SEPARATIONS WITH PARTITION SOLVENTS ON ADSORBENT LOADED PAPER

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SUMMARY

Papers loaded with silica gel adsorbents have been applied advantageously, by utilizing an adsorption mechanism, to the separation of hydrophobic compounds. In this paper the usefulness of the paper with partition solvents has been evaluated using solutes of simple sugars, phenols and amino acids. The results indicate that the paper may be used by the ascending technique for the rapid separation of polar compounds. The advantage of the medium appears to be associated with the neat spot form obtained under these conditions.

Silica gel loaded papers have been commercially available for a number of years. They were introduced to overcome the tedium and variability associated with laboratory prepared samples of this type. In addition, it is apparent that a paper prepared on a continuous basis has potentially greater reproducibility. The Whatman paper has a relatively high loading of silica gel, corresponding to 22% by weight as silica. The silica gel present has the characteristic acidic nature of the material but has a larger pore size than that normally associated with thin layer silica gels.

Silica gel loaded paper is a convenient tool for the chemist as it allows adsorption characteristics to be combined with the economy and convenience of paper. As an adsorption medium, it has been used for the separation of hydrophobic substances on many occasions. In 1962, MICHALEC *et al.*[1] reported the separation of cholesterol esters by a two-dimensional technique and plant pigments were successfully separated by THIRKELL[2] in 1964. MARINETTI[3] and WUTHIER[4] have both applied the paper to the separation of polar lipids and, more recently, SMITH[5] has reported the separations of 2,4-DNP derivatives of keto acids on this paper that are superior to conventional paper chromatography and thin layer chromatography on silica gel.

It was with the object of extending the usefulness of the paper that the separation of polar substances was studied.

The first type of solutes chosen were phenols. Two mixtures were used, the first contained catechol, pyrogallic acid and fluorescein, the second contained, in addition, Rhodamine B.

Fig. 1 compares the silica gel loaded paper with Whatman No. 1 and No. 20 papers and a silica gel layer. The solvent, nitromethane–formic acid–water in the proportions 60:25:15 gave a rapid ascending development corresponding to times of

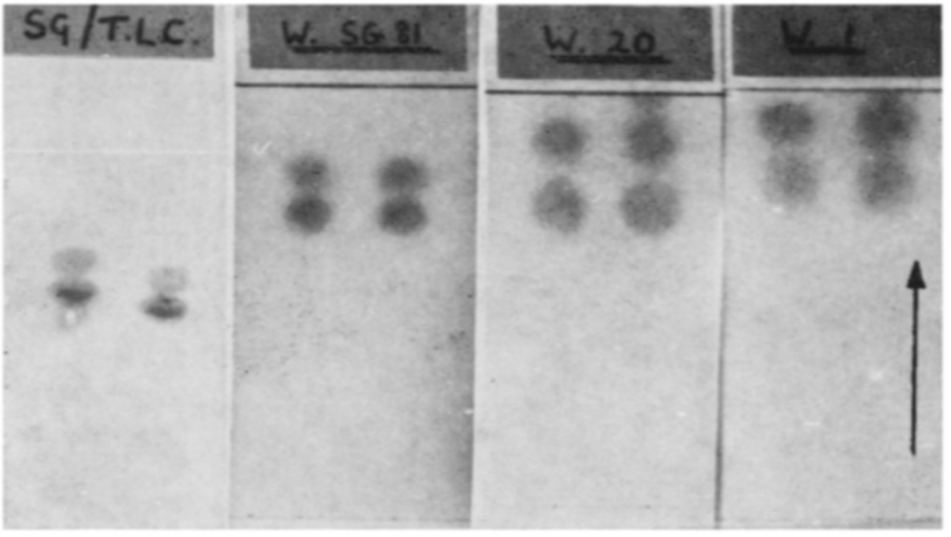

Fig. 1. Phenol separation. Solvent: nitromethane–formic acid–water (60:25:15).

25, 45, and 45 min for a 12.5 cm development on Whatman No. 1, Whatman No. 20 and SG. 81 respectively. Spots are round and distinctly neater on the loaded paper.

With 2% aqueous acetic acid as the developing solvent (Fig. 2) not only are spots more compact on the silica gel paper but it does not show spot elongation to the extent of the other papers. Reproducibility of spot position is as good as conventional chromatography papers and is less susceptible, in this case, to the effect of other constituents in the mixture. This is illustrated here by the lower R_F values for the phenols when Rhodamine B is present in the mixture.

The performance of the paper with respect to acidic and basic solvents has been assessed. Fig. 3 shows the neat spots obtained for amino acids in isopropanol–formic acid–water solvent and an ammoniacal phenolic solvent.

Sugars similarly, give preferred spot forms by development in partitition type

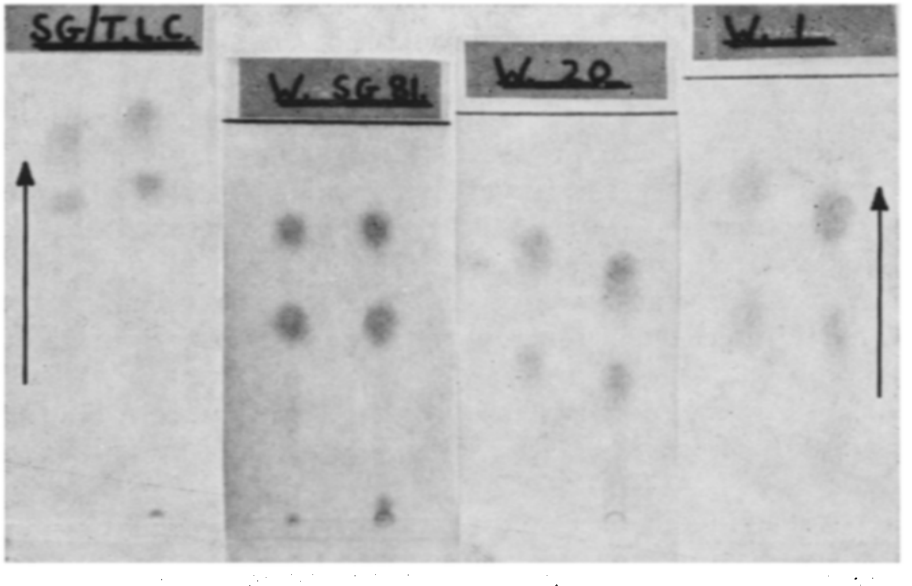

Fig. 2. Phenol separation. Solvent: 2% aqueous acetic acid.

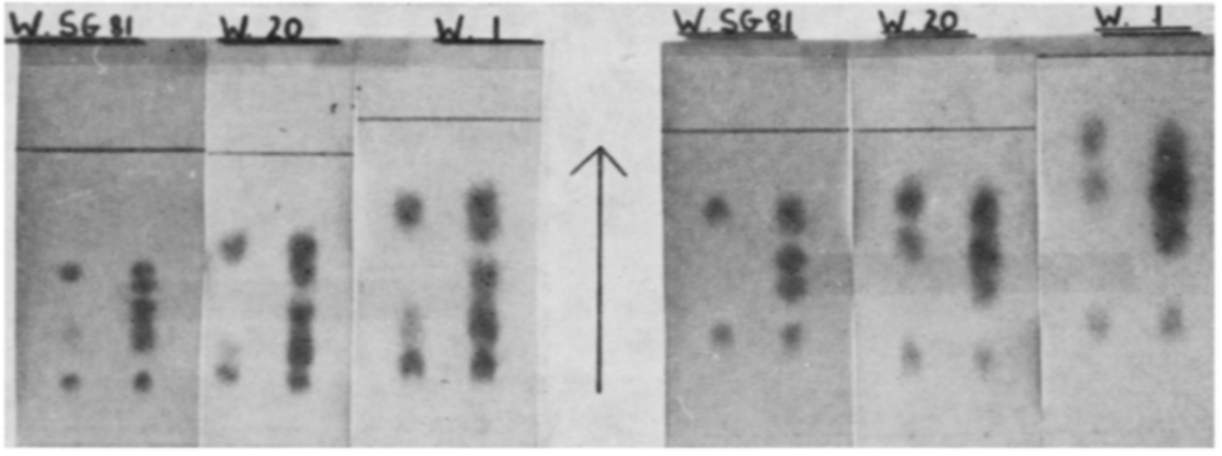

Fig. 3. Separation of amino acids. The left-hand spot in each case is a mixture of three, aspartic acid, leucine and lysine; the right-hand spot contains, in addition, alanine, glycine, threonine and valine. Solvents: left hand, IPA–formic acid–water (80:5:20); right hand, phenol–methanol–4 *N* NH_4OH (25:50:25).

solvents. Complexing components may also be introduced into solvent systems with confidence, which is illustrated in Fig. 4.

Development times are regarded as acceptable for all these solvents, the 2% aqueous acetic acid requiring 50–60 min, the phenolic solvent 2 h 15 min and the IPA–formic acid–water solvent up to 4 h.

A further advantage of this paper is that it shows less fibre orientation effect with respect to solvent flow so that for the development of two-dimensional chromatograms, direction of development has a minimal effect on solvent flow.

The extension of these studies to two-dimensional separations illustrated some interesting aspects of reproducibility. For the development of amino acids with the phenolic and isopropanol solvents it appeared that greater reproducibility was obtainable when the phenolic solvent was used as the second solvent. This was believed to be due to the difficulty in removing phenol from the paper after the first run and the retention of a variable amount of ammonia from this solvent by the silica gel.

In the case of two-dimensional separations of phenols, it was found that neater

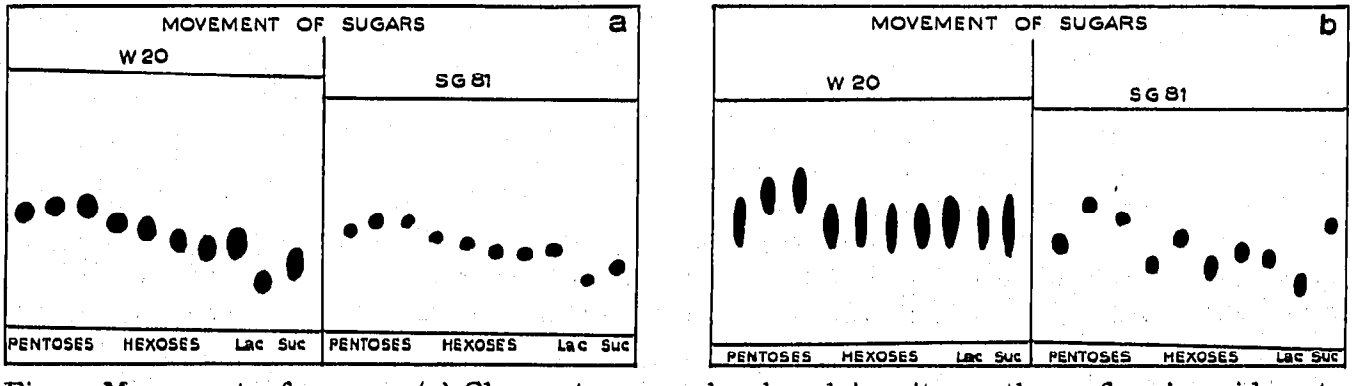

Fig. 4. Movement of sugars. (a) Chromatograms developed in nitromethane–formic acid–water (60:25:15) and (b) chromatograms developed with methanol–4 *N* ammonium hydroxide containing 6 g of boric acid per 100 ml of solvent. The spots were applied in the order: arabinose, rhamnose, ribose, xylose, fructose, galactose, glucose, mannose, lactose and sucrose on each chromatogram.

spots were obtained when the more polar solvent, such as 2% aqueous acetic acid, was used as the second solvent. It was assumed that the more polar solvent was able to open up the cellulose structure to a greater extent, so that if the more polar solvent was used first, the drying of the chromatogram between the developments could in effect 'lock' part of the phenols in the cellulose structure.

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