

This Week's Citation Classic

Rydon H N & Smith P W G. A new method for the detection of peptides and similar compounds on paper chromatograms. *Nature* 169:922-3, 1952.

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The paper describes a simple and sensitive method for the detection of peptides and other compounds containing the $-CO.NH-$ group on chromatograms and electropherograms by chlorination, followed by spraying with an aqueous solution of starch and potassium iodide. [The *SCI*[®] indicates that this paper has been cited in over 660 publications since 1955.]

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This simple but widely used method for the detection of peptides and similar compounds containing $-NH-$ groups was developed under the pressure of sheer necessity, in collaboration with P.W.G. Smith, then working for his PhD at Imperial College, in the course of an investigation of the condensation polymerisation of glycine peptide esters.¹ In order to identify and determine quantitatively the polymerisation products, we needed a method to detect them on paper chromatograms. None of the available methods were suitable, and we were experiencing considerable difficulty, amounting almost to desperation, when I remembered that during World War II I had investigated the *N*-chlorination of nylon, using gaseous chlorine, in the hope of making it impermeable to mustard gas. Since this method worked well on nylon, it seemed likely that it could be extended to peptides (and other compounds containing the $-CO.NH-$ group) to provide the *N*-chloropeptides,

$NC1.CO$, which should be detectable by spraying with potassium iodide solution followed by starch solution to visualise the liberated iodine. The method worked extremely well and not only solved our particular problem but also those of others, who have used it extensively over the past 35 years, no doubt because of its simplicity, sensitivity, and wide applicability.

After the publication of our paper, Helmut Zahn wrote to me, drawing my attention to a paper² in which he had described, a year earlier, an almost identical procedure; this paper had escaped our notice, and no doubt that of other peptide chemists, owing to the highly specialised nature of the journal in which it was published. In Zahn's paper a considerable number of methods for detecting peptides on chromatograms are described, including the chlorine-starch-iodide method—although the latter is not mentioned in the abstract published separately.³

A number of modifications to the original procedure have been published to simplify it or make it more sensitive. These fall into two groups: modification of the chlorination procedure and modification of the method of detecting the *N*-chloroamides. The most widely used modifications of the chlorination procedure are those of S.C. Pan and J.D. Dutcher⁴ in which the gaseous chlorine of the original method is replaced by sodium hypochlorite, and of R.H. Mazur and coauthors⁵ in which the chlorine is replaced by *t*-butyl hypochlorite. The most widely used modified detection procedure is that of F. Reindel and W. Hoppe^{6,7} in which the *N*-chloroamides are detected with benzidine or toluidine/potassium iodide/acetic acid. However, the vast majority of workers still use the original procedure, no doubt because of its simplicity.

1. Rydon H N & Smith P W G. Polypeptides. Part 1. The condensation-polymerisation of some polyglycine esters and azides. *J. Chem. Soc.* 1955:2542-9.
2. Zahn H & Wolf H. Analysis of polyamides and polyurethan by papyrography. *Melliand Textilber.* 32:317-21, 1951. (Cited 55 times since 1955.)
3. ———. Analysis of polyamides and polyurethan by papyrography [paper chromatography]. (Abstract.) *Chem. Abstr.* 45:6847-8, 1951.
4. Pan S C & Dutcher J D. Separation of acetylated neomycins B and C by paper chromatography. *Anal. Chem.* 28:836-8, 1956. (Cited 195 times.)
5. Mazur R H, Ellis B W & Cammarata P S. A new reagent for detection of peptides, nucleotides, and other *N*-H-containing compounds on paper chromatograms. *J. Biol. Chem.* 237:1619-21, 1962. (Cited 200 times.)
6. Reindel F & Hoppe W. Über eine neue Färbemethode zum Nachweis von Aminosäuren, Peptiden und Eiweisskörpern auf Papierchromatogrammen und Elektropherogrammen (On a new colour reaction for the detection of amino-acids, peptides and proteins on paper chromatograms and electropherograms). *Naturwissenschaften* 40:221, 1953.
7. ———. Über eine Färbemethode zum Anfärben von Aminosäuren, Peptiden und Proteinen auf Papierchromatogrammen und Papierelektropherogrammen (Staining methods for identification of amino acids, peptides and proteins on paper chromatograms and paper electrophoretograms). *Chem. Ber.* 87:1103-7, 1954. (Cited 490 times since 1955.)