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# AN IMPROVED METHOD OF PREPARATION OF PLATES AND SHEETS FOR THIN-LAYER ARGENTATION CHROMATOGRAPHY

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## SUMMARY

A simple, relatively inexpensive procedure for the impregnation with silver nitrate of commercially available plates precoated with silica gel is described.

## INTRODUCTION

The utility of thin layer chromatography (t.l.c.) on plates coated with silica gel impregnated with silver nitrate for the resolution of closely related steroids, triterpenes etc., is well documented [1–4]. However the preparation of $AgNO_3$–$SiO_2$-plates with a reproducible resolving capacity is difficult and costly. The recently introduced commercially prepared silver-nitrate–silica gel coated plates are usually discolored, their resolving power is unreliable and they are very expensive. We have developed a simple, relatively inexpensive procedure for the impregnation with silver nitrate of plates precoated with silica gel. For the past three years we have used plates prepared by this method for the analytical and semi-preparative scale resolution of steroids and triterpenes with considerable success.

## EXPERIMENTAL

### *Preparation of the silver nitrate stock solution*

*Method* (*a*). Powdered $AgNO_3$ (4 g), was dissolved with heating in distilled water (10 ml). To the magnetically stirred and heated solution of silver nitrate, acetone (90 ml) was added slowly. The solution was cooled, transferred to a dark (brown) flask and stoppered.

*Method* (*b*). A mixture of powdered silver nitrate (4 g) aqueous acetone (1:9) (100 ml) was magnetically stirred and heated until the solid was completely dissolved. The solution was stored in a stoppered dark (brown) flask.

Solutions of $AgNO_3$ in other solvents (methanol, ethanol etc) were also prepared but the above aqueous acetone solutions gave the best results. Plates impregnated with solutions prepared by methods (a) or (b) had identical chromatographic properties.

### *Impregnation of silica gel plates and sheets with silver nitrate*

Silica gel coated sheets and plates produced by: ICN Pharmaceuticals, Inc. (Woelm-aluminum sheets pre-coated with silica-gel; Woelm plates pre-coated with silica gel F); J. T. Baker Chemical Co. (Baker-Flex-sheets pre-coated with silica gel IB-F): Mallinckrodt Chemical Works, (paper sheets pre-coated with 70% silica AR TLC 7F). Analchem (plates pre-coated with silica gel); Quanta/Gram (plates pre-coated with silica gel) were tested.

Initially most of the experiments were carried with Baker Flex-Silica gel IB-F sheets. The sheets were cut to the required size and dipped in the acetone–$AgNO_3$ solutions: The excess solution was allowed to run-off and the sheets were then air dried for 5 min. The air dried sheets were used immediately or were kept in a dry, dark storage box. When the sheets were stored for long periods (13–15 weeks) as a precaution they were activated by heating for 10–15 min at 80–90° prior to use. No observable changes in physical appearance and chromatographic properties were noted in the stored plates or sheets.

Similar results were obtained with aluminum sheets coated with silica gel (Woelm aluminum sheets; ICN). However on storage for 3–5 months the aluminum coated sheets showed signs of corrosion. The silica gel coated paper sheets (Chrom AR, Mallinckrodt) proved less satisfactory.

The excess acetone–$AgNO_3$ solution used for impregnation was saved and was reused until exhausted.

The silica-gel coated glass plates were impregnated in an analogous manner. However plates produced by several manufacturers showed signs of discoloration after several days of storage. The nature of the binder used by the different manufacturers in the preparation of the plates is not known to us. But, if $CaSO_4$ was the binder the presence of trace

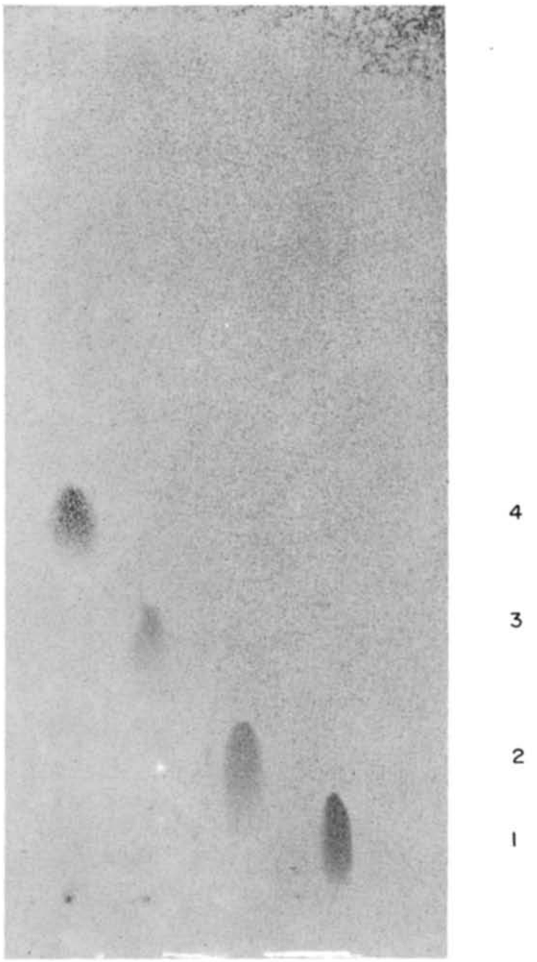

Fig. 1. Chromatographic resolution of 5α-cholest-14-en-3β-ol acetate (**1**). 5α-cholest-9(11)-en-3β-ol acetate (**2**); cholesteryl acetate (**3**) and 5α-cholestan-3β-ol acetate (**4**) on a sheet precoated with silica gel and impregnated with $AgNO_3$. The sheet was developed once with alcohol-free $CHCl_3$.

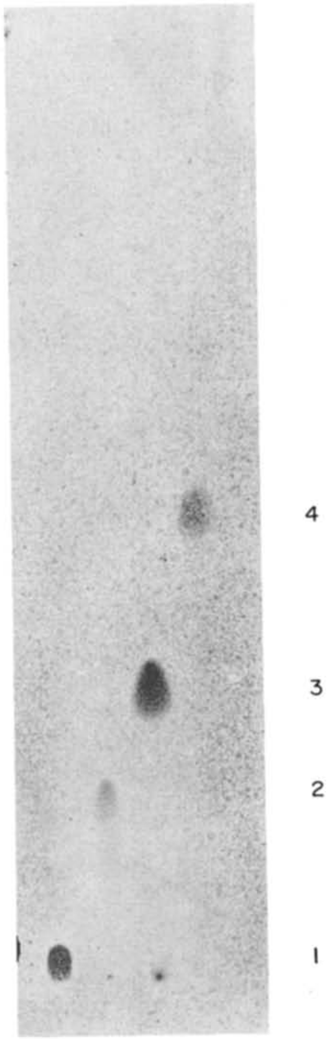

Fig. 2. Chromatographic resolution of ergosteryl acetate (**1**); stigmasteryl acetate (**2**); sitosteryl acetate (**3**) and 5α-stigmastanyl acetate (**4**) on a glass plate precoated with silica gel and impregnated with $AgNO_3$. The plate was developed once with alcohol-free $CHCl_3$.

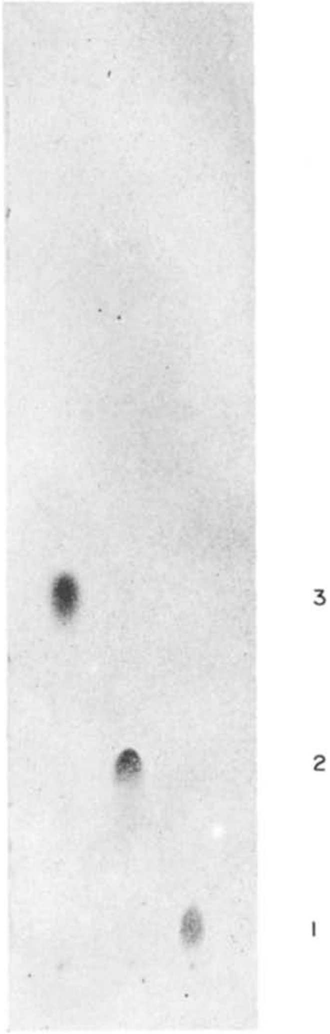

Fig. 3. Chromatographic resolution of lupeyl acetate (**1**); Ψ-taraxasteryl acetate (**2**) and β-amyrin acetate (**3**) on a glass plate precoated with silica gel and impregnated with $AgNO_3$. The plate was developed once with alcohol free $CHCl_3$.

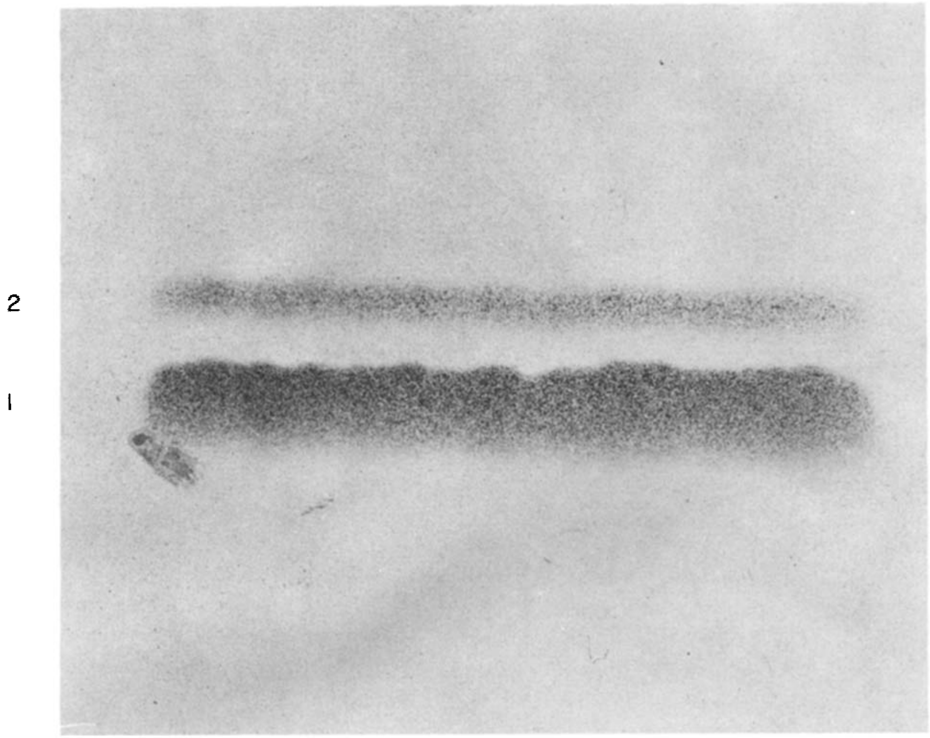

Fig. 4. Semipreparative separation of a mixture of cholesteryl acetate (**1**) (4 mg) and 5α-cholestan-3β-ol acetate (**2**) (1 mg) on a sheet precoated with silica gel and impregnated with $AgNO_3$. The sheet was developed once with alcohol-free chloroform.

amounts of $H_2S$ (or other sulfides) could be responsible for the discoloration. In any event no significant changes in the chromatographic properties of the discolored plates were noted. When the plates were stored for extensive periods of time they were heated at 100°C for 10 min before use.

## RESULTS AND DISCUSSION

At first we tested the influence of the concentration of silver nitrate and water in acetone on the resolving capacity of the impregnated sheets and plates. The optimal results were obtained with the solution described in the experimental section. Increased concentrations of silver nitrate required larger amounts of water for the preparation of homogenous solutions. The higher amounts of water had a detrimental influence on the preparation and handling (e.g. drying) of the plates. Lowering of the concentration of $AgNO_3$ gave plates with a poor resolving capacity.

Figures 1 and 2 show examples of the resolution of several steryl acetates on silver nitrate coated plates prepared by the described procedure. In Fig. 3 the resolution of pentacyclic triterpenic monohydroxy acetates is presented. These resolutions were routinely carried out in our laboratory.

A semipreparative resolution of cholesteryl acetate and of 5α-cholestan-3β-ol acetate on silica-gel–silver nitrate coated sheets is presented in Fig. 4.

The chromatograms described in Figs 1–4 were developed once with alcohol-free chloroform. Development of the chromatograms at *ca.* 0–4°C resulted in better resolutions. It should be mentioned that the mixtures of products shown in Figs 1–4 could not be resolved on plates or sheets not impregnated with $AgNO_3$. The compounds were visualized by spraying the developed chromatograms with 50% aqueous sulfuric acid and heating. Spraying the plates with a solution of rhodamine or exposing them to iodine vapors also resulted in good visualization of the products. The use of rhodamine is recommended for argentation chromatograms on Woelm aluminum sheets (marketed by ICN Co.) which cannot be sprayed with sulfuric acid and heated.

In conclusion we recommend the described procedure for the facile preparation of plates and sheets for argentation chromatography. The obtained plates have a high and reproducible resolving capacity.

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## REFERENCES

1. Vroman H. E. and Cohen Ch. F.: *J. Lipid. Res.* **8** (1967) 150–152.
2. Idler D. R. and Safe L. M.: *Steroids* **17** (1972) 315–324.
3. Sliwowski J. and Kasprzyk Z.: *Phytochemistry* **13** (1974). 1451–1457.
4. Caspi E., Sliwowski J. and Robichaud C. S.: *J. Am. Chem. Soc.* **97** (1975) 3820–3822.