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Separation of iodoamino acids and iodide by paper chromatography with aqueous solvents

Different organic solvent systems have been used for the separation of iodoamino acids by paper chromatography[1,2], but there seems to be no report in the literature on solvent systems that contain only inorganic salt solutions. This communication describes a rapid method of separation of a mixture of iodoamino acids and iodide by the use of 3% sodium chloride solution.

Materials and methods

Standard solutions containing 0.2 mg/ml of monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodothyronine (T_3) and thyroxine (T_4) (all obtained from Sigma Chemicals, U.S.A.) were prepared in a 0.2 *N* solution of ammonia in methanol. An aqueous solution containing 0.16 mg/ml of potassium iodide (guaranteed-reagent grade, Sarabhai Merck, India) was prepared. The solutions were spotted on Whatman No. 4 paper and developed in sodium chloride solutions of different concentrations (1, 2, 3 and 5%) by using the ascending technique. In about 1½ h the solvent front moved 31–32 cm. After development the paper was dried in a chromatogram drying chamber and stained with Ce^{4+}–As^{3+}, methylene blue reagent[3]. Instead of an aqueous solution, a 0.1% solution of methylene blue in 50% ethanol was used.

A mixture of [^{131}I]T_4, [^{131}I]T_3 and [^{131}I]iodide was chromatographed in 3% sodium chloride solution. The radiochromatogram was scanned with a radiochromatogram scanner (Frieseke & Hoepfner GmbH, G.F.R.).

In a separate experiment, [^{131}I]T_4 and [^{131}I]T_3 were added to human serum followed by extraction with acidified butanol and washing with BLAU's reagent[4]. After concentration, the extract was chromatographed in 3% sodium chloride solution.

Results and discussion

It was found from the experiments carried out with sodium chloride solutions of different concentrations that the separation of the mixture of iodoamino acids and iodide is most satisfactory in 3% sodium chloride solution. The mixture is poorly resolved in 1, 2 and 5% sodium chloride solutions as T_3 shows tailing.

The R_F values of the reference compounds in 3% sodium chloride solution are $T_4 = 0.07$; $T_3 = 0.25$; DIT = 0.59; MIT = 0.72 and $I^- = 0.89$.

The separation of the mixture of the above compounds is shown in Fig. 1. Fig. 2 shows a radiochromatogram scan of the mixture of [^{131}I]T_4, [^{131}I]T_3 and [^{131}I]iodide.

Chromatography of the butanol extract of serum mixed with [^{131}I]T_4 and [^{131}I]T_3 gives peaks in the radiochromatogram scan that are with those for the respective markers.

It can be seen from the above results that 3% sodium chloride solution is a suitable solvent system for the separation of iodoamino acids and iodide by one-dimensional ascending paper chromatography, which takes only 1½ h. In contrast, organic solvent systems require 8–12 h for the one-dimensional run and often two-

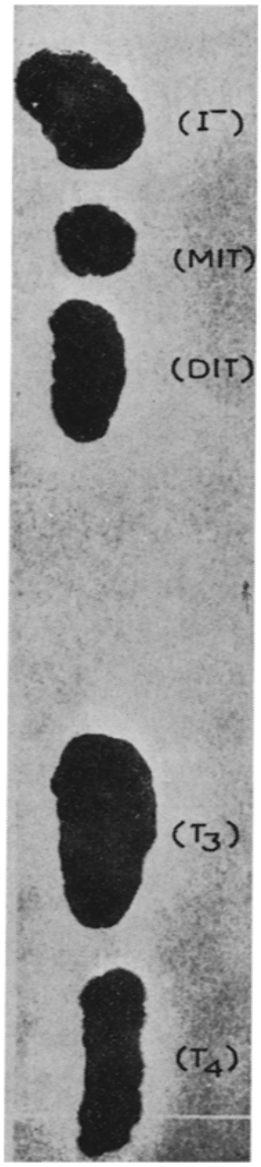

Fig. 1. Separation of a mixture of T_4, T_3, DIT, MIT and I^- in 3% sodium chloride solution.

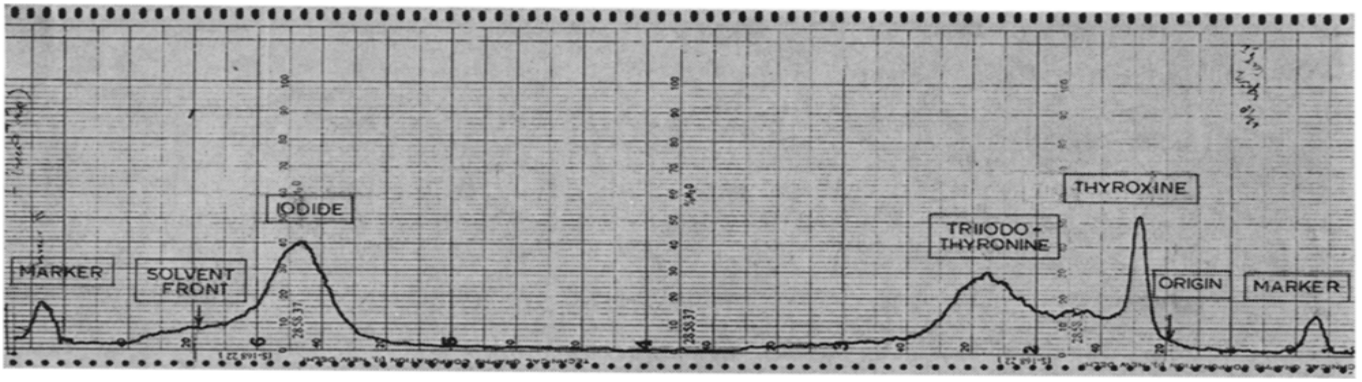

Fig. 2. Separation of [^{131}I]T_4, [^{131}I]T_3 and [^{131}I]iodide in 3% sodium chloride solution.

dimensional chromatography is required for satisfactory separation of the mixture. This short period of chromatographic development with the 3% sodium chloride solution has an additional advantage that there would be considerably less de-iodination of iodoamino acids during chromatography[5]. Furthermore, it is of interest that the rapidity of the method is comparable to that of thin-layer chromatographic procedures.

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