CHROM. 3945

Artefacts in the paper chromatography of D-mannosamine-1-^{14}C hydrochloride

In the course of the analysis of D-mannosamine-1-^{14}C hydrochloride synthesized at the Radiochemical Centre artefacts were detected in the paper chromatographic procedures used as a direct result of the sensitivity of detection by the radioactive method.

D-Mannosamine-1-^{14}C hydrochloride is prepared essentially as the method of KUHN *et al.*[1]. The reaction results in two epimers, D-glucosamine-1-^{14}C hydrochloride and D-mannosamine-1-^{14}C hydrochloride, in the ratio roughly of 4 to 1. These are separated by preparative paper chromatography. The resultant purified material is crystallised from moist methanol by the addition of acetone and dried *in vacuo*.

Several artefacts, notably double-spotting and streaking, have been reported in the paper chromatography of amino sugars[2–4], and low loadings and strongly basic solvent systems have been recommended to counteract them, but these lead to decomposition including the formation of glycosylamines[5].

This note reports the occurrence of artefacts in the paper chromatography of radioactive materials even in the recommended systems. The apparently low radiochemical purity values found are shown to be due to these artefacts, which are undetectable in the paper chromatography of inactive material. The detection of artefacts by conventional (inactive) colour methods depends on the sensitivity of the reagent and this will probably be two or three orders of magnitude less sensitive than radioactive methods.

This compound provides yet another example in which the ideal of a symmetrical "spot" is not attained in any of the systems tried; "streaking" always occurs, even with what is believed to be a pure compound, and with care taken to avoid overloading or underloading of material on the paper.

Materials and methods

D-Mannosamine-1-^{14}C hydrochloride had a specific activity of 45 mC/mmole. Carrier D-mannosamine hydrochloride was purchased from Sigma Chemical Company Ltd., London and was recrystallized from water–methanol–acetone. It had m.p. 178–178.5° (d) $[\alpha]_D^{20}$ — 3.2° (C.1 in water); literature values[6] m.p. 178–180°, $[\alpha]_D^{20}$ —3.0°.

Purified diluted samples in the reverse isotope dilution analysis were obtained by recrystallization to constant specific activity. The samples were dissolved in water and counted by liquid scintillation methods using a Nuclear Chicago Mk. I counter and Triton X-100 scintillant[7]. Counting efficiency was determined by the Channels Ratio method and suitably predetermined background counts were subtracted from all sample counts.

Standard solutions were applied to the chromatograms from calibrated micropipettes and allowed to dry at room temperature. The papers used were all Whatman types and are described in the Tables I and II. Four solvent systems were used for development:

(A) *n*-butanol–ethanol–water(52:33:15),

(B) pyridine–ethyl acetate–acetic acid–water (5:5:1:3),

TABLE I

RADIOCHEMICAL PURITY VALUES DETERMINED IN SOLVENT SYSTEMS A, B, C, D FOR D-MANNOSAMINE-1-^{14}C HYDROCHLORIDE

Chromatogram No.	*Solvent system*	*Paper type*	*Loading (μg)*	*R.C. purity (%)*	*Streaking*
1	A	No. 1	13**	93	+
2	A	No. 1	10	93	+
3	A	No. 1	3	93	+
4	A	No. 1	0.5	93	+
5	A	No. 541*	3	<85	++
6	B	No. 1	13**	95	+
7	B	No. 1	10	96	+
8	B	No. 1	3	96	+
9	B	No. 1	0.5	96	+
10	B	No. 541*	3	<90	++
11	C	No. 1	13**	97	+
12	C	No. 1	10	97	+
13	C	No. 1	3	96	+
14	C	No. 1	0.5	97	+
15	C	No. 541*	3	<92	++
16	D	No. 1	3	95	+

* Acid-washed paper.
** 3 μg active material + 10 μg carrier.

TABLE II

COLOUR YIELDS AND STREAKING OF D-MANNOSAMINE HYDROCHLORIDE IN SOLVENT SYSTEMS A, B, C; WHATMAN NO. 1 PAPER

Solvent	*Loading (μg)*	*Colour intensity of spot**	*Streaking*
A	1	J.V.	—
A	5	L	—
A	10	M	—
A	20	H	—
A	50	H	+
A	100	H	++
B	1	J.V.	—
B	5	L	—
B	10	M	—
B	20	H	—
B	50	H	+
B	100	H	++
C	1	N.D.	—
C	5	J.V.	—
C	10	L	—
C	20	M	—
C	50	H	+
C	100	H	++

* N.D. = not detectable; J.V. = just visible; L = light; M = medium; H = heavy.

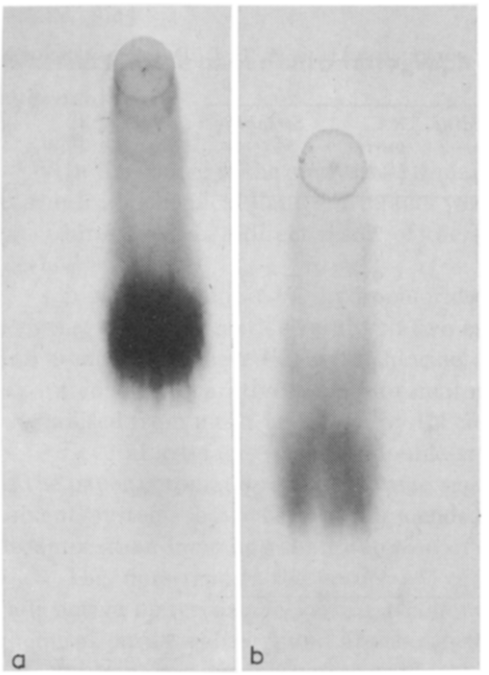

Fig. 1. Autoradiographs of paper chromatograms of D-mannosamine-1-^{14}C-hydrochloride; (a) First run, (b) rerun of active spot eluted from (a). Solvent system A, Whatman No. 1 paper.

(C) ethyl acetate–acetic acid–water (9:2:2), containing 2% phenylboronic acid,

(D) system (A) containing 0.15% w/v ethylenediamine tetraacetic acid, sodium salt.

After development the chromatograms were dried in air at room temperature and the inactive papers sprayed with a 0.5% solution of ninhydrin in *n*-butanol. They were then heated in an oven at 100° for 5 min and the resulting reddish-purple spots noted.

Active chromatograms were scanned to check peak symmetry. They were autoradiographed on "Kodirex" X-ray film and the active areas marked out, cut out and placed into counting vials containing toluene–PPO liquid scintillant. These were counted by liquid scintillation methods. The activity in the major spot was then expressed as a proportion of the total activity along the solvent track.

Results and discussion

Duplicate reverse isotope dilution analysis with D-mannosamine hydrochloride carrier gave a mean radiochemical purity value of 99%. Reverse isotope dilution analysis with D-glucosamine hydrochloride carrier gave a glucosamine content of 2%. Radiochemical purity values from paper chromatography in all four systems are given in Table I.

From the results of the dilution analyses it would appear that the D-mannosamine-1-^{14}C hydrochloride has a radiochemical purity value in the region of 98%. However, paper chromatography systems give consistently low apparent radio-

chemical purities due to heavy streaking of the material on the paper. These purity values are not affected significantly by the use of ethylenediaminetetraacetic acid in the solvent, nor by changing the loading of active material, nor by a fourfold diminution in the specific activity of the active material by dilution with carrier D-mannosamine hydrochloride. The streaking is greatly aggravated by the use of acid-washed papers.

When the principal radioactive spot from chromatogram No. (2) was eluted off and re-run in solvent (A), it gave a radiochemical purity value of 90%, with the same pattern of streaking (see Fig. 1). This was the fourth successive time the material had been chromatographed in similar systems (twice preparatively and twice analytically) and yet it still showed activity streaking back to the origin.

Inactive carrier D-mannosamine hydrochloride was chromatographed with different loadings of material in three systems, as shown in Table II.

It is evident that streaking only becomes apparent with ninhydrin in paper chromatography systems when the loading of material is in the region of 50 μg. But 1 μg of amino sugar (in a spot of normal size) is the threshold of detection by normal colour methods. Hence it is quite possible for streaking to occur at loadings of less than 50 μg and yet for it to be quite undetectable by colour. For example, 20 μg loading with 5% streaking would give 1 μg streaked over a paper area corresponding to, say, ten times the normal spot area.

It is to be noted that neither a fourfold diminution in the specific activity of the active material nor the use of an alkaline solvent (system B) significantly affects the streaking and hence the origin of streaking is only partly due to irreversible absorption onto the paper by an attraction between the mannosammonium ion and the carboxylate ions present in the paper.

CATCH has described several artefacts which can give misleading results in the paper chromatography of radioactive compounds[8]. To these must be added those due to the selection of the solvent system used, since although it may apparently be suitable for inactive materials it may lead to artefacts which can be detected only by the very much more sensitive radioactive methods. The above evidence shows that D-mannosamine-1-^{14}C hydrochloride is a compound which inherently gives rise to streaking.

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