CENTRIFUGAL CHROMATOGRAPHY ON PLASTER OF PARIS

A. AFFONSO
Goa College of Pharmacy, Goa (India)
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The use of thick and thin strips of set plaster of Paris as supporting media in ascending chromatography has been described in an earlier paper[1]. Since ascending chromatography is a slow process limited by the capillary forces causing the ascension, it was decided to investigate the possibility of accelerating the separation, using either a forced positive pressure flow or causing an increased flow rate by the use of centrifugal force. The results of the centrifugal method are reported here.

Centrifugal chromatography was used by McDONALD *et al.*[2] on filter paper discs, sandwiched between plastic discs shaped as saucers. The discs and the paper were rotated at 300 to 1,000 r.p.m., solvent being fed to a point near the center of the filter paper disc. The sample mixture to be separated, was applied at a point a little off-center. The drawbacks of this method are sputtering of the separated bands and also diffuse bands. However, the method is fast and it is possible to make a rapid analysis of comparatively simple mixtures of water soluble compounds[3]. The limitation is that the use of filter paper does not permit the application of sizable amounts of sample mixture and, when volatile solvents are used, evaporation of these interferes with the separation. In contrast to filter paper, a set layer of plaster of Paris does not suffer from these limitations. Apparatuses using the centrifugal force for chromatographic separation have also been adapted for industrial purposes. The chromatofuge described by HOPF *et al.*[4,5] is a centrifuge packed with adsorbent. This paper describes the use of discs of set plaster of Paris in centrifugal chromatography. The sample mixture is applied in a circle away from the center of the disc. The disc is rotated at speeds ranging from 500 to 3,000 r.p.m. and solvent is added at a controlled rate at a point slightly off-center. The circular bands of the separated constituents move towards the periphery, leave the disc due to centrifugal force, and are collected in a special fraction collector. To illustrate the efficiency of the method, separation of two dyes and two alkaloids has been tried and achieved in a matter of minutes.

EXPERIMENTAL

Apparatus and methods

The experimental set up is shown in Fig. 1. The flame proof motor (A) has a speed regulator so that speeds ranging from 500 to 3,000 r.p.m. can be obtained. The fraction collector (H) is a cylinder made of translucent, hard polyethylene. On the inner side of the cylinder 12 closely fitting rings, G_1, G_2, . . . G_{12}, of hard polyethylene having a deep groove on one side were fixed at a slant of 25° to the horizontal. In this

way 12 circular, inclined collection troughs were obtained. At the lowest point of the rings, a hole was made so that pieces of hard polyethylene tubing, E_1, E_2, . . . E_{12}, could be fitted thus making delivery tubes for the liquid that would be collected in the troughs. The slant of the rings makes complete drainage of the liquid collected into 12 small test tubes possible.

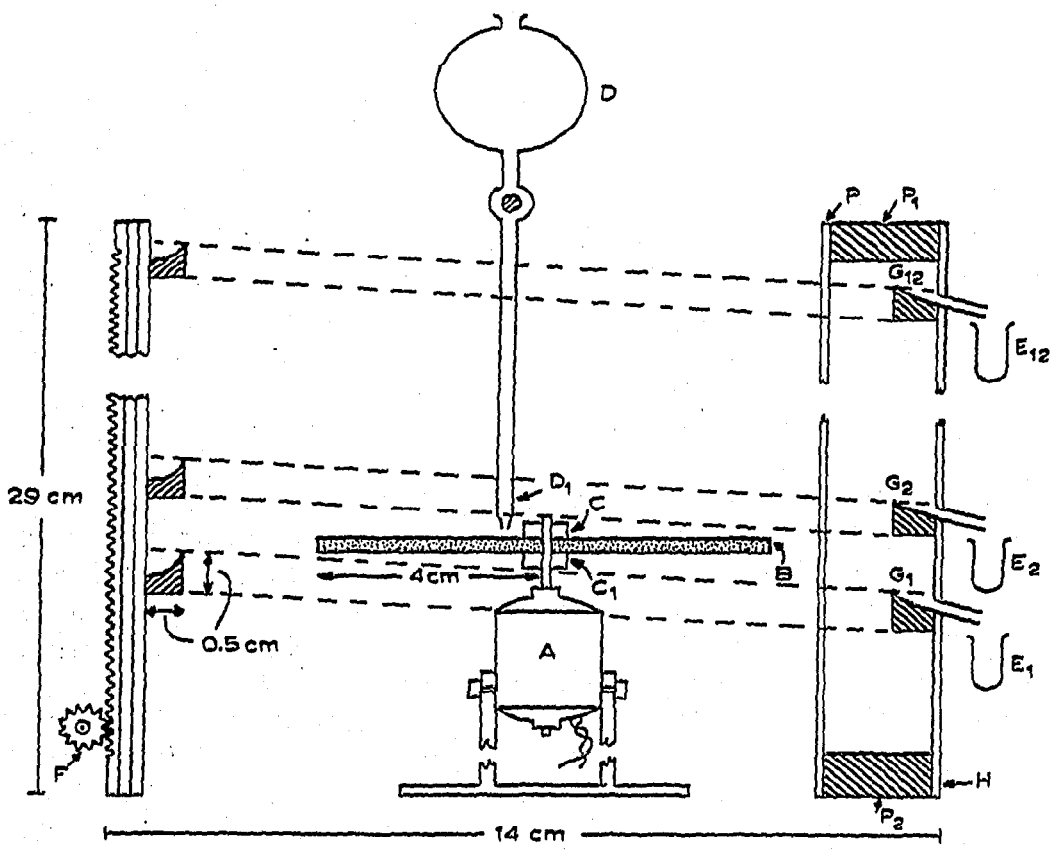

Fig. 1. Set-up for centrifugal chromatography.

The fraction collector was mounted in a wheel and ratchet arrangement (F) so that each collection trough could be brought in line with the set plaster of Paris disc B. The plaster disc was fixed directly to the motor spindle by sandwiching it between two pieces CC_1 of tightly fitting thick pressure rubber or polyethylene tubing. The hole in the disc should fit snugly the motor spindle.

On one side of the cylinder H, two rests P_1 and P_2 were fixed so that a 2 cm broad strip of set plaster of Paris could be fixed with tape. The purpose of the strip was to serve as a pilot for the separation. Since it moves with cylinder H, as the separated fractions leave the disc and are collected in the troughs, a part of each of them impinges on the strip leaving thus a print. When the strip is removed and developed with iodine or Dragendorff reagent, in case of alkaloids, it is possible to fix exactly in which test tube the different constituents have been collected.

The solvent is fed from a separating funnel D having at its end a glass tube D_1 drawn to a point. The solvent could therefore be added in a regulated rate at a place slightly off the center.

Preparation of set plaster of Paris discs and strips

50 g of plaster of Paris manufactured by A.D.T.D. Ltd. England (Calspar Brand) is made into a paste by the addition of 40 c.c. of distilled water. Air bubbles are removed by means of a vibrator and a 3 mm thick layer of set plaster of Paris was obtained by allowing the plaster to set between two 25 cm × 25 cm glass plates separated from each other by 3 mm thick glass strips placed on opposite sides[1]. A circle of 4 cm radius was etched on the dried plate by rotating a sharp pointed divider several times. The depth of the groove should be about ½ mm. A square containing the etched circle was broken off from the original plate in the same way as described previously[1]. A perfectly round disc is obtained by breaking carefully, piece by piece, the plaster along the circular groove. More discs can be obtained in this way from the remaining original plate. Any irregularities were filed off. With the help of a drill bit ½ cm diameter, which was equal to the diameter of the motor spindle, a circular hole was made exactly in the centre of the disc. The disc was now ready to be mounted on the motor spindle as described above. The strip of plaster P (Fig. 1) was cut in the same way as described previously[1]. It was 2 cm broad and 20 cm long.

Preparation of sample mixtures

In order to test the effectiveness of the method, the mixtures used were: (a) Mixture of eosin and malachite green 2 % in acetic acid. (b) Veratrine and atropine hydrochlorides 400 mg/c.c. in 5 % dilute HCl.

Application of sample mixtures

The sample mixtures, 0.05 c.c., were applied to each disc with the aid of the applicator, described previously[1], by rotating the disc at 120 r.p.m. and touching the applicator at a point 1.7 cm away from the centre. In this way a perfectly uniform circular deposition line was obtained. The discs were dried.

Solvent

For the separation of eosin and malachite green, 30 % acetic acid was used. For the separation of atropine and veratrine, the solvent mixture was: benzene–isopropyl alcohol–conc. HCl (15 c.c.:2 c.c.:0.05 c.c.).

Depending on the hydrochlorides of the alkaloids to be separated, the amount of isopropyl alcohol has to be adjusted.

Adjustment of solvent rate

The success of the separation depends on the correct rate of addition of the solvent. In order to fix the solvent addition rate, a blank experiment is carried out in the following way. An identical dry disc of set plaster of Paris is fixed in the centre to a vertical support and the solvent is added at such a rate so that it spreads evenly by capillarity over the whole disc. The surface where the solvent drops should not show excess liquid. Since the centrifugal force helps the distribution of the solvent throughout the disc and since the movement of rotation will evaporate the volatile constituents of the solvent, the rate at which the solvent has to be added in the actual working, can be 1.5 times faster. In the experiment described here, the rates were:

(a) For separation of eosin and malachite green 10 c.c. per min.

(b) For separation of atropine and veratine 15 c.c. per min.

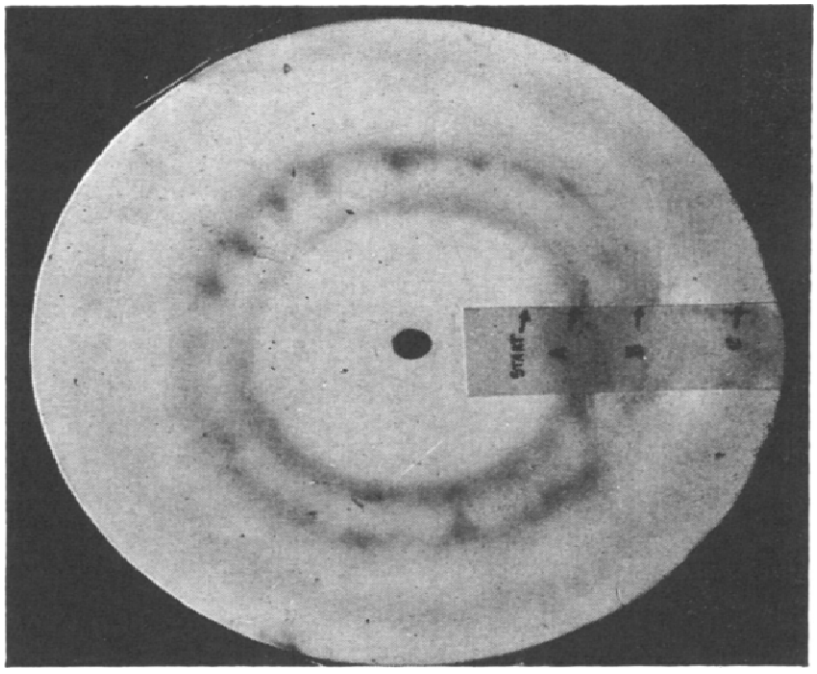

Fig. 3. A = Start; B = atropine; C = veratrine; D = front.

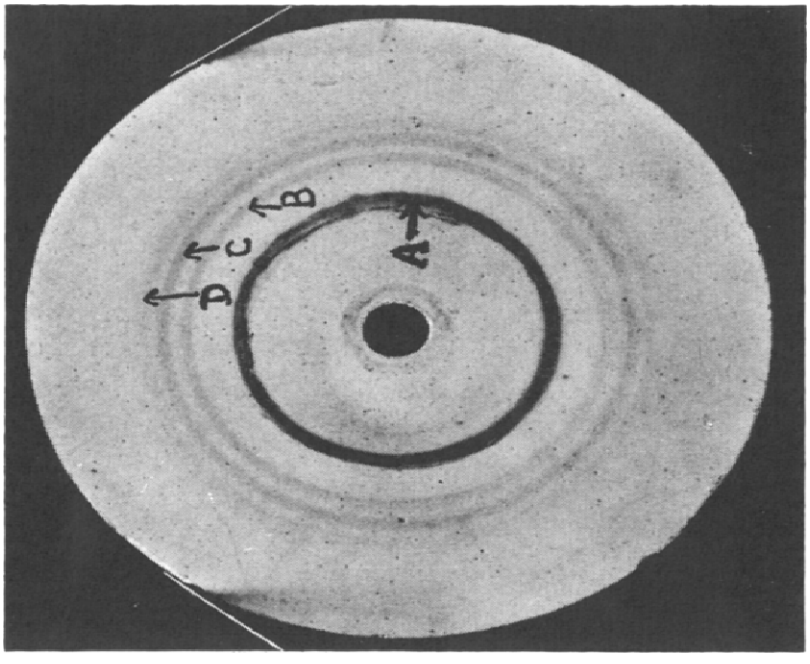

Fig. 2. A = Malachite green; B = eosin; C = eosin impurity.

Collection of separated fractions

The separated constituents in the case of alkaloids are collected in the 12 troughs. Each trough is placed in position for 1 min. In order to find out where the constituents have been collected, the strip P (Fig. 1) is developed with iodine or Dragendorff reagent. The alkaloids appear as sharp bands. By placing the developed strip in its original position, the troughs which contain each alkaloid can be easily located.

RESULTS

Fig. 2 shows a photograph of the progress of separation of eosin and malachite green. The bands are marked A, B, C. The dyes were not very pure and this explains the additional band in case of eosin. Time for separation = 1 min. Time for collection of eosin was 3 min after the start, and for malachite green 4 to 6 min after the start.

Fig. 3 shows a photograph of the progress of the separation of atropine and veratrine. Time for separation = 1½ min. Time for collection was 3 to 5 min after the start for veratrine and 7 to 8 min for atropine.

The purity of each alkaloid was established by re-chromatography on plaster strips as described previously[1].

SUMMARY

A method of centrifugal chromatography using set plaster of Paris discs is described. Solvents suitable for separating a mixture of two dyes and two alkaloids are also described. A specially constructed fraction collector is described and results show that both separation and recovery are possible within a few minutes. The method offers a rapid way to chromatographic separation and studies are in progress on its use for other types of substances and on the use of gradient elution techniques. Studies on industrial applications are also being made.

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