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INCLUSION COMPOUNDS IN CHROMATOGRAPHY

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1. INTRODUCTION

Chemistry used to be described completely in terms of collision theory, where the collision of two molecules could lead to the formation of a chemical bond. The discovery that there are compounds which stereospecifically form "complexes" without chemical bonds with a number of inorganic and organic substances is relatively new, although these "complexes" have been known for over 100 years.

The first attempts to explain their structure and properties using the Werner coordination theory were not successful. Their unusual properties were characterized as late as 1948 and a name was finally proposed — clathrates[1]. A year later, in connection with studies to clarify the structure of the "complexes" of urea, they were termed inclusion compounds[2].

The inclusion process is a result of the ability of one compound, owing to its suitable steric properties and partially also polarity, to enclose spatially another compound. The term "host" and "guest" were used to clarify their function. An important characteristic property of the host is its ability to form a structure with free cavities with dimensions that permit the enclosure of a guest molecule. The for-

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mation of inclusion compounds is not dependent on the chemical affinity or the presence of certain groups, but rather on the spatial arrangement and interactions, where primarily Van der Waals forces and oriented dipole interactions are important.

In conclusion, the following conditions are decisive for the formation of inclusion compounds:

(1) the host structure must contain free cavities of molecular dimensions, which need not be present originally; they are frequently formed in the presence of the guest substance;

(2) the spatial arrangement (dimensions) of the guest molecule must correspond to the dimensions of the free cavity in the host substance.

Generalization of the knowledge on inclusion compounds leads to the following characteristics:

(a) inclusion compounds are formed by combination of whole host and guest molecules;

(b) the formation cannot be explained by common chemical reactions;

(c) the interaction between the host and guest is at the level of Van der Waals forces and corresponds to the energetically most suitable mutual arrangement;

(d) inclusion compounds are stable as solid substances at normal pressures and temperatures; the guest substance cannot leave its position in the host structure;

(e) formation and decomposition depend on a suitable solvent;

(f) some types of host substance are able to interact stereospecifically with the guest molecules in the gaseous phase under certain conditions.

Research in the last few decades has shown that an extensive group of substances fulfill these conditions. Because of their importance with respect to formation, structure, properties and applications, they form an independent research field. Attempts to classify the available knowledge has led to various classification systems[3–7]. One of the possible classifications is given in Table I. The important characteristic here is the shape of the cavity in which the guest is enclosed (cages, channels, layers) and the stability of the crystal lattice of the host compound, *i.e.*, its ability to retain its original crystal structure during the formation or decomposition of the inclusion compound. With inclusion compounds with a constant crystal structure, classification can also be carried out according to the number of molecules forming the inclusion structure.

TABLE I

CLASSIFICATION OF INCLUSION COMPOUNDS

Crystal structure	*Cavity shape*		
	Cages	*Channels*	*Layers*
Variable	Hydroquinone Werner complexes Dianine compounds	Urea Thiourea Tri-*o*-thimotide 4,4′-Dinitrodiphenyl Spirochromanes	Cycloveratryl 4,4′-Dihydroxytriphenylmethane
Permanent	Tri-*o*-thimotide Cyclodextrins	Zeolites Starch Cellulose	Graphite Bentonite
	(Monomolecular)	(Macromolecular)	

This classification is at present the most useful system, although it cannot be considered universal. The great diversity of inclusion compounds sometimes makes it very difficult to classify them unambiguously.

Some inaccuracy and ambiguity follow from the nomenclature used. For example, the terms "inclusion" and "occlusion" are frequently used synonymously, like the expressions "adduct" and "complex". The concept "clathrate", used for polymolecular compounds with a "cage" structure, is sometimes used generally to designate all inclusion compounds. In this paper we use the general term "inclusion" compound, the already established term "clathrates" for the Werner complexes, and the term "adduct" for the inclusion compounds of urea.

2. PROPERTIES OF INCLUSION COMPOUNDS

A number of publications[3–8] have been devoted to descriptions of the properties of inclusion compounds. Here only the properties of those inclusion compounds which have had (or which we feel may find) applications in chromatographic separations will be discussed.

2.1. Formation and stability

The variety of substances forming inclusion compounds reflects the differences in their formation and stability.

Inclusion compounds with variable crystal structure are generally formed by co-crystallization of the host and guest from solution. Inclusion compounds are sometimes formed as a result of prolonged contact of the two mutually dispersed components.

The permanent structure of the host permits simple enclosure as a result of contact in solution (cyclodextrins, cellulose, starch) or in the solid phase (zeolites, bentonite, graphite).

Division into inclusion compounds with permanent and impermanent crystalline structures (see Table I) follows from the fact that the stability of the host lattice is sometimes dependent on the presence of the guest molecule. After removal of the guest, the lattice becomes unstable and frequently recrystallizes (*e.g.*, urea). Nonetheless, most inclusion compounds with variable crystal structure are stable in the solid phase.

A survey of the stability of inclusion compounds is given in Table 2. The stability of inclusion compounds in the solid state, as also follows from the data in Table 2, is dependent on temperature and pressure. Decomposition is usually accompanied by diffusion of the guest molecule away from the inclusion structure.

The stability of many inclusion compounds changes abruptly in the presence of a solvent. On dissolution in water the inclusion compound generally decomposes into its components. An exception to this rule are the cyclodextrins, cholic acid and some macromolecular substances. In solution cyclodextrins form two phases (the liquid phase and the phase in the cavity in their rings) between which substances capable of enclosure form an equilibrium.

TABLE 2
STABILITY OF INCLUSION COMPOUNDS

Host	*Stability in solid state*	*Stability in solution*	*Reference*
Urea	Increases with chain length (50–150°C)	Completely dissociates in water; partially, *e.g.*, in C_6H_6	9
Thiourea	Similar to urea	—	10
Desoxycholic acid	Very high m.p. above that of the acid	Dissolves in water without decomposition	4 (p. 33)
Tri-*o*-thimotide	High decomposition temperature (~170°C)	Partially dissociates	4 (p. 47)
Cyclodextrins	High (100°C *in vacuo*)	Dissociates in water at 60–70°C	4 (p. 51)
Werner complexes	Various: decomposes at 120°C with C_6H_6	Low, dissociates	11
4,4′-Dinitrodiphenyl	High decomposition temperature (200°C)	—	12
Zeolites	High with H_2O, otherwise low	—	13
Bentonites	Decomposes at 100°C	—	3
Cellulose	Some substances stable at 80–100°C, also *in vacuo*	Fairly stable	14
Amylose starch	Unstable at high temperatures		15
Hydroquinone	Unstable at normal temperatures	—	16

2.2. Spatial properties

An important property is the ability of the substances forming the inclusion compounds to interact selectively with molecules with a particular spatial arrangement. This selectivity results primarily from the spatial properties of the host (see Table 3).

A knowledge of the host structure sometimes (*e.g.*, for channel-type compounds) allows the prediction of those substances with which inclusion compounds could be formed. It is sometimes not clear, however, in what form the inclusion compound will crystallize, whether with channel or cage cavities (*e.g.*, tri-*o*-thimotide).

As the character of the interaction between the guest and the host generally assumes the action of dispersion forces, the Van der Waals radii of the guest must be taken into account in spatial considerations; where bi- or polyatomic molecules are involved, the diameter perpendicular to the longitudinal axis must be taken into account.

The spatial grouping of the guest and host corresponds to formation of an energetically favourable structure in which the Van der Waals distances between the guest and host are maintained. Consequently, the host lattice cannot be stabilized with small guest molecules. For example, thiourea (channel diameter 6.1 Å) preferably forms an adduct with isoalkanes rather than with *n*-alkanes, and γ-cyclodextrin preferentially encloses only bromobenzene and iodobenzene and not chlorobenzene.

The stabilization of channelled lattices in the host during the formation of inclusion compounds is dependent on the length of the carbon chain in the guest. With an increasing number of carbon atoms in the chain, the number of Van der Waals

TABLE 3

SPATIAL PROPERTIES OF INCLUSION COMPOUNDS

Host	*Shape and dimensions of cavity (Å)*	*Possible guests*	*Reference*
Urea	Channels (5.25)	*n*-Alkanes *n*-Alcohols *n*-Fatty acids	17
Thiourea	Channels (6.1)	*n*-Alkanes (C_{16} and higher) Isoalkanes naphthalene	18 19
Desoxycholic acid	Channels (5–6)	*n*-Alkanes *n*-Alcohols *n*-Fatty acids Aromatics	20
Tri-*o*-thimotide	Channels (4.8) Cages (6.9)	*n*-Alkanes Isoalkanes Naphthalene	21 4 (p.46)
α-Cyclodextrin β-Cyclodextrin γ-Cyclodextrin	Channels (4.5) or (7) Cages (8.5)	Many organic and some inorganic complexes	4 (p. 49), 22 (p. 10)
Amylose starch	Channels (6)	Fatty acids Branched alcohols Branched alcohols etc.	23 24 24
Cellulose	Channels	*n*-Alkanes Iodine, benzene	4 (p. 59) 25
Molecular sieves:			
4A	Channels (≈4)	Kr, Xe, CH_4, CO_2, CS_2	13
5A	Channels (≈4.9)	C_3H_8, CF_4, B_2H_2	
10X	Channels (≈10)	SF_6, C_6H_6, $C_{10}H_8$	
13X	Channels (≈10)	$1,3,5\text{-}(CH_3)_3C_6H_3$	
Ca, Ba mordenite	Channels (≈3.8)	He, Ne, CO, H_2, NH_3, H_2O	
Spirochromanes	Channels (5.5)	*n*-Alkanes Isoalkanes	26
4,4'-Dinitrodiphenyl	Channels (≈5) 11 Å long	*n*-Alkanes Diphenyls	27
β-Hydroquinone	Cages	SO_2, H_2S, HCN, HCOOH, HCl, HBr, C_2H_2	28
Werner complexes	Cages	Aromatics	29
Dianine complexes (phenol + mesityl-oxide)	Cages	SO_2, Ar, alkanes, aromatics organic acids	30
Bentonite	Layers	Phthalene type complexes	31
Cycloveratryl	Non-planar layers	Aromatics, CS_2, Acetone, chloroform	32
2-Methylnaphthalene	Layers similar to graphite	*n*-Alkanes up to C_{16} Isoalkanes	33

interactions between the ends of the chains decreases and the inclusion compound becomes more stable. This fact also affects the selectivity of mutual interactions. For example, urea does not form adducts with *n*-alkanes up to C_6.

The formation of inclusion compounds with cage lattices is affected by spatial conditions and also by the π-donor–acceptor interactions. The aromatic nucleus of the amine of Werner complexes is an acceptor of the π-electrons of the aromatic nucleus of the guest. Consequently, these compounds do not form, for example, clathrates with cyclohexane; aromatics with electronegative groups are accepted unwillingly by them. If the dimensions of the substituents on the aromatic nucleus of the guest are large, steric factors begin to predominate. On this basis, it becomes possible to explain the selectivity of the interactions of Werner complexes with xylene and other compounds[6].

A change in the enthalpy on formation of inclusion compounds of cyclodextrin that is greater than the entropy change can be explained by (in addition to Van der Waals interactions) the formation of hydrogen bonds between the guest and hydroxyl groups of the host, the dissociation of water molecules on the formation of the inclusion compound and relaxation of the molecular ring of cyclodextrin. On this basis it becomes possible to explain various specific properties of these compounds.

3. GENERAL APPLICATIONS OF INCLUSION COMPOUNDS

The formation of inclusion compounds can be utilized both at the laboratory level and in industrial applications. For example, the isolation of unbranched fatty acids from various animal and vegetable oils can be carried out successfully through the formation of the adduct with urea[34–36], which has also been used for the extraction crystallization of *n*-alkanes from crude oil fractions[37]. Extraction crystallization finds wide application both in industrial applications and in analytical determinations. This is also reflected in the large number of patents on this topic[38,39].

Branched compounds can be successfully isolated using thiourea[40,41], cyclodextrins[42,43] (also enabling benzene isomers[44] and a number of other substances to be separated) and desoxycholic acid (which preferentially forms inclusion compounds with phenanthrene in the presence of anthracene, and with oleic acid in the presence of elaidic acid[45]), etc.

Acidic gases, unsaturated compounds and various impurities (H_2O, O_2, CO) can be separated from gaseous mixtures and from pure gases using molecular sieves[46].

The formation of inclusion compounds has been used in the fractionation of oils and fats[47,48]. The individual fractions of the adducts can be decomposed with water at 90°C, freeing the separated hydrocarbon fractions[49]. Simultaneous use of thiourea and urea also allowed the fractionation of ozokerite[50].

Counter-current distribution has proved a very useful separation technique[51]. Fatty acids were separated almost completely on the basis of the different distribution coefficients of their urea adducts in non-aqueous medium.

Selective separation of the isomers of xylene has been carried out using Werner complexes[29].

In addition to the use of inclusion compounds in the separation of organic substances and some gases, other applications are described in the literature.

The blue colour of iodine which appears on the formation of inclusion compounds with a number of substances (including starch) has long been known[52]. As the formation of inclusion compounds of unstable substances is frequently accompanied by their stabilization, this method could be used for stabilization of the enol forms of

carbonyl compounds[53], prostaglandines[54,55], hydroxyperoxides[56] and isopropylazulene[57] by cyclodextrins. Desoxycholic acid has been used similarly[58].

Prolonged storage of gases (CO_2, SO_2, H_2S, Ne, Kr) in inclusion structures of hydroquinone or of cyclodextrins allowed the measurement of their magnetic susceptibilities at low temperatures[59,60]. Similarly, the adducts of urea can be used for determination of the bond length and dimensions of molecules by measuring their X-ray diffraction patterns[61]. Inclusion of krypton-85 in the hydroquinone lattice leads to the formation of a very sensitive system that reacts to trace amounts of SO_2, O_3 and ClO_2 by freeing of the radioactive krypton[62].

Because of their catalytic effect, which is very similar to enzyme catalysis, cyclodextrins are frequently used as simple models for enzymes[22,63,64].

The stereospecific interaction between the host and guest is reflected in the separation of the optical antipodes. This property was found for cyclodextrins[65] containing optically active molecules of glucose, urea[66] (through crystallization of the hexagonal lattice in the dextro- or levorotatory form), tri-*o*-thimotide[67] (an optically inactive substance that can be pictured as a racemate of two atropoisomeric substances which rapidly interconvert) and desoxycholic acid[68].

4. APPLICATION OF INCLUSION COMPOUNDS IN CHROMATOGRAPHIC METHODS

The selective separation of substances, made possible by the formation of inclusion compounds, has also been employed in multi-stage chromatographic separation processes (LLC, GLC, GSC, GPC and TLC). The work published so far indicates that the chromatographic application of inclusion processes allows the solution of specific analytical problems and that chromatography also frequently becomes a very effective and important method for the study of inclusion compounds.

Of the many types of inclusion compounds, molecular sieves have found the widest use in chromatographic methods; here the inclusion structure of the host is permanent and independent of the content of guest molecules. The study and use of these compounds have therefore been the subject of many publications, which have not always been connected with the inclusion process[46,69,70]. Consequently, greater attention wil be paid here to other types of inclusion compounds, whose use in chromatography has not yet been systematically treated. The following survey is ordered primarily according to decreasing stability of the host lattice. We feel that this property is one of those which determine the possibility of using the host structure as a stationary phase.

4.1. Zeolites and bentonites

It is apparent from the many examples of the use of zeolites that the dominant factor in separation is the size of the cavity or channel in the molecular sieve structure and the size and shape of the molecule of the separated substance[71]. Molecular retention does not, however, always have a purely inclusion character. Considering the heteropolarity of zeolites as adsorbents, it can be assumed that many gases and vapours that have permanent dipoles are subject to electrostatic interactions during separation[72]. The choice of a suitable cation for the zeolite lattice while retaining the

lattice spatial parameters can affect the magnitude of these interactions and thus also the retention of the separated substances[73].

A bentonite treated with an octadecylammonium salt as the selective phase in GLC[74] has found broad applicability, especially in the separation of the isomers of many aromatic compounds[75,76]. To improve the peak symmetry, the bentonite is applied to the support together with a common stationary phase (*e.g.*, silicone oil[77,78]).

4.2. Cyclodextrins

The inclusion properties of cyclodextrins [cyclohexa(hepta, octa)amyloses], which are apparent even in aqueous solutions, are used primarily in liquid chromatography.

Because of the marked solubility of cyclodextrins in water and lower alcohols, the separating phase is an insoluble cyclodextrin–epichlorohydrin resin which retains the inclusion properties of cyclodextrins. Comparison of the distribution isotherms of cyclodextrin resin and Sephadex G-25 for a number of different substances capable of forming inclusion compounds with free cyclodextrin molecules unambiguously demonstrates the inclusion character of the cyclodextrin resin and thus also the possibility of its application in chromatography[79].

The suitability of cyclodextrin resin for gel chromatography (or gel inclusion chromatography) has been demonstrated on the separation of benzoic and chlorobenzoic acids[80]. Comparison of the results obtained in the separation with Sephadex confirms this inclusion character of the separation process, reflected primarily in the high separation efficiency. The differences found for the separation properties of columns with α- and β-cyclodextrin are in agreement with the isotherms measured.

Gel chromatography on a column containing cyclodextrin resin has also been used successfully for the separation of nucleic acids[81]. It was found by measuring the differential UV spectra that the β-cyclodextrin molecule is capable of preferentially forming inclusion compounds with adenine nucleotides. This finding has been used for their chromatographic separation. The interactions are dependent on the pH, position of the phosphate group and degree of polymerization of the nucleotide.

Using amino acids as model substances, chromatographic behaviour of cyclodextrin gels (α-, β- and γ-), resolution and its dependence on the experimental conditions have been studied[123,134]. Cyclodextrin gels have been prepared in the presence of polyvinyl acetate by cross-linking with ethyleneglycol–di(epoxypropyl)ether. Gels with medium swelling capacity, well defined composition and other prominent properties proved to be useful column packings for resolution of aromatic amino acids (primarily on a column packed with β-cyclodextrin gel).

Gels of α- and β-cyclodextrin have also been used for the chromatographic separation of racemic mandelic acid and its derivatives[82]. On the basis of the finding that the gel of β-cyclodextrin preferentially forms inclusion compounds with the L-(+)-isomer, the DL-methyl ester of mandelic acid was separated. The separation efficiency for the other derivatives studied decreased in the order *o*-methylmandelic acid, ethyl ester of mandelic acid, mandelic acid.

The possible stereospecific separation of the diastereoisomers of CrATP was studied by Cornelius and Cleland[83]. The use of a Sepharose column, to which α-cyclodextrin was applied, permitted the separation of β-amylase from albumin[84]. The

same packing without cyclodextrin did not display this separation effect. The use of cyclodextrin as a component of the mobile phase in ion-exchange chromatography has led to separation of prostaglandins E, A and B on a column of strongly acidic anion exchanger[85].

4.3. Tri-o-thimotide and desoxycholic acid

The fact that a saturated solution of tri-*o*-thimotide in tritolyl phosphate permits the inclusion of unbranched but not branched molecules has been employed in gas–liquid chromatography for the selective separation of a number of substances[86]. Comparison of the retention on a column with tri-*o*-thimotide and di-*o*-thimotide, which does not display these properties, confirmed that the selectivity is affected by inclusion in the channels of tri-*o*-thimotide.

A similar study with desoxycholic acid[87] dissolved in tritolyl phosphate or benzyldiphenyl indicated, among other things, that the inclusion is affected by the choice of solvent and solution saturation. The selectivity of desoxycholic acid, forming a saturated solution with tritolyl phosphate, for *n*-alkanes and partially also *n*-alkenes is, however, accompanied by a decrease in column efficiency with increased retention (the peaks are asymmetric and very broad).

4.4. Werner complexes

A detailed study of Werner complexes of the type $MB_4(NCS)_2$ (where M = Fe, Co, Ni and B = 4-methyl, 4-ethyl) and Ni(1-arylalkylamine) $(NCS)_2$ under GSC conditions[88,89] indicated the possibility of affecting the retention by steric factors and also by the charge-transfer interaction. The fact that linear molecules have much longer retention times on a column with M(4-methylpyridine)$_4(NCS)_2$ (the separation factor for *p*- and *m*-xylene is 2.42 when M = Ni) is in agreement with the results of Kemula and Sybilska[90] and suggests that purely steric factors permit clathration and strong Van der Waals effects between the host and guest molecules. The complex with 4-ethylpyridine is different; here selective retention disappears and, as a result of loss of hyperconjugation of the alkyl group and the pyridine nucleus, compared with the previous complex, charge transfer occurs between the metal and the π-electrons of the aromatic nucleus. The weak π-donor produced then changes the character of the retention, although the loss of selectivity is also increased by changes in the lattice parameters, leading to larger cavity diameters.

With the second type of complex studied, Ni(1-arylalkylamine)$_4(NCS)_2$, it was not possible to demonstrate the effect of the π-donor–acceptor system on the separation in the gaseous phase, although electron interactions are a decisive factor for the formation of clathrates of this type of complex. We assume that, under gas chromatographic conditions, factors such as the vapour pressure and diffusion do not allow weak electronic effects to become sufficiently perceptible.

Use of complexes of the M(4-methylpyridine)$_4(NCS)_2$ type in gas–solid chromatography is limited by the temperature (see Table 2) and stability of the packing (after use for 1 week the column loses the ability to separate effectively).

Polish workers[90–109] have studied Werner complexes as a separating phase primarily in liquid chromatography for almost 20 years.

The original work[91] described the use of the clathrate Ni(4-methylpyridine)$_4$(NCS)$_2$ for the separation of the *ortho*-isomers of nitrophenol, nitroaniline, chloronitrobenzene and nitrotoluene. In further work this phase was used as well as the Ni[4-methylpyridine, 3-methylpyridine (3:2)]$_4$(NCS)$_2$ complex and the separated substances included *p*-disubstituted derivatives of benzene[92], *o*-, *m*- and *p*-nitrotoluenes[93–95], isomers of methylnaphthalene[96], *syn*- and *anti*furazolidine[97], nitronaphthalene isomers[98], mononitroethylbenzene[90] and 1- and 2-methylnaphthalene[99]. Sybilska *et al.*[100] applied the Ni[(4-methylpyridine)$_4$(NCS)$_2$]·0.6(hydroquinone) complex of benzoic acid to Celite, the separation being carried out under gas–solid chromatographic conditions. In this way, the isomers of xylene, ethylbenzene, diethylbenzene, benzene and thiophene were separated; hydroquinone molecules clathrated in the complex lattice make the clathration of the eluted substances possible. The same phase applied to plaster allows the separation of the isomers of cresol by TLC[101]. The separation of a number of derivatives of naphthalene using the Ni[(4-methylpyridine)$_4$(NCS)$_2$]·0.7(4-methylpyridine)·0.4(aqueous methanol) phase demonstrated the selectivity of this phase for 2-isomers[102–104]. The Co(4-ethylpyridine)$_4$(NCS)$_2$ complex was used for separation of nitrotoluene isomers[105]; *m*-xylene is added to the separated substances to improve the clathration of 3- and 4- or 2- and 4-nitrotoluene. A study of the effect of an auxiliary guest present in the eluent on a column of Ni(4-methylpyridine)$_4$(NCS)$_2$ complex indicated that irreversible adsorption on the column occurs in the presence of *p*-nitrophenol[106]. The use of 2,5-dimethylpyridine instead of *m*-xylene yielded similar results. The use of this type of compound indicated a high separation efficiency (*e.g.*, the separation of 1- and 2-methylnaphthalene was carried out on a 15-mm column with an I.D. of 6 mm[103]) and stereospecificity; on the other hand, these important properties are limited by the stability of the host structures, which is dependent on the composition of the mobile phase and frequently also on the concentration of the clathrated substances.

The latest papers by Sybilska and co-workers are devoted to study of the selectivity and efficiency of columns with the Ni(NCS)$_2$(4-methylpyridine) guest clathrate[107,108]. Generalization of the experimental data led to conclusions for (a) the optimal composition of the mobile phase, which affects the structural parameters (dilation or contraction of the lattice cavities) and thus also the chromatographic selectivity of this type of Werner complex, and (b) optimal column efficiency, which can be attained at small flow-rates (<10 ml/h), particle size (5–10 μm) and column length (3–4 cm).

A theoretical study[109] clarifying the formation of clathrates in very dilute solutions and thus also the conditions for their chromatographic application suitably completes the above work. Considerations on equilibrium states for inclusion of one, two or *n* various guests are based on the assumption that the chemical potential of the inclusion-active modification of the Werner complex is a decreasing function of the overall degree of occupation of its cage cavities. It has further been shown that the guests can form inclusion compounds in dilute solutions, assuming that an auxiliary guest is present in solution. (The auxiliary guest can be any substance forming clathrates with the given host.) Considering these properties, the following conditions were suggested under which any host can be chromatographically active: (a) the separated substances must form clathrates with the same host; (b) the eluent must contain an auxiliary guest (or guests) in a sufficient concentration for the existence of

at least a three-phase equilibrium; and (c) the crystal structures of the clathrate with the separated substance and of the clathrate with the auxiliary guest must be similar.

This study, which follows from the use of Werner complexes as the stationary phase in liquid chromatography, is especially important because its theoretical conclusions can be applied to other types of inclusion compounds.

4.5. Benzenesulphonates

The benzenesulphonates of some alkali metals[110,111] have proved to be very selective stationary phases for the separation of polar compounds. Studies of the behaviour of alcohols on this type of phase have shown that the retention is affected by the clathration and also by hydrogen bonds and interactions of the molecule with the lone electron pairs of the metal ion. That the size of the lattice cavity is a limiting factor is indicated, *e.g.*, by the elution of diisopropyl ether before diethyl ether and by the thermal collapse of the lattice, appearing as a loss of selectivity properties.

4.6. Urea and thiourea

Adducts of urea (or thiourea) are inclusion compounds in which the host structure is formed in the presence of the guest substance. In contrast to the tetragonal lattice of urea, urea in inclusion compounds crystallizes in a hexagonal arrangement with channels with a diameter of 5 Å, which allows, *e.g.*, the ready incorporation of *n*-alkanes with straight chains, whereas hydrocarbons with side chains or larger substituents are not bound (as with molecular sieves).

A complex evaluation of the selectivity and stability of urea and its adducts as stationary phases in GSC toward *n*-alkanes was given by Mařík and Smolková[112–114]. The effect of the chain length of the guest molecule, temperature and vapour pressure of the guest were studied under chromatographic conditions and it was found that the selectivity of unstable adducts is dependent on the content of guest molecules and is always greater than that for the stable inclusion compounds. Multiple separation processes on the chromatographic column enabled a detailed study to be made of the effect of the sorbate structure on the inclusion properties, *e.g.*, of *n*-alkanes with one or two methyl groups in various positions. The results obtained were used for solving some analytical problems.

Further works employ the relative stability of the urea adduct with *n*-hexadecane[115,116]. Gas chromatography was employed to study the formation and decomposition of inclusion compounds with host substances of various structural types, the course of phase changes and the selectivity[116]. The dependence of the retention data on temperature yielded information on the decomposition of the adduct accompanied by an increase in the retention of the eluted substance by one order of magnitude. The urea adduct with *n*-hexadecane decomposes in the temperature range 90–102.5°C. At lower temperatures the interaction of *n*-alkanes, olefins and *n*-alcohols depends on the inclusion mechanism, in contrast to aromatics and monomethylalkanes, which represent sorbates that are inactive toward the inclusion process. At higher temperatures (above 100°C) the *n*-hexadecane freed from the adduct acts as a liquid stationary phase.

These results are connected with work on other urea adducts, primarily the

inclusion compounds of urea with *n*-hexadecanol (cetyl alcohol)[117–119]. Measurements were carried out over the temperature range 40–100°C on a wide range of substances, including aromatic and aliphatic hydrocarbons, halogen derivatives of hydrocarbons, alcohols, ethers, esters, amines and organic acids, and the results were interpreted by considering possible interactions and contributions by individual intermolecular forces.

Urea (or thiourea) applied to wide-pore silica gel or Chromosorb W[120] was used to study the inclusion process with a selected set of substances. The contribution of inclusion was demonstrated on measurements carried out over the temperature range 40–140°C, *e.g.*, for *n*-alkanes and the halogen derivatives of hydrocarbons. From an analytical point of view, rapid and selective separation of a number of substances was achieved, even though they could not always be unambiguously attributed to the formation of inclusion compounds[118].

The separation of branched and unbranched fatty acids on urea under liquid chromatographic conditions[121] is based on the concept of the linear fatty acid molecules forming a stable adduct with urea, while branched acids can be eluted from the column.

Thiourea has also been used as a stationary phase for the separation of a mixture of branched and unbranched compounds of the fluoro derivatives of pentene[122]. The branched derivatives were eluted before the unbranched derivatives.

5. CONCLUSIGN

The range of applications of inclusion compounds in chromatography is dependent on their stability and selectivity properties.

A primary condition for chromatographic separation is the existence of an interaction between the substance forming the stationary phase and the eluent, which with inclusion compcunds is realized at the level of Van der Waals forces (urea, thiourea, desoxycholic acid, tri-*o*-thimotide), donor–acceptor interactions (Werner complexes, benzenesulphonates), electrostatic interactions (zeolites, bentonites) and hydrogen bonds (cyclodextrins). The interaction is often of mixed character (*e.g.*, cyclodextrins).

The spatial character of all types of interaction frequently leads to a high degree of stabilization of the guest and of the host lattice, which contributes to marked stereoselectivity; this factor can, however, lead to slow diffusion and peak broadening.

Stabilization of the host structure through formation of inclusion compounds sometimes requires the direct use of inclusion compounds as the stationary phase (*e.g.*, adducts of urea, thiourea, Werner complexes). Chromatographic separation is then achieved on the basis of the different stabilities of the individual inclusion compounds formed during the chromatographic process. With stable host structures (zeolites, bentonites) the formation and disappearance of inclusion is unambiguous, which is favourable for their chromatographic application. Similar properties are exhibited by cyclodextrin gels in solution.

The great diversity of substances capable of forming inclusion compounds substantiates the conclusion that they can be favourably employed with wide scope for selective chromatographic separations and that, on the other hand, chromatographic methods can be used to study their unusual properties. Research carried out so far has

shown that chromatography can be used to follow very weak interactions (*e.g.*, formation of inclusion compounds with guests in the gaseous phase) and to study selectively compounds of low stability, and that this knowledge can, in turn, be employed in analytical applications.

6. SUMMARY

This review summarizes the present knowledge on inclusion compounds in general and on the application of their specific properties in analytical chemistry, especially in chromatography. The use of various inclusion compounds in liquid–liquid, gas–liquid, gas–solid, gel permeation and thin-layer chromatography for analytical purposes and the possibility of studying inclusion compounds chromatographically are discussed.

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