AN APPARATUS FOR CENTRIFUGAL ACCELERATION OF PAPER CHROMATOGRAPHY

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The period of several hours generally necessary for the development of paper chromatograms can become a major handicap in biochemical problems involving the use of radioactive isotopes of short half-lives. Recently, acceleration of paper chromatography by the application of centrifugal force has been described by McDONALD and his colleagues[1]. Their method consists of revolving horizontally a circular sheet of filter paper while the developing solvent is delivered continuously in a fine jet near the centre of the disc. Circular chromatograms tend to produce arc-shaped patterns, the size of the arc being proportional to the R_F of the substance. Our determinations of radioactivity were made in an automatic strip counter which carries strips 2.5 cm in width. The spreading mentioned above would preclude any quantitative assessment of the radioactivity on such a cut from the centre of a circular chromatogram. A constant rate of flow of solvent is essential for satisfactory chromatographic separation and the difficulty in regulating solvent feed from an external reservoir might be an additional source of error. Both the above objections have been eliminated in our apparatus, described below. The circular filter paper sheet has been replaced by paper strips laid out in the form of independent "wheel spokes" while a wad of filter paper soaked in the solvent and placed in the development dish is all that is necessary for a solvent reservoir.

APPARATUS

The apparatus consists of a flat, circular aluminium dish, 50.0 cm in diameter and 5.0 cm deep, mounted on bearings and rotated by means of a variable speed electric motor through a belt drive. The dish is coated on the inside with "Araldite" coating resin 985/C3 to prevent corrosion. At the centre of the dish is fixed an aluminium cup to hold a stack of 150–200 filter paper (Whatman No. 1 or 3) discs, 7 cm in diameter, which act as the reservoir. An adaptor to hold discs of larger diameter could be fitted to this cup. An annular ring of thick filter paper (Whatman No. 3, or 3 MM), soaked in the developing solvent, is placed at the bottom of the dish and around the inner cup to maintain a saturated atmosphere. The filter paper strips (60 × 2.5 cm) bearing the samples to be analysed are held in position by clamping a 0.5 cm thick Perspex lid to the rim of the dish which is 2.6 cm wide. A good seal is obtained by a ring of "Rubazote" 1.0 cm in thickness glued under the Perspex lid. The contact between the centre of the paper

strips and the filter paper discs of the solvent reservoir is made by pressure from a small Perspex disc (5.5 cm in diameter and 0.25 cm thick) joined under the centre of the lid. A small length (3.5 cm) of capillary tubing surrounded by a Perspex tube is inserted through a hole in the centre of the lid and pressure disc to be used for introducing additional amounts of solvent after the dish has been sealed. A diagrammatic view of the apparatus is given in Fig. 1.

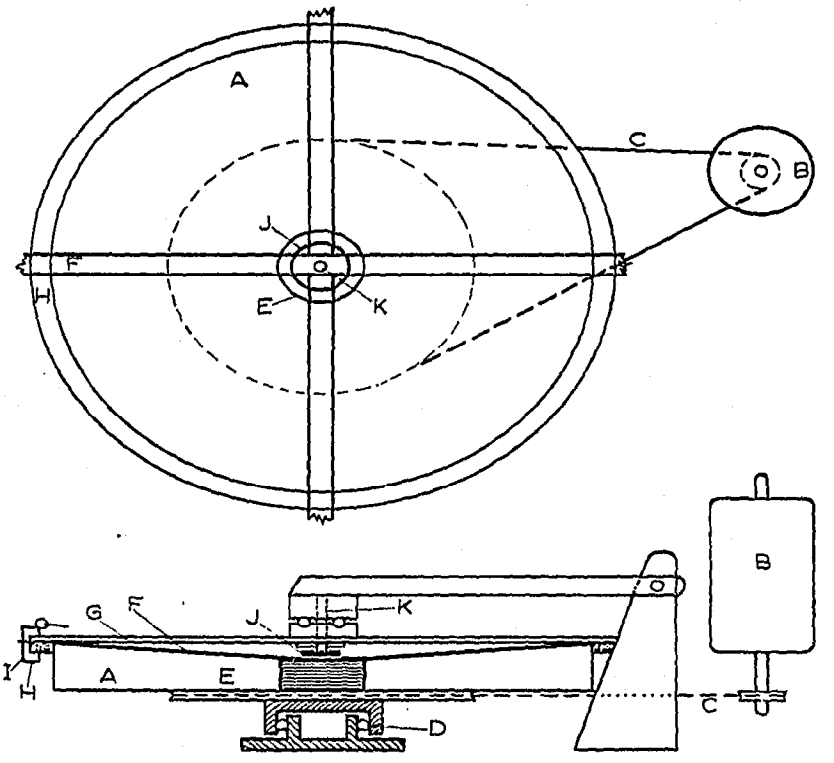

Fig. 1. Top and side views of the apparatus. A: aluminium dish; B: motor; C: belt; D: bearings; E: inner cup for filter paper solvent reservoir; F: chromatogram strips; G: Perspex lid; H: dish rim; I: clamp; J: Perspex disc; K: hole for capillary tubing.

METHOD

The strips are prepared by applying and drying the mixture to be analysed at 7 cm on either side of the centre of the dish. To the wad of filter paper discs in the reservoir is added enough solvent (about 35–50 ml) to saturate it but not enough for the accumulation of any free liquid. The filter paper strips are laid diametrically across the dish and overlapping one another over the top of the reservoir. Up to 12 strips (and hence 24 samples) can be run simultaneously, without any contact between the points of application of samples or any part of the strip beyond 3–4 cm from the centre. A wad of 15 Whatman No. 1 filter paper discs, 7 cm in diameter, are soaked in the solvent and laid on top of the strips directly above the reservoir. The strips are maintained in a taut position by pulling them at both ends while the lid is being clamped on. An additional 5–7 ml of solvent are slowly introduced on to the top layer of filter paper discs through the capillary tubing in the central Perspex tube; the latter is then sealed with a rubber bung. Saturation of the atmosphere in the dish with solvent vapours is

reached in 5–10 min by which time the solvent front on the strips has nearly reached the point of application of samples. The dish is then rotated at speeds varying between 150–400 r.p.m. for 15 to 60 min after which the strips are removed, dried and cut into two at the centre. If the samples are radioactive, the radioactivity distributed along the strip is directly measured with an automatically scanning ratemeter device and without any trimming of the filter paper. Later, the position of markers or carriers is determined by appropriate staining reactions described elsewhere[2].

RESULTS

(*a*) *Adsorption chromatography*

Most of the examples given by McDONALD *et al.*[1] concerned adsorption rather than partition chromatography; the results of similar trials carried out with our apparatus, in order to compare the two methods, are shown in Table I. For this purpose, a mixture

TABLE I

THE EFFECT OF INCREASING CENTRIFUGAL FORCE ON THE SEPARATION OF BROMOPHENOL BLUE, METHYL RED AND METHYL ORANGE WITH 0.075 *M* VERONAL BUFFER, pH 8.6

Speed r.p.m.	*Time run min*	*Length of chromatogram cm*	R_F *values of*		
			MeO	*MeR*	*BPB*
0	300	8.9	0.26	0.63	0.89
200	15	4.8	0.25	0.62	0.89
	30	5.6	0.25	0.66	0.92
	45	6.9	0.28	0.67	0.93
400	15	5.1	0.26	0.66	0.90
	30	6.5	0.29	0.68	0.93
	45	7.7	0.29	0.67	0.91

of bromophenol blue (BPB), methyl red (MeR) and methyl orange (MeO) was resolved into 3 components, using 0.075 *M* veronal buffer, pH 8.6. It will be seen that centrifugal acceleration had little effect on the R_F of the 3 dyes. Also, the constancy in R_F values in our trials is comparable to that described by McDONALD *et al.*[1] for chromatography on circular filter paper.

(*b*) *Partition chromatography*

The acceleration of partition chromatography was the main purpose in designing this apparatus, especially as applied to the separation of halogenated tyrosines, thyronines and their derivatives. In general, better results were obtained with acidic or neutral solvent systems than those equilibrated with ammonia. Some typical results obtained with the accelerated separation of radioactive bromide, 3,5-dibromotyrosine, 3,5,3′,5′-tetrabromothyronine, iodide, 3,5-diiodotyrosine and thyroxine in *n*-butanol–acetic acid–H_2O as the solvent system* are presented in Fig. 2. Comparison of the patterns

* Bromide-82 and iodide-131 were obtained from A.E.R.E., Harwell; ^{82}Br-labelled tetrabromothyronine was synthesized by a modification of YAGI's method[3] and ^{131}I-labelled diiodotyrosine and thyroxine were obtained from Abbot Laboratories, Inc., Oak Ridge, Tenn.

obtained with conventional ascending chromatography and the centrifugally accelerated process confirm that a shorter length of chromatogram and an increased rate of flow of solvent did not appreciably alter the R_F values of these halogenated substances.

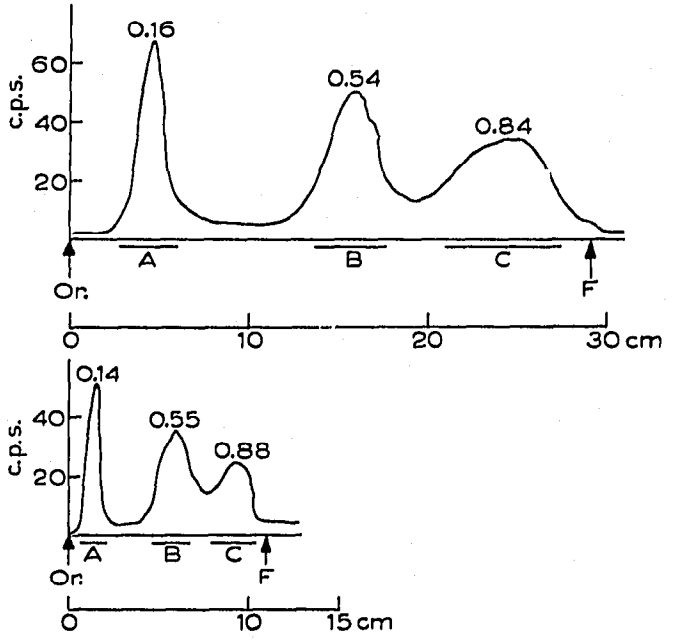

Fig. 2. Distribution of radioactivity of ^{82}Br-labelled substances separated by conventional ascending (top) and centrifugally accelerated (bottom) paper chromatography. Solvent: *n*-butanol–acetic acid–water (78 : 10 : 12). A = bromide ion; B = 3,5-dibromo-L-tyrosine; C = 3,5,3′,5′-tetrabromo-L-thyronine. The figures above the peaks represent their respective R_F values. Or = point of application of the mixture; F = solvent front. Chromatogram development times: 16.5 h for conventional chromatography and 22 min for centrifugal accelerated procedure. Time taken for recording of radioactivity = 62 and 23 min respectively.

In most cases, accelerated separation actually resulted in sharper resolution; at the same time, the shorter centrifugal chromatograms resulted in an important reduction in the time necessary for measuring radioactivity.

DISCUSSION

With the apparatus described above it is possible to reduce the development time of chromatograms from several hours to a few minutes. Centrifugal acceleration of both adsorption and partition chromatography with this apparatus does not result in any distortion of patterns normally obtained with conventional chromatography. The design of our apparatus makes it more convenient to operate and more suitable for separation of radioactive materials than the apparatus for chromatography on circular sheets of filter paper. Besides the ease of handling paper strips (now available commercially in various sizes) arranged in the form of spokes of a wheel, the risk of contamination of radioactive samples due to sideways diffusion is completely eliminated. The solvent reservoir remains in the development dish and the system of filter paper discs soaked in the solvent ensures a constant and reproducible rate of flow of

the solvent. Another advantage of our design of the solvent reservoir is that the starting gravitational force applied to draw the solvent can be varied by varying the diameter of the filter paper discs in the reservoir.

Results presented above show that centrifugal acceleration could be profitably applied in the chromatographic separation of substances labelled with radioisotopes of short half-lives. In this way, more information has been obtained on enzymic debromination of bromothyronines than has been hitherto possible[4]. As an actual example, chromatographic analysis of substances labelled with ^{82}Br ($T_{1/2} = 35.9$ h) by the conventional ascending method required 16 h for development and 24 h for measuring the distribution of radioactivity in 24 strips (automatic scanning at 7.5 cm/h) making a total of 40 h. With centrifugal chromatography the same operation was completed in 8.5 h (30 min development time and only 8 h for radioactivity measurements because of smaller chromatograms). This reduction in the time required for chromatographic analysis has meant a higher "workable" life for ^{82}Br; it has also enabled us to increase the number of sequential experiments performed with ^{131}I-labelled thyroid hormones and analogues. The advantage of speed is not restricted to chromatography of radioactive substances but could also be valuable in the separation of other materials, notably antibiotics and unstable substances.

SUMMARY

The design and manipulation of an apparatus for centrifugal acceleration of paper chromatography has been described. The main novel features consists of using filter paper strips instead of circular sheets and a constant flow solvent reservoir enclosed in the chromatography tank.

Our arrangement is particularly suited for the quantitative analysis of materials labelled with radioactive isotopes of short half-lives and examples are given of the separation of some ^{82}Br- and ^{131}I-labelled substances. Advantages of the above method over circular paper chromatography are discussed.

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