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IMPURITIES IN SILICA GEL AS A CRITICAL FACTOR IN THE QUALITATIVE EVALUATION OF AN ORGANIC COMPOUND

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SUMMARY

Evidence is marshalled which shows that silica gel impurities can cause erroneous results in the evaluation of the purity or identification of an organic compound after column and/or thin-layer chromatography.

INTRODUCTION

Silica gel is one of the most widely used adsorbents for column and thin-layer chromatography. Unfortunately, although it may give the desired separation, impurities present in the silica gel may produce concomitant difficulties for the qualitative identification of the separated compounds.

Several workers have made mention of the annoying interferences caused by silica gel impurities. MILLER AND KIRCHNER[1] in 1952 found that 50–100 mg of a yellow oily material was eluted from 50 g of silicic acid with acetone or ethyl acetate. They also mention that the solvent-extracted silicic acid which was dried in air or in a mechanical convection oven readily picked up additional quantities of oily material. Since that time, most manufacturers have improved the purity of their adsorbents although this problem is still present to a significant extent. MATTHEWS, PEREDA AND AQUILERA[2] found it necessary to extract the silica gel with boiling methanol (redistilled) or else erratic or high results would be obtained for the quantitative determination of certain steroids. They found the lowest blank absorbance readings were obtained after extraction with methanol. Other workers[3,4] advocate a chromatographic development of the chromatoplate with a suitable solvent prior to use. BROWN AND BENJAMIN[5] have cleverly modified this approach by allowing diethyl ether–methanol (20:80) to flow across the plate at right angles to the intended direction of solvent flow prior to chromatographic development. HONEGGER[6] found it necessary to pre-purify both silica gel and the solvents used in the extraction of the chromatographed constituents when quantitative analysis was performed.

The presence of extractable impurities has been substantiated by gas chromatography although the nature of these impurities was not determined[7,8]. MA[9] has made a significant contribution to the possible nature of the contaminant in silica gel. Nuclear magnetic resonance (N.M.R.) indicates that the material is aliphatic in nature. He

also found that those samples of silica gel supplied in plastic containers produced a higher yield of the contaminant as compared to silica gel stored in glass bottles. An N.M.R. spectrum of a plastic container produced a similar spectrum as compared to the silica gel contaminant. The materials are claimed to be high-boiling hydrocarbons with molecular weights of about 550 as determined by mass spectrometry.

It is a common practice in our laboratory to perform column chromatographic separations of our active ingredients from the remainder of the formulation constituents, especially with samples in our aging program. The desired compound in the effluent is quantitatively determined and also is tested by thin-layer chromatography (TLC) to show that there are no degradates or other interfering compounds which might cause an error in the analysis. Moreover, this approach helps to reinforce the specificity of the method.

It has been found that if one passes a chloroform–ethyl acetate–acetic acid solution through a silica gel column and a long-chained aliphatic or aromatic acid is allowed to come into contact with this effluent and air-evaporated, the thin-layer chromatogram from this mixture produces an extraneous spot at the origin of unknown character. Experimental evidence is given which shows that this phenomenon is common to several different compounds.

EXPERIMENTAL

Adsorbents

Eastman Chromatogram Sheet 6060 (silica gel with fluorescent indicator) was employed for TLC.

Silica gel (30–70 mesh ASTM) from E. Merck AG, Darmstadt and Bio-Sil A (200–325 mesh) from Bio-Rad were employed for column chromatography.

Solvent systems

Eluent I	ethyl acetate–chloroform (80:20).
Eluent II	ethyl acetate–chloroform–acetic acid (80:20:5).
Solvent III	chloroform–ethyl acetate–acetic acid (50:40:2).

Reagents

All chemicals were of the best available grade. An 0.03 % solution of the following compounds was prepared in Eluent I: anthranilic acid, benzoic acid, salicylic acid, 1-naphthoic acid, α-naphthol, stearic acid, 1-octadecanol, adipic acid, succinic acid and maleic acid.

Apparatus

Chromatographic columns (1 cm I.D. × 15 cm) were equipped with a teflon stopcock and sintered glass disc. A 125 ml separatory funnel was connected to the top of the funnel with a straight union reducer (Beckman No. 830513).

Chromatographic procedures

Column chromatography. The chromatographic column was filled to a height of 5 cm with silica gel previously washed with Eluent I and conditioned by passing 25 ml of Eluent I through the column. Four milliliter aliquots of 0.03 % solutions of anthra-

nilic acid, benzoic acid, salicylic acid, 1-naphthoic acid, α-naphthol, stearic acid, 1-octadecanol, adipic acid, succinic acid and maleic acid were placed on separate columns and eluted with 35 ml of Eluent I followed immediately by 30 ml of Eluent II. Each fraction was collected separately and diluted to 50 ml with its respective eluent.

Thin-layer chromatography. Five milliliter aliquots of the diluted column fractions were air-evaporated to dryness in 10 ml beakers. Approximately 50 μl of Eluent I was added to the beakers with a hyperdermic syringe. The liquid was drawn up and released several times to dissolve the residue. Each solution was applied to an Eastman sheet (unactivated) and developed with Solvent III. When the solvent front had moved a distance of 10 cm from the origin, the sheet was removed from the development chamber and examined under ultraviolet light (254 nm). After recording the location of the spots, the chromatogram was usually sprayed with iodine (1 % in chloroform) and again the spots were recorded. The chromatogram was then placed in an oven to remove the iodine and also any acetic acid (from Solvent III) which was still on the silica gel sheet. The sheet was then sprayed with Brom Cresol Purple according to STAHL[10]. Many of these systems were duplicated except that the iodine detection step was not employed.

Comparison standards were also prepared by placing two 0.4 ml aliquots of each of the 0.03 % working solutions, in 10 ml beakers, adding separately, 4.6 ml of Eluent I and Eluent II to each solution. The same procedure as performed for the column effluents was carried out starting with the air-evaporation step.

Addition of compounds to column effluents

Elutions were performed on both types of adsorbents according to the same column chromatographic procedure as previously described except that no samples were added to the column. In effect, these effluents represent column blanks.

Five milliliters of each effluent were placed in a 10 ml beaker and 0.4 ml of each working solution (0.03 %) was added. The same TLC procedure was performed as mentioned under the heading *Thin-layer chromatography* starting from the air-evaporation step.

Extraction of silica gel

A 50 g sample of silica gel was placed in a column and eluted with 200 ml of Eluent II. A significant amount of a yellow oily residue was obtained upon evaporation of the effluent. This residue was subjected to infrared analysis. Evaporation of the same quantity of Eluent II produced a trace amount of residue.

RESULTS AND DISCUSSION

Originally, a compound having an indazole acid moiety structure was subjected to column chromatography as part of a preliminary study for the separation of this compound from an ointment. The same techniques as in the section *Chromatographic procedures* were applied. The first eluent, Eluent I, did not elute this compound, but TLC of this effluent produced one spot near the solvent front. This was experimentally shown to be due to solvent impurities in the ethyl acetate and agrees with that found by CROSBY AND AHARONSON[11]. Eluent II was passed through the same column and

it eluted the aromatic acid. Application of TLC to this effluent showed three spots at 254 nm, one near the solvent front (the solvent impurities), one at the origin, and one at an R_F value of 0.5 (the aromatic acid). The extraneous spot at the origin appeared at 254 nm to be deep blue to purple in color; in fact, it was noticeable under the ultraviolet light after spotting but before chromatographic development. Spraying the chromatogram after development with Brom Cresol Purple produced a definite blue spot at the origin while the aromatic acid spot turned yellow.

The aforementioned column chromatographic work was performed with a batch of silica gel which was in our laboratory for some time. The same experiment was repeated with a newly acquired batch of silica gel in order to eliminate the theory of possible air contamination of the silica gel or other types of pollution. Again, one observed the same number of spots as with the older batch of silica gel.

Since Eluent II eluted the aromatic acid from the column and TLC of this effluent produced the unknown spot at the origin, it was originally postulated that the compound might be displacing some impurity from the silica gel column. No spot was observed at the origin with the first effluent which did not contain the aromatic acid. The same phenomenon was observed with anthranilic acid, 1-naphthoic acid, salicylic acid and benzoic acid. Repeating the entire elution sequence with a second sample, using the same column which performed the first set of elutions, produced the same TLC results. It is interesting to note that elutions with 1-octadecanol, α-naphthol (which were chosen because they are similar in structure to their respective acids) and aliphatic acids of lower molecular weight (adipic, succinic and maleic), did not show any spot formation at the origin after TLC. Standard working solutions (1-naphthoic, benzoic, anthranilic, salicylic and stearic acid) which were separately air-evaporated in Eluents I and II did not show this unknown spot; however, placing these standards in column blank effluents I and II produced the same chromatograms as compared to these compounds that were placed in the column and eluted with each eluent. No extraneous spot was formed at the origin with the compounds in effluent I, but it was formed with the compounds in effluent II, regardless of sample addition to the column blank effluent or after sample elution. Spotting of each column blank effluent did not produce a spot at the origin. These cogent results seem to indicate that the organic compound does not displace the unknown impurity from the silica gel, but that the unknown impurity is eluted by the second solvent system (Eluent II). Since the only major difference between Eluents I and II is that Eluent II contains acetic acid, this acid is probably responsible for eluting this impurity from the silica gel.

The foregoing experiments were also performed with unactivated Bio-Sil A. Unlike the other brand, no significant spot at the origin was obtained after adding the compounds to a column blank effluent or after actual elution of the compound through the column. It is interesting to note that the compounds were eluted with Eluent I, unlike the Merck brand, which was eluted only with Eluent II. These contrasting results are probably due to the differences in the activity and type of adsorbent[12].

It is also interesting to note that if one evaporates 5 ml of each column blank effluent (I, II) to near dryness and spots them on an Eastman Silica Gel Sheet followed by spotting 1-naphthoic acid on top of these spots, the resulting chromatogram after TLC shows no spot at the origin. It is possible that the air-evaporation step and/or contact time is a critical factor for causing the unknown spot to be formed at the

origin. Further investigation was discontinued although many facets of this artifact formation are yet to be explored.

An infrared spectrum of the yellow residue after extraction of 50 g of silica gel with Eluent II revealed the presence of (1) a mixture of long-chained aliphatic hydrocarbons, (2) a phthalate ester (strongly resembling di-octyl phthalate) and (3) a trace of an acidic component. The presence of the phthalate ester seems to indicate that it is being removed from the plastic container by the silica gel. These impurities can cause a major problem, especially when one is trying to recover and identify a trace component from the effluent.

CONCLUSION

At present, there is no clear indication of the cause of extraneous spot formed at the origin by TLC of the column effluent to which an aromatic or long-chained aliphatic acid is added. However, from the results reported here, one should not hastily assume that the formation of a secondary spot by TLC of a column effluent containing the eluted compound is due to some impurity or degradation product from that compound. This can cause a serious error in judgement especially upon evaluation of the purity of a substance or in a degradation or aging study of a compound. Likewise, the recovery of trace amounts of a compound which is separated by column chromatography from its components can be erroneously identified or complicated, due to the eluted impurities from the silica gel.

Preliminary investigations indicate that silica gel impurities are present in an amount sufficient to interfere in the qualitative identification of trace components after they have been separated and extracted from silica gel plates. Results will be presented at a later date.

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