QUANTITATIVE PAPER RADIOCHROMATOGRAPHY USING TOLLENS REAGENT

II. AMMONIACAL SILVER NITRATE REAGENTS CONTAINING LESS THAN EQUIMOLAR PROPORTIONS OF AMMONIA FOR THE OXIDATION OF SACCHARIDES ON PAPER CHROMATOGRAMS

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INTRODUCTION

The application of Tollens reagent to radiometric determination of saccharides on paper chromatograms was described in a previous paper[1]. According to that method, silver deposited in spots on paper as a result of the oxidation of separated substances is converted into $Ag^{131}I$ and determined radiometrically.

Oxidations carried out on paper with silver compounds are also accompanied by oxidation of the cellulose of the support and elementary silver is deposited in the background of the chromatogram. It was shown that this effect does not interfere in principal with radiochromatographic determinations, it is, nevertheless, the critical factor limiting the sensitivity of the method.

Because of the solubility of sugars aqueous solutions of Tollens reagent in common use can be added to paper chromatograms of sugars only by spraying. This fact involves further difficulties, as the background formed after spraying exhibits a non-uniformity which not only decreases the sensitivity of the determination, but also, in many cases, appears to be the source of serious errors.

Since attempts to improve the spraying techniques have been unsuccessful, our attention was turned to other methods of oxidation of sugars, especially methods employing the technique of adding silver reagents to the chromatograms by dipping.

The appropriate experiments were performed to test the methods described by TREVELYAN, PROCTOR AND HARRISON[2] and DEDONDER[3]. It was found that the strongly alkaline reaction conditions prevalent in the first method accelerated the oxidation very markedly but, on the other hand, caused very serious difficulties in the course of the removal of the excess reagent. This did not occur in DEDONDER'S method, but the rate of the reaction under the conditions described by him was very slow.

During these experiments a very marked positive effect of minute amounts of ammonia on sugar oxidations with silver nitrate was observed. This observation led to the development of a new type of ammoniacal silver nitrate reagent, the preparation and properties of which will be described in the present paper.

EXPERIMENTAL

Reagents

Saccharides. Glucose, fructose and inositol, commersial preparations, analytical grade were used in all experiments.

Silver reagents. Silver nitrate solution (SNS): 1 ml of saturated aqueous $AgNO_3$ solution was added to 200 ml of acetone. The precipitated salt was dissolved by dropwise addition of a minimal amount of water (about 5 ml)[2].

Ammoniacal silver nitrate solution (ASNS): 1 ml of saturated aqueous $AgNO_3$ solution was converted into ammoniacal silver nitrate with concentrated ammonia (about 1.6 ml). This solution was then added to 197.4 ml of acetone and the precipitated salt was dissolved with water as above.

Both solutions were used for the preparation of the thirteen reagents listed in Table I.

TABLE I

SILVER NITRATE REAGENTS CONTAINING DIFFERENT AMOUNTS OF AMMONIA

Reagent	$\frac{V_{ASNS}}{V_{ASNS} + V_{SNS}} \times 100\%$
SNS	0
R 8	8.4
R 17	16.7
R 25	25.0
R 33	33.3
R 42	41.7
R 50	50.0
R 58	58.3
R 67	66.7
R 75	75.0
R 83	83.4
R 92	91.7
ASNS	100.0

Note. Stock solutions of SNS and ASNS can be stored in a dark place over a period of several days. All reagents containing deficient amounts of ammonia are unstable and must be freshly prepared before use.

Chromatography

Whatman No. 1 paper strip chromatograms, 350 mm long and 15 mm wide, were prepared. The substances to be tested in amounts of 4.10^{-2} μmoles were spotted on the starting point located 120 mm from the lower end of the strip. The chromatograms were developed by the ascending technique for 18 h at room temperature, using the system *n*-butanol–acetic acid–water (6:1:2)[4]. The developed chromatograms were air dried for 72 h before further operations.

Oxidation reaction

Chromatograms were drawn quickly through the reagents and air dried. This manipulation was repeated three times, but at the third time the drying was omitted and the wet chromatograms were placed horizontally[5] in an oven. The reaction was carried out at 50° in a humid atmosphere produced by placing flat vessels filled with water on the bottom of the oven. A fan mounted inside the oven provided adequate circulation of the air.

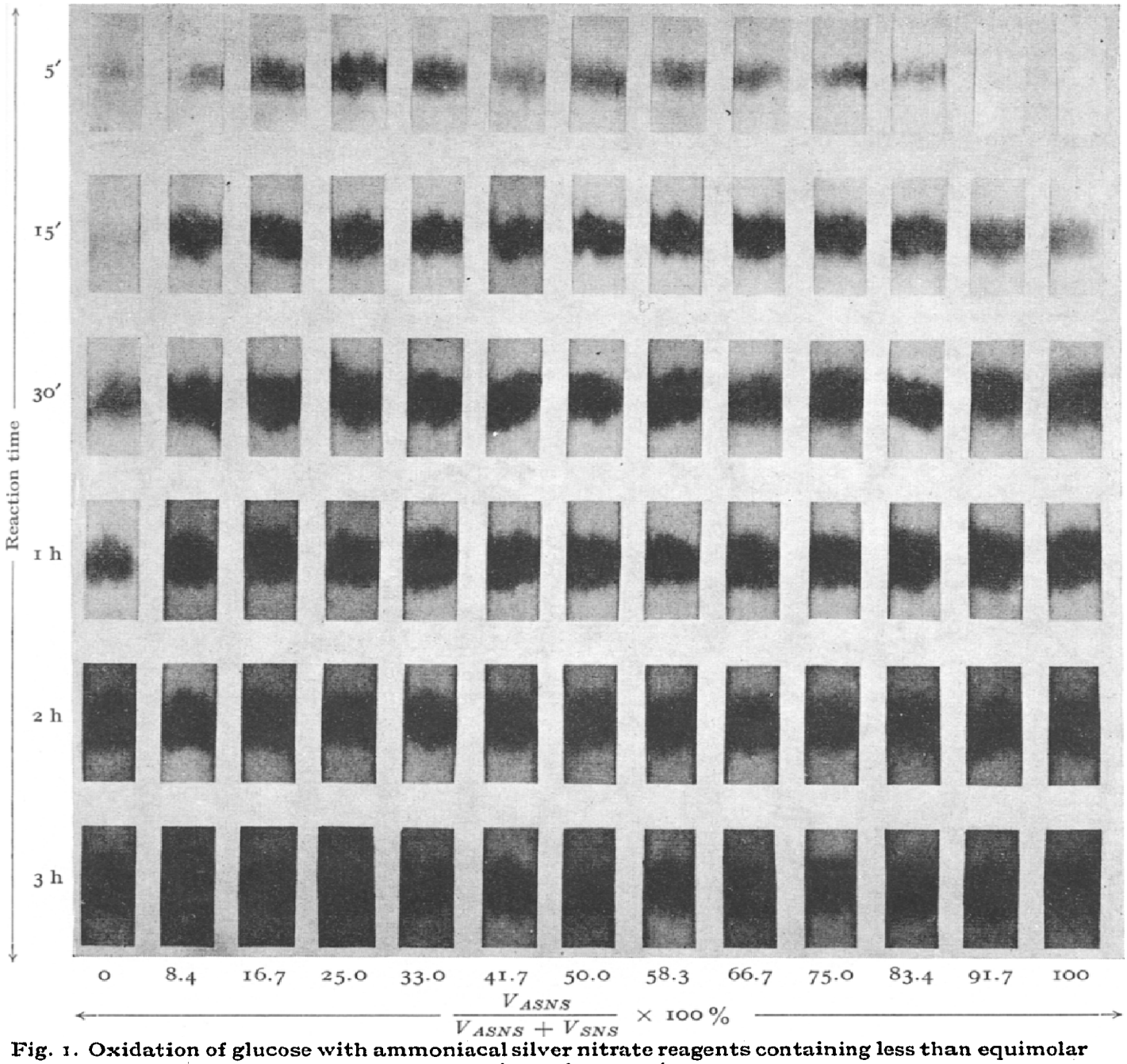

Fig. 1. Oxidation of glucose with ammoniacal silver nitrate reagents containing less than equimolar proportions of ammonia.

Immediately after the reaction, excess reagents were removed from the chromatograms by washing for 2 min with 10 % sodium thiosulphate followed by three washings for 5 min with water.

RESULTS AND DISCUSSION

The oxidizing properties of silver nitrate reagents containing different amounts of ammonia were tested in reactions with glucose, fructose and inositol. The thirteen reagents listed in Table I were used. The reactions were interrupted at intervals of 5, 15 and 30 min, 1, 2 and 3 h. The results of the experiments are presented in Figs. 1–3.

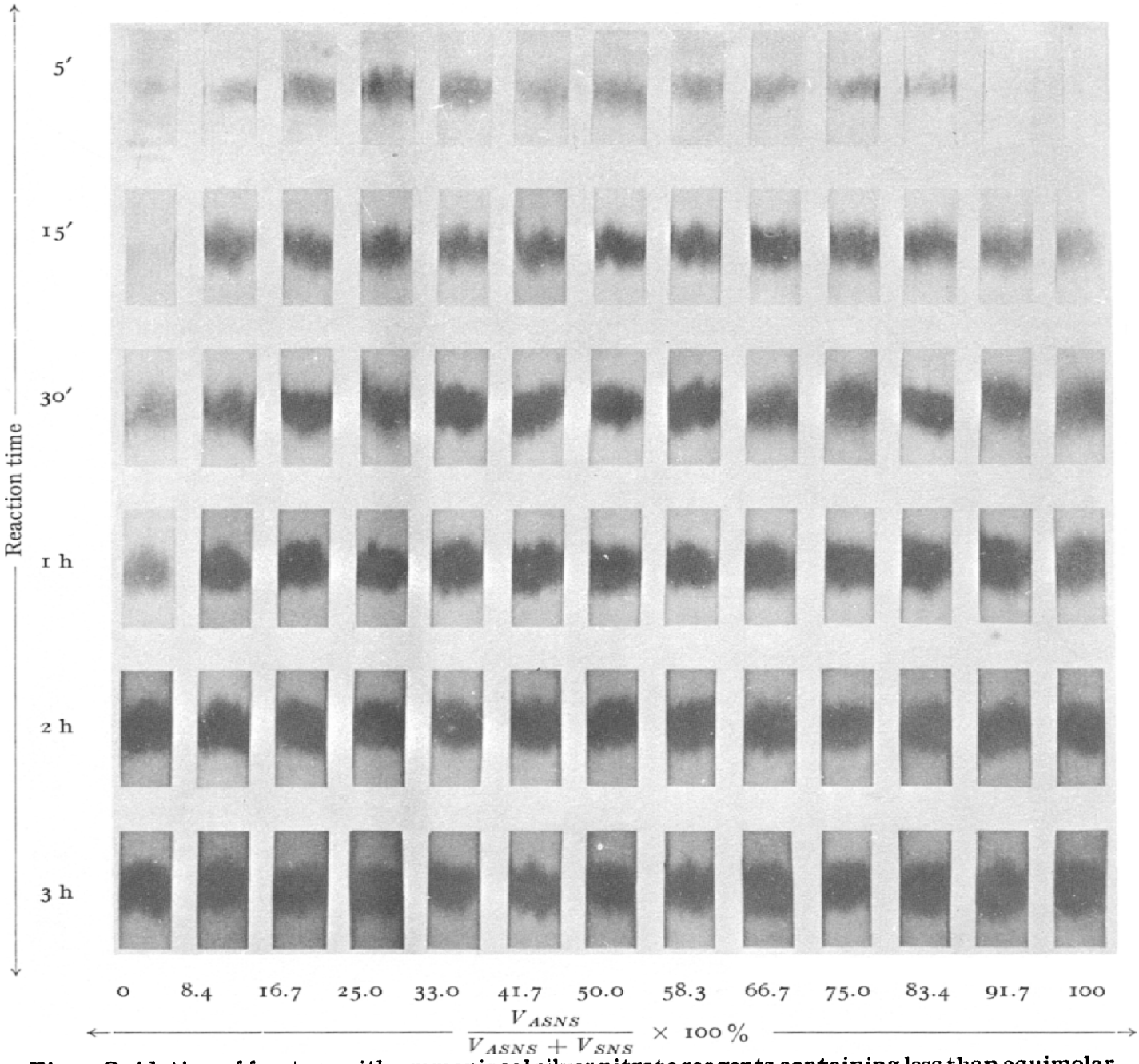

Fig. 2. Oxidation of fructose with ammoniacal silver nitrate reagents containing less than equimolar proportions of ammonia.

It can be seen that all ammoniacal silver nitrate solutions containing less than equimolar proportions of ammonia show higher reactivity towards the tested compounds than do SNS or ASNS. The early stages of the reaction may be used to determine the most reactive mixtures. These were reagents R25–R58 in the case of glucose and reagents R42–R58 in the case of fructose and inositol.

In evaluating the reagents tested, the most important criterion is the comparison of the results obtained with glucose and inositol—compounds representing the highest and the lowest susceptibility towards oxidizing agents among low-molecular sugar compounds. Distinct silver spots could be seen with glucose after only 5 min reaction.

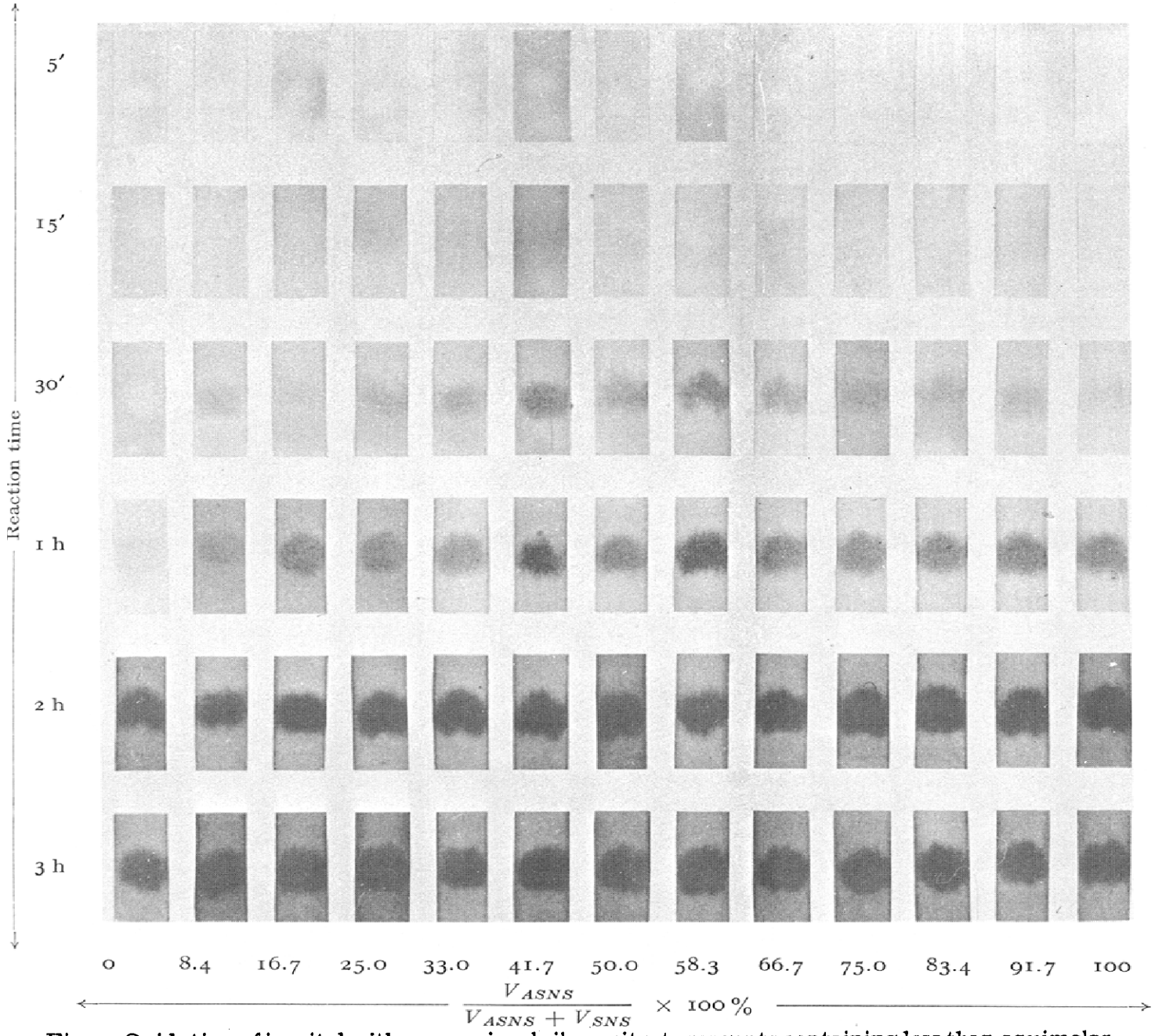

Fig. 3. Oxidation of inositol with ammoniacal silver nitrate reagents containing less than equimolar proportions of ammonia.

A similar picture was obtained with inositol after 30 min. The ratio, however, of the times needed to carry the reactions to completion was much less than six to one. In the case of glucose 1 h was needed whereas for inositol the limit was in the range of 2 h. The behaviour of inositol shows that the conditions described are suitable for analysis of the whole range of monosaccharides and related compounds.

The higher reactivity of mixtures containing smaller amounts of ammonia than is necessary to form the diamminesilver complex—$[Ag(NH_3)_2]^+$— may be connected with the existence in solution of a dynamic equilibrium of the kind

$$Ag^+ + [Ag(NH_3)_2]^+ \rightleftharpoons 2[Ag(NH_3)]^+$$

and the higher reactivity of the monoamminesilver cation. This hypothesis is supported by the fact that maximal reactivity of the tested reagents occurred in the case of solutions containing ammonia in the range of 50 % compared to that calculated on the basis of the diamminesilver complex.

It should be mentioned that the inhibiting influences of an excess of ammonia or ammonium salts on the oxidizing ability of Tollens reagent was observed by DREYER[6]. The same effect seems to occur with DEDONDER's method[3].

Further studies on the application of the modified ammoniacal silver nitrate reagents in radiochromatography of sugars are in progress.

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SUMMARY

Ammoniacal silver nitrate reagents possessing higher reactivity towards saccharides than Tollens reagent are described. The highest reactivity is shown with unstable reagents containing about equimolar amounts of silver nitrate and ammonia. Acetone–water solutions of the reagents can be added to paper chromatograms of saccharides by dipping.

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