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IN SITU CHEMICAL REACTIONS ON THIN LAYERS IN THE IDENTIFICATION OF ORGANIC COMPOUNDS

INTRODUCTION

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SUMMARY

The literature on the subject of *in situ* reactions on thin layers is reviewed and a simple scheme for classifying the techniques involved is put forward. The review deals with those organic reactions which aid identification of substrates, but not with reversible complexing reactions (*e.g.* with silver nitrate), where the applied material can be recovered unchanged. Spontaneous or unintended reactions are distinguished from organised reactions with which most of the review is concerned.

The idea of carrying out a reaction on chromatography paper and then separating the products of the reaction on the same piece of paper has long been known. It was therefore not a great step to using the same principle on thin-layer chromatoplates. The range of the technique has been considerably extended on thin layers, since the latter are much better suited to the more corrosive reagents often necessary.

In this review attention is confined to those cases involving organic substrates, where the reaction takes place *in situ* on the chromatoplate, though it is often possible to carry out the same reaction on a microgram scale in a small vessel and then to apply the reaction products to the chromatoplate. Reaction *in situ*, where it is possible to apply it, helps to preserve the advantage of speed possessed by TLC, though it may be somewhat lacking in degree of control compared with reaction in a flask or capillary. It should also be pointed out that, in this review, no attempt has been made to include reversible as well as irreversible reactions (*e.g.* complexing with silver nitrate, boric acid, etc., which are essentially an aid to separations), though they can play a significant role in the identification of substances.

Two main classes of reaction *in situ* on thin-layer plates may be distinguished, organised or intended reactions (O) and spontaneous or unintended reactions (S). Particular attention will be paid to organised reactions, since these are of particular value for the purpose of identification. However, it should be pointed out that good reproducibility in organised reactions can be jeopardised by the effects of simultaneous but unintended reactions.

The first application of *in situ* reactions on thin layers to the identification of substances was probably that described by MILLER AND KIRCHNER[1] in 1953. Since then the technique in various modes has been applied to a wide variety of compounds, though the literature is not particularly extensive. A good deal of the work up to 1965 has been reviewed in a book by KIRCHNER[2], who covered reactions involving oxidation, reduction (or hydrogenation), dehydration, hydrolysis, esterification, bromination, enzyme action and the formation of certain derivatives. In the past few years the range of reactions has not been extended much, but there have been an appreciable number of new applications. Both one-dimensional (X1) and two-dimensional (X2) techniques have found new applications and the various types of techniques are summarised in Table I and in Fig. 1.

TABLE I

CLASSIFICATION OF TECHNIQUES

Code	*Method type*	
O	Organised, intended	Reaction types
S	Spontaneous, unintended	
D	Defined reaction	
F	Fingerprint method	
AR	Radiation, heat, light	Methods of reactant application
AG	Gas, vapour	
AL	Liquid spray	
AM	In mobile phase	
AS	In stationary phase	
X1	One-dimensional	TLC
X2	Two-dimensional	
X2 RSS	Two-dimensional with reaction before first development	
X2 SRS	Two-dimensional with reaction between developments (diagonal chromatography)	
X2 RSRS	Two-dimensional with reaction before each development	
M	Miscellaneous	

One may broadly distinguish two types of reaction product. In the first case (D) the product is one of a few simple and identifiable compounds via a defined reaction as in, for instance, the formation of cinnamyl alcohol from cinnamaldehyde by reduction with lithium aluminium hydride[1]. In the second case (F) the product is complex and unidentified as in the technique described by MATHIS AND OURISSON[3] and by WILK AND BRILL[4]; this may be classed as a 'fingerprint' method and is the one less commonly used (see Fig. 1b).

A summary of the various reactions which have been reported as being possible to carry out *in situ* is given in Table II. In the paragraphs which follow the application of these reactions and techniques to particular problems is briefly discussed.

CARBOHYDRATES, PROTEINS AND AMINO ACIDS

There have been comparatively few reports on the application of reaction techniques to these classes of compound. PATAKI *et al.*[5,6] have prepared the dinitro-

TABLE II

SUMMARY OF REACTIONS

No.	*Reaction*	*Reference*
1	Acetylation	3, 20, 21, 25, 27
2	Dehydration by H_2SO_4	1, 26
3	Derivative formation	
	(a) Acetate	See reaction 1
	(b) Dinitrobenzoate	1
	(c) Dinitrophenyl (of amino acid)	5, 6
	(d) Dinitrophenylhydrazone	21, 25, 26, 29
	(e) Methyl ester	See reaction 5
	(f) Phenyl isocyanate	1
	(g) Semicarbazone	1
4	Diazotisation	30, 31
5	Esterification of acids	20, 21
6	Halogenation	4, 10, 11, 18, 25, 28
7	Hydrogenation, catalytic	10, 11, 25
8	Hydrolysis with acid	14, 15, 16, 17, 33
9	Hydrolysis with alkali	1, 20, 25
10	Isomerisation	23
11	Methanolysis	12, 13, 16, 17
12	Nitration	25, 32
13	Oxidation with chromic acid	1, 24, 25, 26
14	Oxidation with nitric acid	24
15	Oxidation with oxygen, ozone	28
16	Oxidation with peroxide, peracid	3, 8
17	Photochemical reaction	7, 22, 23
18	Pyrolysis	34
19	Reduction with $FeSO_4$	18
20	Reduction with $LiAlH_4$	1
21	Reduction with $NaBH_4$	24, 25
22	Reduction with Sn + HCl	31
	Reduction with hydrogen	See reaction 7
23	Miscellaneous	9, 25, 28

phenyl derivatives of amino acids *in situ* on silica gel by spraying with dinitrofluorobenzene reagent (method types O/D/AL/X1, X2 RSS and X2 SRS) and RIVETT AND WILSHIRE[7] have studied the photochemical decomposition of certain short-chain peptides on silica gel (method types O/D/AR/X1, X2 RSS).

LIPIDS AND ASSOCIATED COMPOUNDS

Several reactions have been applied in the analysis and identification of fatty acids; MALINS AND MANGOLD[8] have oxidised unsaturated methyl esters with peracetic acid (method type O/D/AL/X1); JOHNSON *et al.*[9] have made use of the specific reaction with silver nitrate to identify and determine cyclopropene acids (method type O/D/AS/X1) and KAUFMANN and coworkers[10,11] have described the catalytic hydrogenation of unsaturated fatty acids on kieselguhr[10] or gypsum[11] (method types O/D/AG/X1, X2 SRS). The same authors[10,11] have also described the bromination of both fatty acids and triglycerides (method types O/D/AM/X1, X2 SRS).

The methanolysis by 12% KOH in methanol of phospholipids on silica gel has been described by KAUFMANN *et al.*[12] (method types O/D/AL/X1, X2 SRS) for both qualitative and quantitative analysis of mixtures and OETTE AND DOSS[13] have

compared quantitatively the '*in situ*' and 'off plate' methanolysis of lipids (method type O/D/AL/X1).

Several papers have reported the use of *in situ* reaction for the identification and analysis of plasmalogens; SCHMID AND MANGOLD[14] and HORROCKS[15] have described two-dimensional procedures for identification of plasmalogens and the aldehydes formed from them on acid hydrolysis (method type O/D/AG/X2 SRS). Recently a very interesting two-dimensional procedure, involving both hydrolysis of the plasmalogens and the methanolysis of the acyl phosphatide residues *in situ*, has been developed by LUNDBERG and co-workers[16,17] (method type O/D/AG + AL/X2 RSRS).

SCOTNEY AND TRUTER[18] have used *in situ* reduction of peroxides by ferrous salt (method type O/D/AL/X2 SRS) in order to identify the products of autoxidation of lanostenyl acetate. COPIUS PEEREBOOM AND BEEKES[19] have shown how cholesterol can be distinguished from its critical pair, brassicasterol, by development in solvent containing bromine (method type O/D/AM/X1).

The identification of aliphatic alcohols, esters and acids, using *in situ* acetylation, hydrolysis and esterification with methanol, has been described by PURDY AND TRUTER[20] (method type O/D/AL/X1); similar acetylation and esterification reactions have also been employed by HOLLOWAY AND CHALLEN[21] in the identification of natural waxes (method type O/D/AL/X1).

SUBSTANCES OF PHARMACOLOGICAL INTEREST

A paper by STAHL[22] concerning the effect of light and air on pyrethrins is the first describing the use of *in situ* reaction between developments in two dimensions, the method usually referred to as diagonal chromatography (also SRS or TRT technique) (see Fig. 1d). This method was later used by SCHUNACK AND ROCHELMEYER[23] to study the photochemical *cis–trans* isomerisation of annulolins on silica gel (method type O/D/AR/X2 SRS).

An important paper by KAESS AND MATHIS[24] discusses the reduction *in situ* by sodium borohydride and oxidations by nitric acid or by chromic acid in the identification of alkaloids (method type O/D/AL/X1); several other reactions in capillaries (before application to the chromatoplate) are mentioned in this same paper. The identification of alkaloids by reaction with iodine vapour, a 'fingerprint' method, has been described by WILK AND BRILL[4] (method type O/F/AG/X1).

A very useful paper by ELGAMAL AND FAYEZ[25] should also be mentioned; this describes methods for many *in situ* reactions on silica gel or alumina, as applied to a wide variety of model compounds of pharmacological interest (method type O/D/AL + AG/X1).

MISCELLANEOUS SUBSTANCES

The identification of certain terpene alcohols, employing oxidation by chromic acid, dehydration by sulphuric acid and the formation of DNPH derivatives of carbonyls, has also been described by MATHIS[26] (method type O/D/AL/X1) and the author himself[27] has recently described the partial acetylation of polyglycerols *in situ* (method type O/D/AL/X2 SRS).

WILK *et al.*[28] have discussed the use of reaction with various gases or vapours (O_2, O_3, N_2O_3, NO_2, NO, Cl_2, Br_2, I_2, SO_2 and HNO_3) in a fingerprint method for the identification of various aromatic bases and polynuclear aromatic hydrocarbons (method type O/F/AG/X1) and the formation of DNPH derivatives of the bromophenacyl esters of some aromatic acids has recently been employed by STEDMAN[29] (method type O/D/AL/X1). The diazotisation of aromatic amines *in situ* on silica gel and the coupling of the diazo compounds with a phenol has been described by MARCUS *et al.*[30] in a study of dye products from commercial amines (method types O/D/AL/X1, X2 SRS) and THAWLEY[31] has shown that aromatic nitro compounds can be reduced on silica gel by tin and hydrochloric acid and the amine formed diazotised. POLESUK AND MA[32] have also recently described the nitration of aromatic compounds on thin layers and a method for the partial hydrolysis of *p*-aminobenzoic esters and of sulphonamides on silica gel by hydrochloric acid vapour has been reported[33] (method type O/D/AG/X1).

Finally mention should be made of a new technique[34] involving TLC in one dimension of the pyrolysis products of a substance (not formed *in situ*); a pseudo two-dimensional modification, with the exit port of the pyrolysis chamber being moved along one axis of the chromatoplate as the pyrolysis temperature increases, is likely to give a two-dimensional pattern, which is highly characteristic of any given substance, provided it is reproducible.

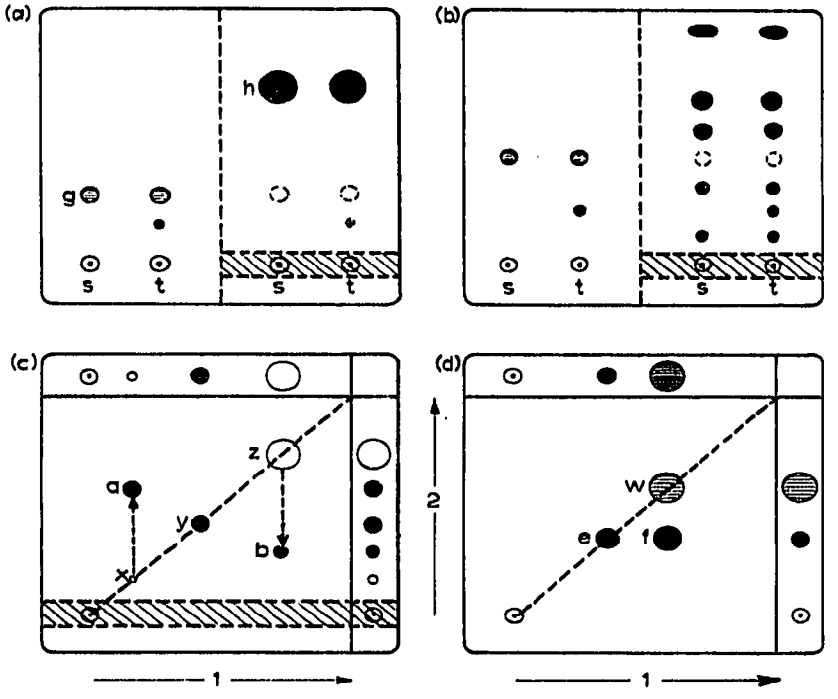

Fig. 1. (a) One-dimensional organised defined reaction (type O/D/X1). s = standard, t = test material. h is formed from g (*e.g.* a carbonyl by oxidation of an alcohol). (b) One-dimensional organised fingerprint reaction (type O/F/X1). s = standard, t = test material. (c) Two-dimensional organised defined reaction (type O/D/X2 SRS). Reagent is applied to shaded area between developments; a is formed from x, y does not react and b is formed from z. (d) Two-dimensional spontaneous defined reaction (type S/D/X2 RSRS); e is formed from w before first development and f is formed similarly between developments in the same solvent, if e = f. Hatched spot, original substance; black spot, product from *in situ* reaction; the shaded area is the area to which reagent is applied.

SPONTANEOUS REACTIONS

It is not the author's intention to review also the subject of the spontaneous or unintended reaction *in situ* (type S), but some mention of it seems necessary. Many of the secondary reactions that can be caused by the adsorbent have been reviewed by LEDERER AND LEDERER[35] and there have been a number of recent publications dealing with instability of lipids[36–38], of olefines[39], of steroids[40–42], of carotenoids[43], of pyrazolones[44], aflatoxins[45], and silyl ethers[46] on thin layers, due to the action of the adsorbent, of light or of atmospheric oxygen.

As PENNER *et al.*[42] point out, the appearance of several spots on a normal chromatoplate and the conclusion that the original substance is impure may be an erroneous one. It must therefore be necessary to take care to ensure that the products of an organised reaction *in situ* are the result only of that reaction on the substrate and not partly the result of a spontaneous reaction. STAHL[47] and others[42] have clearly described how one may detect instability of a compound on a chromatoplate using a simple two-dimensional procedure (see Fig. 1d).

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DISCUSSION

DE ZEEUW: When diagonal chromatography (separation–reaction–separation procedures, S–R–S) is applied, the nonreacting substances are expected to lie on the diagonal if the same solvent is used for development. However, is there an influence due to the direction in which the layer was spread and is it possible that when working with complex mixtures displacement will occur in the direction of the first development?

DALLAS: The position of the spots can vary very slightly with the direction of development, but I have found this unimportant in practical interpretation of the two-dimensional chromatograms. The effect of overloading on the distortion from the true diagonal is more important from my experience.

HAIS: If the position of the spot is modified in the first dimension by the presence of other components of the sample, especially if displacement occurs, then in the second dimension the substance may move without being affected (displaced) by other components, from which it was already separated in the first run. This will result in off-diagonal spots and should be borne in mind in the interpretation of the pattern.