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CHROMATOGRAPHY OF SUBSTITUTED ANILINES ON 3,5-DINITRO-BENZOYL PAPER

AN EXAMPLE OF ORGANIC CHARGE-TRANSFER CHROMATOGRAPHY

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SUMMARY

Simple and substituted anilines are adsorbed by 3,5-dinitrobenzoyl (DNB) paper, a potential polymeric charge-transfer acceptor. The formation of distinct colors observed when DNB paper was contacted with various anilines (donors) is consistent with the hypothesis that charge-transfer interactions play an important role in the binding of these components to DNB paper. The R_F values from organic solvents could be correlated with the Hammett substituent constant (σ), in the case of electron-releasing substituents. Differences in R_F values were sufficient to permit chromatographic separation of some of the substituted anilines, indicating that DNB cellulose is a useful chromatographic medium.

INTRODUCTION

In a recent communication, we reported that a number of aromatic donors were adsorbed by 3,5-dinitrobenzoyl (DNB) cotton cellulose¹. The adsorption was attributed to the formation of discrete charge-transfer complexes between pendant DNB groups as electron acceptors and various mobile phase solutes as donors. A typical example was the complex formed when DNB cotton was contacted with an anisidine solution.

Distinct color change that the DNB cotton underwent when immersed in donor solutions supported charge-transfer complexation as the principal mechanism

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for the adsorption process. The special properties of DNB cotton suggested that an appropriate form of DNB cellulose might prove useful as a chromatographic agent.

The principle of selective adsorption via charge-transfer complexation on electron-acceptor stationary phases has been applied by previous investigators. For example, silica gel impregnated with trinitrofluorenone was used to separate polynuclear aromatic hydrocarbons². Columns of tetrachlorophthalic anhydride³ and nitrobenzylpolystyrene⁴ are examples of homogeneous materials that have been employed as stationary phases.

The present communication reports the results of our initial experiments employing DNB paper for the chromatographic separation of substituted anilines. We have found a definite relationship between the donor strengths of anilines with electron-releasing substituents and the experimentally determined R_F values.

EXPERIMENTAL

Synthesis of DNB paper

Whatman No. 2 filter paper was cut into 2-in. \times 8-in. strips. A 37.7-g quantity of the strips was immersed in a stirred solution of 100 g DNB chloride, 900 ml dimethylformamide (DMF) and 100 ml pyridine. The paper was allowed to react at room temperature for 18 h. The DNB paper strips were washed several times with DMF followed by 95% ethanol and equilibrated at ambient conditions for 24 h prior to weighing. The total product weighed 71.7 g. This weight increase corresponded to a degree of substitution (DS) of 0.75 dinitrobenzoyl groups per anhydroglucose unit. The calculated nitrogen content for this DS is %N = 6.69, which compares with a nitrogen content of 6.18% found by Kjeldahl analysis.

Development of chromatograms

Chromatograms were developed using a 2:5 mixture of ethyl acetate-cyclohexane. Development times ranged from 20 to 30 min. The frontal displacement was 10-11 cm. In order to reduce the spreading of the spots, the origin was placed at a distance of about 5 cm above the solvent contact point. Since the anilines employed formed colored complexes with the DNB paper, it was not generally necessary to employ a detection reagent. However, in some instances yellow complexes were formed which became quite diffuse after development of the chromatogram (for example, when aniline was employed), and confirmation of the position of the spot was facilitated by spraying the chromatogram with a 0.5% solution of 1,2-naphthoquinone-4sulfonic acid.



Fig. 1. Typical chromatogram on DNB cellulose paper showing the separation of p-NH₂, p-OCH₃ and p-Cl substituted anilines. The solvent was ethyl acetate-cyclohexane (2:5).

RESULTS AND DISCUSSION

TABLE I

An obvious advantage of using a polymeric acceptor such as DNB cellulose as a chromatographic agent is that in many instances colored complexes are formed. In such cases, the development of a chromatogram can be monitored visually. The color of the spots circumvents the need for a detection reagent and greatly simplifies the task of finding appropriate experimental conditions for separation.

Fig. 1 shows a typical chromatogram. Satisfactory separation of mixtures of substituted anilines could be achieved when the R_F values differed by about 0.2 R_F units.

The R_F values found for the anilines employed in this study are presented in Table I. Inspection of Table I shows that for anilines having electron-releasing sub-

Substituent	R _F	Color	σ*
p-NH2	0.05	dark brown	0.66
p-OH	0.11	brown	-0.37
p-OCH ₃	0.33	reddish brown	-0.27
p-CH ₃	0.64	light brown	-0.17
m-CH ₃	0.68	yellow	0.07
Н	0.57	pale yellow	0.00
p-Cl	0.57	yellow	+0.23
p-SO ₂ NH ₂	0.04	bright yellow	+0.57 ·
p-CN	0.29	yellow	+0.66

*R*_F VALUES FOR SUBSTITUTED ANILINES ON DNB PAPER

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* Substituent constant values from ref. 8.

stituents (*m*-CH₃, *p*-CH₃, *p*-OH, *p*-OCH₃, *p*-NH₂) the R_F values decrease as the electron-releasing power of the substituent increases. No such correlation is observed for electron-withdrawing substituents (H, *p*-Cl, *p*-SO₂NH₂, *p*-CN). A decrease in R_F values might be expected for anilines substituted with increasingly electron-releasing groups provided the principal mechanism by which the anilines are adsorbed by the stationary phase used with the DNB paper involves charge-transfer complex formation.

In adsorption chromatography the fundamental expression relating R_F to the strength with which a particular solute is bound by the stationary phase, as reflected in the association constant K, is⁵

$$R_F = \frac{1}{1 + aK} \tag{1}$$

In this expression a is the ratio of the quantities of stationary and mobile phase donor and is constant at any point behind the solvent front. Eqn. 1 indicates that those donors that interact strongly with the adsorbent, and therefore have large Kvalues, will have correspondingly low R_F values. Foster and Morris⁶ have shown that the formation constants for the trinitrobenzene charge-transfer complexes of a series of benzenes substituted with electron-releasing groups could be correlated by means of the Hammett equation⁷, while those benzene derivatives with electron-withdrawing groups could not be correlated. The Hammett equation is:

$$\log K = \sigma_{\ell} + \text{constant}$$
(2)

The symbol σ is the substituent constant and is a measure of the electronreleasing or withdrawing power of the substituent; ϱ is the experimentally determined reaction constant and is a measure of the sensitivity of K to a change of substituent. Combining eqn. 2 with eqn. 1 in logarithmic form, and assuming $\alpha K \gg 1$, a Hammett relationship is obtained which predicts a linear dependence of $-\log R_F$ on the substituent constant (σ):

$$-\log R_F = \sigma \varrho + \text{constant} \tag{3}$$

Fig. 2 shows a Hammett plot of the R_F values determined for anilines substituted with electron-releasing groups. No linear dependence of $-\log R_F$ versus σ was found when the substituents were electron-withdrawing. From a least-squares treatment of the data in Fig. 2, a ρ value of -2.0 was calculated. The simple correlation coefficient between σ and $\log R_F$ was -0.955, which, in spite of the small number of observations, is significant at the 95% level. The correlation of R_F values with eqn. 3 found for anilines with electron-releasing substituents is consistent with the hypothesis that charge-transfer interaction plays an important role in the binding of these compounds to the stationary phase. Apparently, the breakdown in the Hammett correlation when anilines substituted with electron-withdrawing groups were used results from the diminished importance of charge-transfer interactions as compared with other forces involved in adsorption (such as dipole-dipole interactions).

The chromatography of anilines on DNB paper might be viewed as a type of



Fig. 2. Hammett-type plot showing the relationship between $-\log R_F$ and σ .

small molecule "affinity" chromatography since a specific complex is formed on the stationary phase between an immobilized acceptor and a mobile phase donor. We believe that the application of the principle of charge-transfer chromatography on celluloses modified with various donor or acceptor groups may be useful for the separation of a wide variety of chemical and biochemical substances.

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