Chromatography on plaster of Paris

The use of plaster of Paris as a supporting medium for chromatography is reported in this paper. Although the use of calcium sulphate has been studied as a medium for chromatography[1,2] and electrophoresis[3], the layer of set plaster of Paris ($CaSO_4 \cdot 2\ H_2O$) differs essentially from most carriers because it offers a continuous mesh of supporting medium with controllable porosity. Although the results so far obtained with the plaster medium in the qualitative separation of widely differing substances are consistently better than the usual media used in thin-layer techniques, it seems that the advantages of using thin and thick strips of set plaster of Paris in preparative chromatography are far more important and of immediate usefulness. The separation and recovery of individual alkaloids from a mixture of alkaloids using thin and thick strips of set plaster of Paris is reported in this paper to illustrate the point that the medium is capable of handling amounts of sample which make it useful for preparative purposes.

The mechanism of setting of plaster of Paris has already been studied[4]. Plaster of Paris contains $Ca(SO_4) \cdot 2\ H_2O$, $Ca(SO_4) \cdot {}^1/_2\ H_2O$ (density 2.6 to 2.75 and 6.2 % water) and a soluble anhydride (density about 2.44). When water is added, the latter two substances, being more soluble in water than the first, form a saturated solution from which the $Ca(SO_4) \cdot 2\ H_2O$ crystallises in fine or coarse needles depending on the amount of water added. Essentially, the setting of plaster of Paris is a hydration reaction represented as follows[5]:

$$Ca(SO_4) \cdot {}^1/_2\ H_2O + 1{}^1/_2\ H_2O \longrightarrow Ca(SO_4) \cdot 2\ H_2O \ (\Delta H = -3.800)$$

Experimental

Preparation of thin plates of set plaster of Paris. 30 g plaster of Paris manufactured by A.D.T.D. Ltd, England (Calsparbrand) is made into a paste by the addition of 24 c.c. distilled water. Air bubbles are removed by means of an electric vibrator. The paste is poured uniformly on to a glass plate 25 cm × 25 cm along a length of approx. 15 cm. The glass plate has two strips of glass 25 cm × 1 cm and 1 mm thick placed on opposite sides so that when another glass plate 25 cm × 25 cm is placed on top of the first plate a gap of 1 mm is left between the two plates. After the plaster has been poured on the bottom plate, the top plate is placed in position so that the plaster is pressed into a strip. Care is taken to see that no air bubbles remain and this can be achieved by vibration before the tip plate is placed in position. The plaster is allowed to set completely (approximately 6 h). The gap between the glass plates is then wetted with distilled water when the plates can be separated easily and a strip of plaster obtained. The strip is then dried in an oven at 100° for 3 h. It is then cut to size by marking grooves with a razor blade and ruler and then breaking the plaster along the grooves. In this way, we have prepared strips of plaster of Paris 15 cm × 3 cm and 1 mm thick.

Preparation of thick strips of plaster. The technique used was the same as the one described for thin strips. The thickness of the strip is regulated by using strips of glass 25 cm × 1 cm and 3 mm thick to separate the top and bottom plates of glass. In this way we obtained strips of 15 cm × 3 cm and 3 mm thick. Even thicker plates can be obtained by using thicker separating strips of glass.

Application of sample on thin and thick strips. The mixture of hydrochlorides of alkaloids to be separated was applied to thin strips in the following way: A calibrated capillary tube (0.5 mm I.D.), the end of which was wrapped with a piece of cotton wool to make a swab, was filled with 0.1 c.c. of the sample containing 6 mg of each alkaloid. The capillary tube with the cotton wool end was used like a pen to apply the sample along a straight line on the plaster strip. The application was stopped when 0.05 c.c. had been applied representing 3 mg of each alkaloid. For thick strips prepared as described above 0.2 c.c. were applied representing 12 mg of each alkaloid. The deposits of alkaloids were in this way uniform and the strip was dried for 2 h in an oven before development of the chromatogram.

Solvent. For the separation of the alkaloidal salts the solvent consisted of: chloroform (5 c.c.), amyl alcohol (0.15 c.c.), toluene (0.15 c.c.), and conc. HCl (0.025 c.c.).

The solvent becomes slightly turbid on shaking but the turbidity disappears after some time. Toluene helps in clear definition of zones but is not essential.

Chromatography of thin and thick strips. The thin strips were chromatographed using the above solvent in a standard ascending chromatography chamber. Separation of alkaloids took place in 1 h but the chromatography was allowed to proceed for 4 h to obtain more widely separated bands.

Thick strips were similarly chromatographed but the length of time was 6 h. The total distance travelled by the solvent on both thin and thick strips at the end of these periods was not more than 15 cm. We found that the distance travelled by the solvent depends on the porosity of the plaster which in turn depends on the amount of water used to prepare it.

After chromatography the strips were dried in an oven at 100° for 2 h.

Extraction of the separated alkaloids. The procedure was the same as that described below for both the thin and thick strips.

From the original dried strip after chromatography a narrow strip $^1/_2$ cm wide was cut off longitudinally by making a groove with a razor blade and then breaking the plaster along it. The narrow strip was immersed in iodine solution or Draggendorf's reagent, whereby the alkaloids became visible as brown zones. By placing the narrow strip alongside the original strip, it was possible to mark the zones on it with a pencil. The zones were cut off, crushed and extracted with acidified ethanol. The alkaloids so obtained were estimated quantitatively and percentage recovery determined. Instead of cutting the strip, the alkaloidal zones can also be eluted by suction on the strip with the help of a Buchner flask fitted with a cork having a slit. The eluting acidified ethanol is dropped with a pipette along the zone to be eluted.

Results

Fig. 1 shows a thin strip (15 cm × 3 cm and 1 mm thick) with the separated alkaloids. The sample used was a mixture of hydrochlorides of atropine, brucine, aconitine, and codeine (60 mg/c.c.); the size of the sample was 0.05 c.c., containing 3 mg of each alkaloid.

The percentage recovery after extraction with acidified ethanol was as follows: atropine, 98 %; brucine, 97 %; aconitine, 96 %; and codeine, 93 %.

On the thick strip (15 cm × 3 cm and 3 mm thick) the sample mixture was the same as for the thin strip; the size of the sample was 0.2 c.c., containing 12 mg of each alkaloid.

The percentage recovery after extraction with acidified ethanol was as follows: atropine, 98 %; brucine, 98 %; aconitine, 93 %; and codeine, 90 %.

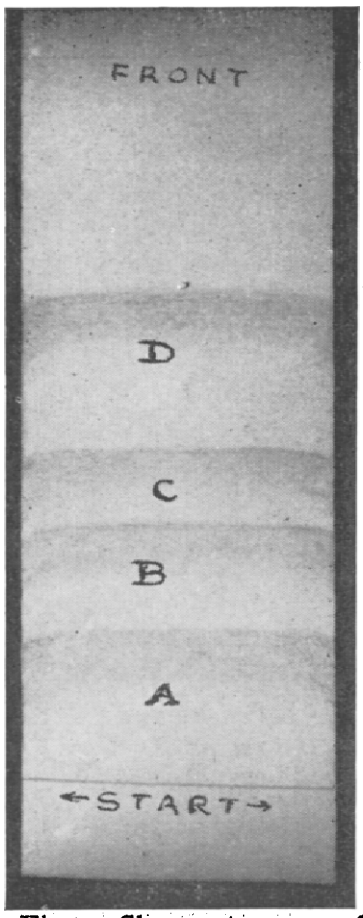

Fig. 1. Chromatogram of a mixture of four alkaloids on a plaster of Paris strip 15 cm × 3 cm and 1 mm thick. A = Atropine; B = aconitine; C = codeine; D = brucine.

Conclusion

The method is useful for preparative chromatography. By using thicker strips the size of the sample to be separated can be increased. Up to 5 mm thick strips have been used. The alkaloids separated are very pure in nature and even purer than those used initially. The method is being studied for separation of sugars and amino acids in sizable quantities.

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