Urea clathrates of fatty acids in thin-layer and paper chromatography

Clathrate formation has been suggested as a method of fractionation in chromatography and CASON *et al.*[1] succeeded in separating straight chain from branched chain fatty acids by means of urea columns. Though clathrate formation cannot be taken as a general method of fractionation, its application to thin-layer and paper chromatography of fatty acids may be of certain interest and provide a quick evaluation of the nature of an unknown acid and achieve separations which may simplify the determinations of complex mixtures of various acids.

The investigation has been carried out by means of cellulose papers impregnated with urea and with urea–calcium sulphate thin layers. The former were strips of cellulose paper (Whatman No. 3) impregnated with a 20% methanolic solution of urea and dried under normal atmospheric conditions, and the latter glass plates coated with a mixture of urea and calcium sulphate. Urea thin layers cannot be obtained unless a binding material such as calcium sulphate is used. The binding agent (25 g) was mixed in a mortar with 60 ml urea solution to make a paste which was then spread on glass plates. Uniform and smooth layers were obtained when 20 to 40% urea was used with the above amount of calcium sulphate. The plates were dried at room temperature.

The chromatograms were run at room temperature by the ascending technique. After the eluant had reached a certain height, the strips were taken out of the chromatographic vessel and the acids detected as yellow spots on a blue background by spraying with alcoholic bromocresol purple and exposing for a short time to ammonia vapours.

A large number of acids were investigated. Identical results were obtained with the impregnated papers and the chromatoplates. The main features of the investigation are summarized below:

1. The fatty acids up to C_{10} moved towards the front. Similar behaviour was observed when the clathrate forming material (urea) was not used.

2. Fatty acids with a number of carbon atoms higher than C_{16} did not travel at all and stayed at the starting point. Lauric and myristic acids were found near the starting point.

3. *Trans* and *cis* fatty acids could be separated as the former were clathrated easily while the latter did not enter the clathrate channels with ease. This was observed using a mixture of elaidic and oleic acids which yielded two spots, one for elaidic acid at the starting point and the other for oleic acid travelling almost with the front. Other unsaturated fatty acids (linoleic, linolenic, etc.) behaved in the same way.

4. Oxyacids, with short or long carbon chains, were not clathrated, *e.g.* ricinoleic, citric, tartaric acids etc. travelled with the front.

5. The introduction of a certain group in an organic molecule did not affect the efficiency of clathrate formation, no matter what kind of functional group, *e.g.* hydroxyl, carbonyl or halogen, was present. The longer the C chain the better the clathration. If there was branching or a cyclic ring at the end of an aliphatic chain the formation of the clathrate depended on the chain length and unsaturation.

Chromatography thus provides another method of clathrate formation besides the already existing conventional solution techniques, in which clathrates are prepared by addition of a small amount of the compound to be clathrated to a saturated

methanolic solution of urea. When there was no clathrate formation, the guest molecule remained a free-moving sample and moved along with the front. Clathrate formation is not considered to be a stepwise equilibrium reaction but a molecular addition between the host and the guest molecules where the shape and size of the components are the determining factors.

The results clearly show that oleic and the lower saturated fatty acids could be separated from other saturated fatty acids, at room temperature, by taking advantage of the preferential formation of urea clathrates by the higher saturated components of the mixture. Separation of fatty acid is mainly concerned with chain length and unsaturation, while branched chains are seldom involved. The difference in clathrate formation is particularly pronounced with acids up to capric acid and from lauric to acids with longer chains.

It is interesting to note the different behaviour of oleic and elaidic acids. The *cis* double bond seems to cause a slight distortion in the long hydrocarbon chain and thus increases the diameter to a point where the fatty acid will not easily enter the host molecule as a planar molecule without considerable bending. Thus, this slightly greater spatial requirement in oleic acid differentiates it from other fatty acids in clathrate formation.

We suggest that the urea clathrate formation (or in general, this clathrate technique) may be successfully employed for fractionation of mixtures of organic acids as it gives a fairly sharp separation into two classes of acids.

We also investigated fatty acids using a 20% methanolic solution of thiourea. This clathrating agent shows a similar selectivity, reacting easily with cyclohexane or branched chain molecules but not with straight chain molecules. The fatty acids moved to the top of the thin layers and paper chromatostrips, and no clathrate formation was observed. This permits comparison of urea and thiourea for fractionations of the same compounds.

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