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(54) Title: CHROMATOGRAPHIC STATIONARY PHASE FOR SEPARATION OF CYCLODEXTRINE AND DERIVATIVES THEREOF

(57) Abstract: The present invention relates to chromatographic stationary phase characterized by the general formula M-BG-SP-SE, wherein M is a chromatographic carrier matrix; BG is a group of $(Z)_3Si$, wherein Z is, hydroxyl, alkyl, alkoxy group or a bond binding to M; SP is optionally substituted C_{1-6} alkylene, SE is -W-R_2-Ar, wherein W is -NH-CO-NH-, -NH-CO- or -NH-CO-O-, R_2 is optionally substituted C_{1-6} alkylene, Ar is C_{5-16} optionally substituted aromatic group optionally containing O-, N- and S-atoms; with the proviso that if SP is propylene and Ar is phenyl optionally substituted with -NO_2, then W is different from -NH-CO-. Further subjects of this invention are the use of the above stationary phases, the starting compounds applied in the preparation of the characteristic surface of the above stationary phases and the use thereof.

Chromatographic stationary phase for separation of cyclodextrin and derivatives thereof

The present invention relates to chromatographic stationary phases for examination of cyclodextrines (CDs) and their derivatives and for preparative separation thereof. Further subjects of this invention are the use of the above stationary phases, the starting compounds applied in the preparation of the characteristic surface of the above stationary phases and the use thereof.

CDs are known since about a century as enzyme-modified starch-derivatives having toroidal shape and consisting of 6, 7 or 8 glucopyranose units. There are CDs with higher number of glucopyranose units (delta etc.), but they are rarely used in practice. The 6-member CD is named as α-CD, the 7-member CD is named as β-CD and the 8-member, which has the greatest cavity, is named as γ-CD.

The CD molecules have an hydrophobic inner cavity due to the position of glucopyranose units, while the outer surface has hydrophilic character due to the high number of hydroxyl groups. This feature makes CD molecules capable to encapsulate apolar, therefore poorly water soluble, mainly aromatic guest molecules into the apolar cavity, making possible the preparation of homogenous aqueous solutions containing the guest molecules in high concentration. Beyond the fact that the solubility of the guest molecules is modified, the presence of CDs influences other physical and chemical properties of the guest molecules, which may result in that chemically stable solutions can be prepared from compounds which are, otherwise, ready for decomposition (J. Szejtli, Cyclodextrines and Their Inclusion Complexes, Akadémiai kiadó, Budapest, 1982.).

This phenomenon can be exploited for example in the following fields:

1. In the pharmaceutical industry, when the poor solubility of the active agent does not allow to achieve the appropriate concentration and stability (injections, infusions, eye drops).
2. In the cosmetic industry for the preparation of compositions containing non-polar, natural active agents, fat-soluble vitamins.
3. In case of plant protecting compositions, beyond the above advantages, CDs ensure the controlled release of the active agents.
4. Analysis and preparative scale separation of optical isomers. CDs, covalently bonded to an inert support (HPLC, SMB) or applied in eluent (HPLC, CE, chiral extraction), act as chiral selectors.

5. Purification of waste waters with CD-containing absorbents.
6. Catalysis.

(J. Szejtli, Cyclodextrine Technology, Kluwer Publ. Co., Dordrecht Holland, 1988.)

CD-derivatives show similar behavior than natural CDs. The use of the derivatives has a further advantage, namely, the physical properties of the formed inclusion complexes can be adjusted finely in accordance with the purpose of the application (A. P. Croft, R. A. Bartsch, Tetrahedron 39, 1417, 1982).

While natural CDs and their complexes can be prepared in pure and standard form, which can be characterized easily, this statement is not true for CD-derivatives, due to several reasons coming from the chemical structure of CDs. The number of the hydroxyl groups, standing in position 2, 3 and 6 in each glucopyranose unit, is 6x3 in α-CDs, 7x3 in β-CD and 8x3 in γ-CD, which are mainly randomly substituted, depending on the reaction conditions (the unsubstituted α-CDs, β-CDs and γ-CDs are summarized in the name "basic-CDs" in the following parts). The aggregate substitution is described by the term "degree of substitution" (DS). This parameter relates to the average number of substituted hydroxyl groups per CD molecule. The monosubstituted derivatives can be prepared as a relatively uniform product (but in this case the yield is low) or a uniform product can be prepared, if all the hydroxyl groups are modified. If a substitution degree is aimed to achieve being between the above mentioned two extremities, the product will be the mixture of isomers (wherein several hundred isomers may present).

The determination of the ratio of the isomers has not been solved yet because there is no such high performance analytical method which ensures the separation of such a great number of materials having very similar physical-chemical properties.

Further analytical problem can be the quantification of the unsubstituted basic-CDs being present in small amount in the product. Beyond the fact that the amount of the basic-CDs is a characteristic feature of the quality, this value has a considerable practical importance, particularly in therapeutical (intravenous) application, because the unsubstituted basic-CDs may precipitate in the blood, i.e. may cause embolism.

Usually high pressure liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) are applied as analytical methods to solve the above analytical problems. The information obtained by these methods can be completed — if necessary — with additional data obtained by mass-spectrometry or NMR. Presently these latter methods are used mainly for scientific purpose and are not applied in the daily practice.

Various HPLC methods have already been reported for analysis of CDs and CD-derivatives. Aminopropyl silica column is one of the most frequently applied columns for analysis of carbohydrates and CDs using acetonitrile-water eluent. The authorized method No. USP26-NF21 requires this method for determination and qualification of β-CDs and for the examination of other impurities (USP26-NF21, Official Monograph, pp. 2698-2699).

This normal phase HPLC method is suitable for characterization of hydroxypropyl-substituted β-CD-derivatives and for the separation of β-CD contamination (G. Liu, D. M. Goodall, J.S. Loran; Chirality, 5, p. 220 , 1993; N. Rabearimonjy, S. Ounnar, M. Righezza, M. Dreux; Proc. Int. Symp. Cyclodextrines, 9th, 1999, pp. 19-22).

The well-known disadvantage of the aminopropyl stationary phase is that — coming from the chemical properties of the amino group — the life-time of the column is short, the efficiency of the column decreases quickly and the reproducibility is getting poorer.

European Pharmacopoeia prescribes HPLC column containing octadecyl (C18) silica stationary phase for identification and quantification of α-CD and β-CD and for the examination of impurities (European Pharmacopoeia 4.).

Reverse phase chromatography can be applied with success for the characterization of methylated CDs where octyl (C8) or octadecyl (C18) stationary phase is applied (K. Koizumi, Y. Kubota, T. Utamura, S. Horiyama: J. Chromatogr. 368, p. 329, 1986; Y. Kubota, T. Tanimoto, S. Horiyama, K. Koizumi: Carbohydr. Res., 192, p. 159, 1989; G. Schomburg, A. Deege, H. Hinrichs, E. Hübinger: J. High Resolut. Chromatogr., 15, p. 579, 1992; I. Caron, C. Elfakir, M. Dreux: J. High Resolut. Chromatogr. 21(10), pp. 554-560, 1998).

The separation of α-CD and γ-CD may cause difficulties, because the efficiency depends strongly on the properties of the C18 media. Wide variety of C18 columns are available on the market with very different properties (surface coverage, amount of the so-called residual silanol groups, average pore size etc.).

Phenylpropyl silica stationary phase proved to be effective in the separation of methylated β-CD-derivatives both in HPLC and SFC methods. Caron et al. showed that the separation potency of phenyl-containing stationary phases is remarkably different depending on the manufacturer (I. Caron, C. Elafkir, M. Dreux: Chromatographia 47(7/8), pp. 383-390, 1998).

In spite of the fact that great number of applicable HPLC methods are disclosed in the literature for analysis of CDs and CD-derivatives, there is no column on the market dedicated specifically for CD analysis.

The above-mentioned stationary phases have the following limits on the basis of our experience:

1. It is very difficult (sometimes it is not possible) to find suitable HPLC column for the separation basic-CDs from each other and for the determination of basic-CD contamination of the CD-derivatives. The substantially better separation achieved by the stationary phases according to the present invention is shown in Figures 1 to 2c (details are given below).
2. For the characterization of statistically substituted CD-derivatives the „fingerprint" chromatograms obtained on the above columns are not enough detailed, they are not proper for quantitative analysis and the reproducibility is very poor. The substantially better resolution achieved by the stationary phases according to the present invention is shown in Figures 2a to 6b (details are given below).
3. CDs and their derivatives show very weak interactions with the stationary phase, thus the retention times are very short. Therefore, the unsubstituted basic-CDs, CD derivatives and decomposition products thereof can not be determined with acceptable resolution in one run. The substantially better separation achieved by the stationary phases according to the present invention is shown in Figures 7a and 7b (details are given below).

In the course of our experiments we found that most of the above problems can be solved by the use of the chromatographic media according to the present invention. The novelty of the stationary phases according to the present invention comes from the special groups covering the surface of the carrier (support). These covalently bonded groups contain a nitrogen-containing, preferably carbamide-containing spacer part (on the one hand, having a distance keeping function and, on the other hand, being responsible for the selectivity) and connected to this part a group having an aromatic part (which is responsible for the strength of the selective connection, called as selector in the following parts). The interaction of these two parts improves the efficiency of the separation in an unexpected manner.

There are solutions disclosed in the literature where the stationary phase contains carbamide group, however, in these cases this group functions as a spacer primarily and the other parts of the stationary phase are different from that of the present invention, moreover, the field of utility is also different.

For example, in US Patent No. 6 117 325 such a covering group is applied for separation of optical isomers, wherein a substituted urethane group is attached to a polysaccharide.

In WO 01/98370 A1 a process is disclosed for binding cyclodextrines to silica support through carbamide group. The chromatographic stationary phase prepared by this method can be applied for separation of enantiomers.

Silica-based adsorbents are known, too, wherein an aromatic group, for example a nitrophenyl group, is bonded to a spacer consisting of propyl group and amide group [see Si-Nitrobenzamide (R64030B) mentioned in catalogue "Synthesis 2003-04" of SiliCycle Inc., wherein a 3-(4-nitrobenzamido)propyl group is attached to the silica]. We would underline that this adsorbent is proposed for immobilization of enzymes having essentially different structure, and not for separation.

In the above catalogue an adsorbent is proposed for immobilization of cationic detergents, which contains 3-carbamidopropyl group (product No.: R670030B).

The separation of the components of statistically substituted CD-derivatives can be solved much better by the use of the stationary phases according to the present invention, acknowledged here the fact that the absolute separation of the isomers is theoretically impossible. Moreover, the fingerprint chromatograms obtained by the separation of the main homologue/isomer groups can be applied for quick and simple analysis, which can be useful for identification of batches, carrying out stability assays, optimization of technological steps etc. The stationary phases according to the present invention can be applied effectively both analytical and preparative purposes.

The first subject of the present invention is providing a chromatographic stationary phase for the separation of cyclodextrines and derivatives thereof and their impurities, which can be characterized by the general formula M-BG-SP-SE, wherein

M is a chromatographic carrier matrix;

BG is a group of general formula $(Z)_3Si$, wherein Z is, independently from each other in every occurrence, hydroxyl, C_{1-6} alkyl, C_{1-6} alkoxy group or a valence bond binding to M;

SP is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$, wherein

R_1 is, independently from each other in every occurrence, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl),

X^- is negatively charged counter ion;

SE is $-W-R_2-Ar$, wherein

W is -NH-CO-NH-, -NH-CO- or -NH-CO-O-,

R_2 is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$,

Ar is C_{5-16} aromatic group optionally containing one to four heteroatoms selected from O-, N- and S-atoms, and optionally substituted with one or more substituents selected from the following group: -OH, -SH, $-NO_2$, halogen, -CN, C_{1-6} alkyl, C_{1-6} alkoxy, -COOH, $-SO_3H$, $-NH-CO-NH_2$, $-[N(R_1)_3]^+X^-$ or $-N(R_3)_2$, wherein R_3 is, independently from each other in every occurrence, hydrogen, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl);

with the proviso that if SP is propylene and Ar is phenyl optionally substituted with $-NO_2$, then W is different from -NH-CO-.

The M chromatographic carrier matrix is a material which is insoluble under chromatographic conditions, e.g. organic polymer, aluminium oxide, zirconium oxide, polysaccharide, and macroporous gels made of these materials, glass beads, irregular silica (polysilicic acid xerogel), preferably spherical, porous silica having a particle size of 0.003 to 0.01 mm, preferably 0.005 mm for analytical purpose, and having a particle size of 0.015 to 0.06 mm, preferably 0.02 mm for preparative purpose, and the pore size is 10 to 50 nm, preferably 15 nm.

In this specification the meaning of C_{1-6} alkyl embraces all the linear or branched C_{1-6} alkyl groups including all the possible spatial isomers thereof. Preferred groups are C_{1-4} alkyl groups, especially methyl and ethyl.

In this specification the meaning of C_{1-6} alkoxy embraces all the linear or branched C_{1-6} alkoxy groups including all the possible spatial isomers thereof. Preferred groups are C_{1-4} alkoxy groups, especially methoxy and ethoxy.

In this specification the meaning of C_{1-6} alkylene embraces all the linear or branched bivalent C_{1-6} alkylene groups including all the possible spatial isomers thereof. Preferred groups are the linear groups, e.g. $-(CH_2)_3-$, $-(CH_2)_4-$, $-(CH_2)_5-$ groups. Especially preferred group is the $-(CH_2)_3-$ (propylene group).

The X^- counter ion is a monovalent anion, e.g. halogenide ion.

The meaning of C_{5-16} aromatic group embraces the phenyl, naphthyl, anthryl and phenanthryl, where the phenyl is preferred. Further groups belonging to this meaning are the groups derived from pyrene and fluoroanthrene.

Preferred halogen atoms are the chloro and bromo atoms.

In the preferred stationary phases the symbols have the following meanings:

a) M is a silica (silicon dioxide) based chromatographic carrier, SP is propylene group, W is -NH-CO-N- and Ar is phenyl or naphthyl optionally substituted with $-NO_2$ or -OH;

b) those from the above mentioned groups, where Ar is phenyl substituted with $-NO_2$.

For the separation of CDs having ionic character the following stationary phases according to the present invention can be applied:

a) SP is C_{1-6} alkylene substituted with $-NH_2$, -COOH , $-SO_3H$ or salts thereof or $-[N(R_1)_3]^+X^-$;

b) R_2 is C_{1-6} alkylene substituted with $-NH_2$, -COOH , $-SO_3H$ or salts thereof or $-[N(R_1)_3]^+X^-$;

b) SP and R_2 is C_{1-6} alkylene substituted with $-NH_2$, -COOH, $-SO_3H$ or salts thereof or $-[N(R_1)_3]^+X^-$.

The coverage of the stationary phases according to the invention is usually 0.1 to 1.0 mmole covering group/g silica, practically 0.2 mmole covering group/g silica.

The preparation of stationary phases according to the present invention can be carried out by processes which are well-known from the prior art. Basically the following two methods are used in the practice for the preparation of chromatographic stationary phases.

1. Covering the surface of the carrier with an appropriate silylating agent containing a reactive group suitable for the formation of the spacer, which reactive group is able to react with an appropriate group of the selector, which latter is an aromatic ring system in this case.
2. The silylating agent used in the previous method is coupled at first with the aromatic compound and the surface of the silica is covered with this derivative.

In our present work we applied the second method, because this makes possible the homogeneous formation of a chemically modified surface. The reaction between the starting silylating agent and the aromatic compound, which results the spacer, is a quantitative homogeneous reaction, while it is not true when the same reaction is made with the starting silylating agent previously attached to the silica surface. This latter reaction is heterogeneous and the conversion is not complete due to spatial hindrance coming from the structure of the silica.

In the preparation of the silylating agents according to the present invention we applied two methods for coupling the spacer and the aromatic group which are disclosed more specifically in Examples 1 and 2.

Method A) disclosed in example 1 can be characterized by the reaction of an aromatic amine having the appropriate substituents and basicity with a 3-isocyanatoalkyl-trialkoxysilane, preferably 3-isocyanatopropyl-triethoxysilane (see Reaction Scheme 1 with the preferred compounds). In general, this reaction is fast, quantitative and selective with respect to the amino group, even in that case, too, when the aromatic group contains hydroxyl substituent.

Method B) disclosed in example 2 can be applied when electron-withdrawing substituents are connected to the aromatic ring, therefore the basicity of the amino group is decreased which results in reacting with isocyanates slowly and with poor yield.
In this case we formed an isocyanato group in the appropriate position of the aromatic ring and this was reacted with an 3-aminoalkyl-trialkoxysilane, preferably 3-aminopropyl-triethoxysilane (see Reaction Scheme 2 scheme with the preferred compounds).

Silylating agents prepared by the above mentioned methods are reacted with the appropriately pretreated carrier (preferably silica) preferably in an aprotic solution (preferably in toluene), at a temperature between 50 °C and the boiling point of the solution (for instance at 110 °C), for 10 to 36 hours, preferably for apprx. 24 hours. The pretreatment is carried out preferably for apprx. 24 hours, in vacuum, over 100 °C, preferably at 105 to 120 °C. The coupling reaction is disclosed in Reaction Scheme 3 by the use of a nitrophenyl analogue. Here we would mention that the ideal coupling with three valence bonds is given in Reaction Scheme 3, but the silylating agent according to the invention may bind only with two or one valence(s) to the carrier, as a consequence of either spatial hindrance or having alkyl in the meaning of one or two Z' group(s).

A further subject matter of this invention are the silylating agents prepared by the above methods which agents can be used directly to cover the surface of the carrier.
These compounds can be characterized by general formula BG'-SP-SE, wherein

BG' is a group of general formula $(Z')_3Si$, wherein Z' is, independently from each other in every occurrence, leaving group or C_{1-6} alkyl;

SP is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$, wherein

R_1 is, independently from each other in every occurrence, C_{1-6} alkyl or

hydroxy(C_{1-6} alkyl),

X^- is negatively charged counter ion;

SE is -W-R_2-Ar, wherein

W is -NH-CO-NH-, -NH-CO- or -NH-CO-O-,

R_2 is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: -NH_2, -COOH and -SO_3H and salts thereof and -$[N(R_1)_3]^+X^-$,

Ar is C_{5-16} aromatic group optionally containing one to four heteroatoms selected from O-, N- and S-atoms, and optionally substituted with one or more substituents selected from the following group: -OH, -SH, -NO_2, halogen, -CN, C_{1-6} alkyl, C_{1-6} alkoxy, -COOH, -SO_3H, -NH-CO-NH_2, -$[N(R_1)_3]^+X^-$ or -$N(R_3)_2$, wherein R_3 is, independently from each other in every occurrence, hydrogen, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl);

with the proviso that if SP is propylene and Ar is phenyl optionally substituted with -NO_2, then W is different from -NH-CO-.

The meaning of the phrases used in these definitions is the same as that of disclosed above. Furthermore, the phrase "leaving group" denotes a group which split off during the reaction of the silylating agent and the carrier, thus the silylating agent is able to react with the silanol groups of the carrier. Halogens and C_{1-6} alkoxy groups are preferred groups, chloro atom, methoxy and ethoxy groups are more preferred.

Preferred silylating agents are the covering groups of the above mentioned preferred stationary phases with the further note that preferred leaving group is the ethoxy group.

The invention is disclosed by the following examples, too, but without the intention that the claim should be limited to the disclosure thereof because several further variants can be implemented by a skilled person on the basis of the teaching of the present specification.

Example 1

Method A)

0.420 g (2,64 mmol) 3-hydroxy-β-naphthylamine was dissolved and stirred in 10 cm^3 water and peroxide free tetrahydrofurane. Thereafter 0.653 g (2.64 mmol) 3-isocyanatopropyl-triethoxysilane was slowly added to the above stirred solution at 25 °C. The reaction mixture was stirred at room temperature for further 30 minutes under inert gas atmosphere. The solution was clarified with active carbon, if necessary, and the

tetrahydrofurane was removed by distillation under reduced pressure. The corresponding 1-(3-triethoxysilylpropyl)-3-(3-hydroxy-2-naphthyl)-urea (Compound 1a, 1.09 g) was obtained as a thick oil which solidifies upon standing. The yield is quantitative. The product can be used for derivatization of silica without further purification.

Thin-layer chromatography was applied for the verification of the compound and its purity (Merck Silica gel 60F254, eluent: dichloromethane-hexane-methanol 20:10:2 v/v, Rf: 0.37).

By this method the following compound was prepared yet:

Compound 1b: 1-(3-triethoxysilylpropyl)-3-(2-naphthyl)-urea. Rf.: 0.62

Example 2

Method B)

0.442 g (2.0 mmol) 3-Aminopropyltriethoxysilane was dissolved in 10 cm^3 tetrahydrofurane. Thereafter 0.328 g (2.0 mmol) of 4-nitro-phenylisocyanate was dissolved in 10cm^3 tetrahydrofurane and this solution was slowly added to above amine solution. The tetrahydrofurane was water and peroxide free in both solutions. The reaction mixture was stirred at room temperature for further 30 minutes under inert gas atmosphere. The solution was clarified with active carbon, if necessary, and the tetrahydrofurane was removed by distillation under reduced pressure. The corresponding 1-(3-triethoxysilylpropyl)-3-(4-nitrophenyl)-urea (Compound 2a, 0.77 g) was obtained as a waxy material which solidifies quickly. The yield is quantitative. The product can be used for derivatization of silica without further purification.

Thin-layer chromatography was applied for the verification of the compound and its purity (Merck Silica gel 60F254, eluent: dichloromethane-hexane-methanol 20:10:2 v/v, Rf: 0.53).

By this method the following compound was prepared yet:

Compound 2b: 1-(3-triethoxysilylpropyl)-3-(phenyl)-urea. Rf.: 0.57 .

Example 3

Preparation of silica based stationary phase

Silylating agents prepared according to Example 1 or 2 were reacted with pretreated silica in toluene at 110 °C for 24 hours (the silica was dried before for 24 hours in vacuum at 110 °C).

The silica based stationary phases prepared by the above method can be packed into HPLC-column after filtration, washing and sedimentation.

The following stationary phases were prepared by this method:

Stationary phase 3a, where the carrier is Hypersil Silica-HS (5μm) and the covering group derives from compound 1a.

Stationary phase 3b, where the carrier is Hypersil Silica-HS (5μm) and the covering group derives from compound 1b.

Stationary phase 3c, where the carrier is Hypersil Silica-HS (5μm) and the covering group derives from compound 2a.

Stationary phase 3d, where the carrier is Hypersil Silica-HS (5μm) and the covering group derives from compound 2b.

RESULTS

The excellent separation results achieved by the stationary phases according to the present invention are shown by Figures 1 to 7b.

— Figure 1 illustrates the retention times of unsubstituted basic-CDs on different stationary phases, using methanol-water 30:70 v/v as eluent. The detection was carried out with a refraction index (RI) detector and the flow rate was 1,0 cm^3/min. One grade on the time axis represents 1 minute in all chromatograms.

The upmost chromatogram illustrates a separation which was performed on a stationary phase having 4-nitrophenyl-amide selector which embodies the closest structure known from the prior art. (The preparation of this stationary phase was carried out according to Examples 2 and 3, among the same conditions applied for the preparation of the other stationary phases. In case of the other Figures this type of column was applied when a column having 4-nitrophenyl-amide selector is mentioned.) Chromatograms 2 to 5 illustrate separations performed on different stationary phases (containing urea group) according to this invention (the stationary phases are as follows: 3d, 3a, 3b, 3c, respectively). It can be seen that the stationary phases according to this invention generate substantially higher difference in the retention time which has a considerable advantage in the practice.

The unexpected superior effect of the present invention can be seen most clearly from the comparison of chromatograms 1 (upmost) and 5 (bottom) because the only difference between the groups covering the silica carrier (more specifically: between the spacers) is the

use of urea group instead of amide group, while the difference between the retention times increased significantly.

— Figures 2a to 2c illustrate chromatograms of acetyl-γ-CD performed on different columns.

Conditions:

Figure 2a

Stationary phase: Nucleosil 120-7 NH_2 (Macherey-Nagel GmbH & Co.), 4x100 mm, eluent: 75 v/v% acetonitrile, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 2b

Stationary phase: Nucleosil C18 (Macherey-Nagel GmbH & Co.), 5μm, 4.6x250 mm, eluent: 50 v/v% methanol, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 2c

Stationary phase: stationary phase 3b according to this invention containing 2-naphthyl-urea selector, 5μm, 4x250 mm, eluent: 58 to 42 v/v% methanol-water, flow rate: 1.0 cm^3/min, temperature: 30°C.

It can be seen that the resolution is significantly better on the stationary phase according to the invention (the peaks are separated at the base-line, i.e. the overlapping is less), moreover, as an unexpected effect, the contaminant basic-CD (γ-CD) can be found at the start of the chromatogram (it elutes with small elution time). This phenomenon meets that requirement that, advantageously, contaminant being present in a small quantity elutes before the peak of the main component, thus the evaluation becomes easier and more precise (a sharp peak can be seen instead of a vague sign).

— Figure 3 illustrates that quite a small difference in the degree of substitution (DS) can be detected by the use of stationary phase according to the present invention (stationary phase 3c; 3,5-hydroxypropyl-β-CDs having DS=3 and DS=3.5 were used) and the underivatized β-CD contaminant can be quantified with good accuracy.

— Figures 4a and 4b illustrate comparative chromatograms for the determination of composition of hydroxypropyl-β-CD.

Conditions:

Figure 4a

Stationary phase: Nucleosil with nitrophenylpropyl selector (Macherey-Nagel GmbH & Co.), 5 μm, 4x250mm, eluent: 35 v/v% acetonitrile, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 4b

Stationary phase: stationary phase 3c according to invention having 4-nitrophenyl-urea selector, 5μm, 4x250 mm, eluent: methanol-dioxane-water 35:5:60, flow rate: 1.0 cm^3/min, temperature: 30°C.

It can be seen (see Figure 4b) that the resolution is significantly better on the stationary phase according to the invention (more and sharper peaks with bigger distance from each other, i.e. with less overlapping), and the underivatized β-CD contamination can be assayed easily and precisely.

— Figures 5a and 5b illustrate the influence of the substituents in case of two selectors according to the invention through the examination of randomly methylated γ-CD.

Conditions:

Figure 5a

Stationary phase: stationary phase 3a according to invention having (3-hydroxy-2-naphthyl)urea selector, 5μm, 4x250 mm, eluent: acetonitrile-water 26:74, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 5b

Stationary phase: stationary phase 3b according to invention having (2-naphthyl)urea selector, 5μm, 4x250 mm, eluent: acetonitrile-water 26:74, flow rate: 1.0 cm^3/min, temperature: 30°C.

It can be seen that the presence of the hydroxyl substituent improved the separation.

— Figures 6a and 6b illustrate the influence of the N-containing group bonded to the spacer in case of two selectors according to the invention through the examination of randomly methylated α-CD.

Conditions:

Figure 6a

Stationary phase: 4-nitrophenylamide containing selector, 5μm, 4x250 mm, eluent: acetonitrile-water 26:74, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 6b

Stationary phase: stationary phase 3c according to invention having 4-nitrophenyl-urea selector, 5μm, 4x250 mm, eluent: acetonitrile-water 26:74, flow rate: 1.0 cm^3/min, temperature: 30°C.

The unexpected effect observed in Figures 2a to 2c is proven by these chromatograms: a minimal change in the structure caused a substantial increase in the effectiveness of the separation (much more peaks which are much sharper).

— Figure 7 demonstrates that the stationary phase according to the invention can be applied effectively for the examination of the degradation of randomly methylated β-CD.

Figure 7a: chromatogram of randomly methylated β-CD containing no contamination.

Eluent: acetonitrile-water 20:80, flow rate: 1.0 cm^3/min, temperature: 30°C.

Figure 7b: chromatogram of randomly methylated β-CD artifically degraded with hydrochloric acid.

Eluent: acetonitrile-water 26:74, flow rate: 1.0 cm^3/min, temperature: 30°C.

CLAIMS

1. Chromatographic stationary phase characterized by the general formula

M-BG-SP-SE, wherein

M is a chromatographic carrier matrix;

BG is a group of general formula $(Z)_3Si$, wherein Z is, independently from each other in every occurrence, hydroxyl, C_{1-6} alkyl, C_{1-6} alkoxy group or a valence bond binding to M;

SP is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$, wherein

R_1 is, independently from each other in every occurrence, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl),

X^- is negatively charged counter ion;

SE is $-W-R_2-Ar$, wherein

W is -NH-CO-NH-, -NH-CO- or -NH-CO-O-,

R_2 is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$,

Ar is C_{5-16} aromatic group optionally containing one to four heteroatoms selected from O-, N- and S-atoms, and optionally substituted with one or more substituents selected from the following group: -OH, -SH, $-NO_2$, halogen, -CN, C_{1-6} alkyl, C_{1-6} alkoxy, -COOH, $-SO_3H$, $-NH-CO-NH_2$, $-[N(R_1)_3]^+X^-$ or $-N(R_3)_2$, wherein R_3 is, independently from each other in every occurrence, hydrogen, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl);

with the proviso that if SP is propylene and Ar is phenyl optionally substituted with $-NO_2$, then W is different from -NH-CO-.

2. Chromatographic stationary phase according to claim 1, wherein M is silica-based chromatographic carrier, SP is propylene, W is -NH-CO-NH- and Ar is phenyl or naphthyl optionally substituted with $-NO_2$ or -OH.

3. Chromatographic stationary phase according to claim 2, wherein Ar is phenyl substituted with $-NO_2$.

4. Chromatographic stationary phase according to claim 1, wherein SP is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

5. Chromatographic stationary phase according to claim 1, wherein R_2 is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

6. Chromatographic stationary phase according to claim 1, wherein SP and R_2 is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

7. Compounds of general formula BG'-SP-SE, wherein

BG' is a group of general formula $(Z')_3Si$, wherein Z' is, independently from each other in every occurrence, leaving group or C_{1-6} alkyl;

SP is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$, wherein

R_1 is, independently from each other in every occurrence, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl),

X^- is negatively charged counter ion;

SE is -W-R_2-Ar, wherein

W is -NH-CO-NH-, -NH-CO- or -NH-CO-O-,

R_2 is C_{1-6} alkylene, optionally substituted with one or more substituents selected from the following group: $-NH_2$, -COOH and $-SO_3H$ and salts thereof and $-[N(R_1)_3]^+X^-$,

Ar is C_{5-16} aromatic group optionally containing one to four heteroatoms selected from O-, N- and S-atoms, and optionally substituted with one or more substituents selected from the following group: -OH, -SH, $-NO_2$, halogen, -CN, C_{1-6} alkyl, C_{1-6} alkoxy, -COOH, $-SO_3H$, $-NH-CO-NH_2$, $-[N(R_1)_3]^+X^-$ or $-N(R_3)_2$, wherein R_3 is, independently from each other in every occurrence, hydrogen, C_{1-6} alkyl or hydroxy(C_{1-6} alkyl);

with the proviso that if SP is propylene and Ar is phenyl optionally substituted with $-NO_2$, then W is different from -NH-CO-.

8. Compounds according to claim 7, wherein SP is propylene, W is -NH-CO-NH- and Ar is phenyl or naphthyl optionally substituted with $-NO_2$ or -OH.

9. Compounds according to claim 7, wherein Ar is phenyl substituted with $-NO_2$.

10. Compounds according to claim 7, wherein SP is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

11. Compounds according to claim 7, wherein R_2 is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

12. Compounds according to claim 7, wherein SP and R_2 is C_{1-6} alkylene substituted with $-NO_2$, -COOH, $-SO_3H$ groups or salts thereof or $-[N(R_1)_3]^+X^-$.

13. Use of chromatographic stationary phase according to any of claims 1 to 6 for separation of basic-CDs and/or CD-derivatives or degradation products thereof.

14. Use of compounds according to any of claims 7 to 12 for the preparation of chromatographic stationary phase.

15. Use according to claim 14 wherein the stationary phase is for separation of basic-CDs and CD-derivatives.

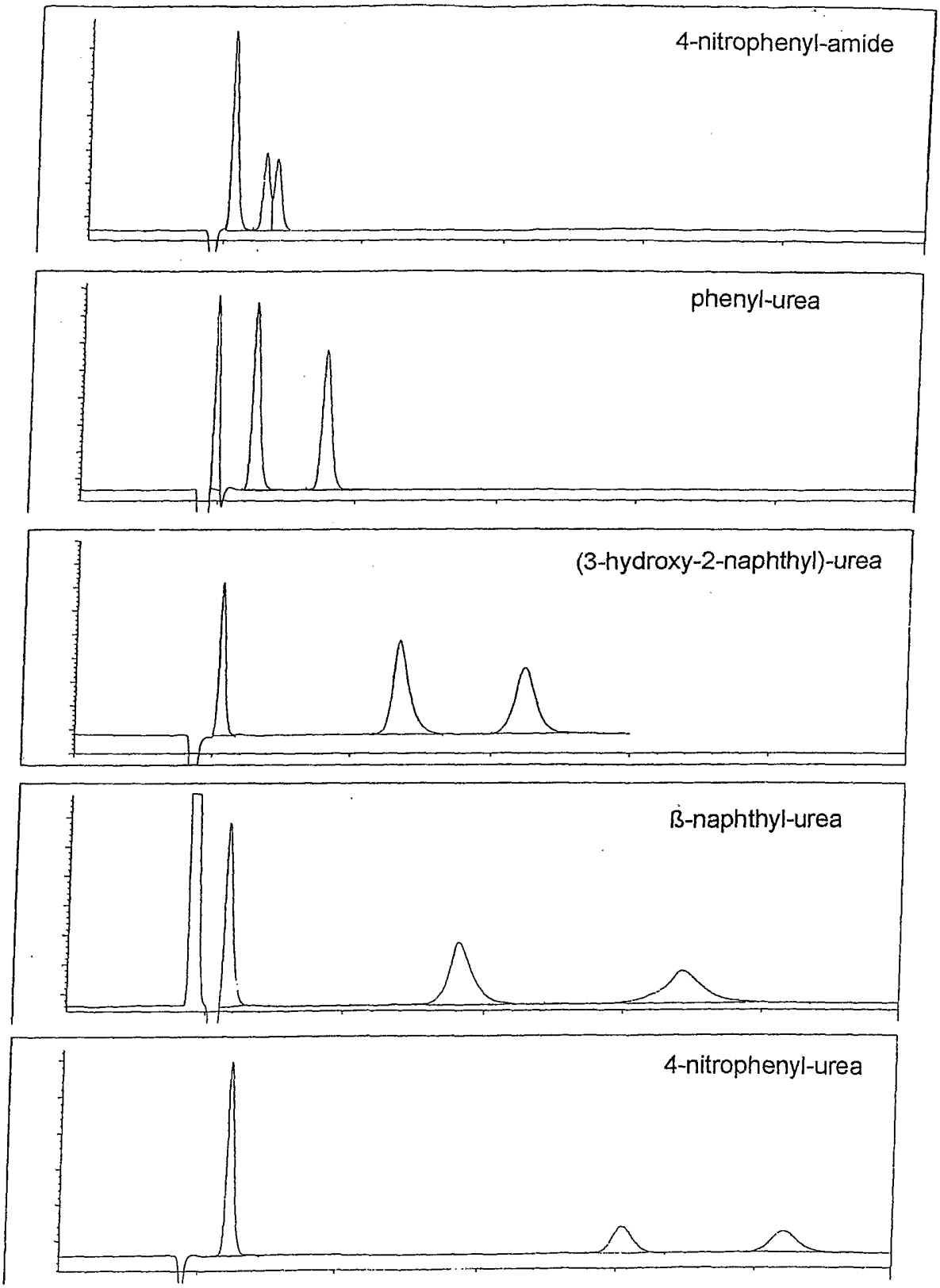

4-nitrophenyl-amide
phenyl-urea
(3-hydroxy-2-naphthyl)-urea
ß-naphthyl-urea
4-nitrophenyl-urea

Fig. 1

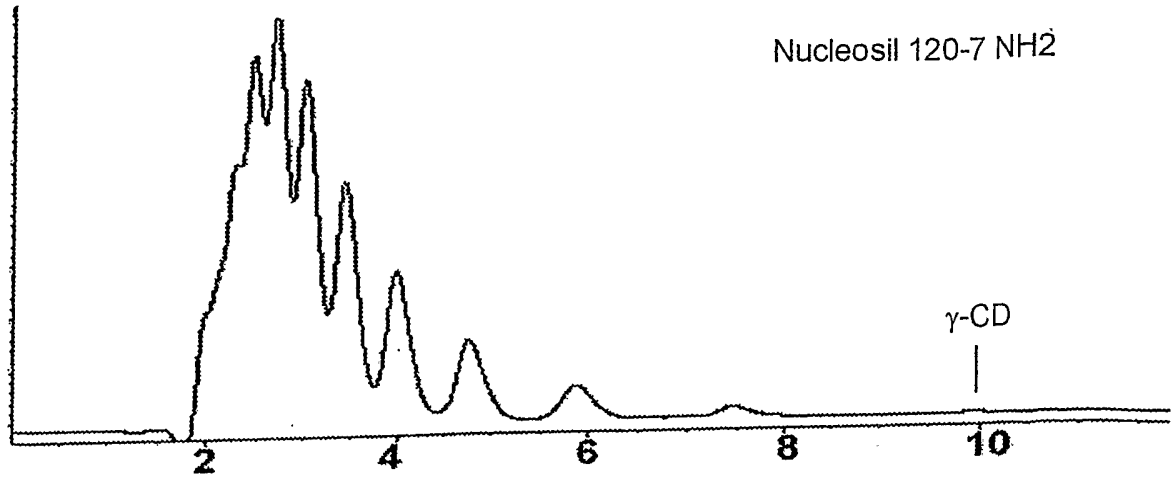


Fig. 2a

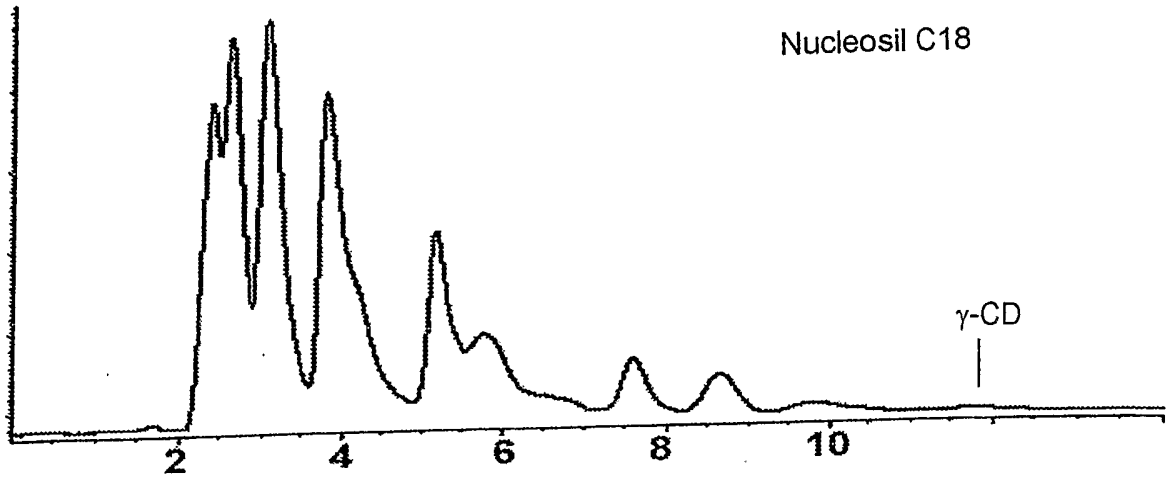


Fig. 2b

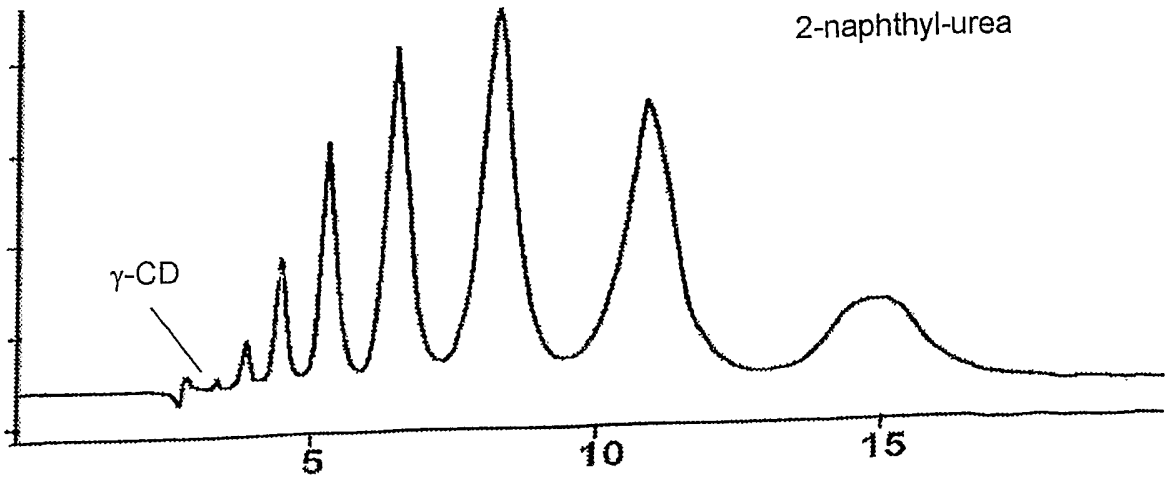


Fig. 2c

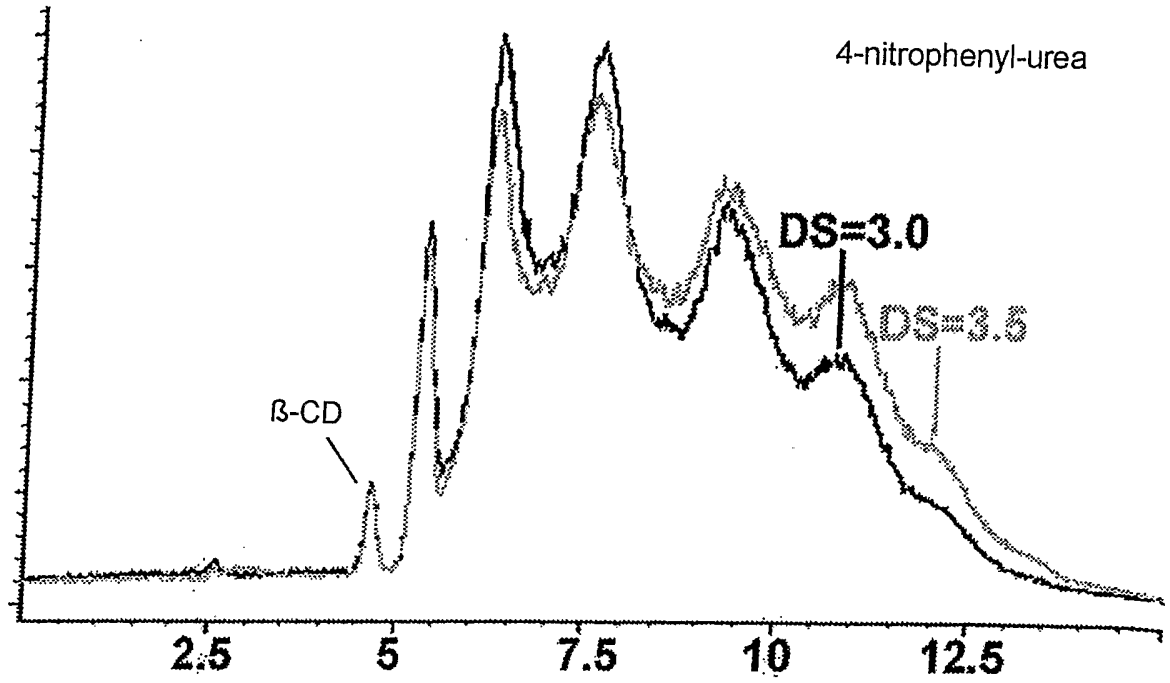


Fig. 3

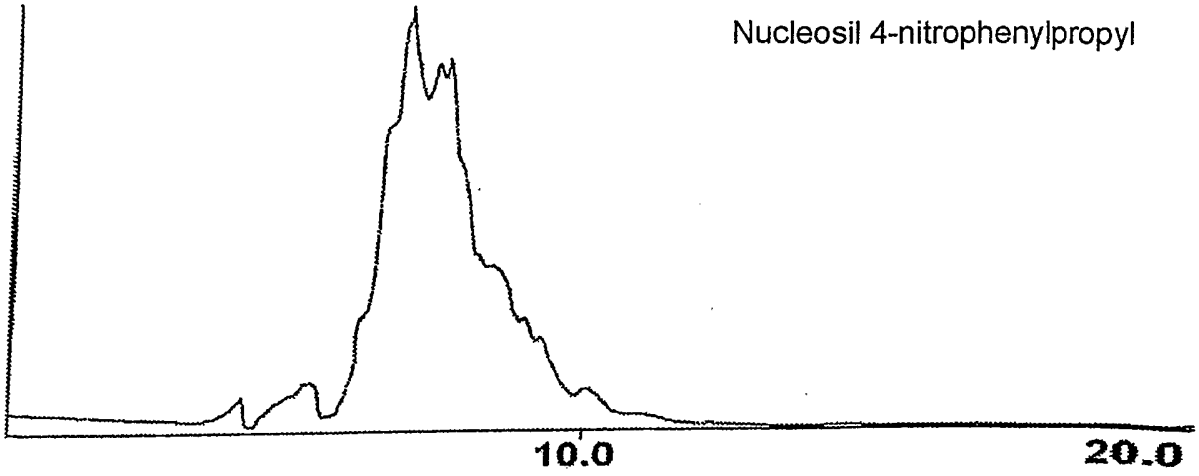

Nucleosil 4-nitrophenylpropyl
10.0
20.0

Fig. 4a

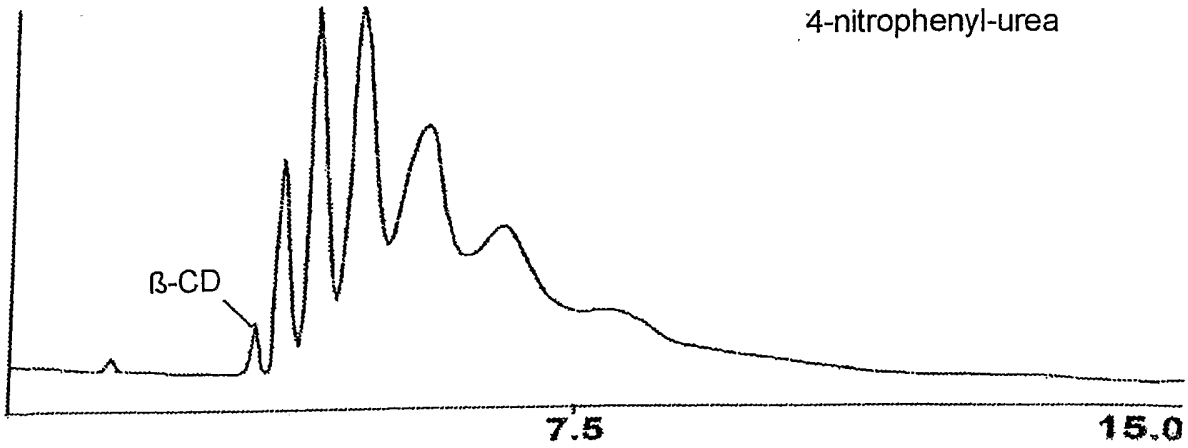

4-nitrophenyl-urea
ß-CD
7.5
15.0

Fig. 4b

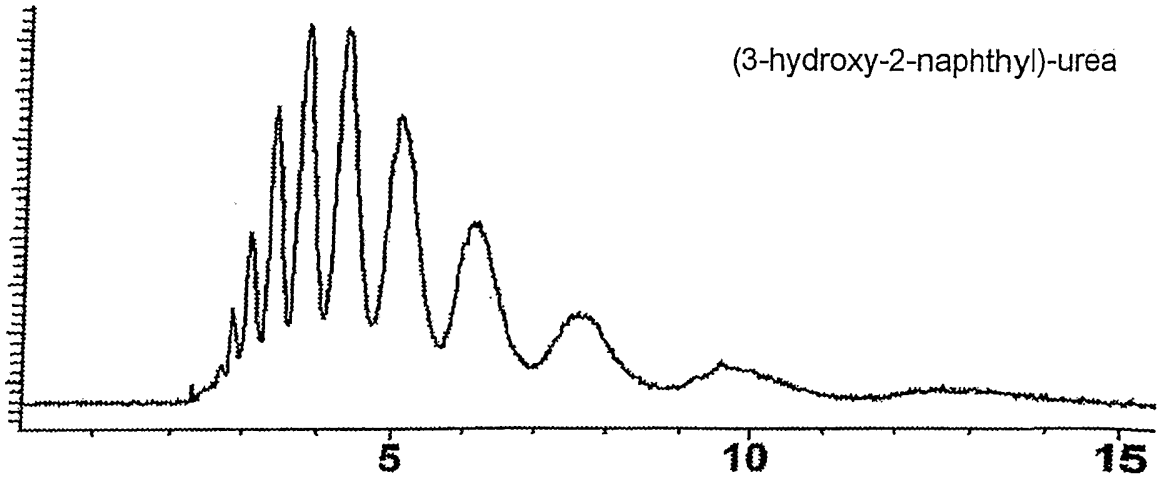


Fig. 5a

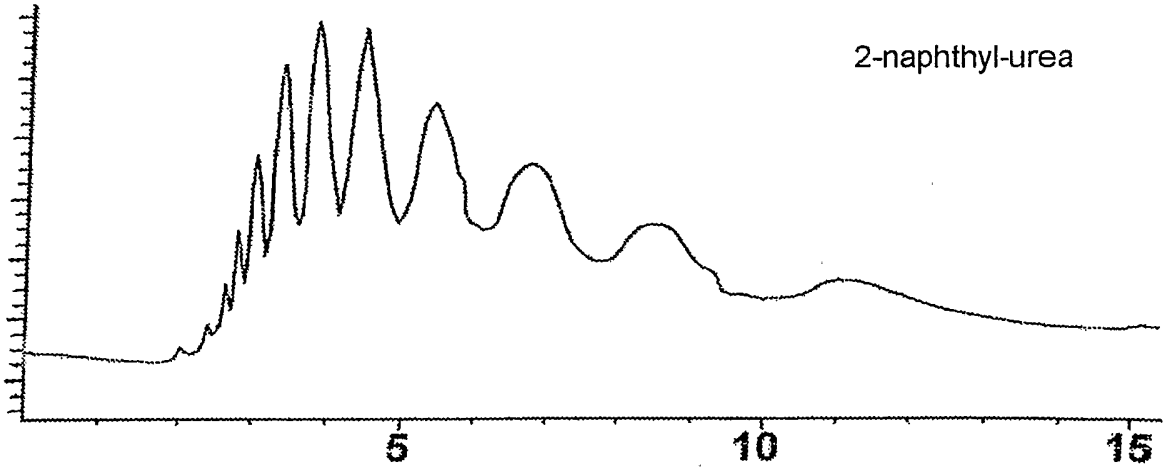


Fig. 5b

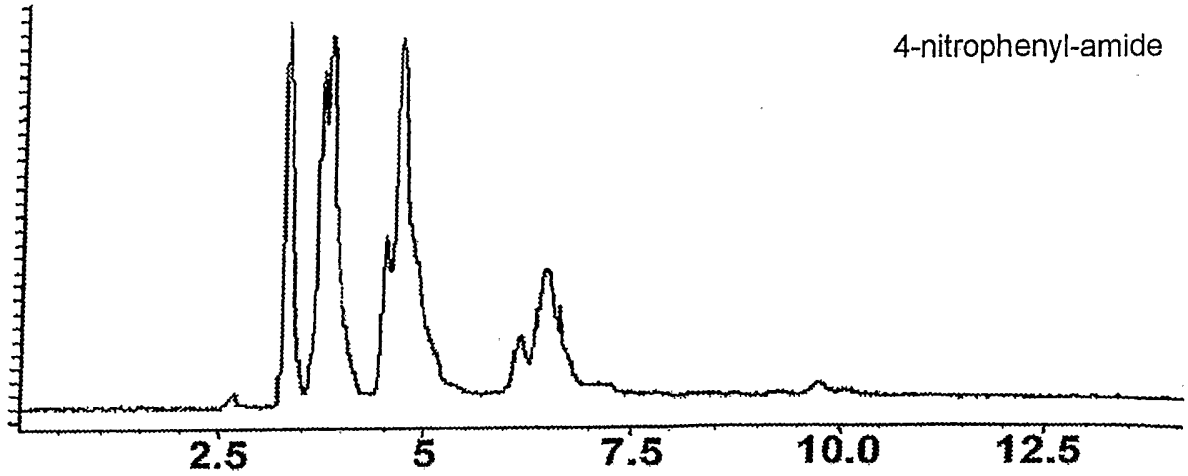

4-nitrophenyl-amide
2.5
5
7.5
10.0
12.5

Fig. 6a

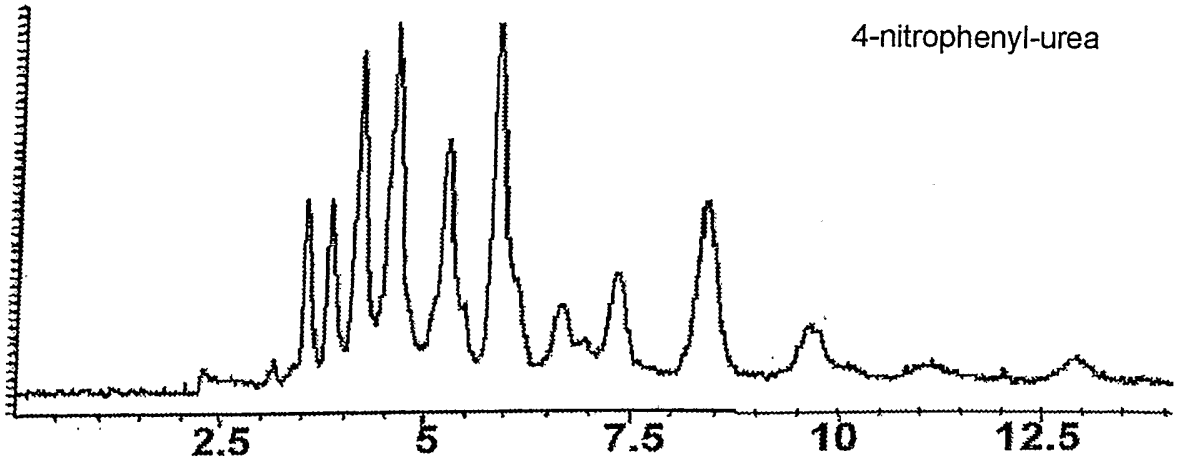

4-nitrophenyl-urea
2.5
5
7.5
10
12.5

Fig. 6b

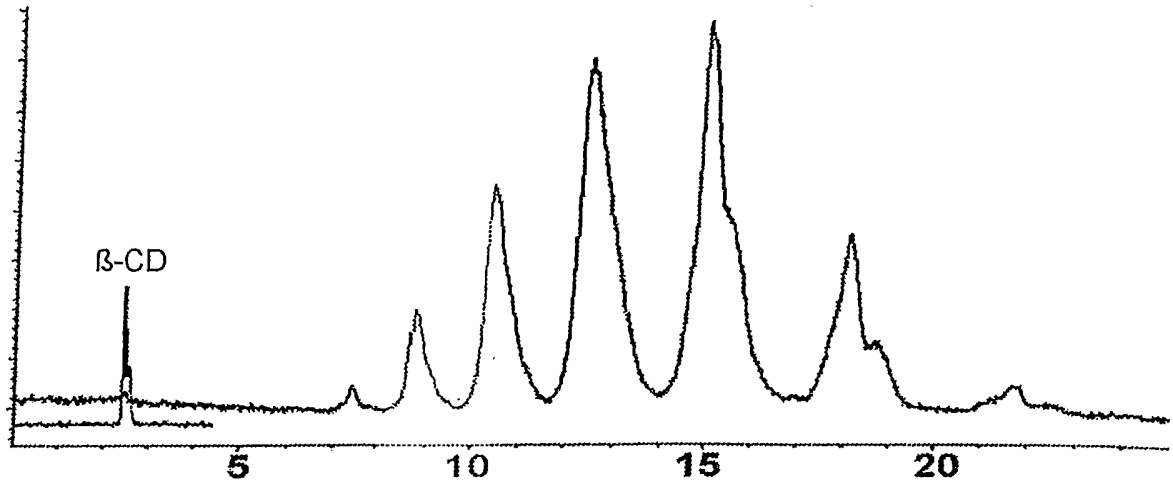


Fig. 7a

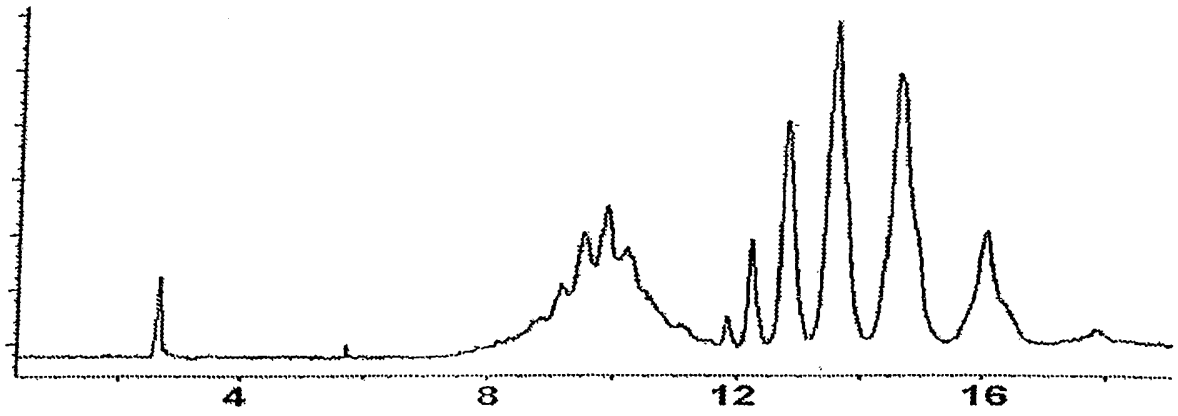


Fig. 7b

Reaction scheme 1

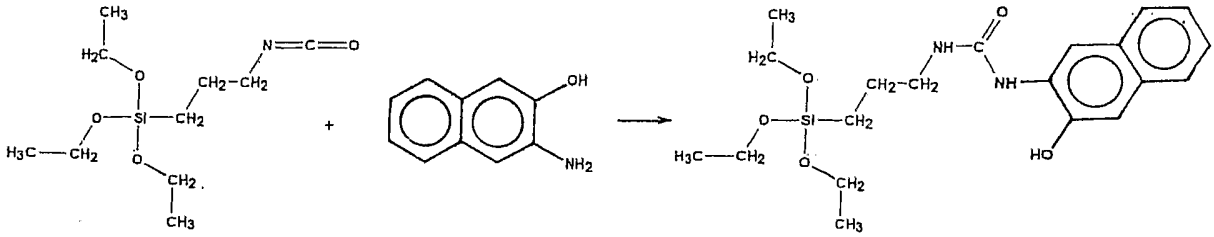


Fig. 8

Reaction scheme 2

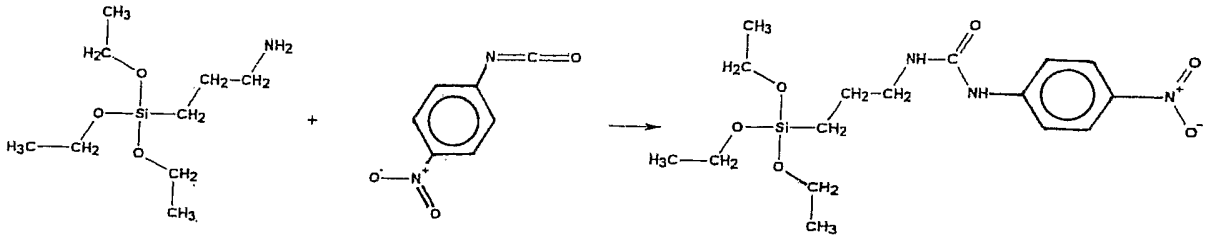


Fig. 9

Reaction scheme 3

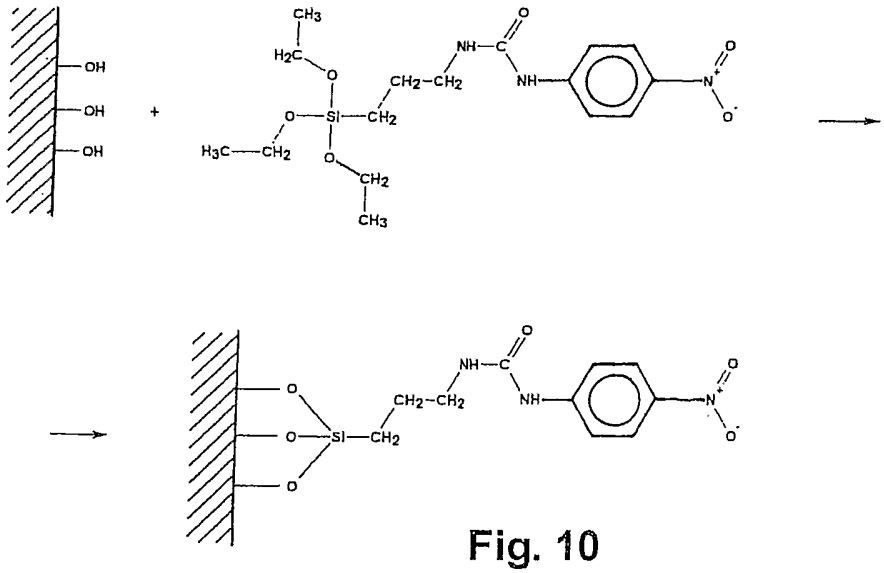


Fig. 10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU2005/000043

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 B01D15/08 C08B37/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 B01J G01N B01D C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 322 310 A (HOUSE ET AL) 30 March 1982 (1982-03-30) column 3, line 35 - column 4, line 61; claims 1,3,4,7-9	1-3,7-9, 14,15
X Y	US 4 512 898 A (OI ET AL) 23 April 1985 (1985-04-23) column 3, line 4 - line 25 column 3, line 56 - line 63 column 4, line 23 - line 35 column 5, line 1 - line 6	1,2,7,8, 14,15 13

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 September 2005	23/09/2005

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/HU2005/000043

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 781 977 A (YAGI ET AL) 1 November 1988 (1988-11-01) column 4, line 20 - column 6; table 1 column 40, line 51 - column 41, line 7 column 6, line 25 column 10; examples 11,12 column 16; examples 33,35 column 37, line 15	13
A	US 4 867 884 A (RENDLEMAN, JR. ET AL) 19 September 1989 (1989-09-19) column 3, line 35	13-15
A	US 4 997 966 A (LOHMANN ET AL) 5 March 1991 (1991-03-05) column 5, line 1 - line 20	1,7,14

INTERNATIONAL SEARCH REPORT

Information on patent family members

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