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QUANTITATIVE THIN-LAYER CHROMATOGRAPHY WITH THE USE OF DIFFERENT TYPES OF ADSORBENTS

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SUMMARY

In quantitative thin-layer chromatography, the accuracy of measurements is highly dependent on the type of adsorbent and liquid mobile phase used. The present work was undertaken in order to establish the degree to which the type of adsorbent and the specific surface area affect the results of quantitative measurements in thin-layer chromatography. The problem was studied by separating a series of mixtures of substances with different adsorption energies and abilities to form hydrogen bonds, on different adsorbents (including silica gel) with different specific surface areas.

The type of mobile phase solvent has an effect on the chromatographic parameters examined. The spots of the separated substances were examined with a densitometer. Planimetric measurements of the spots obtained were also made. It was found that the spot areas as well as the peak areas of the densitometric curves of the developed substances depend on many factors, among which the specific surface area of the adsorbents, the mobile phase, their viscosity coefficient and the migration rate of the phase on the adsorbent layer are of special importance.

Generally, the areas of the spots of substances and the peak areas increase in parallel with increases in the specific surface area of the adsorbent. Some deviations from regularity are caused by some factors that are involved in the chromatographic process.

INTRODUCTION

Quantitative thin-layer chromatography (TLC) is used not only as a rapid and convenient method for the separation and identification of the components of mixtures, but also for the determination of the components. Accordingly, the establishment of optimal conditions for quantitative analysis by TLC is very important. Apart from the high quality of the measuring equipment used, such as fluorimeters and densitometers, the accuracy of measurements is highly dependent on the type of adsorbent and liquid mobile phase.

In a series of experiments, it was found out that only by examining adsorbents with different specific surface areas and with different microporous structures can the optimal conditions of chromatographic separation be established.

The present work was undertaken in order to establish the degree to which

the type of adsorbent and the specific surface area affect the results of quantitative measurements in TLC.

EXPERIMENTAL

The problem was studied by separating a series of mixtures of substances on different adsorbents (including silica gel) with different specific surface areas. The adsorbents and their characteristics are listed in Table I. The type of mobile phase solvent has an effect on the chromatographic parameters examined. For the development of the chromatograms, solvents were used that differed in their abilities to form hydrogen bonds and in their elution parameter, ϵ° (ref. 1). Table II lists the solvents and their characteristics. Various compounds which differed in their adsorption energies¹ and in their ability to form hydrogen bonds were used as model substances, and were chosen according to the classification of PIMENTEL AND McCLELLAN² (Table III).

TABLE I

ADSORBENTS USED

Particle sizes of the adsorbents (diameter) = 0.08–0.10 mm.

<i>Absorbent</i>	<i>Amount of water for preparing sorbent suspension (ml per 25 g)</i>	<i>Specific surface area, B.E.T. (water vapour) (m²/g)</i>	<i>Average radii of capillaries, r (Å)</i>
Silica Gel G	50		
Aluminium oxide	50		
Magnesium silicate	50		
Kieselguhr G	50		
Polyamide	—		
Silica Gel A	59	58	7
Silica Gel B	59	122	8
Silica Gel C	45	161	30
Silica Gel D	39	337	10
Silica Gel E	38	565	10

TABLE II

COMPARISON OF AN ELUOTROPIC SERIES OF SOLVENTS FOR SiO₂ USING ϵ° VALUES AND THEIR CLASSIFICATION ACCORDING TO THEIR ABILITY TO FORM HYDROGEN BONDS

<i>Solvent</i>	<i>Eluent strength parameter, ϵ°, according to SNYDER¹</i>	<i>Class according to PIMENTEL AND McCLELLAN²</i>
Methanol	0.73	AB
Acetone	0.47	B
Chloroform	0.26	A
Cyclohexane	0.03	N
Carbon tetrachloride		N

TABLE III
SOLUTES USED

Solute	Concentration		Classification according to PIMENTEL AND McCLELLAN ³
	Mole/l	Vol. %	
<i>o</i> -Nitroaniline		3	B
<i>m</i> -Nitroaniline		3	B
<i>p</i> -Nitroaniline		3	B
<i>m</i> -Nitrophenol	0.14		AB
<i>p</i> -Nitrophenol	0.14		AB
5-Aminoquinoline	0.10		AB
Fluorenone	0.08		B

During the experiments, suspensions of the adsorbent that had a carefully chosen consistency³ were placed on glass plates. The layers, 3 mm in thickness, were activated for 2 h at 135°. The solutions of the substances were spotted on to the plates with calibrated pipettes of 4 μ l volume. The chromatograms were developed by the ascending technique to a distance of 16 cm and the spots of the substances were either made visible with colour reagents or were examined in transmitted light.

The spots of the separated substances were examined with a Zeiss-Jena Schnell-photometer Model III densitometer equipped with a GIBI compensation register, which recorded, on the tape, changes in light intensity in relation to the amount and distribution of the substance in a spot. The spots were measured along the line of development according to SHELLARD⁴. Using a relationship between the amount of substance in a spot and the peak area⁴, the results are expressed on graphs in which the ordinate represents the peak area in square centimetres and the abscissa the parameter of solvent elution, ϵ° .

Planimetric measurements of the spots obtained were also made. Their surface areas were measured in square millimetres with an accuracy of $\pm 5\%$. The results are given in graphs in which the magnitude of the surface areas of the spots is plotted as the ordinate and the specific surface areas of the adsorbents as the abscissa.

RESULTS AND DISCUSSION

Sometimes, during the quantitative determination of substances by TLC, the operating conditions made changes in the chromatographic systems necessary, usually of the adsorbent, although some substances in mixtures were determined. In such instances, changes also occur in the shape and the size of the spots of the substances involved.

In order to investigate this problem, photometric determinations of the spots on various adsorbents were made, with isomeric nitroanilines used as model substances. The results are shown in Fig. 1.

It can be seen from Fig. 1 that the same substances, on different types of adsorbents, have different spot areas and different peak areas (PA) on the densitometric curves. PA values for individual substances differed most on magnesium silicate and least on Kieselguhr. The reason for these differences lies in the different adsorp-

tion abilities of the various adsorbents and the type of mobile phase, chloroform being of great importance. Chloroform is able to form hydrogen bonds with separated substances and adsorbents, especially when Kieselguhr, which is a weak adsorbent, is used.

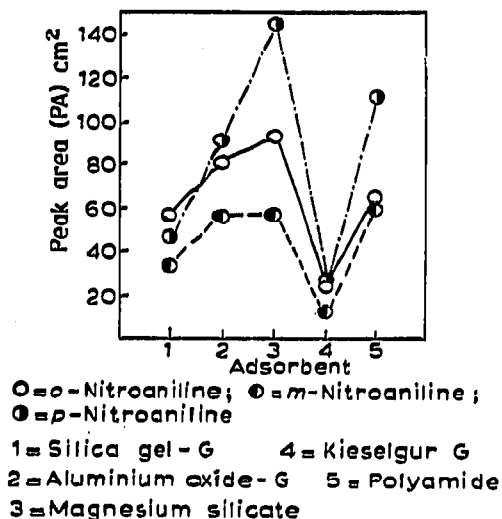


Fig. 1. Relationship between the peak areas of the densitometric curves of *o*-, *m*- and *p*-nitroanilines and the type of adsorbent. Mobile phase: chloroform.

The PA values are also influenced by the tailing of substances on the chromatogram, and tailing accounted for most of the differences in PA values. Fig. 1 shows that the differences in PA values were least on Silica Gel G. These PA values indicate that the spots are compact and that there is no blurring of the spots, which affects the accuracy of densitometric quantitative measurements.

In our experiments, adsorbents with different surface areas were used. Such stationary phases mostly permitted the optimal conditions for the separation of mixtures of substances to be used. Accordingly, it was necessary to examine the extent to which the magnitude of the specific surface area of the adsorbent and its microporous structure influence the shape and size of the spots and the magnitude of the peak area.

Fig. 2 gives the results of an investigation in which various aromatic compounds with different sizes, structures and chemical affinities were used as model substances. The mobile phase was chloroform, a solvent which is able to form hydrogen bonds with separated substances. Fig. 2 shows that the size of the spots of the substances examined decreases with an increase in the specific surface area of the adsorbent. On adsorbents with specific surface areas greater than 300 m²/g, the sizes of the spots were much smaller than those on adsorbents with small specific surface areas. Changes in the spot areas take place on adsorbents with specific surface areas less than 200 m²/g and it is supposed that such adsorbents do not have such high adsorption interactions, and the mobile phase is able to induce a greater extent of diffusion of the substance in a given "volume" of the adsorbent in the layer on the plate. With adsorbents with specific surface areas greater than 200 m²/g, the adsorption of the

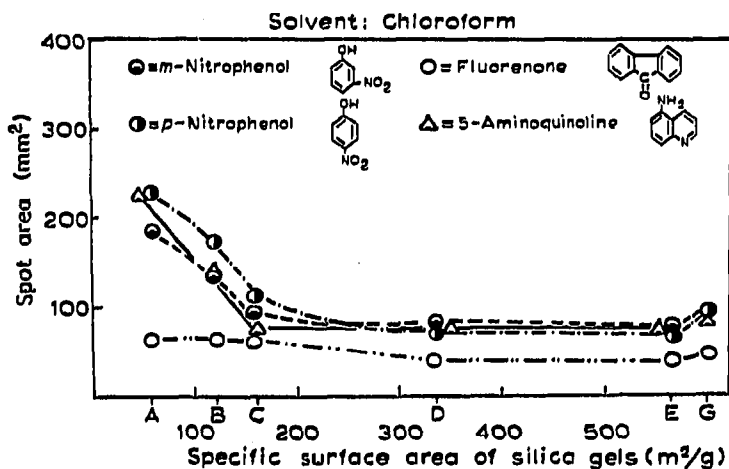


Fig. 2. Effect of the specific surface area of the silica gel adsorbents on the spot areas of *m*-nitrophenol, *p*-nitrophenol, fluorenone and 5-aminoquinoline using chloroform as the mobile phase. A-E indicate adsorbents with different specific surface areas. G indicates the adsorbent used for comparison (Silica Gel G according to STAHL).

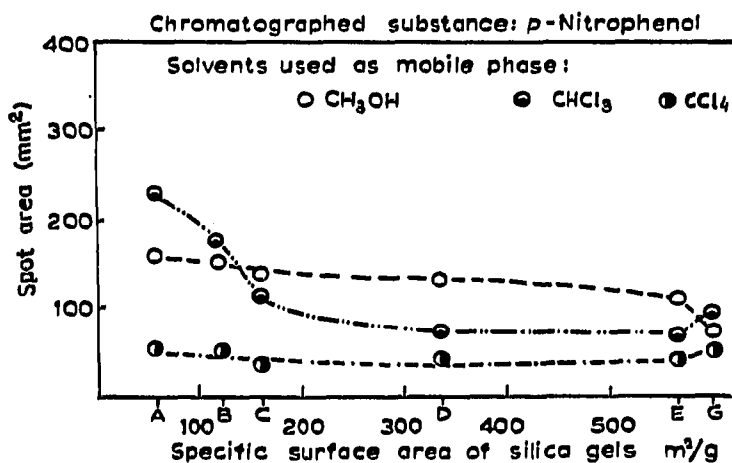


Fig. 3. Effect of the solvent and specific surface area of silica gel adsorbents on the spot area of *p*-nitrophenol. A-E indicate adsorbents with different specific surface areas. G indicates the adsorbent used for comparison (Silica Gel G according to STAHL).

adsorbents is much stronger and so the extent of diffusion decreases. The size of the spot was also influenced by the ability of a given substance to form hydrogen bonds with hydroxyl groups on the surface of the adsorbent. Nearly identical interaction capacities of a group of substances for the adsorbent surface¹ caused the formation of spots on the chromatograms, the sizes of which did not differ to a great extent. This effect was especially observed for substances (from the AB group, according to PIMENTEL AND McCLELLAN²) that are able to form such surface compounds.

Fig. 3 shows to what extent the solvent that is used as mobile phase affects the size of spots of the separated substances. The size of spots of *p*-nitrophenol on silica

adsorbents with different specific surface areas, when different mobile phases were used, was determined. This compound was used because it was the isomer with the highest adsorption affinity of the nitrophenols¹.

Fig. 3 shows that the dependence of the spot size of *p*-nitrophenol on the specific surface area is in agreement with the classification according to PIMENTEL AND McCLELLAN², *i.e.*, in agreement with the ability of the solvents used to form hydrogen bonds. The spot areas in the solvents generally decreased in the order methanol, chloroform, carbon tetrachloride. With carbon tetrachloride, the surface areas of the spots were nearly identical on all the adsorbents used, because the *p*-nitrophenol can form very strong hydrogen bonds with hydroxyl groups of the adsorbent surface owing to the very low adsorption activity of carbon tetrachloride. The porosity of the adsorbent has no great influence on the relationships described.

The accuracy of quantitative measurements in TLC is most influenced by a relationship between the peak area of the densitometric curve of the chromatographed substance and the specific surface area of the adsorbent used and the type of the mobile phase.

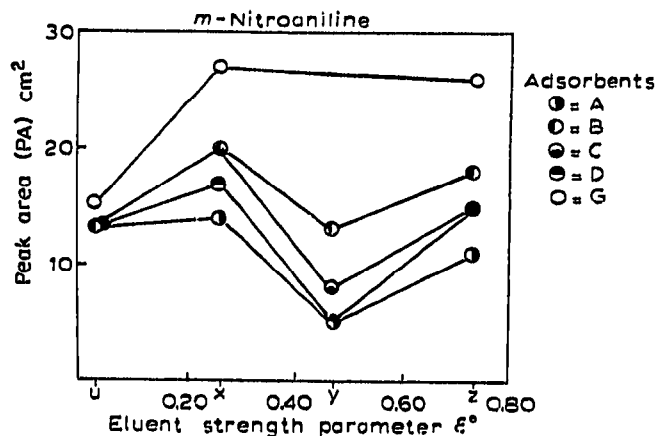


Fig. 4. Relationship between the peak area of the densitometric curve of *m*-nitroaniline and the elution parameter ϵ° of the solvents used as mobile phases, for adsorbents with different specific surface areas. U = cyclohexane; X = chloroform; Y = acetone; Z = methanol. Different adsorbents are marked by letters. Specific surface areas of the gels: (A) 58 m²/g; (B) 122 m²/g; (C) 161 m²/g; (D) 337 m²/g. G indicates the reference gel.

Fig. 4 shows curves of the relationship between the peak area of *m*-nitroaniline (taken as a model substance because of its intermediate features between *o*- and *p*-nitroaniline, for comparison with previous studies) and the elution parameter ϵ° of various solvents, and the specific surface area of the adsorbents used.

It can be seen from Fig. 4 that PA values depend, to a great extent, on the adsorbent that is used as the stationary phase. Therefore the PA values, as the intensity of transmitted light, of the substance are highest for relatively small specific surface areas, and they decrease in parallel with an increase in the specific surface areas of the adsorbents. This effect is possibly due to some differences in the adsorption ability of the substance on the different adsorbents. *m*-Nitroaniline is strongly adsorbed on adsorbents with large specific surface areas and it is therefore concentrated in a small volume on the adsorbent layer. Accordingly, the spot is not large.

When adsorbents with smaller specific surface areas are used, the adsorption of this model substance is much less. The total adsorption of this substance takes place on a larger area of the adsorbent layer and is accompanied by an increase in the volume of the adsorbent. One observes spots with larger areas, often with tailing, which entails an increase in peak area.

A different course of the above relationship for Silica Gel G is possibly caused by a specific method of preparation and different adsorption affinities.

This general tendency towards an increase in spot area on chromatograms and an increase in peak area with decreasing adsorption can differ, depending on the adsorbent used. These changes were observed with different solvents having different ε° values. For cyclohexane ($\varepsilon^\circ = 0.03$), the PA values are nearly equal on all adsorbents, because *m*-nitroaniline has small R_f values in the solvents and the adsorption of this substance, which is high in comparison with that of the solvent, does not permit the substance to expand in the thin adsorbent layer. Chloroform ($\varepsilon^\circ = 0.26$) has a different diffusion coefficient in the adsorbent layer and a different concentration of molecules on the adsorbent surface (hydrogen bonds), which results in changes in the PA order on the curve (see Fig. 4).

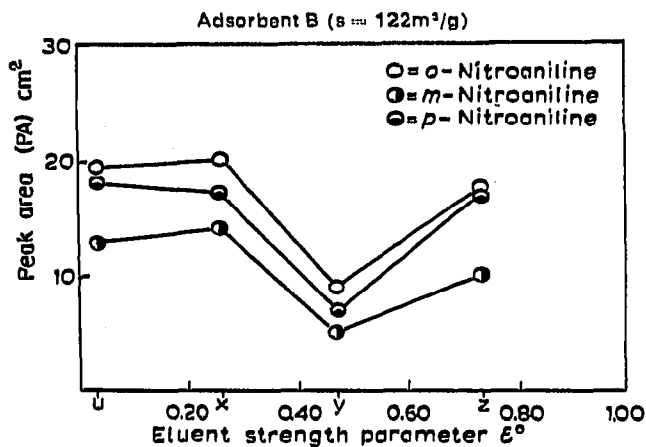


Fig. 5. Relationship between the peak areas of the densitometric curves of *o*-, *m*- and *p*-nitroanilines and the elution parameter ε° of the solvents used as mobile phases, obtained on Silica Gel B with specific surface area, $s = 122 \text{ m}^2/\text{g}$. Different solvents are marked by letters (see Fig. 4).

Different interactions of acetone ($\varepsilon^\circ = 0.47$) and methanol ($\varepsilon^\circ = 0.73$) with the adsorbent surface result in differences in their PA values. In some instances, the absence of a linear function⁵ course is observed, $\xi = zf^2/t$, where ξ is the solvent constant, zf is the distance of migration of the solvent on the adsorbent and t is time (depending on the specific surface area of the adsorbent). Therefore, the differences in the migration rates of solvents are considered to be responsible for the deviation of the function $\text{PA} = f$ (adsorbent surface). This can explain the large differences in PA values when chloroform and acetone are used as the mobile phase.

In order to show the extent to which the PA values depend on the type of substance used, curves were constructed to show the relationships between the PA values and the solvents used for *o*-, *m*- and *p*-nitroanilines on silica gel with a specific

surface area $s = 122 \text{ m}^2/\text{g}$ when using the above solvents. Fig. 5 shows that the PA values of all the isomers depend on their structures. The lowest PA values were obtained for *m*-nitroaniline which, according to SNYDER¹, can be one of the most highly adsorbed isomers. Higher PA values were obtained for *p*-nitroaniline and the highest for *o*-nitroaniline, which can be explained by the "ortho" effect.

The relationships between the PA values of the three substances and the type of the solvent used are also similar to those shown in Fig. 4. The observed differences in PA values can be explained by differences in the migration rates of the mobile phases: at a particular time, a different amount of substance is being transferred to the mobile phase than that being transferred to the adsorbent surface.

In general, both the spot sizes and the peak areas of the chromatographed substances depend on many factors, among which the specific surface area of the adsorbent, the mobile phase, its viscosity coefficient, and the migration rate of the phase on the adsorbent layer are of special importance.

Generally, the spot areas and the PA values decrease in parallel with increases in the specific surface area of the adsorbent. Some deviations from this regularity are caused by some factors that are involved in the chromatographic process.

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