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## An improved procedure for thin-layer and column chromatography of xanthones on silica gel

Analytical and preparative chromatography of naturally occurring xanthones* is usually carried out on commercial types of silica gel using either neutral eluants of low or medium polarity or the same eluants with the addition of an acid, *e.g.* acetic acid[1–3]. Although no difficulties in using these procedures have been reported, in the author's experience trouble may arise owing to the presence of metal ions as impurities in the commercial silica gel.

Irreversible adsorption owing to chemisorption has thus been found to occur with compounds having a grouping suitable for chelation, *e.g.* primarily 1-hydroxyxanthones, and also to some extent with compounds having an *ortho* dihydroxy grouping. Chemisorption leads to poor reproducibility in analytical work and to losses where preparative methods are concerned. Chelation may be diminished by addition of an acid to the eluent but small differences in adsorptive properties of the compounds to be separated may at the same time be overwhelmed by the polarity increase.

The presence of metal impurities has also been found to accelerate the decomposition of certain compounds on thin layers leading to the formation of artefacts as shown in the example given below.

These problems are substantially diminished through the use of purified silica gel combined with the addition of a complexing agent to the adsorbent. The complexing agent chosen is EDTA disodium salt which is found not to have any unwanted effects on the chromatographic process besides being virtually insoluble in organic solvents. The disodium salt is slightly acidic and will therefore not give rise to salt formation with phenolic compounds.

A single example of the utility of this method is the separation of a naturally occurring mixture (ratio 5:4) of the isomeric xanthones gentisin and isogentisin from *Gentiana Lutea* L. These compounds, originally separated by fractional crystallization[4], were recently isolated as a mixture by a chromatographic process[5]. Using commercial silica gel and the usual solvent systems both compounds showed pronounced ability to chemisorb and were not separable. Furthermore, a pure sample of isogentisin was found to decompose fairly rapidly on the thin layers showing a change in the dark absorbance (short wave UV) of the compound to a light blue fluorescence. The artefact formed was found to be more polar than isogentisin; its nature has as yet not been elucidated.

On the EDTA-impregnated silica gel layers described below, the compounds were neatly separated using methylene chloride–ethyl acetate (19:1) as the eluent. No tailing was found to occur and decomposition of isogentisin was appreciably retarded. In a column process using EDTA-impregnated silica gel (ratio to compound

* The method described herein may readily be applied to other phenolic compounds. Thus it has been found that the flavonoid compounds catechin, galangin, kaempferol, quercetin, and myricetin all show more or less pronounced tailing when chromatographed on commercial types of silica gel. Moreover catechin, quercetin, and myricetin decompose on the silica gel layers. These unwanted effects may be abolished by the use of EDTA-impregnated silica gel (P. ARENDS AND J. GRY NIELSEN, unpublished results).

100:1) the naturally occurring mixture was resolved to an extent of 80–90 %. Elution was effected with methylene chloride to which 0–10 % of ethyl acetate was added.

*Method*

Since the purification of silica gel for thin-layer chromatography is tedious a rather pure commercial product (*e.g.* Silica Gel HR, Merck) without any added Plaster of Paris is used. An appropriate slurry is made up with the addition of 2 % (based on the silica gel) of EDTA disodium salt. A 2:1 mixture of corn starch and tapioca flour in an amount of 2.5 % is used as a binder[6], 5 min heating of the slurry on a steam bath being sufficient to hydrolyze the starch. Activation is carried out as usual. The thin-layer plates prepared with the starch binder are very fast-running but this has hitherto only proved convenient.

Silica gel for column chromatography is purified using hydrochloric acid. A commercial product (*e.g.* silica gel 0.05–0.2 mm, Merck) is slurried in 2 parts by weight of 2 *N* hydrochloric acid and heated for 1/2 h on a steam bath. The silica gel is then washed with 2 *N* hydrochloric acid, preferably in a column operation, until ferric ion cannot be detected, then with water to neutral reaction. The majority of the water is removed on a suction filter and the silica gel is slurried in an appropriate amount of water containing 2 % (based on the original amount of silica gel) of EDTA disodium salt. The slurry is concentrated *in vacuo* until powdery and dried at 120° for 24 h. After passing through a mesh-45 sieve to remove any lumps present the silica gel is impregnated with 10 % of water and is stable during storage.

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