The Purification of Synthetic ^{14}C Labelled Compounds by the Use of Preparative Paper Chromatography

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Received August 19, 1969.

SUMMARY

Preparative paper chromatography (PPC) for the purification of synthetic compounds labelled with ^{14}C : choline, chlormequat, glutamic acid, indoleacetic acid and urea is described. The influence of some parameters as time, amount of substances used and geometry factors (dimensions of paper sheets) on the repeatibility of PPC are given.

INTRODUCTION.

The separation of substances by means of preparative paper chromatography (PPC) was applied as one of the methods of the purification and separation of organic compounds [1–3]. The aim of the present work was applying PPC to the purification of some compounds labelled with ^{14}C as it has been done for the products of biosynthesis of the labelled compounds [4], especially obtained from the algae *Chlorella vulgaris* [5]. Now the adaptation of PPC for the purification of synthetic compounds labelled with ^{14}C is offered.

The subject seemed trivial but only in strictly definite conditions one obtain the good reproducibility of the PPC process. Purification of small amounts of compounds labelled with ^{14}C is important for both the producer, as it solves him the problem of storing these compounds which are unstable owing to their autoradiolysis, and the user who receives the method of purification of aged compounds.

CHROMATOGRAPHIC TECHNIQUE.

The glass chamber commonly available (Chropa Entwicklungskammer - 002, Glaswerke, Illmenau, Deutsche Demokratische Republik) was used for the ascending chromatography. Whatman No. 3 filter papers, standard

TABLE 1.

Parameters of PPC			Value obtained for					
No.	Parameter	Denomination	Urea-^{14}C	Choline-^{14}C	Chlormequat-^{14}C[a]	Ammonium glutamate-5-^{14}C	Glutamic acid-1-^{14}C	Ammonium indolilo, acetate-2-^{14}C
	Time							
1	Conditioning	h	2	0	0	1	1	0
2	Period of ageing of the system	days	3-7	3-7	3-7	0	3-7	0
3	Developing the chromatogram	h	7	10	10	6	7	10
4	Autoradioradiography	h	to 1 μCi/cm current of the sheet of filter paper — 60 — for all substances 1-10 μCi/cm current of the sheet of filter paper — 24 — for all substances 1-20 μCi/cm current of the sheet of filter paper — 6 — for all substances					
5	Elution	h	10	10	10	10	10	3
	Amount							
6	Sample spotted	μg/cm curr.	600	500	500	300	500	600
7	Overloading	μg/cm curr.	1,000	700	500	200	200	700
8	Diffusion	μg/cm curr.	2,000	1,000	1,200	800	800	1,100
	Geometry							
9	Start	cm	5	7	7	5	7	5
10	Solution front	cm from the start	16	18-20	18-20	15	15	18-20

[a] The amount of the sample spotted corresponded to the amount of chlormequat-^{14}C for separation pure compound ^{14}C chromatographically analysed (11).

cut 28.5 × 35 cm in size, previously washed in pile by water during 6 days were used. The amount of impurities in paper was determined gravimetrically by water elution and evaporation. Before purification the paper contained c. 20 μg impurities (consists mainly of the binder) on cm^2 of the filter paper. The purity of the paper after washing was proved by the second washing and only 2 $\mu g/cm^2$ of impurities were eluted. They consist of cellulose powder only, caused by destruction of cellulose fiber. The substances developed in chosen systems gave generally $R_f = 0.3\text{-}0.5$. The choice of the systems of those R_f for purified compounds gave the possibility of elimination of the impurities which remained in the filter paper and usually have $R_f = 0.6\text{-}1.0$ [6]. The impurities from the substances purified ($R_f = 0.0\text{-}0.25$ and $R_f = 0.6\text{-}1.0$) were also removed.

The amount of the substance purified varied from 10-100 mg per the glass chamber used. This amount was regulated by the number of standard sheets of filter paper on which the substance was dropped (Table 1).

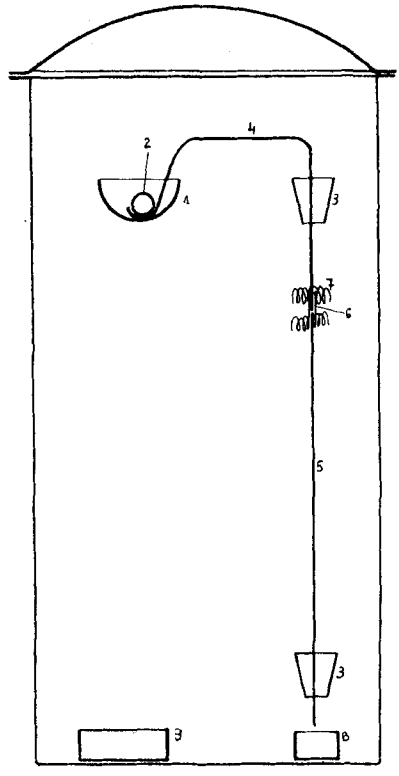

FIG. 1. The chamber for elution of the substance from the strips cut out of sheets of filter paper after developing of chromatogram. 1. kuvette; 2. weights; 3. filter paper guide; 4. feeding filter paper; 5. filter paper strip for elution; 6. slide; 7. nickel springs; 8. vessel with water; 9. vessel for eluted solution.

Acidic and alkaline developing systems were used. According to Pasieka's notices on cellulose hydratation [2] the systems containing about 15 % of water were used. The R_f value obtained in the PPC by the use of standard sheets of filter paper was 0.6-0.7 of the R_f obtained on the 2 cm wide paper strips. The chromatograms were developed at 22-23° C, afterwards the sheets were horizontally dried, and the spot positions were determined by autoradiography or under the UV light. The main spot was cut off and water eluted (Fig. 1).

The speed of elution was regulated by the width of the feeding filter paper and the amounted solution evaporated *in vacuo*. The cellulose powder eventually present in the solution was centrifugated off.

The chromatographic chambers used for elution were washed twice a month to prevent the growing moulding.

The following compounds : (2-hydroxyethyl)-trimethyl-$^{14}C_3$-ammonium chloride (choline), (2-chlorethyl)-trimethyl-$^{14}C_3$-ammonium chloride (chlormequat) (7), urea-^{14}C [8], ammonium glutamate-5-^{14}C [9], ammonium 1*H*-indolilo, 3-acetate-2'-^{14}C [10] and glutamic acid-1-^{14}C were purified. Glutamic acid-1-^{14}C was stored several years before purification. The yield of the single purification of these compounds was about 80 % with the exception of ammonium 1*H*-indolilo, 3-acetate-2'-^{14}C, for which the yield was a little higher (90 %). This could be explained by the lower adsorption of the substance on the filter paper. The last substance was eluted just after developing without drying. The purity of each compound was at least 97-98 % and was checked chromatographically and autoradiographically. The details of the analytical results will be published elsewhere.

Chromatographic phenomena.

On the ground of the nonanalytical and preparative purpose only, it could be emphasised that the great importance in this, PPC method of separation, is to know the terminal amounts of the substances spotted on the strips. Two phenomena were associated with these amounts : overloading and diffusion.

Overloading of the spots amounted from 200 to 2,000 μg/cm current of the strip (Table 1) and caused the region of readibility of chromatogram (after autoradiography). Sometimes this phenomenon was observed directly after developing as bright shining spots with sharp edges on the wet sheet of the filter paper.

Diffusion of the spot was connected with the least permissible value of the amount of the substance spotted by which an enormous enlarging and the diffusion of the edges of the spot after developing began, and began from 800-2,000 μg/cm curr. depending of the substance spotted.

In this work care was taken to limit the amounts of the substances spotted on paper strips to these caused overloading only.

ACKNOWLEDGEMENT.

The author wish to thank Dr A. Pasternak for revising this manuscript.

EXPERIMENTAL.

Choline-^{14}C was obtained from formaldehyde-^{14}C [7] using ethylene chlorohydrine instead of ethylene-1,2-dichloride. Purification of chlormequat-^{14}C [7] was described previously and recently collected experimental data concerning PPC enclosed in Table 1. Glutamic acid-5-^{14}C was obtained from potassium cyanide-^{14}C by condensation with benzamido, γ-butyrolactone [9] and benzoic acid separation, glutamic acid-1-^{14}C several years stored was purificated by PPC. 1*H*-indolilo, 3-acetic-2′-^{14}C acid was obtained from formaldehyde-^{14}C [7, 10].

The following developing systems were used :
No. 1. *n*-butanol : acetic acid : water = 4 : 1 : 5 (upper layer)
No. 2. *n*-butanol : acetic acid : water = 5 : 4 : 4
No. 3. *s*-butanol : *t*-butanol : 25 % ammonium hydroxide = 5 : 4 : 1
No. 4. *s*-butanol : *t*-butanol : 25 % ammonium hydroxide = 5 : 2 : 1
No. 5. *i*-propanol : 25 % ammonium hydroxide : water = 10 : 1 : 1
No. 6. *n*-butanol : ethanol : water = 4 : 1 : 1.

Choline-^{14}C was purified in the system No. 1 (R_f = 0.22). The purity of the product was above 99 %.

Urea-^{14}C [8] was purified by developing in the systems No. 1 and 6 (R_f = 0.44, 0.33 resp.) successively, obtaining compound of 98-99 % purity.

Glutamic acid-5-^{14}C was dissolved in a 5 % ammonium hydroxide and purified in the following two manners : by developing in systems No. 3 and 4 successively obtaining ammonium glutamate-5-^{14}C (R_f = 0.05, 0.015, resp.) and nonlabelled homoserine (R_f = 0.15, 0.11, resp.); by developing in systems No. 3 and 2 (R_f = 0.05, 0.25, resp.) especially after storing the raw product for some years. The free glutamic-5-^{14}C acid was obtained by the action of Dowex 50 W × 2 (H^+ form), 200-400 mesh. The purity of the compound in both cases was 97-98 %.

Glutamic acid-1-^{14}C was twice developed in the system No. 2 (R_f = 0.25). The purity of the product was 98-99 %.

1*H*-indolilo, 3-acetic-2′-^{14}C acid was purified by developing in the system No. 5 (R_f = 0.4). The wet sheets of the paper were taken out from the chamber, controlled under a mercury lamp with an UV filter, and the presence of the main spot was marked with a soft pencil. The main spots cut out in the form of long strips from the still wet filter papers were kept temporarily in the chamber saturated with the vapour of ammonia and afterwards eluted (Fig. 1) with a 5 % ammonium hydroxide; the solution thus obtained was lyophylizated.

The free acid was obtained by treatment with formic acid *in vacuo* below 0° C and subsequent ammonium formate sublimed at 60° C/0.01. The purity of the product was 98-99 %.

REFERENCES

1. PASIEKA, A. E., LOGAN, J. E. — *Can. J. Biochem.*, **44** : 149 (1966).
2. PASIEKA, A. E. — *Can. J. Biochem. Physiol.*, **38** : 1137 (1960).
3. ROCKLAND, L. B. — *J. Chromatog.*, **16** : 547 (1964).
4. SALA, E., MAIER-HÜSER, H., FROMAGEOT, P. — *J. Labelled Comp.*, II : 391 (1966).
5. LIEBSTER, J., KOPOLDOVA, J., KOZEL, J., DOBIASOWA, M. — *Coll. Czech. chem. Comm.*, **26** : 1582, 1694, 1700 (1961).
6. SCHWANE, R. A., KOWKABANY, G. N. — *Anal. Chem.*, **35** : 1160 (1963).
7. FALEÇKI, J. — *Bull. Acad. Polon. Sci. Ser. sci. chem.*, XVI : 29 (1968).
8. FALEÇKI, J. — *Bull. Acad. Polon. Sci., Ser. sci. chem.*, XVI : 35 (1968).
9. PICHAT, L., MIZON, J., HERBERT, M. — *Bull. Soc. chim. France*, 1787 (1963).
10. STUTZ, R. E., ATKINSON, D. E., GORDON, S. A. — Contract AECR, ANL-4710, *Nucl. Sci.*, **6** : 162 (1952).
11. CHWALA, Ch. — *J. Labelled Comp.*, IV : 10 (1968).