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THE APPLICATION OF PAPER CHROMATOGRAPHY TO FORENSIC CHEMISTRY*

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Paper chromatography since its rediscovery barely ten years ago has been applied in every branch of chemistry for the separation, purification, and identification of a great range of compounds. The number of subjects and materials that fall within its sphere of influence increases daily. While there is no general theory that covers all aspects of the separation of compounds on paper it is useful to think of this separation as being due to differences in the partition coefficients of the compounds between the eluent phase and the stationary phase held in the paper fibres. The method offers a rapid means of comparison, and in many cases an identification, on a few micrograms of material and is obviously a very powerful tool in the hands of the forensic chemist.

It is not possible here to review all the literature that is of interest in this field; indeed, it is the intention only to give a few examples in which from personal experience paper chromatography has proved to be an invaluable aid.

The problems that face the forensic chemist are peculiar in that it is rare for him to have sufficient material of high enough purity to obtain such data as % carbon, % hydrogen, % nitrogen, mixed melting points, and all the usual criteria required for a complete analysis. Instead, the materials with which he deals are more often mixtures of similar oils, greases, drugs from natural sources whose active constituent has not yet received the attention of the research worker, or minute quantities of drugs and metabolites isolated from viscera. He is consequently forced to resort in many cases to such tests as empirical colour reactions and physical comparisons in an effort to compare or contrast his sample with supplied control substances or a sample of known origin. It is in this field that paper chromatography provides a valuable addition to his armoury. Its advantages may be summarised as follows.

1. The method offers a ready separation and comparison of similar

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compounds on a microgram scale. Consequently in the case of toxicological specimens, for example, traces of drugs and their metabolites are easily separated from one another and from such impurities as fat, and normal body breakdown products.

2. The Rf values, i.e. $\frac{\text{distance of travel of spot}}{\text{distance of travel of solvent,}}$ of the compounds under investigation in several solvent systems provide a set of numerical values that taken into account with other considerations are very often specific.

3. After the separation on paper many colour tests on the now pure material designed to show up functional groups, as well as such physicochemical properties as quantitative UV adsorption, provide valuable data as to chemical identity.

4. Only micrograms of material are required and, while the method as usually performed is only roughly quantitative, it is very easily made highly quantitative.

5. Where compounds having unknown physical properties are involved the active fraction can easily be traced on the micro scale prior to a macro work up.

6. Because such a small quantity of material is used up the bulk of the sample can often be retained—always a matter of great importance in forensic work.

The mechanics whereby the solvent is caused to flow over the paper are largely governed by personal choice coupled with a desire to obtain maximum resolution of the spots. The solvent can flow vertically—ascending or descending—or horizontally. Sufficient is it to say that no more than the paper, the solvent, and some type of container are required.

The Rf values and techniques for a vast number of different classes of compounds have appeared in the literature, but, in the author's opinion, it is essential to use published Rf values only as a guide and to run one's own control specimens side by side with the unknown. This will have particular appeal to the forensic chemist.

The paper itself usually requires little pre-treatment although the author invariably washes before use with acetic acid followed by distilled water, as advocated by Hanes and Isherwood(1).

Care must be taken in the interpretation of chromatograms as it is not unknown for a pure compound to give more than one spot(2, 3) or to change in Rf with the pH of the solution in which it is applied to the paper(3, 4). This emphasises the importance of running exact controls. While the solvents and types of paper used for the actual

development are largely governed by experience, it is possible here to give some general headings for the techniques of identification. These are:

1. Natural colour of substances under investigation.
2. Behaviour in ultraviolet light.
3. Use of chemical reagents designed to produce colour or changes in behaviour under UV.
4. Biological tests on the paper or on eluted spots.
5. Radioactivity detected by counts or autoradiography.

These methods are largely self explanatory, but it is perhaps rewarding to consider some of the types of spraying or dipping agents that are used to show up the position of the spots as visible colours. These can be generally regarded as non-specific and those that show varying degrees of specificity. The former include potassium permanganate-sulphuric acid(5), and the forensic scientist will not be surprised that iodine both as vapour and as a spray is often used. Showing more specificity are ninhydrin for amino acids, certain classes of amines(6) and ammonium salts of fatty acids(7); ferric chloride for phenols(8) and penicillin derivatives(9); and ammoniacal silver nitrate for reducing compounds. Indicator solution is also used to show up acidic or basic spots. In the more highly specific class may be mentioned that for α glycols where a sodium periodate cleavage to the dialdehyde is followed by the appearance of a pink colour at the site of the spots on spraying with Schiff's reagent(10).

From these few examples it will be seen that while the separation of compounds on paper in several solvent systems, in one and two dimensions, provides highly characteristic Rf values, there can be coupled with these values an ever increasing number of spot tests so that the degree of final identification is very high indeed.

To turn from the general field to the subjects of interests in forensic chemistry is not a big jump as the following examples will prove. The original experiments in paper chromatography took place nearly one hundred years ago and were used to separate dyestuffs. This provides a link with present day practice where compounds that have no colour are converted into coloured derivatives either in solution before development or actually on the paper itself. The separation of the individual cresols and phenols in that favourite suicide draught—disinfectant—may be accomplished by diazotisation followed by a separation of the coloured diazo derivatives(11). It may in some cases be necessary to decide whether the cresol mixture recovered from the stomach has come from a certain bottle, and a rough quantitative analysis is easily

carried out by visual inspection after development of the chromatogram. Alcohols (12), aldehydes, and ketones (13) may also be converted to coloured, non-volatile derivatives before chromatography. The separation of the dyes in writing inks on paper is an additional aid in the hands of the document examiner (14). The larceny of some sweets by a youth provided an opportunity for the comparison with the stolen sweets of sugars present in three minute crystals and some gummy material in the suspect's pocket. The chromatographic separation of different sugars and their appearance in toffees and sweets has been accomplished (31), and the exact similarity of the paper chromatographic pictures of this gum with the sweet not only showing the component sugars but their relative concentrations is an example of how paper chromatography may give information unobtainable by any other means. While the presence of natural or induced colour in the compounds is an advantage, it is by no means essential. Compounds that absorb or fluoresce in UV radiation reveal their presence on the chromatogram where they can be photographed by placing the paper chromatogram on a sheet of photographic paper and exposing to ultraviolet (15). Adsorbent spots on developing the photograph show up as white spots on a black background. A comparison of oils and greases transferred in motor accidents from the vehicle to the victim is easily accomplished by a paper chromatographic separation followed by inspection or photography in UV light. The method was also used by the author recently in a case involving the larceny of bicycles where a comparison of oil and grease scrapings was desired. In most of the work done on paper chromatography the paper holds the water rich phase, but it is sometimes necessary to reverse the procedure and make the paper hold the water poor phase—such is the case in the separation of different resins reported recently (16).

It is, however, to the toxicologist that the applications of paper chromatography provide a very material aid. The advent of new drugs having a very high specific activity and consequently a relatively low lethal dose has presented the toxicologist with the need for the classification of poisonous material not on a milligram but on a microgram scale. The use of paper chromatography provides the answer. The identity of the poison can be found with a higher degree of certainty and using at the very most only one tenth the quantity by paper chromatography than by the older means of arbitrary colour tests. This is because one has not only a set of colour tests on the pure material on the paper but also a set of very highly characteristic numerical Rf values. The importance of a paper chromatographic separation of the

wanted material from all impurities, before the colour tests are applied, cannot be overestimated. Some measure of the resolving power of paper chromatography is revealed by a study of the amino-acids and peptides separated on a single two dimensional chromatogram by Dent(17) where no less than 61 very similar compounds are resolvable using this technique. Another example is the separation of the 2′ and 3′ phosphates of various nucleosides on paper(18). The necessity of extracting as pure a sample as possible is not removed by the use of paper chromatography. It is with the final gum or oil, perhaps less than 100 μg in quantity that paper chromatography will be of most use and may well provide the answer as to its identity.

From the acid ether extraction the main bulk of toxicological specimens are made up by the barbiturates. This class of compounds often cause coma, and death may be delayed for several days. The quantities of drug remaining in the organs is often hence reduced to a very low level. The separation and identification of some barbiturates has been accomplished(19), and a method based on these reports has been in use in the author's laboratory for some time now. In one case a person died shortly after the ingestion of an anti-asthmatical drug containing phenobarbitone (⅛ gr.). From 100 g of liver was isolated sufficient barbiturate to show on paper chromatography the presence not only of phenobarbitone but also of another barbiturate very probably nembutal. From the densities of the spots it was possible to say that the quantities agreed with the consumption of two different barbiturates in ⅛th grain quantities. It will be noted that these quantities (40 μg and 20 μg) are well below the values necessary for the cobalt acetate / isopropylamine reaction. In another case the victim died from strangulation, and the pathologist was of the opinion that this occurred during sleep. Confirmation of this view was obtained by the isolation from 10 g of blood (only sample available) of a specimen that behaved on paper exactly as amytal and was in therapeutic concentration. The presence of barbiturate metabolites, as well as the unchanged drug, has been demonstrated in several specimens in which death had been delayed, and this suggests that colourimetric comparison methods for the quantitative determination of barbiturate in toxicological specimens will be unreliable if done prior to initial purification by chromatography. In the ether extraction from acid solution may also be found the sulphonamides, and their separation and identification has also been demonstrated using paper chromatography(20). The absence of a sulphonamide from a whole chicken was shown in a case involving the larceny of some birds, that, it was alleged, had been given a proprietary sulphona-

mide administered as a bactericide. The estimated maximum quantity in the whole bird was of the order of 40 μg. And while this is much too small a quantity for classical analysis it would have been sufficient to provide not only an identification of the particular sulphonamide, had it been present, but also a quantitative assay, by the use of paper chromatography.

In the chloroform extract from alkaline solution may be found among others the alkaloids and many basic synthetic preparations. Different classes of alkaloids have been examined by this technique(21), and general considerations of the paper chromatographic separation of alkaloids have been reviewed(22). The selection of suitable solvent systems and identification reagents for use by the toxicologist with this class of compound is being reviewed in this laboratory and sufficient has already been achieved to provide an unambiguous identification of very many alkaloids on a 2-5 μg scale. Also included are such compounds as the cocaine type of benzoic esters(23) and many compounds having amino groups in the molecule such as ephedrine, amphetamine(24) and also adrenaline(24, 2a, 25). One example in which paper chromatography was used with advantage was in a case where the suicide had the choice of atropine or one of the new anti-Parkinsonian synthetic drugs. It was found to be extremely simple to show not only the absence of atropine in the organs but also to confirm the presence of the synthetic drug plus several of its metabolites. No reliance could in this case be placed on the colours with classical alkaloidal reagents for two reasons—the minute quantity available because death had been delayed, and also the absence of any data on the results of these colour tests with the synthetic drug in the presence of its metabolites.

While the compounds mentioned above that have been extracted by the acid ether and the alkaline chloroform provide the bulk of the specimens there are other examples in which paper chromatography has been used in this laboratory, or of which the author feels use can be made in the future. In cases of greyhound doping the smallness of the dose, perhaps coupled with death after the strain of running, reduces the quantity available for examination once again to microgram amounts. Digitalis provides a good example of this type of drug and also how its metabolism to the genin alters the significance of a biological test. Paper chromatography however has been used to separate and identify the members of this family(25), and has been used in this laboratory as an aid in this type of investigation. Other sterols of interest to the forensic chemist that have been investigated include

natural oestrogens(26) and sapogenins(27). The antibiotics have not been neglected, and in one case the presence of both procaine(28) and penicillin(9) was demonstrated in the dregs of a sample of procaine penicillin where death followed the injection of a therapeutic dose.

The amino acids provide an example to illustrate the potentialities of the method, and disclose an increasing number of natural products that can be compared after their chromatographic separation on paper. The separation of amino acids in urine(29) offers perhaps the first hope of an easy comparison of this type of body fluid and its relation to the individual. The amino acid constituents of gastric juices and saliva have been shown to be different(30), and soon no doubt examples will appear of this type of work being used by the forensic chemist.

In the inorganic field there have been many papers on the separation of both anions and cations, and there are many applications of this type of separation that will interest the forensic chemist and the toxicologist.

It is hoped that these few examples and suggestions will draw attention to the potentialities of the technique and the horizon of the forensic chemist will by its use be even further extended.

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