic and basic mobile phases, it is suggested that they have a neutral character with respect to their chromatographic behavior pattern.

Table II. Physical and Chemical Parameters of FCDE, FCDE-I, and Si-60GF<sub>254</sub>

Adsorbent	Specific surface area ( $\alpha_s$ )	Surface hydroxyl group density ( $\alpha\text{OH}_{(s)}$ )
FCDE	1.55	0.00
FCDE-I	1.39	0.00
Si-60GF <sub>254</sub>	306	4.84

Table III. Pore Diameter and Volume of FCDE-I and Si-60GF<sub>254</sub>

Adsorbent	Pore diameter ( $\mu\text{m}$ )	Pore volume (mL/g)
FCDE-I	6.9432	2.7859
Si-60GF <sub>254</sub>	0.0066	1.2999



## Conclusion

This work was carried out on commercial ink samples to better understand the systematic effects of stationary phase properties such as  $a_s$  and  $\alpha_{OH(s)}$  on  $R_f$  values and the separability of components.

As a first step, FCDE-I was prepared by flux calcinations 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<sub>254</sub>, 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<sub>254</sub>-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 and the separability of ink components, it is necessary to prepare layers from Si-60GF<sub>254</sub> and FCDE-I mixtures at systematically altered ratios. (iii)  $R_f$  values of the ink components increased when Si-60GF<sub>254</sub> was replaced with FCDE-I-Si-60GF<sub>254</sub> (3:1, w/w), while the mobile phase was constant.  $R_f$  values increased because the

interactions responsible for retention of ink components weakened because of the decrease in activity of the layer. (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<sub>254</sub> mixtures were quite suitable for separating the ink components. The activity of the stationary phase can be changed systematically by adding FCDE-I to Si-60GF<sub>254</sub>. Thus the chromatographic properties of the adsorbent can be optimized by FCDE-I. (v) Replacing of the acidic mobile phase with the basic phase changed the  $R_f$  value significantly for the pink component only, with  $R_f$  values for the yellow, dark blue and green components unchanged. When a similar comparison was done for other layers, the same result was produced. Considering that all the components are water-soluble dyes, it may be possible to attribute this behavior pattern to acid-base properties of the components.

**Table IV. Data for the Red and Blue Ink Components Obtained Using a Butanol–Acetic Acid–Water Mixture (12:3:5, v/v/v) as the Mobile Phase on Various Stationary Phases**

Stationary phase	$R_f$ (Mean $\pm$ SD)			
	Yellow	Pink	Dark blue	Green
Si-60GF <sub>254</sub>	0.38 $\pm$ 0.02	0.91 $\pm$ 0.05	0.49 $\pm$ 0.03	0.56 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (1:3, w/w)	0.42 $\pm$ 0.02	0.91 $\pm$ 0.05	0.62 $\pm$ 0.03	0.67 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (1:2, w/w)	0.43 $\pm$ 0.02	0.94 $\pm$ 0.05	0.63 $\pm$ 0.03	0.69 $\pm$ 0.04
FCDE-I-Si-60GF <sub>254</sub> (1:1, w/w)	0.55 $\pm$ 0.03	0.93 $\pm$ 0.05	0.68 $\pm$ 0.03	0.74 $\pm$ 0.04
FCDE-I-Si-60GF <sub>254</sub> (2:1, w/w)	0.58 $\pm$ 0.03	0.95 $\pm$ 0.05	0.70 $\pm$ 0.04	0.78 $\pm$ 0.04
FCDE-I-Si-60GF <sub>254</sub> (3:1, w/w)	0.62 $\pm$ 0.03	0.98 $\pm$ 0.05	0.78 $\pm$ 0.04	0.82 $\pm$ 0.04
FCDE-I	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05

**Table V. Data for the Red and Blue Ink Components Obtained Using a Butanol–Ethanol–2M Ammonia Mixture (3:1:1, v/v/v) as the Mobile Phase on Various Stationary Phases**

Stationary phase	$R_f$ (Mean $\pm$ SD)			
	Yellow	Pink	Dark blue	Green
Si-60GF <sub>254</sub>	0.40 $\pm$ 0.02	0.71 $\pm$ 0.04	0.56 $\pm$ 0.03	0.56 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (1:3, w/w)	0.46 $\pm$ 0.02	0.77 $\pm$ 0.04	0.61 $\pm$ 0.03	0.61 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (1:2, w/w)	0.53 $\pm$ 0.03	0.79 $\pm$ 0.04	0.65 $\pm$ 0.03	0.65 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (1:1, w/w)	0.56 $\pm$ 0.03	0.80 $\pm$ 0.04	0.68 $\pm$ 0.03	0.68 $\pm$ 0.03
FCDE-I-Si-60GF <sub>254</sub> (2:1, w/w)	0.61 $\pm$ 0.03	0.85 $\pm$ 0.04	0.73 $\pm$ 0.04	0.73 $\pm$ 0.04
FCDE-I-Si-60GF <sub>254</sub> (3:1, w/w)	0.68 $\pm$ 0.03	0.94 $\pm$ 0.05	0.81 $\pm$ 0.04	0.81 $\pm$ 0.04
FCDE-I	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05	1.00 $\pm$ 0.05

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