

# SEPARATION IN ORGANIC CHEMISTRY

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## ABSTRACT

Separation methods used in organic chemistry are briefly reviewed, emphasis being placed on aspects that are frequently ignored, and special attention being paid to the analytical applications of separation. The relevance of physical, inorganic and analytical chemistry for organic chemistry is stressed.

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The term 'separation' can be used in analytical chemistry in both a literal and figurative sense (an idea that is due to Professor Čůta<sup>1</sup>). In figurative separation, use is made of some difference in properties of the individual components of a mixture in order to determine one of them without physical separation from the others. In this sense, a selective titration or a spectrophotometric measurement may be a 'separation' in that no other species is measured together with the one of interest. The object of this paper is to present a survey of both literal and figurative methods of separation in organic chemistry, particular attention being paid to certain features which are often forgotten in application of the methods.

## PHYSICAL SEPARATION

Physical separation may be used for isolation, purification, identification and determination of organic compounds. It is, of course, generally a two-phase process, and can be classified into solid-liquid, liquid-liquid, gas-liquid and gas-solid systems. An exception is electrophoresis, which takes place in a single phase. Applications of physical separation may cut across these categories—for example, phase titrations may result in separation of a solid or a liquid phase from the initial liquid phase. Similarly, some of the factors associated with physical separation have implications for figurative separation.

### Solid-liquid systems

A major application of these is in precipitation methods, which can be taken to include crystallization techniques, and they will mainly be used for isolation and purification. It is instructive to consider what degree of purity is likely to be required. If we take an organic compound containing 60 per cent carbon, then for an analysis (assumed to be made without other errors) to fall within the accepted limits of  $\pm 0.3$  per cent carbon the compound must contain not more than 0.5 per cent of a non-carbonaceous compound, i.e. it must be at least 99.5 per cent pure. If it is contaminated with carbonaceous material, however, a lower level of purity may occur without detection by analysis. Thus such a

compound recrystallized from glacial acetic acid (40 per cent carbon) could contain 1.5 per cent of the solvent and still show a 'correct' result; for benzene (92 per cent carbon) as solvent, one per cent of it could escape detection. This is an oversimplification, because the addition of experimental error to the bias introduced by the impurity would be as likely as not to make the result fall outside the limits of error, but there is still a 50 per cent chance that the impurity would escape detection. Only if the amount of impurity were doubled would it be fairly certain to be found. It follows that for application of classical microanalysis, a purity of 99.5 per cent or better is required. We shall return to this point later. A further complication is the possibility of segregation of impurity so that the sample taken for analysis may contain more or less than the average amount. The smaller the sample, the less the chance of choosing a more heavily contaminated portion, but the more disastrous the consequences if such a portion is chosen.

There is a considerable body of information and instruction available on crystallization of organic compounds<sup>2</sup>, with emphasis on the art of inducing crystallization to occur when it does not want to. There is sometimes a feeling that the art is dying and science left out, and that some organic chemists, especially research students, do not think hard enough about what they are doing. For example, although the nature of the precipitation process and of contamination have been extensively studied in inorganic and physical chemistry, the results are not always applied as they might be in organic chemistry. It is ironic to reflect that the technique of precipitation from homogeneous solution was first used in organic chemistry, for example in precipitation from aqueous alcohol by addition of water to the pure alcohol solution until a precipitate appears (i.e. a phase titration, see below) followed by warming to redissolve the precipitate and then cooling until crystals appear, yet an organic chemist has been heard to observe that a compound *must* be pure because it was obtained by precipitation (irrespective of the conditions used). Very often, the same solvent system is used for repeated recrystallization in an attempt at purification, even though the solvent itself may be strongly adsorbed (especially glacial acetic acid) or the adsorption isotherm for an impurity favours extensive co-precipitation. The reason usually given is that the compound is too soluble in other solvents; for analytical purposes, however, it is purity that counts, not the yield, and recovery of a small fraction of pure material could be achieved from almost any solvent, and the remainder could be recovered by evaporation of solvent. Glacial acetic acid is notoriously hard to remove if it has been used as solvent, but recrystallization from another solvent will often reduce the contamination to an acceptable level. Mixed solvent systems for precipitation from homogeneous medium might also be explored rather more extensively. Another possibility for improving the purity of an analytical sample is due to the late Professor Gordon<sup>3</sup>, and consists of careful control of the crystallization process and separation of that fraction which will be least contaminated. For example, co-precipitation may occur extensively on the first portion of precipitate formed, or may be delayed until precipitation is almost complete (*Figure 1*). True, this is difficult to realize in practice, but it might be worth exploration.

The second major application is in chromatography, whether by adsorption on columns or thin layers, by ion-exchange, or by gel-filtration. Here it may

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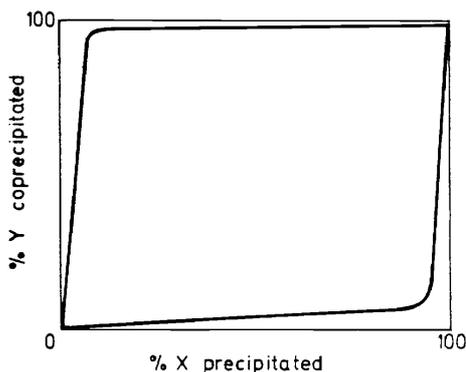


Figure 1. Diagrammatic representation of two extreme types of co-precipitation of Y with X. Collection of only a fraction of the precipitate will give a product that is less contaminated.

happen that a large volume of solvent is necessary for elution of a small amount of compound, followed by evaporation of the solvent. The solvent may contain sufficient impurity itself, or extract enough material from a column of support, to leave a significant amount of contamination behind when it is removed, and it is certainly desirable to use a further purification process before use of the material for analysis. The presence of 1 p.p.m. of impurity in 500 ml of solvent yields  $\sim 500 \mu\text{g}$ —ten per cent of a 5 mg recovery! The possibility of specific interaction with the solid phase must not be forgotten—*isomerization, cyclization, oxidation, condensation* etc, and even total destruction, may occur. All too often a single and unsuitable solvent is applied to develop a chromatogram and the resultant single spot of unresolved mixture is regarded as evidence that a single substance is present. At least two solvents of differing polarity should be tried, and the starting materials and likely byproducts tested individually under the same conditions.

Zone refining<sup>4</sup> is a technique for purification that is perhaps not used as much as it might be, probably because the usual scale of working in organic chemistry is too small. Some interesting possibilities have been suggested by recent work on organometallic complexes, notably metal  $\beta$ -diketonates<sup>5</sup>, and it is interesting to speculate on the possibilities of deliberate addition of ions to remove impurities that can form metal complexes.

### Liquid-liquid systems

Solvent extraction is the obvious example of these systems, with its variants such as countercurrent extraction and partition chromatography. Although adjustment of acidity has long been exploited for separation of compounds containing suitable functional groups, and extraction of organometallic compounds is commonplace in analysis, little seems to have been done to utilize metal complexes for isolation and purification of organic compounds containing suitable ligand groups and atoms. A similar approach that *has* been used is the formation of extractable ion-association compounds between large cations such as tris (1,10-phenanthroline) iron(II) or tetraphenylarsonium and organic acid anions<sup>6</sup>. Extraction of small amounts of impurity is fairly easily dealt with by washing with the initial solvent. If A is 99 per cent extracted into C from D,

but B is only one per cent extracted, then washing the extract with pure D will lower the concentration of B in C to 0.01 per cent of its original concentration in C.

Possibilities for exploration include the use of low-melting solids for extraction at temperatures above their melting point, followed by cooling and separation of the solid extract<sup>7</sup>, and the exploitation of polarity by use of mixed solvents (cf. the mixotropic solvent series in partition chromatography).

Phase titrations are an example of two-phase systems used in analysis, and can be either liquid-liquid or liquid-solid systems. Such methods are laborious because they require a considerable amount of calibration, and are restricted in scope to simple mixtures of known qualitative composition, but can be very useful for routine analysis of suitable systems<sup>8,9</sup>.

### **Gas-liquid systems**

Partition gas chromatography has become of much greater importance than distillation, and when coupled with other techniques such as mass spectrometry, infra-red spectroscopy etc, offers a very powerful tool indeed. For some purposes, however, distillation may still be the better method, especially if relatively involatile compounds are to be separated, when distillation at reduced pressure may solve problems of thermal instability.

As remarked above, chromatography of any kind, as a test of purity, should be used with caution, and the behaviour of likely impurities and byproducts and of the starting materials should be tested under the same conditions as the test compound; alternatively, but perhaps less satisfactorily, the test sample alone should be chromatographed on more than one stationary phase. In preparative gas chromatography, the possibility of bleed of stationary phase from the column must be considered, and the fractions collected may need further purification.

### **Gas-solid systems**

Adsorption chromatography and sublimation come under this heading. Interaction with the absorbent must be watched for in the former, and in the latter attention must be paid to the possibility of thermal decomposition giving volatile products that may be condensed along with the sublimate. It should also be remembered that hygroscopic substances can still sorb water from the atmosphere after sublimation, unless the operation is done in a dry atmosphere.

### **Mass spectrometry**

Although mainly used as a tool for analysis and for structural investigation, the mass spectrometer could in principle be used for isolation of certain species, though the process would be rather expensive, limited in scope and wasteful because of fragmentation of the parent compound. A major analytical application is the detection of impurities; if the peaks corresponding to these are well separated from the main spectrum, detection limits of the order of parts per million may be attained. It is easier to detect impurities if they have molecular

weights higher than that of the major component; lower homologues may not always be detected as impurities unless present in relatively large amount. The substance to be determined must have a certain degree of volatility, of course, but even involatile compounds can be examined if flash pyrolysis or a suitable chemical reaction can provide volatile products, and the presence of volatile impurities is easily detected. A more recent use of mass spectrometry, and one that has gained acceptance by the journals, is for determination of elemental composition of compounds.

### FIGURATIVE SEPARATION

Figurative separation involves a means of measurement which will also provide differentiation between components of a mixture, and this usually means use of spectroscopy or electrochemical methods.

#### Spectroscopic methods

Ultra-violet, visible region and infra-red absorption spectroscopy are well tried techniques in organic chemistry, and problems really arise only when mixtures with overlapping spectra are to be analysed, or if impurities contribute a background spectrum. Traditionally such problems were solved by use of simultaneous equations applied to a series of measurements at suitable wavelengths, but naturally errors were propagated rather rapidly as the number of measured components increased. The advent of the computer and the cathode-ray oscillograph has led to the development of spectrum simulators in which a series of spectra can be varied at will and combined until they match the observed spectrum, but it is still necessary to have a good idea of how many contributory spectra are important ones, as otherwise a family of solutions might be possible. One approach to the problem is to treat the individual absorption peaks as Gaussian in shape and to analyse the peaks one by one across the spectrum. Again a certain amount of caution is necessary in interpretation, lest an impurity should have a single peak that coincides with a peak from the major component, and so not only escape detection itself but cause an error in the determination of the other component. It is frequently assumed that if a background spectrum appears superimposed on that of the substance examined, all that is needed is to compare the spectrum with that of pure substance and assume that the contribution of the impurity to the peak of interest is a linear function of the wavelength. This assumption may not always be justified, however; and various mathematical methods have been proposed for resolving the problem, usually by a trial and error fitting of other simple mathematical functions of absorbance with respect to wavelength<sup>10</sup>.

A further problem arises in connection with the sensitivity of the methods as a means of detecting impurities. The relationship between absorbance and concentration is a logarithmic one, and the reading errors are minimal over a relatively short portion of the absorbance scale. Even when differential methods are used, the experimental error in determining the concentration is likely to be a few parts per thousand, and the method will not detect amounts of impurity much below say 0.5–1 per cent.

Fluorescence spectroscopy is a much more potent tool for detection of impurities, since the intensity of the light emitted is a function of the intensity of the exciting radiation, and very small amounts of fluorescing impurity (in the ng region) can be detected in a non-fluorescent matrix. A somewhat similar potential lies in laser-excited emission of infra-red radiation, since a very intense radiation can be realized and a long excitation pathlength used<sup>11</sup>. There are also several possibilities for improving the selectivity of fluorescence methods. Enhancement or quenching of fluorescence can be achieved by change of solvent medium, change of pH, coupling and redox reactions etc, and both the excitation and emission spectra can be used for identification purposes<sup>12</sup>.

Nuclear magnetic resonance spectroscopy is now a favourite tool for elucidation of structure, especially the identification of functional groups and determination of the number of them present. The snag is that while the method is invaluable for obtaining information about the major component(s) of a mixture, it is not sufficiently sensitive to yield information about minor or trace components (i.e. present in amounts below about two per cent). Consequently it may happen that a research worker prepares a compound of sufficient purity to yield the n.m.r. spectrum of the major component only, decides that the compound is the one intended, and submits the unpurified product for elemental analysis only to find that the results do not agree with the expected ones within experimental error. The reason is obvious. As pointed out above, the minimum purity required for a sample suitable for analysis is at least 99.5 per cent, and although the research worker knows that he has made a particular compound, the analyst must perforce analyse the material supplied as a sample, and if this happens to be a mixture the results will be number averages derived from the relative amounts and compositions of the components of that mixture. In such a case both the analyst and the research worker are 'right'—the one in providing analytical results that are not those expected, and the other in having correctly identified the main component of the sample.

### **Electrochemical methods**

Polarography and its ancillary techniques such as oscillographic polarography, voltammetry etc, are useful in many applications in organic chemistry and analysis. The anodic and cathodic reactions possible with many functional groups provide considerable scope for differentiation of components of mixtures, and the sensitivity of the methods is very useful in trace analysis.

### **Differential titration**

By suitable methods, such as spectroscopic or conductometric determination of the end-points, it is possible to titrate a strong and a weak acid in succession, and mixtures of such organic acids can be analysed. The strength of acids which are too weak for normal titration can be increased by various means, such as change of solvent, or use of a metal ion to form a complex with accompanying release of protons. In some circumstances differential redox reactions may be possible.

## COMBINATION OF METHODS

When one or other of the two types of separation cannot alone resolve the composition of a mixture, a judicious combination of the two may provide the answer to the problem. For example, solvent extraction of a metal complex of an organic compound has been followed by atomic-absorption determination of the metal in order to determine the organic moiety of the complex<sup>13</sup>. Again, the products of electrolysis at the dropping mercury cathode have been identified by submicroanalysis and gas-liquid chromatography<sup>14</sup>. Selective extraction or chromatographic separation is often used to simplify complex mixtures before spectroscopic or other determinative methods are applied. Precipitation is often used to remove unwanted material before working up the main product, or using an analytical procedure with which the foreign material would interfere. Many reactions in which organic reagents are used for inorganic analysis can be turned round and used the other way, as Feigl has repeatedly shown.

To sum up, while organic chemistry has often provided fruitful ideas for inorganic and analytical chemistry, there are many respects in which it may learn from these branches of chemistry, and two-way traffic of this kind can only be of benefit to chemistry as a whole.

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