Beta-Cyclodextrin

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The first published reference to cyclodextrins was by Villiers in 1891. Subsequent work by Schardinger in 1903 is still acknowledged by the continued use of the term Schardinger dextrins. A detailed reference to the research conducted prior to 1957 has been published by French.[1]

The few people in manufacturing industry who have heard of cyclodextrins know of them as expensive fine chemicals. Recent advances in enzyme technology, coupled with increased scale production, have brought β-cyclodextrin (BCD) to a point where it can be used economically in food and cosmetic applications, although it cannot be considered as a cheap carrier. Many workers, however, are finding applications for its unique properties and stabilising ability. In such cases it is cost effective.

Cyclodextrin Chemistry

Cyclodextrins are produced by the action of the enzyme cyclodextrin transglycosylase (CTG) on a maltodextrin solution, produced by the digestion of starch with α-amylase. Three cyclodextrins are obtained: α-, β- and γ having 6, 7 and 8 glucopyranose units, respectively. The most common and most suitable for flavour application is β-cyclodextrin.

The 7 glucopyranose units are all connected in the 1:4 positions (figure 1). The glucosyl /O\ bridges point into the centre of the molecule. Figure 2 shows the BCD molecule as a torus shape with a high electron density in the centre. The primary -OH groups project from one edge, and the secondary -OH groups from the other. These factors combine to produce a molecule with a hydrophobic centre and relatively hydrophilic outer surface. Hydrogen bonding limits the solubility of BCD.

In solution both the outer and inner surface of the BCD molecule will attract water. Hydrogen bonding of the water molecules on the inner surface, however, causes distortion of the molecule. The hydrated BCD represents a highly energetic state and will readily accept a guest molecule in place of the water (figure 3).

The complex formation is effected by three factors:

—loss of water from the inner surface of the torus accompanied by reduction in energy
—ability of whole or part of guest molecule to fit into BCD torus
—reduction of energy state on transfer of guest from solution to hydrophobic environment.

As a rule the more hydrophobic the guest mole-

The Third International Symposium on Cyclodextrins will be held July 20-25 at the University of Lancaster, U.K. in conjunction with the International Meeting on Inclusion Phenomena. For further information, contact Dr. John F. Gibson, Royal Society of Chemistry, Burlington House, London W1V 0BN, U.K.

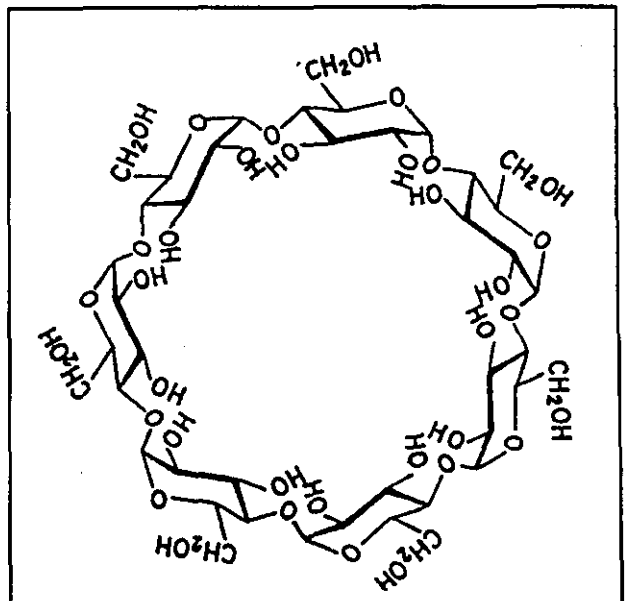

Figure 1. Chemical structure β-cyclodextrin[3]

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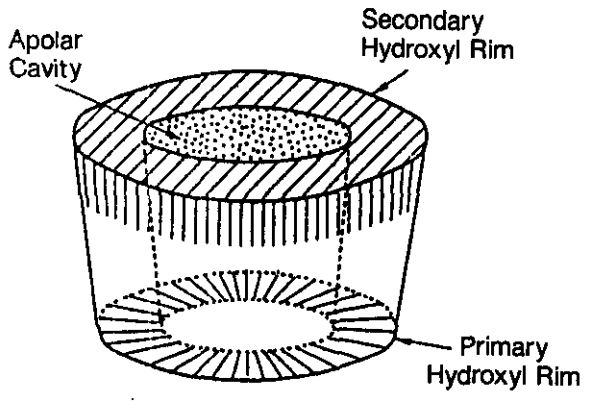

Figure 2. Molecular Shape β-Cyclodextrin[3]

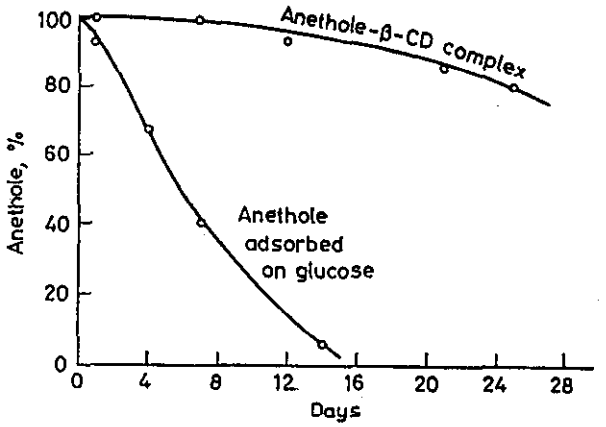

Figure 4. Loss of (a) Anethole (10%) (b) Vanillin (6.2%) From 2mm Layer Exposed to the Atmosphere at Ambient Temperature[3]

cule, or more insoluble, the more readily it will complex.

Typically ethyl alcohol or acetic acid, being readily soluble in water, will complex with BCD. In the presence of an essential oil, however, the less soluble oil molecules would complex in preference. An essential oil with limited solubility will complex easily providing it can be taken into solution. This can usually be achieved by continuous stirring or shaking. An insoluble product such as β-carotene would need to be dissolved by addition of a water soluble solvent. The more hydrophobic β-carotene will complex in preference to the water soluble solvent.

Complexes are normally less soluble than the BCD itself; the majority will crystallise out of solution at ambient temperature. They can be filtered and dried by most normal drying methods—fluid bed, vacuum and spray drying. In the few cases where a complex will not crystallise out, the solution can be freeze-dried or spray-dried.

The dry, crystalline complex will not release its guest molecule unless it is either heated above 200°C or dissolved. Once dissolved a portion of the complex will dissociate; the degree of dissociation depends on the nature of the guest molecule.

Complex Stability

Complexation will protect a guest molecule from loss by evaporation, attack on oxygen, light and UV, and intra or intermolecular reactions. A true crystalline complex of an odourous chemical should be odourless. Up to 20% of a guest, however, can be trapped within the crystal lattice but not complexed. This is not as stable as the bulk of the product and may give rise to limited losses.

The stability of both anethole and vanillin is shown in Figures 4 and 5. Samples of both products as BCD complexes and as mixtures with glucose were exposed to the atmosphere at ambient temperature. Complexation will "fix" a volatile material until such time as the complex encoun-

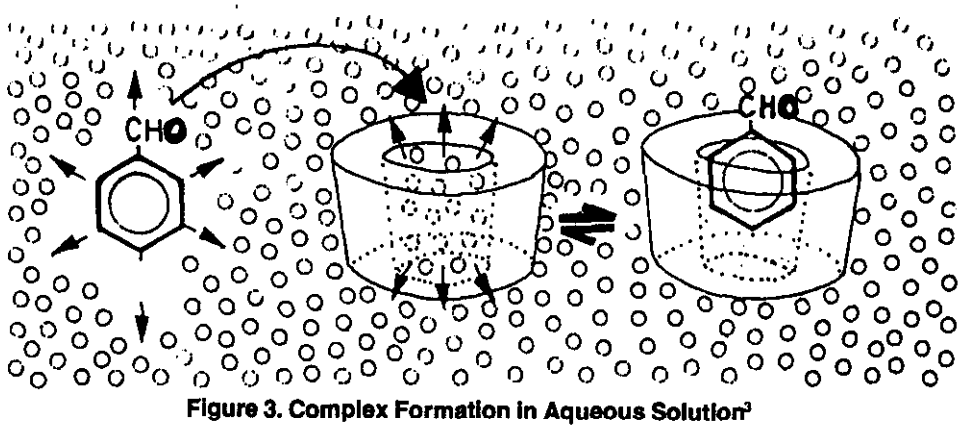

Figure 3. Complex Formation in Aqueous Solution[3]

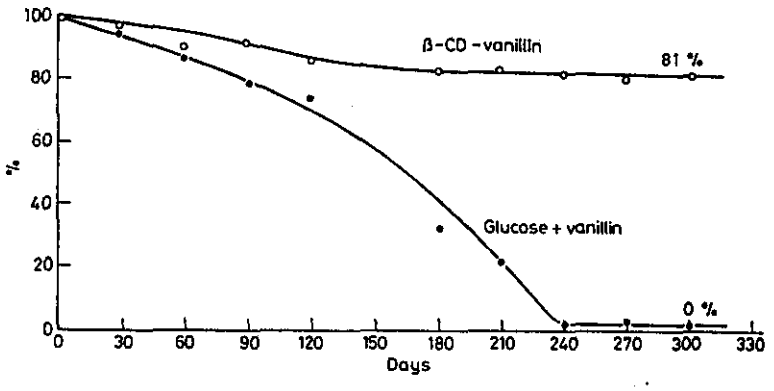

Figure 5. Loss of (a) Anethole (10%) (b) Vanillin (6.2%) From 2mm Layer Exposed to the Atmosphere at Ambient Temperature[3]

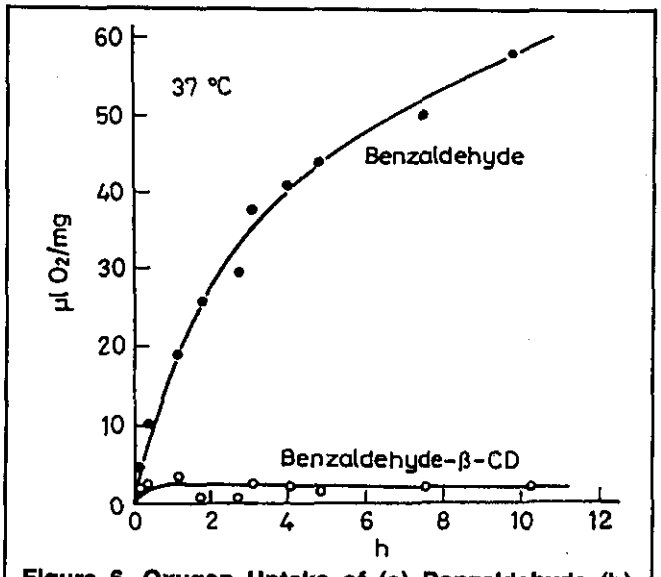

Figure 6. Oxygen Uptake of (a) Benzaldehyde (b) Lemon Oil. As measured in a Warburg Apparatus at 37°C in neat Oxygen

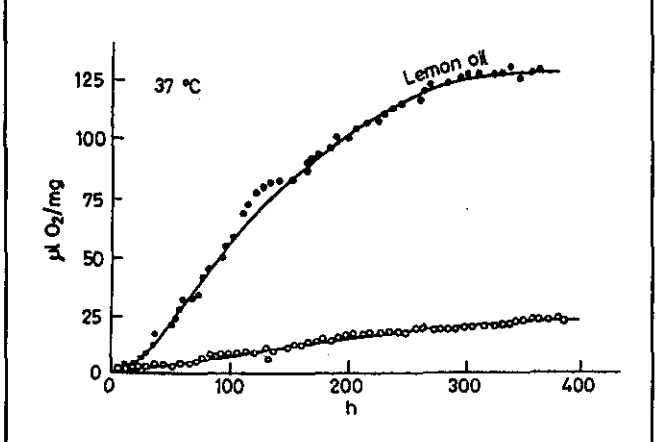

Figure 7. Oxygen Uptake of (a) Benzaldehyde (b) Lemon Oil. As measured in a Warburg Apparatus at 37°C in neat Oxygen[3]

ters a warm moist environment. It will then dissolve in the moisture and dissociate: for example, a flavour complex in the mouth or an aroma complex in talcum powder.

Resistance of complexes to oxidation is shown in Figures 6 and 7. The oxygen uptake in a Warburg apparatus is plotted at 37°C for both benzaldehyde and lemon oil. Complexation considerably reduces loss by oxidation.

The composition of a recovered flavour and/or aroma shows only minor changes when compared to the original. Figure 8 shows the chromatograms of an onion oil before and after complexation.

Complex Preparation

There are three common ways of preparing a BCD complex. In each case it is essential to dissolve the BCD and guest molecules. They will only be complexed in solution.

1. *Liquid–Liquid.* Stir or shake a solution of BCD (hot or cold, acid or alkaline) with the guest. If the guest is insoluble then it should be dissolved in a water soluble solvent. The solubility of BCD rises with temperature (figure 9). A typical complex of an essential oil could be made by preparing a 15% BCD solution—at 70°C—adding the essential oil and cooling while stirring or shaking. The crystalline complex can then be filtered and dried.

2. *Liquid–Solid.* Solid BCD and guest can be blended in a powerful mixer. It is necessary to add sufficient water to form a paste and mix for sufficient time to allow all of the BCD and guest to dissolve. Solvents should not be used in this method as there is no opportunity for an equilibrium to be established. This method is suitable for use with products such as oleoresins.

3. *Gas–Liquid.* Passing a vapour through a

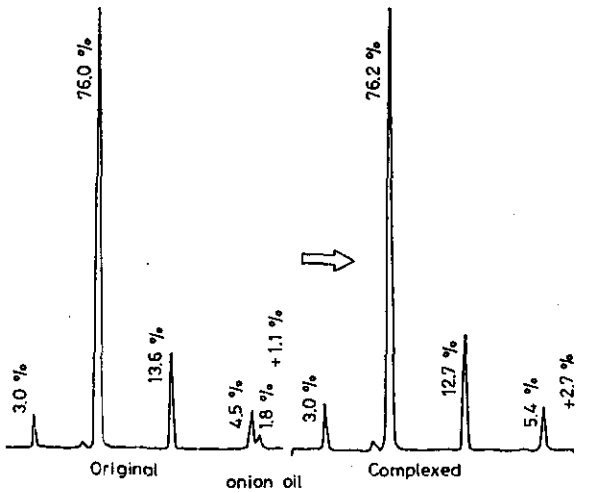

Figure 8. Gas Chromatograms of (a) Original (b) Recovered from Complex Onion Oil[3]

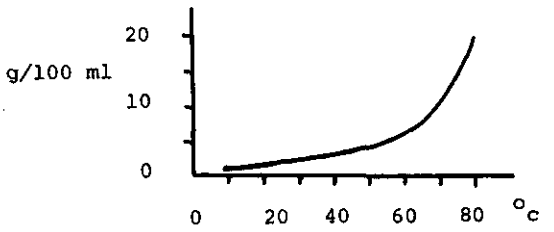

Figure 9. β-Cyclodextrin Solubility in Water[3]

BCD solution, hot or cold, is sufficient to complex many solvents or flavour chemicals present. The complex can either be separated by filtration or the volatiles recovered by steam distillation.

Szejtli et al.[2] has described equipment suitable for removing trace amounts of methylene chloride from large volumes of air (figure 10). This principle could equally be used to recover volatiles from an evaporation process.

The composition of a complex will depend on the molecular weight of the guest material. Normally complexes are 1:1, BCD having a molecular weight of 1134. If an essential oil has an average molecular weight of 110 then a 10% complex can be formed. Table I lists some common natural flavour materials and Table II some chemicals which form BCD complexes. With some materials it is not possible to form the theoretical 1:1 complex. Dimethyl sulphide should form a 5.5% complex; to date only a 2% complex has been reported. Hydrochloric acid will form a stable complex at between 1:1.5 and 1:2.

Cyclodextrin Status as a Food Ingredient

BCD is currently being manufactured in both Hungary and Japan. In Hungary it is permitted for use in food, and products containing BCD complexes are on sale. The Ministry of Health and Welfare in Japan regards BCD as a "natural food additive," and it has been permitted without restriction since 1976.

Under current U.K. legislation BCD can be

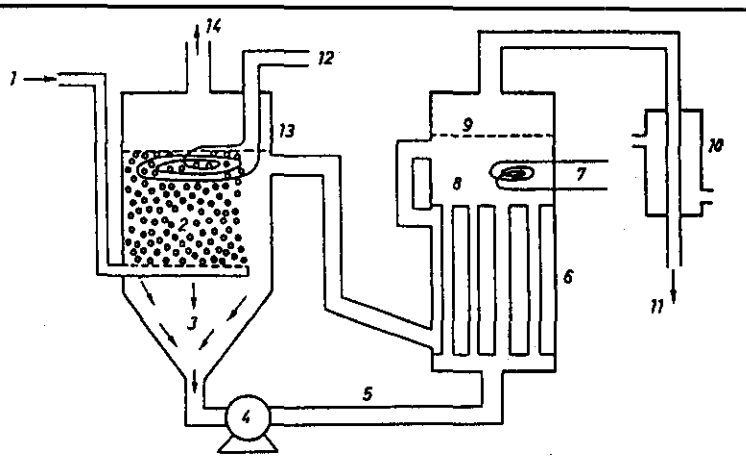

Figure 10. Schematic representation of absorption of solvent vapours by cyclodextrin solution.
1 = Gas/vapour phase of large volume; 2 = saturated cyclodextrin solution; 3 = settled crystals; 4 = pump; 5 = cold slurry; 6 = heat exchanger; 7 = supplementary calorifer; 8 = warm solution; 9 = solvent vapours; 10 = condenser; 11 = condensed solvent; 12 = supplementary condenser; 13 = cold cyclodextrin solution; 14 = gas phase of large volume with a highly reduced solvent content[3]

Table I. β-Cyclodextrin Complexes[5]

Flavour	% Content Complex
Vanillin	6.20
Dill oil	6.92
Marjoram oil	8.00
Benzaldehyde	8.70
Lemon oil	8.75
Cinnamon oil	8.76
Anise oil	9.00
Peppermint oil	9.70
Garlic oil	10.20
Caraway oil	10.50
Mustard oil	10.92

used in food, however new legislation is expected during the next two years. Application has been made to the Ministry of Agriculture, Fisheries and Food for the inclusion of BCD in the new proposed list.

It is understood that the French government has been asked to approve BCD and that U.S. FDA approval is also actively being sought.

Toxicology of Cyclodextrins

Orally administered BCD can be considered as a nontoxic substance. According to FAO Nutrition Meetings Report, Series No. 46A WHO/FOOD AD/70.36, in the case of enzymatically modified starches, toxicological tests are not necessary.

The following results have been published for BCD:[3]

Intravenous toxicity	LD_{50}	0.788gm/kgm (rats)
Per os toxicity	LD_{50}	12,500mg/kgm (mice)
	LD_{50}	12,000mg/kgm (rats)
	LD_{50}	5,000mg/kgm (dogs)
Intraperitoneal toxicity	LD_{50}	438mg/kgm (rats)
Subcutaneous toxicity	LD_{50}	505mg/kgm (rats)

The distribution of ^{14}C in male rats fed on ^{14}C-labeled starch and ^{14}C-labeled BCD was found to be very similar.[3] There was however, a significant difference in the time taken. With starch the maximum $^{14}CO_2$ was exhaled within one hour, whereas with BCD the maximum was attained in the 8-9th hour.

The slow degradation of BCD is to be expected. The absence of any terminal groups will prevent normal enzyme attack.

BCD in Flavour Applications

Many flavour chemicals and essential oils are expensive and/or difficult to use in processed food products. This is due to their volatility and tendency to oxidise.

Table II. β-Cyclodextrin Complexes[4]

Aroma Components	Mmass*	Molecular Size Length (nm)	Molecular Size Diameter (nm)
Allicin	162.27	1.2	0.5
Allyl-isothiocyanate	99.19	0.7	0.4
Diallyl-disulfide	146.26	1.2	0.5
Anethole	148.20	1.0	0.6
Benzaldehyde	106.12	0.9	0.6
Benzyl alcohol	108.13	0.9	0.6
Benzoic acid	122.12	0.9	0.6
Borneol	154.24	1.1	0.7
Cineol	154.24	1.0	0.7
Citral	152.23	1.0	0.6
Citronellol	156.26	1.0	0.6
Cinnamaldehyde	132.15	0.9	0.7
Cinnamic acid	148.16	0.9	0.7
Eugenol	164.21	1.0	0.6
Fenchone	152.23	1.1	0.7
Geraniol	154.24	1.0	0.7
Camphene	136.23	0.9	0.7
Carvone	150.21	1.0	0.7
Linalool	154.24	1.0	0.7
Menthol	156.27	0.9	0.6
Oenanthic acid ethyl ester	158.00	1.3	0.5
Pelargonic acid ethyl ester	186.30	1.5	0.5
alpha- and beta-Pinenes	136.23	1.0	0.7
Salicylic acid methyl ester	152.00	1.1	0.7
Terpineol	154.24	1.0	0.6
Thymol	150.22	0.9	0.6

* Divide by 11.34 to give theoretical content of BCD complex.

It has not been possible, for instance, to use benzaldehyde in a baking process or lemon oil in a coating for deep fat frying. The use of pyrazines through an extruder has often resulted in total loss and onion or garlic oils cannot be used to surface-coat snack foods.

In countries where cyclodextrins are permitted for use in food, all of the above problems can be solved. A BCD complex is stable during baking or extrusion, yet the flavour is instantly available on eating. A typical example would be a 10% onion oil BCD complex. Such a product should be almost odourless, not requiring special packaging or isolated storage and production areas. An onion oil complex used as part of a potato crisp flavour would retain its flavour for beyond the shelf life of the crisp.

The preparation of BCD flavour complexes is a

simple process, already being carried out on an industrial scale.[6-8] When stored in a sealed container, such complexes lose less than 5% of their active ingredient, even after two years.

The use of BCD to complex the more odourous flavour substances—followed by subsequent blending with a powder flavour—will eliminate the problems encountered when spray-drying many flavours. It would reduce the very expensive losses of flavour components and improve relationships between the company and its neighbors.

BCD in Perfumery Applications

BCD complexed fragrances can be utilised in solid perfumes, fragrant candles,[9] incenses[10] and detergents.[11] Fragrance and other chemical BCD complexes will release their active ingredient in the presence of warm moisture. This can be utilised in a variety of products where release is triggered by perspiration, urine or any other body fluid.

BCD in Tobacco Products

Tobacco aromas are normally dusted or sprayed onto the tobacco. They are by their very nature volatile and are lost during processing and storage. BCD complexes will remain unchanged in the tobacco mix until liberated by ignition.[12,13]

BCD has been used in cigarette filters with some success.[14] There is considerable work yet to be done on the incorporation of BCD into a polymer matrix such as cellulose acetate.

Extraction with BCD

The milling of a hot slurry of vegetable matter and BCD, followed by washing with hot water, will remove many volatiles as BCD complexes. Recovery would be effected by hot filtration followed by crystallisation of the complex on cooling. Steam distillation of the complex will yield the volatiles.

Such a process would yield solvent free extracts with a minimum of heat treatment. It is not difficult to envisage an extraction plant based on this principle. The BCD would be recycled.

The extraction of materials from solution is also possible using BCD. For example, it should be possible to extract flavour from a wine or spirit without extracting the alcohol. The author has used this technique on several occasions to remove strong or unwanted flavours from home-made wine.

Selective extraction can also be used to purify materials. The treatment of mustard oil with BCD will yield purified allyl isothiocyanate (figure 11).

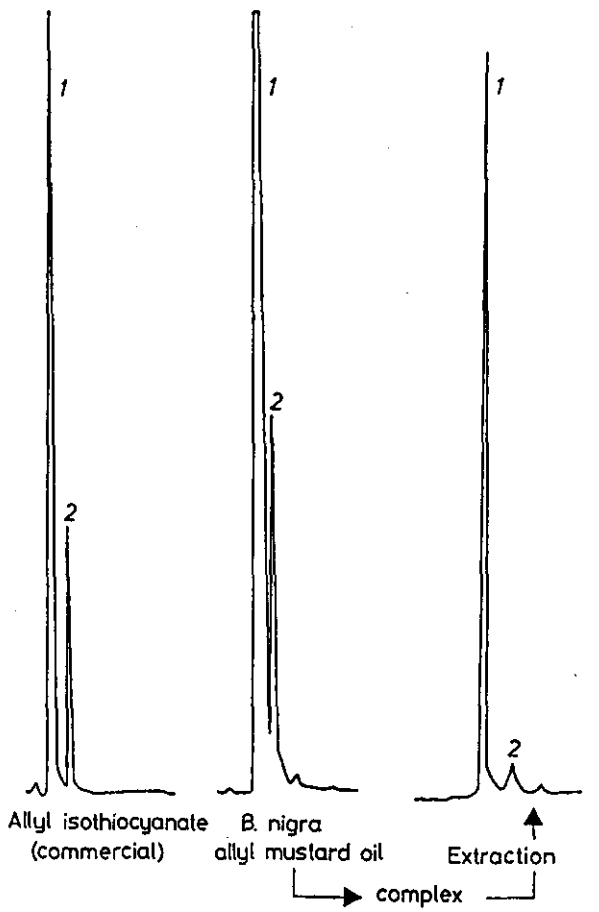

Figure 11. Gas Chromatograms of Allyl Isothio cyanate extracted from mustard oil[5]

Vapour Scrubbing with BCD

Figure 10 illustrates a method for the removal of vapour from large volumes of air. This vapour may be a solvent from an extraction plant; if the outgoing air is treated in this way, then refrigerated cooling will probably not be needed on the guard condensers. Alternatively the vapour may be from a juice concentration plant. Recovery and return of such volatiles will upgrade the finished juice.

There are many possible applications for this technique where pollutants can not only be removed but recovered and used.

BCD Polymers

Some possible applications for BCD become impractical due to the large volumes of liquid from which complexes must be filtered. The removal of trace components from effluent, or the concentration of orange juice aroma, would require extensive filtration equipment.

The problem may be overcome by the use of an insoluble BCD polymer. Such a polymer can be packed into a column and used to treat large volumes of liquid. Material can be recovered by steaming the column.

Each BCD molecule contains twenty-one hydroxyl groups. Some of these can be used to crosslink and polymerise the product. An alternative approach would be to incorporate BCD molecules in some other polymer such as CMC or cellulose acetate.

In a polymeric form the hydrophobic BCD cavity is still active. Complexes which normally form and dissociate in water can be produced. Such polymers and their complexes may find application in the slow release of chemicals into the atmosphere.

Other BCD Applications

BCD complexes of vitamins, colours and fatty acids can be formed and used in food products. There are also many applications in the drug, agrochemical and chemical industries. For a comprehensive list of these applications and references, I would direct the reader to Szejtli's book, Cyclodextrins and Their Inclusion Complexes.[3]

There is little doubt that many people, having read this paper, will think of at least one possible application for BCD. You may not find a published reference to that application; not because it will not work, but rather because there are so many uses yet to be discovered.

Acknowledgement

The author would like to thank Professor J. Szejtli for his kind permission to reproduce the diagrams in this paper.

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