Sorbent comprising on its surface an aliphatic unit having an anionic or deprotonizable group for the purification of organic molecules

The present invention relates to a sorbent comprising a solid support material, the surface of which comprises a residue of a general formula (I), wherein the residue is attached via a covalent single bond to a functional group on the surface of either the bulk solid support material itself or of a polymer film on the surface of the solid support material. Furthermore, the present invention relates to the use of the sorbent according to the invention for the purification of organic molecules, in particular pharmaceutically active compounds, preferably in chromatographic applications.

Chromatography media for organic molecules and biomolecules have traditionally been categorized according to one or more of the following possible modes of interaction with a sample:

- hydrophobic interaction (reversed phase)
- hydrophilic interaction (normal phase)
- cation exchange
- anion exchange
- size exclusion
- metal ion chelation.

The provision of new chemical compounds, either by its discovery in plant extracts or animals or, by chemical synthesis, always demands the provision of new chromatographic materials, the further development of known chromatographic materials or the finding of a new way for the purification of

the chemical compounds which is simple and cost-effective. That is, there is always a demand for new highly selective downstream purification technologies capable of handling large capacities without up-scaling the required volumes of liquid by the same factor.

Traditional stepwise application of the above chromatographic categories to a given separation problem was accordingly mirrored in a step-by-step, steady improvement of the product purity but also in product losses at every stage which accumulate seriously in the end, not to mention the operational time and cost of goods. Introduction of affinity chromatography at an early stage into the downstream process could be an answer to this demand since the reduction of a consecutive series of sequential chromatography steps into only one could thus be demonstrated many times. Affinity chromatography is sometimes regarded as a class of its own although, from a chemical point of view, it is based on the same interaction modes as above, but usually on a combination of two or more modes. By using affinity chromatography the specific interactions between an analyte and the sorbent may be verified both between the analyte and active residues bound on the surface of a matrix of the chromatographic material and between the analyte and surface characteristics of the matrix itself.

Affinity chromatography has mostly been carried out with bulk gel-phase resins. Pre-eminent gel-forming materials are medium-crosslinked polysaccharides, polyacrylamides, and poly(ethylene oxides). Such hydrogels often ensure a compatible interface which can well accommodate both the active residue of the ligand and the analyte interacting therewith due to their softness (conformational flexibility, elastic modulus), large pore systems, high polarity and high

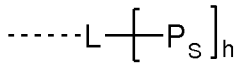
water content, as well as the absence of reactive or denaturing chemical groups. They are able to retain analytes, such as proteins, in their native state, i.e. preserve their correctly folded, three-dimensional structure, state of association, and functional integrity, or do not chemically change the structure of a complex pharmaceutically active compound. The mechanical resistance of these media is, however, much weaker than that of inorganic support materials since they are compressible under an applied pressure and do not tolerate shear stress caused by agitation, column packing or high liquid flow rates. Affinity sorbents that are fully compatible with robust HPLC process conditions are therefore rare.

Only in the recent past it has been recognised that the mechanical resistance of the stationary phase is a bulk property of the sorbent support whereas only a thin layer at the interface between the stationary and the mobile phases is responsible for mass exchange and for the interaction with the biological analyte. Therefore the concept of combining the function of a mechanically very rigid and dimensionally stable, porous 3-dimensional core, and a biocompatible, gel-like interface layer which carries the active residues for binding the analyte has been brought up, and the associated synthetic problems have been technically solved. Such hybrid materials employ loosely crosslinked polymers of high polarity on a base of either an inorganic oxide or a densely crosslinked polymer of low polarity.

It was an object of the present invention to provide a new sorbent for chromatographic applications which allows the simple and cost-effective purification of organic molecules, even when used in chromatographic applications which demand a high stability of the material either with regard to the

mechanic stress or in view of the solution characteristics of the eluent.

The present invention therefore provides a sorbent comprising a solid support material, the surface of which comprises a residue of the following general formula (I):



formula (I),

wherein the residue is attached via a covalent single bond represented by the dotted line in formula (I) to a functional group on the surface of either the bulk solid support material itself or of a polymer film on the surface of the solid support material, depending on whether the solid support material comprises a polymeric film or not; and

wherein the used symbols and parameters have the following meanings:

L is a (h+1)-valent aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms, wherein

one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S;
one or more CH-moieties in said groups may be substituted by N;
said groups may comprise one or more double bonds between two carbon atoms; and
one or more hydrogen atoms may be substituted by D, F, Cl or OH;

P_S represents independently at each occurrence either a deprotonizable group or an anionic group;

h is 1, 2 or 3, more preferred 1 or 2 and most preferred 1.

An (h+1)-valent linear aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms preferably is one of the following groups: methylene, ethylene, n-propylene, iso-propylene, n-butylene, iso-butylene, sec-butylene (1-methylpropylene), tert-butylene, iso-pentylene, n-pentylene, tert-pentylene (1,1- dimethylpropylene), 1,2-dimethylpropylene, 2,2-dimethylpropylene (neopentylene), 1-ethylpropylene, 2-methylbutylene, n-hexylene, iso-hexylene, 1,2-dimethylbutylene, 1-ethyl-1-methylpropylene, 1-ethyl-2-methylpropylene, 1,1,2-trimethylpropylene, 1,2,2-trimethylpropylene, 1-ethylbutylene, 1-methylbutylene, 1,1-dimethylbutylene, 2,2-dimethylbutylene, 1,3-dimethylbutylene, 2,3-dimethylbutylene, 3,3-dimethylbutylene, 2-ethylbutylene, 1-methylpentylene, 2-methylpentylene, 3-methylpentylene, cyclopentylene, cyclohexylene, cycloheptylene, cyclooctylene, 2-ethylhexylene, trifluormethylene, pentafluorethylene, 2,2,2-trifluorethylene, ethenylene, propenylene, butenylene, pentenylene, cyclopentenylene, hexenylene, cyclohexenylene, heptenylene, cycloheptenylene, octenylene or cyclooctenylene.

It is preferred that L is an (h+1)-valent linear aliphatic hydrocarbon group having 1 to 20 carbon atoms, even more preferred 1 to 10 carbon atoms, or branched or cyclic aliphatic hydrocarbon group having 3 to 20 carbon atoms, even more preferred 3 to 10 carbon atoms,

wherein

one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S;
one or more CH-moieties in said groups may be substituted by N;
said groups may comprise one or more double bonds between two carbon atoms; and
one or more hydrogen atoms may be substituted by D, F, Cl or OH.

It is further preferred that L comprises at least one of the above heteroatoms. Most preferred is that L comprises at least one unit -C(O)- which preferably binds to the surface of the solid support material or the polymer covering the solid support material.

Examples of the linking unit L are the following:

-(C_{1-10}-alkylene)-,
-(C_{1-6}-alkylene)-NH-,
-C(O)-,
-C(O)-NH-,
-C(O)-CH(OH)-,
-C(O)-NH-NH-C(O)O-,
-C(O)-(C_{1-12}-alkylene)-,
-C(O)-NH-(C_{1-6}-alkylene)-,
-C(O)-(C_{1-12}-alkylene)-C(O)-,
-C(O)-(C_{1-12}-alkylene)-NH-C(O)O-,
-C(O)-(C_{1-6}-alkylene)-C(O)-NH-,
-C(O)-(C_{1-6}-alkylene)-C(O)-NH-(C_{1-6}-alkylene)-,
-C(O)-O-(C_{1-6}-alkylene)-,
-C(O)-(C_{1-6}-alkylene)-Y-, wherein Y is NH, O or S,
-C(O)-(C_{1-3}-alkylene)-O-(C_{1-3}-alkylene)-C(O)-NH-,
-C(O)-(C_{1-3}-alkylene)-O-(C_{1-3}-alkylene)-C(O)-NH-(C_{1-6}-alkylene)-,

$-C(O)-(C_{1-6}\text{-alkylene})-C(O)-NH-(C_{1-6}\text{-alkylene})-NH-C(O)-NH-$,

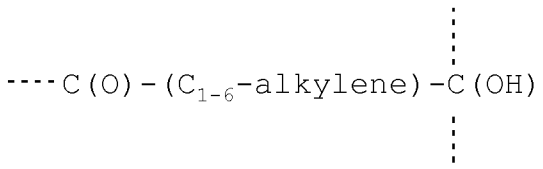
$-CH_2-CH(OH)-CH_2-(OCH_2CH_2)_m-O-$, wherein m is 1, 2, 3, 4, 5 or 6;

$-(C_{1-6}\text{-alkylene})-Y-(C_{1-6}\text{-alkylene})-$, wherein Y is S, O, NH or $-S(O_2)-$;

$-C(O)-(CH(CH_2CH(CH_3)_2))-NH-C(O)-$,

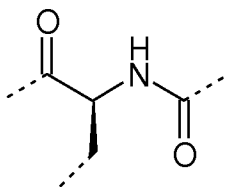
$-C(O)-NH-(C_{1-6}\text{-alkylene})-NH-C(O)-$,

$-C(O)-(C_{1-6}\text{-alkylene})-NH-C(O)-(CH(CH_2CH(CH_3)_2))-NH-C(O)-$,



,

and



,

wherein the following groups are more preferred

$-(C_{1-6}\text{-alkylene})-$,

$-C(O)-(C_{1-6}\text{-alkylene})-C(O)NH-(C_{1-6}\text{-alkylene})-$,

$-C(O)-(C_{1-6}\text{-alkylene})-$,

$-C(O)-CH(NH(C(O)OC(CH_3)_3))-(C_{1-3}\text{-alkylene})-$,

$-C(O)CH(NH_2)(C_{1-3}\text{-alkylene})-$,

$-C(O)-CH(NH(C(=NH)(NH_2)))-(C_{1-6}\text{-alkylene})-$,

$-C(O)-(C_{1-3}\text{-alkylene})-C(=CH_2)-$,

$-C(O)C(=CH_2)-(C_{1-3}\text{-alkylene})-$,

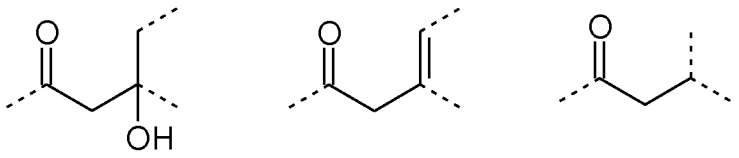
$-C(O)CH=CH-$,

$-C(O)-(C_{1-3}\text{-alkylene})-CH(OH)-(C_{1-3}\text{-alkylene})-$,

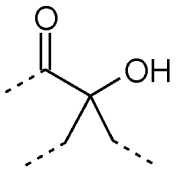
$-C(O)-(C_{1-3}\text{-alkylene})CH=CH-$,

$-C(O)-(C_{1-3}\text{-alkylene})CH(CH_2OH)-$,

$-C(O)-(C_{1-3}\text{-alkylene})-C(=CH_2)-$,



and



and wherein

The following groups are even more preferred:

$-CH_2CH_2CH_2-$,

$-C(O)CH_2-$,

$-C(O)CH_2CH_2-$,

$-C(O)CH_2CH_2CH_2-$,

$-C(O)CH_2CH_2C(O)NHCH_2CH_2-$,

$-C(O)-CH(NH_2)CH_2-$,

$-C(O)-CH(NH(C(O)OC(CH_3)_3))CH_2-$,

$-C(O)CH_2OCH_2-$,

$-C(O)CH_2C(=CH_2)-$,

$-C(O)C(=CH_2)CH_2-$,

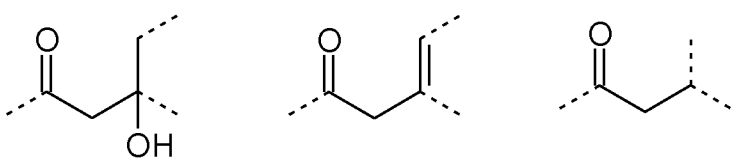
$-C(O)CH=CH-$,

$-C(O)CH_2CH(OH)CH_2-$,

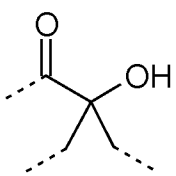
$-C(O)CH_2CH=CH-$,

$-C(O)CH_2CH(CH_2OH)-$,

$-C(O)CH_2C(=CH_2)-$,



and



,

wherein the dotted lines in all above listed linkers L represent the bonds to the functional group of the solid support material or the polymer film and P_S, and wherein in all above listed linkers L it is preferred that the first mentioned atom having a free ending line is connected in this position to the solid support material.

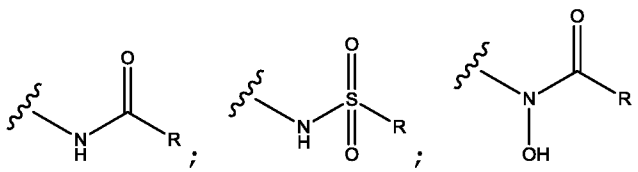
L is even more preferred -C(O)-(C_{1-6}-alkylene)-, and most preferred $-C(O)CH_2CH_2-$.

The group P_S is either an anionic group or a deprotonizable group, i.e. a group which may become an anionic group in solution. It is preferred that these groups are totally or partly present as anionic groups in a ph range of between 6 and 8. But nevertheless the groups P_S may also be polar groups having a hydrogen atom which can be split off by means of stronger bases, wherein these hydrogen atoms are preferably bound to a heteroatom.

Examples of the groups P_S are as follows:

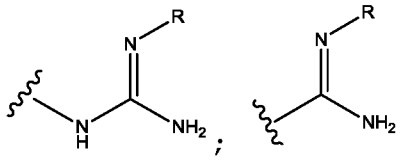
a) $-COOH$, $-SO_3H$, $-CONH_2$, $-CONHNH_2$, $-SO_2NH_2$, $-PO_3H_2$, $-PO(OH)(NH_2)$, $-CO(NHOH)$, $-CO(NH(O-C_{1-4}\text{-Alkyl}))$, $-CSNH_2$, $-NHCONH_2$, $-N(OH)CONH_2$, $-NHCSNH_2$, $-CSNHNH_2$;

b)



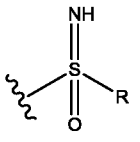
, wherein R = -(C_{1-4}-alkyl), -O(C_{1-4}-alkyl), -NH(C_{1-4}-alkyl), (substituted) aryl, (substituted) O-aryl, (substituted) NH-aryl, -CF_3 and other fluorated alkyl groups;

c)



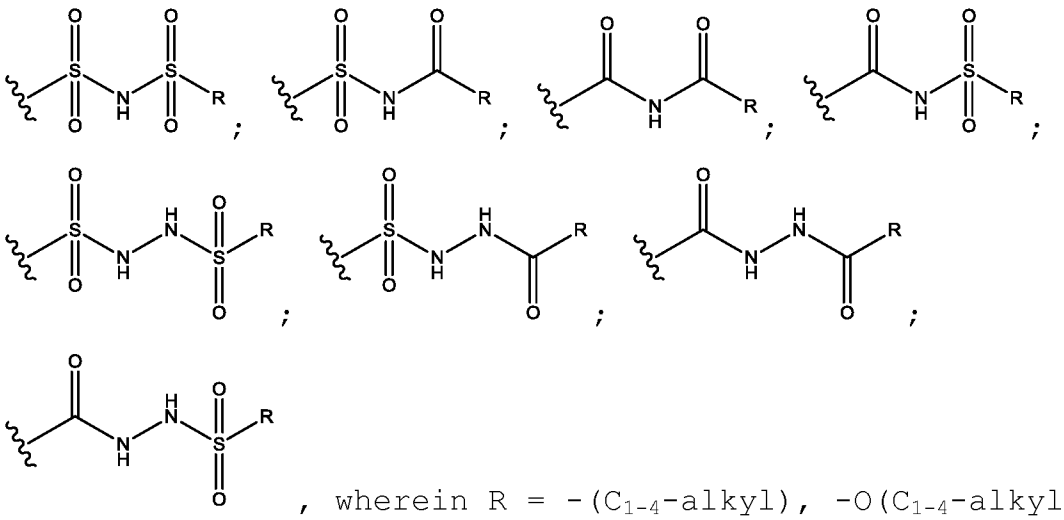
, wherein R = -OH, -CN, -NO_2;

d)



, wherein R = (C_{1-4}-alkyl), (substituted) aryl, -CF_3 and other fluorated alkyl groups;

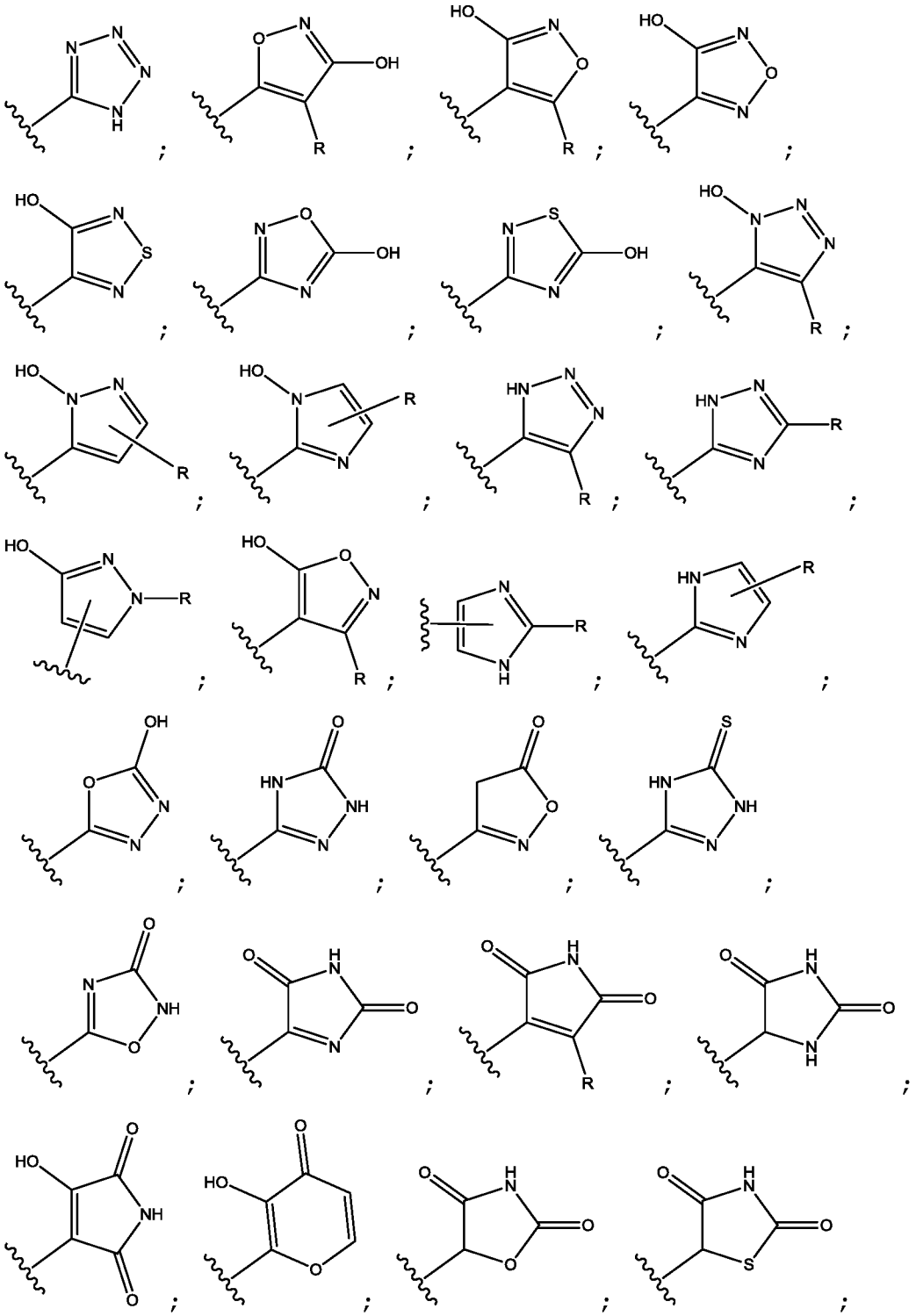
e)

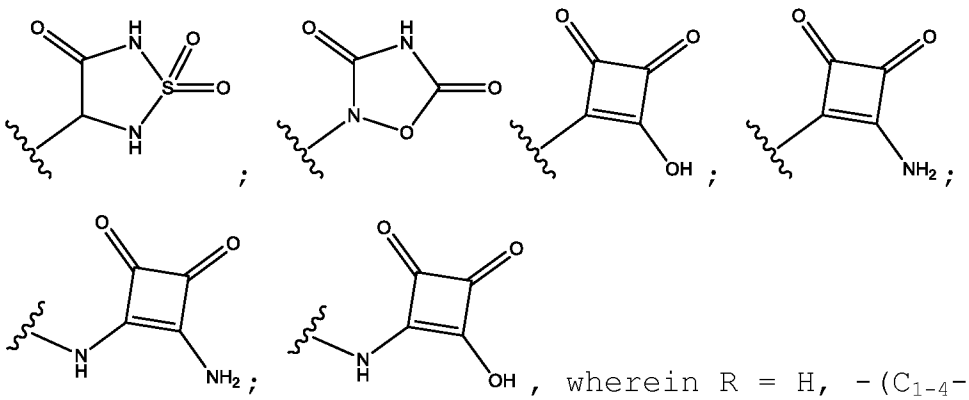


, wherein R = -(C_{1-4}-alkyl), -O(C_{1-4}-alkyl), -NH(C_{1-4}-alkyl), -NH(C_{2-4}-alkenyl), (substituted) aryl,

(substituted) O-aryl, (substituted) NH-aryl, $-CF_3$ and other fluorated alkyl groups;

f)





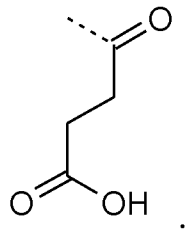
, wherein R = H, -(C_{1-4}-alkyl), -CF_3 and other fluorated alkyl groups;

g) -OH and -SH.

In the sorbent according to the invention it is further preferred, that, if one or more hydrogen atoms of the linker L are substituted by -OH, the group P_S is different from -OH.

It is more preferred that the group P_S is -SO_3H, -COOH or -PO_3H_2, even more preferred -SO_3H or -COOH, and most preferred -COOH.

The most preferred residue according to formula (I) is the following:



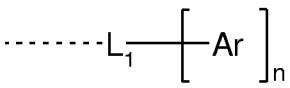
.

In one embodiment according to the invention the sorbent comprises no further residue than the residue according to formula (I).

In another embodiment of the present invention the sorbent according to the invention comprises beneath the residue according to formula (I) a further residue. The further residue is preferably a residue with a hydrophobic group, such

as a mono- or polycyclic aromatic ring system having 6 to 28 aromatic ring atoms or a linear aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms.

In one embodiment the further residue is preferably a residue according to the following formula (II):



formula (II),

wherein the residue is attached via a covalent single bond represented by the dotted line in formula (II) to a functional group on the surface of either the bulk solid support material itself or of a polymer film on the surface of the solid support material, depending on whether the solid support materials comprises a polymer film or not; and

wherein the used symbols and indices have the following meanings:

L_1 is an (n+1)-valent linear aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms, wherein
one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S,
one or more CH-moieties in said groups may be substituted by N,
said groups may comprise one or more double bonds between two carbon atoms, and
one or more hydrogen atoms may be substituted by D, F, Cl or OH;

Ar represents independently at each occurrence a monovalent mono- or polycyclic aromatic ring system having 6 to 28 aromatic ring atoms or a monovalent mono- or polycyclic heteroaromatic ring system having 5 to 28 aromatic ring atoms, wherein one or more hydrogen atoms of the aromatic or heteroaromatic ring system may be substituted by D, F, Cl, OH, C_{1-6}-alkyl, C_{1-6}-alkoxy, NH_2, $-NO_2$, $-B(OH)_2$, -CN or -NC; and

n is an index representing the number of Ar-moieties bound to L_1 and is 1, 2 or 3.

It is particularly preferred that, if the residues of formula (I) are bound to the functional group which is on the surface of the solid support material itself, the sorbent according to the invention comprises the further residue according to formula (II).

The (n+1)-valent linear aliphatic hydrocarbon group has the same meaning as the (h+1)-valent aliphatic hydrocarbon group defined above except for the substitution of the parameter h by n.

It is preferred that L_1 is an (n+1)-valent linear aliphatic hydrocarbon group having 1 to 20 carbon atoms, even more preferred 1 to 10 carbon atoms, or branched or cyclic aliphatic hydrocarbon group having 3 to 20 carbon atoms, even more preferred 3 to 10 carbon atoms,

wherein

one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S,
one or more CH-moieties in said groups may be substituted by N,

said groups may comprise one or more double bonds between two carbon atoms, and
one or more hydrogen atoms may be substituted by D, F, Cl or OH;

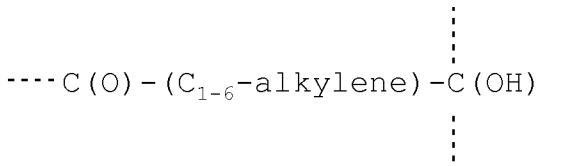
Furthermore, the linking unit L_1 preferably comprises at least one -C(O)-, preferably directly connected to the support material or the polymer film covering the support material.

Examples of the linking unit L_1 are the following:

-(C_{1-10}-alkylene)-,
-(C_{1-6}-alkylene)-NH-,
-C(O)-,
-C(O)-NH-,
-C(O)-CH(OH)-,
-C(O)-NH-NH-C(O)O-,
-C(O)-(C_{1-12}-alkylene)-,
-C(O)-NH-(C_{1-6}-alkylene)-,
-C(O)-(C_{1-12}-alkylene)-C(O)-,
-C(O)-(C_{1-12}-alkylene)-NH-C(O)O-,
-C(O)-(C_{1-6}-alkylene)-C(O)-NH-,
-C(O)-(C_{1-6}-alkylene)-C(O)-NH-(C_{1-6}-alkylene)-,
-C(O)-O-(C_{1-6}-alkylene)-,
-C(O)-(C_{1-6}-alkylene)-Y-, wherein Y is NH, O or S,
-C(O)-(C_{1-3}-alkylene)-O-(C_{1-3}-alkylene)-C(O)-NH-,
-C(O)-(C_{1-3}-alkylene)-O-(C_{1-3}-alkylene)-C(O)-NH-(C_{1-6}-alkylene)-,
-C(O)-(C_{1-6}-alkylene)-C(O)-NH-(C_{1-6}-alkylene)-NH-C(O)-NH-,
-CH_2-CH(OH)-CH_2-(OCH_2CH_2)$_m$-O-, wherein m is 1, 2, 3, 4, 5 or 6;
-(C_{1-6}-alkylene)-Y-(C_{1-6}-alkylene)-, wherein Y is S, O, NH or -S(O_2)-;
-C(O)-(CH(CH_2CH(CH_3)$_2$))-NH-C(O)-,

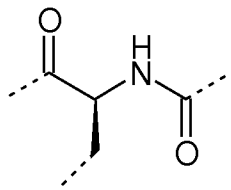
-C(O)-NH-(C_{1-6}-alkylene)-NH-C(O)-,

-C(O)-(C_{1-6}-alkylene)-NH-C(O)-(CH($CH_2CH(CH_3)_2$))-NH-C(O)-,



,

and



,

wherein the following units are more preferred:

-C(O)-,

-C(O)CH_2-,

-C(O)CH_2CH_2-,

-C(O)$CH_2CH_2CH_2$-,

-C(O)-CH=CH-,

-C(O)CH(OH)-,

-C(O)$CH(CH_3)$-,

-C(O)CH_2O-,

-C(O)NH-,

-C(O)$NHCH_2$-,

-C(O)$NHCH(CH_3)$-,

-CH_2CH_2-,

-$(CH_2)_4$-NH-,

-C(O)CH_2CH_2C(O)-,

-C(O)CH_2CH_2C(O)-NH-,

-C(O)CH_2CH_2C(O)$NHCH_2$-,

-C(O)CH_2CH_2C(O)$NHCH_2CH_2$-,

-C(O)CH_2CH_2C(O)$NHCH_2CH_2CH_2$-,

-C(O)CH_2CH_2C(O)$NHCH_2CH_2NHC$(O)NH-,

-C(O)OCH_2-,

-C(O)OCH_2CH_2-,

-C(O)CH_2S-,

-C(O)CH_2OCH_2C(O)$NHCH_2$-,

-CH_2CH_2S(O)$_2CH_2CH_2$-,

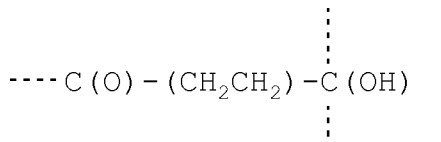
-CH_2CH(OH)$CH_2OCH_2CH_2OCH_2$CH(OH)CH_2-,

-CH_2CH(OH)CH_2(OCH_2CH_2)$_5$O-,

-C(O)(CH_2)$_{10}$-,

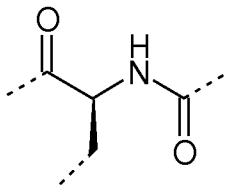
-C(O)(CH(CH_2CH(CH_3)$_2$))-NH-C(O)-,

-C(O)($CH_2CH_2CH_2$)-NH-C(O)-(CH(CH_2CH(CH_3)$_2$))-NH-C(O)-,



,

or



,

wherein the following units are even more preferred:

-C(O)-,

-CH_2CH_2-,

-C(O)NH-,

-C(O)$NHCH_2$-,

-C(O)CH_2O-,

-C(O)CH_2CH_2-,

-C(O)$CH_2CH_2CH_2$-,

-C(O)CH_2CH_2C(O)NH-,

-(CH_2)$_4$-NH-,

-C(O)CH_2CH_2C(O)NH-CH_2-,

-C(O)CH_2CH_2C(O)NH-CH_2CH_2,

-C(O)CH_2CH_2C(O)$NHCH_2CH_2$NHC(O)NH-,

-C(O)OCH_2-,

-C(O)CH_2OCH_2C(O)$NHCH_2$-,

-CH_2CH(OH)CH_2(OCH_2CH_2)$_5$O-,

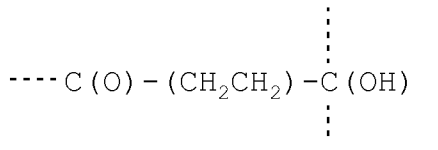
-C(O)-(CH(CH_2CH(CH_3)$_2$))-NH-C(O)-,

-C(O)CH(OH)-,

-C(O)CH(CH_3)-,

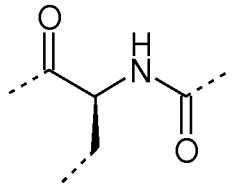
-C(O)NHCH(CH_3)-,

-C(O)-($CH_2CH_2CH_2$)-NH-C(O)-(CH($CH_2CH(CH_3)_2$))-NH-C(O)-,



,

or



,

wherein the dotted lines in all above mentioned definitions of L_1 represent the bonds to the functional group of the solid support material or of the polymer film and Ar, and wherein in all above listed linkers L_1 it is preferred that the first mentioned atom having a free ending line is connected in this position to the solid support material.

It is even more preferred that L_1 is -C(O)-, -CH_2CH_2-, -C(O)CH_2O- or -C(O)NH-, wherein the units are connected to the functional group via its carbonyl atom, -C(O)- and -C(O)NH- being more preferred and -C(O)- being most preferred.

A (monovalent) mono- or polycyclic aromatic ring system in the sense of the present invention is preferably an aromatic ring system having 6 to 28 carbon atoms as aromatic ring atoms. Under the term „aromatic ring system" a system is to be understood which does not necessarily contain only aromatic groups, but also systems wherein more than one aromatic units may be connected or interrupted by short non-aromatic units (< 10 % of the atoms different from H, preferably < 5 % of the atoms different from H), such as sp^3-hybridized C, O, N, etc. or -C(O)-. These aromatic ring systems may be mono- or

polycyclic, i.e. they may comprise one (e.g. phenyl) or two (e.g. naphthyl) or more (e.g. biphenyl) aromatic rings, which may be condensed or not, or may be a combination of condensed and covalently connected rings. The aromatic atoms of the ring systems may be substituted with D, F, Cl, OH, C_{1-6}-alkyl, C_{1-6}-alkoxy, NH_2, $-NO_2$, $-B(OH)_2$, -CN or –NC.

Preferred aromatic ring systems e.g. are: phenyl, biphenyl, triphenyl, naphthyl, anthracyl, binaphthyl, phenanthryl, dihydrophenanthryl, pyrene, dihydropyrene, chrysene, perylene, tetracene, pentacene, benzpyrene, fluorine, indene and ferrocenyl.

A monovalent mono- or polycyclic heteroaromatic ring system having 5 to 28, preferably 5 to 14, most preferred 5 aromatic ring atoms in the sense of the present invention is preferably an aromatic ring system having 5 to 28, preferably 5 to 14, most preferred 5 atoms as aromatic ring atoms. The heteroaromatic ring system contains at least one heteroatom selected from N, O, S and Se (remaining atoms are carbon). Under the term „heteroaromatic ring system" a system is to be understood which does not necessarily contain only aromatic and/or heteroaromatic groups, but also systems wherein more than one (hetero)aromatic unit may be connected or interrupted by short non-aromatic units (< 10 % of the atoms different from H, preferably < 5 % of the atoms different from H), such as sp^3-hybridized C, O, N, etc. or –C(O)-. These heteroaromatic ring systems may be mono- or polycyclic, i.e. they may comprise one (e.g. pyridyl) or two or more aromatic rings, which may be condensed or not, or may be a combination of condensed and covalently connected rings.

Preferred heteroaromatic ring systems are for instance 5-membered rings, such as pyrrole, pyrazole, imidazole, 1,2,3-

triazole, 1,2,4-triazole, tetrazole, furane,thiophene, selenophene, oxazole, isoxazole, 1,2-thiazole, 1,3-thiazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadiazole, 6-membered rings, such as pyridine, pyridazine, pyrimidine, pyrazine, 1,3,5-triazine, 1,2,4-triazine, 1,2,3-triazin, 1,2,4,5-tetrazine, 1,2,3,4-tetrazine, 1,2,3,5-tetrazine, or condensed groups, such as indole, isoindole, indolizine, indazole, benzimidazole, benzotriazole, purine, naphthimidazole, phenanthrimidazole, pyridimidazole, pyrazinimidazole, chinoxalinimidazole, benzoxazole, naphthoxazole, anthroxazole, phenanthroxazole, isoxazole, benzothiazole, benzofurane, isobenzofurane, dibenzofurane, chinoline, isochinoline, pteridine, benzo-5,6-chinoline, benzo-6,7-chinoline, benzo-7,8-chinoline, benzoisochinoline, acridine, phenothiazine, phenoxazine, benzopyridazine, benzopyrimidine, chinoxaline, phenazine, naphthyridine, azacarbazole, benzocarboline, phenanthridine, phenanthroline, thieno[2,3b]thiophene, thieno[3,2b]thiophene, dithienothiophene, isobenzothiophene, dibenzothiophene, benzothiadiazothiophene or combinations of these groups. Even more preferred are imidazole, benzimidazole and pyridine.

An monovalent linear aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms preferably is one of the following groups: methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, sec-butyl (1-methylpropyl), tert-butyl, iso-pentyl, n-pentyl, tert-pentyl (1,1- dimethylpropyl), 1,2-dimethylpropyl, 2,2-dimethylpropyl (neopentyl), 1-ethylpropyl, 2-methylbutyl, n-hexyl, iso-hexyl, 1,2-dimethylbutyl, 1-ethyl-1-methylpropyl, 1-ethyl-2-methylpropyl, 1,1,2-trimethylpropyl, 1,2,2-trimethylpropyl, 1-ethylbutyl, 1-methylbutyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2,3-

dimethylbutyl, 3,3-dimethylbutyl, 2-ethylbutyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl, n-tridecyl, n-tetradecyl, n-pentadecyl, 1-hexylnonyl, n-hexadecyl, 1-hexyl-decyl, n-heptadecyl, n-octadecyl, n-nonadecyl, $-(CH_2)_{20}CH_3$, $-(CH_2)_{21}CH_3$, $-(CH_2)_{22}CH_3$, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, 2-ethylhexyl, trifluormethyl, pentafluorethyl, 2,2,2-trifluorethyl, ethenyl, propenyl, butenyl, pentenyl, cyclopentenyl, hexenyl, cyclohexenyl, heptenyl, cycloheptenyl, octenyl or cyclooctenyl, wherein one or more, preferably one, CH_2-moieties in said groups may be substituted by a group having a hydrogen donor and/or a hydrogen acceptor moiety, such as O, S, $-S(O)_2-$, -C(O)NH- or -C(S)NH-, and wherein one or more hydrogen atoms may be substituted by F, Cl, Br, -CN or -NC, wherein F and -CN is preferred.

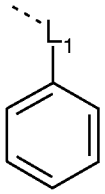
It is, however, preferred that Ar in formula (II) is a (p+1)-valent mono- or polycyclic aromatic rings system.

It is further preferred that Ar in formula (II) is a monovalent aromatic ring system having 6 to 14 aromatic ring atoms, which may be substituted or not. That is, it is more preferred that Ar is phenyl, naphthyl, anthracyl or pyryl, which may be substituted or not. It is even more preferred that either no hydrogen atom of Ar is substituted or one or more hydrogen atoms of Ar is/are substituted by one or more of F or CN. Alternatively, Ar may be substituted with one -CN. In this case Ar may be a phenyl which is substituted with -CN, preferably in para-position with respect to the position of L_1.

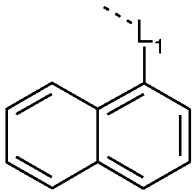
The residues according to formula (II) may in a preferred way be all combinations of preferred and most preferred meanings for L_1 and the most preferred meanings of Ar.

Furthermore, it is preferred that n is 1 or 2, even more preferred 1, so that L_1 is a bivalent linker.

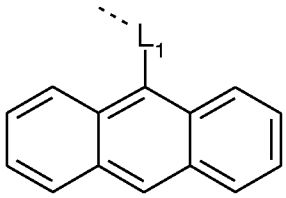
Preferred examples of the residues of formula (II) are the following:



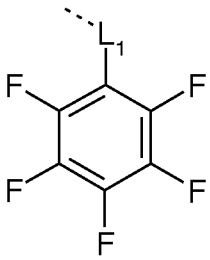
(II)-1



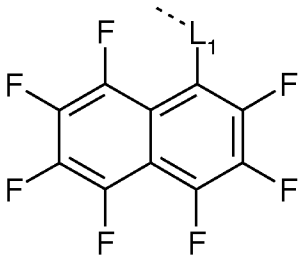
(II)-2



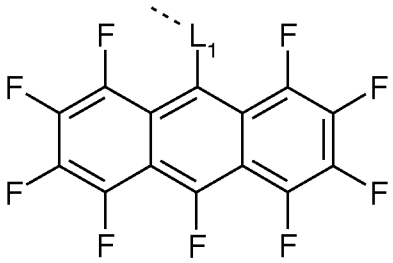
(II)-3



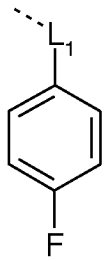
(II)-4



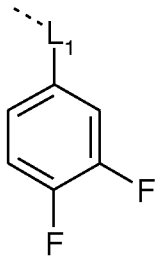
(II)-5



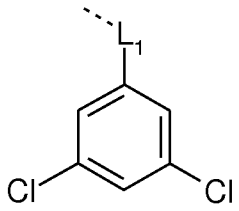
(II)-6



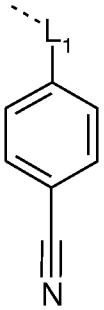
(II)-7



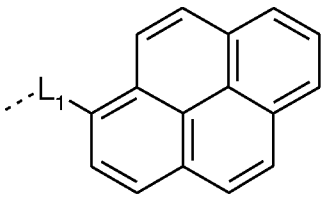
(II)-8



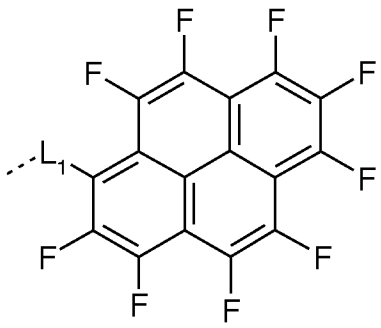
(II)-9



(II)-10



(II)-11

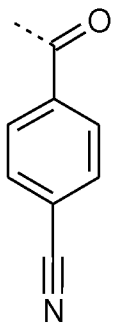


(II)-12

wherein L_1 has the same general and preferred meanings as defined above, and wherein (II)-4, (II)-5, (II)-6, (II)-7,

(II)-8, (II)-9 and (II)-10 are even more preferred, and wherein (II)-4 and (II)-10 are still more preferred and (II)-10 being most preferred.

The most preferred residue of formula (II) is the following:

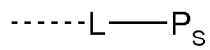


(II)-10-1.

In an embodiment the sorbent according to the present invention only comprises residues according to formula (I).

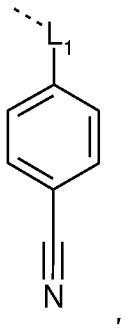
In an embodiment the sorbent of the present invention comprises residues according to formula (I) and residues according to formula (II). In this embodiment it is further preferred that Ar in formula (II) is an aromatic ring system comprising a -CN as substituent, wherein a para-CN-substituted phenyl being more preferred.

In an embodiment the sorbent of the present invention comprises one residue according to formula (I) of the following structure



and

one residue according to formula (II) of the following structure



wherein L, L_1 and P_S independently of each other – but not limited to – have the following meanings:

L_1 is –C(O),

L is $-C(O)-(C_{1-6}\text{-alkylene})-$, wherein $-C(O)CH_2CH_2-$ is most preferred,

P_S is –COOH.

It is further preferred in the before-mentioned embodiment that all of the symbols L, L_1 and P_S have (preferred) meanings as defined.

In case the sorbent according to the invention comprises residues according to formula (I) and residues according to formula (II), the ratio per mole of a residue according to formula (I) to a residue according to formula (II) is preferably in the range of from 0,5 to 2, more preferably from 0,75 to 1,25, still more preferred from 0,9 to 1,1, wherein the amounts of residues are calculated in that the amount of functional groups of the polymer are determined via titration analysis (see Example part) after the residue according to formula (I) has been applied and after the subsequent application of the residue according to formula (II).

According to the present invention a C_{1-6}-alkyl is a linear, branched or cyclic alkyl group. Linear alkyl groups have

preferably 1 bis 6, more preferably 1 to 3 carbon atoms. Branched or cyclic alkyl groups preferably have 3 to 6 carbon atoms. One or more hydrogen atoms of these alkyl groups may be substituted with fluorine atoms. Furthermore, one or more CH_2-groups may be substituted with NR, O or S (R is preferably H or C_{1-6}-alkyl). If one or more CH_2 groups are substituted with NR, O or S, it is preferred that only one of these groups are substituted; even more preferred substituted by an O-atom. Examples of these compounds comprise the following: methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl, t-butyl, 2-methylbutyl, n-pentyl, s-pentyl, cyclopentyl, n-hexyl, cyclohexyl, n-heptyl, cycloheptyl, n-octyl, cyclooctyl, 2-ethylhexyl, trifluormethyl, pentafluorethyl and 2,2,2-trifluorethyl.

A C_{1-6}-alkoxy is a C_{1-6}-alkyl group which is connected via an o-atom.

A C_{1-12}-alkylene, C_{1-10}-alkylene, C_{1-6}-alkylene or C_{1-3}-alkylene is an alkyl groups as defined above, wherein one hydrogen atom is not present and the resulting bivalent unit has two bonds.

A C_{2-4}-alkenyl is a linear or branched alkenyl group with 2 to 4 carbon atoms. One or more hydrogen atoms of these alkenyl groups may be substituted with fluorine atoms. Furthermore, one or more CH_2-groups may be substituted by NR, O or S (R is preferably H or C_{1-6}-alkyl). If one or more CH_2-groups are substituted by NR, O or S, it is preferred that only one of these groups are substituted; even more preferred substituted by an O-atom. Examples of these groups are ethenyl, propenyl and butenyl.

An aryl is a mono- or polycyclic aromatic or heteroaromatic hydrocarbon residue which preferably contains 5 to 20, more

preferred 5 to 10 and most preferred 5 or 6 aromatic ring atoms. If this unit is an aromatic unit it contains preferably 6 to 20, more preferred 6 to 10 and most preferred 6 carbon atoms as ring atoms. If this unit is a heteroaromatic unit it contains preferably 5 to 20, more preferred 5 to 10 and most preferred 5 carbon atoms as ring atoms. The heteroatoms are preferably selected from N, O and/or S. A (hetero)aromatic unit is either a simple aromatic cycle, such as benzene, or a simple heteroaromatic cycle, such as pyridine, pyrimidine, thiophene, etc., or a condensed aryl- or heteroaryl group, such as naphthaline, anthracene, phenanthrene, chinoline, isochinoline, benzothiophene, benzofurane and indole, and so on.

Examples for (hetero)aromatic units are as follows: benzene, naphthalene, anthracene, phenanthrene, pyrene, chrysene, benzanthracene, perylene, naphthacene, pentacene, benzpyrene, furane, benzofurane, isobenzofurane, dibenzofurane, thiophene, benzothiophene, isobenzothiophene, dibenzothiophene, pyrrole, indole, isoindole, pyridine, chinoline, isochinoline, acridine, phenanthridine, benzo-5,6-chinoline, benzo-6,7-chinoline, benzo-7,8-chinoline, phenothiazine, phenoxazine, pyrazole, indazole, imidazole, benzimidazole, naphthimidazole, phenanthrimidazole, pyridimidazole, pyrazinimidazole, chinoxalinimidazole, oxazole, benzoxazole, naphthoxazole, anthroxazole, phenanthroxazole, isoxazole, 1,2-thiazole, 1,3-thiazole, benzothiazole, pyridazine, benzopyridazine, pyrimidine, benzpyrimidine, chinoxaline, 1,5-diazaanthracene, 2,7-diazapyrene, 2,3-siazapyrene, 1,6-diazapyrene, 1,8-diazapyrene, 4,5-diazapyrene, 4,5,9,10-tetraazaperylene, pyrazine, phenazin, phenoxazine, phenothiazine, fluorubine, naphthyridine, benzocarboline, phenanthroline, 1,2,3-triazole, 1,2,4-triazole, benzotriazole, 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-thia-

diazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, 1,3,4-thiadiazole, 1,3,5-triazine, 1,2,4-triazine, 1,2,3-triazine, tetrazole, 1,2,4,5-tetrazine, 1,2,3,4-tetrazine, 1,2,3,5-tetrazine, purine, pteridine, indolizine and benzothiadiazole.

The solid support material is preferably a macroporous material. The pore size of the solid support material is preferably at least 6 nm, more preferably from 20 to 400 nm and most preferably from 50 to 250 nm.

According to an embodiment of the sorbent according to the invention, the solid support material has a specific surface area of from 1 m^2/g to 1000 m^2/g, more preferred of from 30 m^2/g to 800 m^2/g and most preferred of from 50 to 500 m^2/g.

It is preferred that the solid support material has a porosity of from 30 to 80 % by volume, more preferred from 40 to 70 % by volume and most preferred from 50 to 60 % by volume. The porosity can be determined by mercury intrusion according to DIN 66133. The pore size of the solid support material can also be determined by pore filling with the mercury intrusion method according to DIN 66133. The specific surface area can be determined by nitrogen adsorption with the BET-method according to DIN 66132.

The solid support material may be an organic polymeric material or an inorganic material. Especially in case that the sorbent according to the invention comprises more than one residue, the solid support material is preferably an inorganic material.

In case the solid support material is a polymeric material, it is substantially non-swellable. For that reason, it is mostly

preferred that the polymeric material has a high crosslinking degree.

The polymeric material is preferably crosslinked at a degree of at least 5 %, more preferably at least 10 % and most preferably at least 15 %, based on the total number of crosslinkable groups in the polymeric material. Preferably, the crosslinking degree of the polymeric material does not exceed 50 %.

Preferably the polymeric material for the solid support material is selected from the group consisting of generic or surface-modified polystyrene, (e.g. poly(styrene-co-dinvinylbenzene)), polystyrene sulfonic acid, polyacrylates, polymethacrylates, polyacrylamides, polyvinylalcohol, polysaccharides (such as starch, cellulose, cellulose esters, amylose, agarose, sepharose, mannan, xanthan and dextran), and mixtures thereof.

The polymeric material possibly used in the present invention preferably has before the crosslinking has been performed 10 to 10000, particularly preferably 20 to 5000 and very particularly preferably 50 to 2000 repeat units. The molecular weight M_W of the polymeric material before the crosslinking has been performed is preferably in the range of 10000 to 2000000 g/mol, particularly preferably in the range of 100000 to 1500000 g/mol, and very particularly preferably in the range of 200000 to 1000000 g/mol. The determination of M_W can be performed according to standard techniques known to the person skilled in the art by employing gel permeation chromatography (GPC) with polystyrene as internal standard, for instance.

In case the solid support material is an inorganic material, the inorganic material is some kind of inorganic mineral oxide, preferably selected from the group consisting of silica, alumina, magnesia, titania, zirconia, fluorosile, magnetite, zeolites, silicates (cellite, kieselguhr), mica, hydroxyapatite, fluoroapatite, metal-organic frameworks, ceramics and glasses, like controlled pore glass (e.g. trisoperl), metals such as aluminium, silicon, iron, titanium, copper, silver, gold and also graphite or amorphous carbon.

Independent of whether the solid support material is a polymeric material or an inorganic material, the solid support material provides a solid base of a minimum rigidity and hardness which functions as an insoluble support and provides a basis for the enlargement of the interface between stationary and mobile phases which is the place of interaction with the analyte as the molecular basis for the process of the partitioning between said phases, and for an increased mechanical strength and abrasiveness, especially under flow and/or pressurized conditions.

The solid support materials according to the invention may be of homogeneous or heterogeneous composition, and therefore also incorporate materials which are compositions of one or more of the materials mentioned above, in particular multi-layered composites.

The solid support material may be a particulate material, preferably having a particle size of from 5 to 500 µm. The solid support material may also be a sheet- or fibre-like material such as a membrane. The external surface of the solid support material thus may be flat (plates, sheets, foils, disks, slides, filters, membranes, woven or nonwoven fabrics, paper) or curved (either concave or convex: spheres, beads,

grains, (hollow) fibres, tubes, capillaries, vials, wells in a sample tray).

The pore structure of the internal surface of the solid support material may, inter alia, consist of regular, continuous capillary channels or of cavities of irregular (fractal) geometry. Microscopically, it can be smooth or rough, depending on the way of manufacture. The pore system can either extend continuously throughout the entire solid support material or end in (branched) cavities. The rate of an analyte´s interfacial equilibration between its solvation in the mobile phase and its retention on the surface of the stationary phase and thus the efficiency of a continuous flow separation system is largely determined by mass transfer via diffusion through the pores of the solid support material and thus by its characteristic distribution of particle and pore sizes. Pore sizes may optionally show up as asymmetric, multimodal and / or spatially (e.g. cross-sectionally) inhomogeneous distributions.

In one embodiment, the surface of the solid support material may not be covered with a further material, such as a polymer. In this case the residues of formulae (I), and optionally (II), bind to a surface group (functional group) of the solid support material itself. In this case the following solid support materials are preferred: silicagel with alkylsilanol groups containing functional groups, such as a hydroxy group or an amine group, for attaching ligands (i.e. residues according to formula (I) or (II)), aromatic polymers like styrene polymers with functionalized aromatic groups containing amines or carboxylic acids, polymethylmetacrylates with partially cleaved ester groups for attaching ligands.

In one embodiment, the inorganic support is preferred when the residues directly bind to functional groups which are part of the surface of the solid support material itself.

Alternatively, the surface of the solid support material may preferably be covered with a film of a polymer which comprises or consists of individual chains which are preferably covalently crosslinked with each other, but which are preferably not covalently bound to the surface of the solid support material. The inventors of the present invention have surprisingly found that especially for the purification of compounds having both a hydrophobic and a hydrophilic moiety it is important that the polymer is flexible enough to come into a conformation which makes it possible that the both the hydrophobic and the hydrophilic (e.g. ionic interactions) moieties may come into contact with the hydrophobic and hydrophilic moieties of the compound to be purified. In case a polymer film would be used which is covalently bound to the surface of the support material the inventors of the present invention observed that the purification capacity significantly decreased. That is, the use of a non-surface bound cross-linked polymer as a polymer film has three advantages: (1) Flexibility of the polymer due to the fact that it is not surface bound; (2) the cross-linking ensures that the film is adhered to the surface of the support material and is not lost; (3) the thickness of the polymer can be adjusted as thin as wanted, if the polymer is not covalently bound to the polymer.

Furthermore, the polymer covering the surface of the support material is preferably a hydrophilic polymer. The hydrophilic properties of the polymer strengthens the hydrophilic

interactions of the sorbent according to the invention to the compounds to be purified.

The preferred polymer for the crosslinkable polymer is preferably assembled by at least monomers comprising a hydrophilic group, preferably in its side chain. Preferable hydrophilic groups are $-NH_2$, -NH-, -OH, -COOH, $-OOCCH_3$, anhydrides, -NHC(O)- and saccharides, wherein $-NH_2$ and -OH is more preferred and $-NH_2$ is most preferred.

If co-polymers are employed, the preferred co-monomers are simple alkene monomers or polar, inert monomers like vinyl pyrrolidone.

Examples of polymers covering the support material are: polyamines, such as polyvinylamine, polyamino acids, such as polylysin, polyethylene imine, polyallylamine etc. as well as functional polymers other than those containing amino groups, such as polyvinyl alcohol, polyvinyl acetate, polyacrylic acid, polymethacrylic acid, their precursor polymers such as poly(maleic anhydride), polyamides, or polysaccharides (cellulose, dextran, pullulan etc.), wherein polyamines such as polyvinylamine and polyallylamine are more preferred and polyvinylamine is most preferred.

With respect to a superior purification capacity it is further preferred that in the sorbent according to the invention the molar ratio of the residues according to formula (I) to the amount of functional groups of the polymer (derivatization degree) is preferably in the range of 0,25 to 0,6, more preferred in the range of 0,33 to 0,45, wherein the amount of residues according to formula (I) is determined by elemental analysis and the amount of functional groups is determined by

titration (see Example part) of the sorbent before the residues according to formula (I) have been applied.

Furthermore, the sorbent according to the invention preferably contains residues according to formula (I) in the range of from 80 to 220 µmol/mL, more preferred in the range of from 100 to 190 µmol/mL, related to the total volume of the sorbent, wherein the amount is determined by elemental analysis.

The amount of free functional groups of the sorbent according to the invention is in the range of from 10 to 100 µmol/mL, related to the total volume of the sorbent. This amount is determined by titration. The discrepancy between the amount of free functional groups (1) determinable from the molar ratio above and the amount of residues according to formula (I) and (2) the value determined directly by titration is due to the differences in determination via elemental analysis and via titration.

The polymer can be applied to the macroporous support by all means of coating known to a person skilled in the art such as absorption, vapor phase deposition, polymerization from the liquid, gas or plasma phase, spin coating, surface condensation, wetting, soaking, dipping, rushing, spraying, damping, evaporation, application of electric fields or pressure, as well as methods based on molecular self-assembly such as, for example, liquid crystals, Langmuir Blodgett- or layer-by-layer film formation. The polymer may thereby be coated directly as a monolayer or as multilayer or as a stepwise sequence of individual monolayers on top of each other. The ratio of the weight of the polymer covering the support material to the weight of the support material preferably ranges from 0,02 to 0,2, more preferably 0,05 to

0,12, in the sorbent according to the invention. If the above ratio is above the upper limit, the polymer film is too thick and the pores of the support material are totally covered resulting in a sorbent having no available pores. If the above ratio is below the lower limit, the amount of polymer is not enough to cover the entire support material. Furthermore, in the latter case more crosslinking agent would have to be used in order to fix the polymer to the support material, again resulting in a polymer film being not flexible enough.

According to a preferred embodiment of the sorbent according to the invention, the crosslinking degree of the crosslinked polymer is at least 2 %, based on the total number of crosslinkable groups in the crosslinked polymer. More preferred the crosslinking degree is of from 5 to 50 %, more preferred of from 5 to 30 %, most preferred from 10 to 20 %, based on the total number of crosslinkable groups in the crosslinked polymer. The crosslinking degree can easily be adjusted by the stoichiometric amount of the crosslinking reagent used. It is assumed that nearly 100 mol% of the crosslinker reacts and forms crosslinks. This can be verified by analytical methods. The crosslinking degree can be determined by MAS-NMR spectroscopy and quantitative determination of the amount of crosslinker in relation to the amount of polymer. This method is most preferred. The crosslinking degree can also be determined by IR spectroscopy based on e.g. C-O-C or OH vibrations using a calibration curve. Both methods are standard analytical methods for a person skilled in the art.

The crosslinking reagent used for crosslinking the polymer is preferably selected from the group consisting of dicarboxylic acids, diamines, diols, urea and bis-epoxides, such as terephthalic acid, biphenyl dicarboxylic acid, 1,12-Bis-(5-

norbornen-2,3-dicarboximido)-decandicarboxylic acid and ethylene glycol diglycidylether. In one embodiment the at least one crosslinking reagent is a linear, conformationally flexible molecule of a length of between 4 and 20 atoms. Preferred examples of crosslinking reagents are 1,12-Bis-(5-norbornen-2,3-dicarboximido)-decandicarboxylic acid and ethylene glycol diglycidylether.

Preferred molecular weights of the polymers used range from, but are not limited to, 5000 to 50000 g/mol, which is particularly true for polyvinylamine. Polymers having a molecular weight near the lower limit of the range given above have shown to penetrate even narrow pores of the carrier so that solid state materials with high surface areas and consequently with good mass transfer kinetics, resolution and binding capacity can be used in the sorbents of the present invention.

According to a further embodiment the crosslinked polymer carries functional groups.

The term "functional group" means any simple, distinct chemical moiety belonging to the crosslinked polymer on the surface of the solid support material or to the crosslinkable polymer during preparation of a polymer film on the surface of the solid support material. Thereby, the functional group may serve as chemical attachment point or anchor. Functional groups preferably contain at least one weak bond and/or one heteroatom, preferably a group behaving as nucleophil or electrophil.

The preferred functional groups are primary and secondary amino, hydroxyl, and carboxylic acid or ester groups, when taken before the residues of formulae (I) or (II) have been

bound to these groups. When the residues are bound to the functional groups the nature of these groups change with respect to the structure of the residues bound.

The invention also relates to a method for preparing a sorbent, preferably the sorbent according to the invention, comprising:

(i) providing a polymer having functional groups;
(ii) adsorbing a film of said polymer onto the surface of a carrier;
(iii) crosslinking a defined portion of said functional groups of the adsorbed polymer with at least one crosslinking reagent;
(iv) derivatising further defined portions of said functional groups of the crosslinked polymer with one or more residues according to the formulae (I) and/or (II).

The polymer to be adsorbed on the surface of the carrier is preferably solved in an aqueous media wherein the pH is suitably adjusted in order to solve or suspend the polymer. The adsorbing of the polymer on the surface of the carrier is preferably done by dipping the carrier into the solution or suspension containing the polymer. The mixture is then preferably shaked in order to get a complete mix of the ingredients. Capillaric forces make sure that pores of the carrier are soaked with the solution or suspension. Then, the water is preferably evaporated in vacuum at a temperature between 40 and 60°C, thereby depositing the polymer at the walls of the pores in the form of a film. Then, the coated material is preferably suspended in an organic solvent, such as isopropanol or dimethylformamide (DMF), and is preferably crosslinked by means of a crosslinking agent, such as ethylene

glycol diglycidyl ether, preferably at a temperature between 25 and 60°C for 4 to 8 hours.

In the case, wherein the solid support material does not contain a polymeric material on its surface, the residues according to formulae (I) and/or (II) bind directly to functional groups on the surface of the solid support material.

Depending on the kind of functional groups and depending on the residue according to formula (I) or (II) different derivatization strategies of the solid support can be used. If the solid support material contains amino groups as functional groups, residues containing a carboxylic acid group can be attached to the amine nitrogen atom via the carboxylic carbon atom via peptide chemistry using coupling reagents like 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), propylphosphonic anhydride (T3P) etc. or by using reactive groups in the reagent like isocyanates, epoxides on anhydrides. If the solid support material contains amino groups, aliphatic carbon atoms of the residue according to formula (I) or (II) may be bound to the amine nitrogen atom via a nucleophilic aliphatic substitution. In case the residue according to formula (I) or any other residue contains carboxylic acid groups as group P_S, these groups have to be protected in order to ensure that the carboxylic acid group of the linker (before being attached to the solid support material) and not the group P_S binds to the functional group on the surface of the solid support material.

If the solid support material contains hydroxy groups, residues according to formulae (I) and (II) containing a carboxylic acid group before being attached to the functional group may be attached to the oxygen atom of the hydroxy group via the carboxylic carbon atom by using the carboxylic acid chloride or the ester of the carboxylic acid group. If the solid support material contains hydroxy groups, aliphatic carbon atoms of the residue according to formulae (I) and (II) may be bound to the oxygen atom of the hydroxy group via a nucleophilic aliphatic substitution.

If the solid support material contains carboxylic acid groups, carboxylic acid esters or carboxylic acid anhydrides, the residue according to formulae (I) and (II) may be attached via nucleophilic attack of a nucleophilic group, such as $-NH_2$, -OH, -SH at the electrophilic carbon atom of the carboxylic acid group, acid ester or anhydride, thereby forming an amide, ester or thioester.

The sorbent of the present invention may be used for the purification of organic molecules (organic compounds) or the purification of solutions from certain organic molecules. That is, the present invention further refers to the use of a sorbent according to the invention for the purification of organic molecules or the purification of solution from organic molecules.

The term "purification" is referred to as comprising separating, or increasing the concentration and/or purity of an organic molecule from a mixture containing said organic molecule.

In other words the present invention is also directed to a method of purification of organic molecules which also

includes the separation of unwanted organic molecules from a solution by using the sorbent of the present invention.

The use of the sorbent according to the invention for the purification of organic molecules or the method for the purification of organic molecules by using the sorbent according to the invention comprises the following steps:

(i) applying a crude mixture comprising the organic molecules being dissolved or suspended in a liquid on a chromatographic column containing the sorbent according to the invention or a sorbent prepared according to a method of the invention;

(ii) elution of the organic molecule from the column by using an eluent.

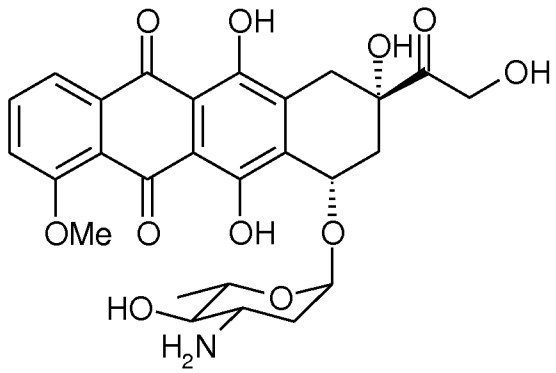
The eluent used in step (ii) may be the same solvent as used for the liquid in step (i), but may also be different, depending on the conditions necessary for the purification of the organic molecules. As liquid in step (i) or eluent in step (ii) every kind of solvent or buffering systems applicable in the field of chromatography may be used. In the present invention aqueous buffering systems also may be used in combination with alcohols having a low molecular weight, such as methanol, ethanol. Other possible organic solvents are for instance heptane, hexane, toluene, dichloromethane, etc. In the present invention it is, however, particularly preferred that the eluent or solvent is pure water or water containing NH_4HCOO or other basic substances.

The organic molecules purified by means of the sorbent of the present invention are preferably pharmaceutically active compounds.

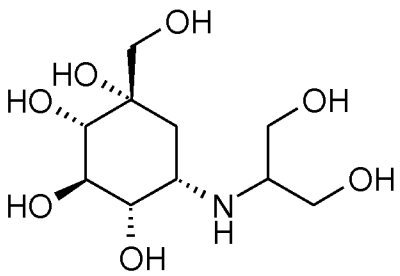
The organic molecules to be purified are preferably compounds having a hydrophilic and a hydrophobic moiety in its molecule. More preferably the organic molecules are compounds having beneath a hydrophobic hydrocarbon moiety groups which are able to act as hydrogen donor or hydrogen acceptor. The organic molecule is preferably a compound having one or more of the moieties selected from the groups consisting of -OH, -O-, -S- and -C(O)-. Most preferred the organic molecule is a compound having 2 or more, preferably 3 or more hydroxyl groups.

The organic molecules have preferably a molecular weight in the range of from 500 to 200000 g/mol, more preferably in the range of from 500 to 150000 g/mol, and most preferred of from 500 to 2500 g/mol.

Particularly preferred as organic molecules used in the use/process of the present invention are epirubicine, voglibose and their derivatives, wherein epirubicine and voglibose have the following structures:



epirubicine, and



voglibose.

Furthermore, the sorbent according to the invention may also be used for separating endotoxines from solutions. The term „endotoxines" as used in the present invention refers to a class of biochemical substances. Endotoxines are decomposition products of bacteria, which may initiate variable physiologic reactions in humans. Endotoxines are components of the outer cell membrane (OM) of gram-negative bacteria or blue-green algae. From the chemical view endotoxines are lipopolysaccharides (LPS) which are composed of a hydrophilic polysaccharide component and a lipophilic lipide component. In contrast to the bacteria endotoxines stem from, endotoxines are very thermally stable and endure sterilisation. The currently most sensitive method of measuring endotoxines is made by means of the activation of the coagulation cascade in the lysate of amoebocytes which have been isolated from limulus polyphemus. This test is commonly known as the so-called LAL-test.

In the case of the purification of epirubicine, voglibose and their derivatives, preferably the epirubicine or voglibose as shown above, it is preferred that a sorbent according to the invention is used which comprises a residue according to formula (I). In this case it is particularly preferred that the residue is $-C(O)-CH_2CH_2COOH$.

In the case of the purification of epirubicine or its derivatives, preferably epirubicine it is preferred that a sorbent according to the invention is used which comprises a residue according to formula (I), more preferred comprising only a residue according to formula (I). It is further preferred that the residue according to formula (I) is $-C(O)-CH_2CH_2COOH$.

In the case of the purification of voglibose or its derivative, preferably voglibose, it is further preferred that a sorbent according to the invention is used which comprises a residue according to formula (I) and a residue according to formula (II). In this case it is particularly preferred that the residue according to formula (I) is $-C(O)-CH_2CH_2COOH$ and that the residue according formula (II) is that of formula (II)-10-1.

The invention also relates to a column for liquid chromatography or solid phase extraction comprising a sorbent according to the invention or a sorbent prepared according to a method according to the invention as a stationary phase within a tubular containment and optionally further components such as frits, filter plates, flow distributors, seals, fittings, screwings, valves, or other fluid handling or connection elements. In one embodiment, the method is further characterised by its physical and chemical resistance against applied pressures up to 20 bar, against applied heat up to 110 °C, as well as against common sanitisation protocols, thus enabling its repetitive use of up to 1,000 times, preferably up to 5,000 times. The invention also relates to a collection of a plurality of the same or different sorbents according to the invention or of sorbents prepared according to a method according to the invention or of columns according to the invention in the format of a microplate or microchip array, or

a multi-capillary or microfluidic device, capable of being processed in parallel.

The invention also relates to a diagnostic or laboratory purification kit comprising a sorbent according to the invention or a sorbent prepared according to a method according to the invention or a column according to the invention or a collection of sorbents or columns according to the invention and, within the same packaging unit, further chemical or biological reagents and / or disposables necessary for carrying out the method according to the invention or a different analytical, diagnostic, or laboratory method different therefrom.

The present invention further refers to the following embodiments:

(i) A method for the purification of organic molecules by using a sorbent according to the invention.

(ii) The method according to embodiment (i), wherein the organic molecules are pharmaceutically active compounds.

(iii) The method according to embodiment (i) or (ii), wherein the organic molecules have a molecular weight in the range of from 500 to 200000 g/mol.

(iv) The method according to any one of embodiments (i) to (iii), wherein the organic molecules are selected from the group consisting of epirubicine, voglibose, their derivatives and endotoxines.

The present invention is further explained by means of the following figures and examples which should however not be understood as being limiting for the scope of the present invention:

Figures:

Fig. 1: Fractionation chromatogram of the purification of epirubicine in Example 3

Fig. 2: Fractionation chromatogram of the purification of voglibose in Example 4

Fig. 3: LC-MS analytics of the fractionated product (3a) and a mixture with the impurities (3b).

Fig. 4: Sample curve for the determination of the amount of amine groups by means of break-through measurement with 4-toluene sulfonic acid (front analysis).

Examples:

Analytical methods:

Determination of the amount of amine groups by means of break-through measurement with 4-toluene sulfonic acid (front analysis) (titration):

The respective sorbent is packed into a column having the dimensions 33.5 x 4 mm (bed volume 0.42 mL). The filled column is then flushed with the following media at a flow rate of 1.0 mL/min:

- 5 mL of water
- 10 mL of a 100 mM aqueous solution of ammonium acetate
- 1 mL of water
- 10 mL of a 100 mM aqueous solution of trifluoroacetic acid

- 10 mL of water

A base line is detected at a HPLC-device having a pump and a UV-detector after water has been pumped through the device for 5 min at 0.5 mL/min. After that a solution of 10 mM 4-toluene sulfonic acid in water is pumped through, whereas the extinction of the eluent is detected at 274 nm. The extinction rises in few minutes to a level of about 700 mAU and remains constant at this level (flush-in curve). After 25 min the column is applied between pump and detector and is flushed with 10 mM of 4-toluene sulfonic acid at 0.5 mL/min. The extinction then drops to 0 mAU since the column is binding 4-toluene sulfonic acid. If the capacity of the column is exhausted, the extinction of the eluate again rises to the starting level of ~700 mAU.

For the determination of the capacity of 4-toluene sulfonic acid the area below the level of the flush-in curve is integrated as comparative area, thereby obtaining the relationship between surface area and the amount of 4-toluene sulfonic acid. After that the area (break-through area) of the toluene sulfonic acid solution absorbed by the column is titrated, and the volume of the device and the dead volume of the column (0.5 mL) are subtracted. The break-through area directly indicates the amount of 4-toluene sulfonic acid bound to the column. Dividing this amount by the volume of the column yields in the capacity of toluene sulfonic acid per mL of the sorbent, also resulting in the amount of amine groups of the sorbent. For the better understanding of this method Fig. 4 shows such an example curve.

Example 1: Method of producing a sorbent according to the invention comprising residues of the following formula: $-C(O)-CH_2CH_2COOH$:

Silicagel SP-1000-10 from DAISO was coated with polyvinylamine using 66.7 g of a 12% polyvinylamine solution in water with adjusted pH between 9.0 to 9.5 for 100 g of silicagel. The mixture was agitated on a sieve shaker until the solution was fully soaked up in the pores of the silicagel. After that the sorbent was dried in vacuum at 50°C until the water was completely evaporated. Afterwards the dried sorbent was suspended in 150 mL N,N-Dimethylmethanamide (DMF) and agitated at 25°C for 16 hours with 1.28 g of 1,12-Bis-(5-norbornen-2,3-dicarboximido)-decandicarboxylic acid. Afterwards the sorbent was filtered off and washed with 230 mL DMF, 390 mL 0.5 M trifluoroacetic acid (TFA) in DMF, 780 mL 0.1 M TFA in H_2O, 230 mL H_2O and 230 mL MeOH. After drying the sorbent is ready for further modification.

The amount of amine groups of the resulting intermediate determinable by titration was about 395 µmol/mL.

For the modification of the polymer 50 g of the coated sorbent was washed with 15 mL triethylamine (TEA) in 250 mL DMF. After washing the sorbent was suspended in 250 mL DMF. 3.98 g of succinic anhydride was diluted in 50 mL DMF, 5.54 mL TEA was added and the mixture was given to the suspended sorbent. After 5 hours of stirring at room temperature, the mixture was filtered off and the sorbent washed with 500 mL 0.1 M TFA in DMF and 200 mL DMF. The second derivatization step was carried out similar to the first but this time the reaction mixture was left stirring for 18 hours. After that the reaction mixture was filtered off and the sorbent was washed with 500

mL DMF, 1000 mL 0.1 M TFA in DMF, 500 mL water and 500 mL methanol. Afterwards the sorbent was dried at 40°C in vacuum.

The resulting sorbent contains about 132 µmol/mL of the residues $-C(O)-CH_2CH_2COOH$, determined via elemental analysis. The ratio of amount of the residues $-C(O)-CH_2CH_2COOH$ (ligand) to the amount of the sorbent without ligand is about 0,34.

Example 2: Method of producing a sorbent according to the invention comprising residues of formula (II)-10-1 and residues of the following formula: $-C(O)-CH_2CH_2COOH$:

The coating and crosslinking of the sorbent was performed according to Example 1.

The amount of amine groups of the resulting intermediate determinable by titration was about 395 µmol/mL.

Further modification was done as follows: 10 g of the sorbent was washed with 150 mL 0.5 M TEA in DMF and suspended afterwards in 30 mL DMF. 640 mg 4-cyanobenzoic acid, 1.72 g 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 590 mg N-Hydroxybenzotriazole (HOBt) and 605 µL TEA were diluted in 15 mL DMF and given to the suspension. The mixture was agitated for 12 hours and subsequently filtered off. The sorbent was washed with 150 mL DMF, 150 mL 0.1 M TFA in DMF, 150 mL DMF, 150 mL 0.5 M TEA in DMF and 150 mL DMF. Afterwards the sorbent was resuspended in 20 mL DMF and 213 mg 4-cyanobenzoic acid, 549 mg HBTU, 196 mg HOBt and 203 µL TEA were added. The mixture was agitated for 24 hours and subsequently washed with 100 mL DMF, 150 mL 0.5 M TFA in DMF, 150 mL DMF, 150 mL 0.5 M TEA in DMF and 150 mL DMF. Afterwards it was suspended in 30 mL DMF. 724 mg succinic

acid anhydride and 1 mL TEA were added and the mixture agitated for 16 hours at 25°C. The washing and reaction step was performed three additional times before the sorbent was washed with 150 mL DMF, 150 mL 0,5 M TFA in DMF, 150 mL 0,5 M TFA in water, 150 mL water, 150 mL methanol and dried in vacuum at 50°C.

The resulting sorbent contains about 169 µmol/mL of the residues $-C(O)-CH_2CH_2COOH$, determined via elemental analysis. The ratio of amount of the residues $-C(O)-CH_2CH_2COOH$ (ligand) to the amount of the sorbent without ligand is about 0,43. The ratio of residues according to formula (I) to residues according to formula (II) is about 1,04.

Example 3: Purification of epirubicine by using the sorbent produced in Example 1:

The crude mixture of epirubicine and several impurities were separated using an Dionex HPLC system consisting of a four channel low-pressure gradient pump (LPG 580, LPG 680 or LPG 3400), auto sampler (Gina 50, ASI-100 or WPS-300), six-channel column switching valves (Besta), column oven and a diode-array uv detector (UVD 170U, UVD 340S or VWD 3400). The sorbent produced in Example 1 was filled in a 250x4 mm steel column. The gradient and flow rate was used as shown in Table 1 below. The fractionation chromatogram is shown in Figure 1. Table 2 shows the analytics of the several fractions taken. Combining the fractions I8 to I13 epirubicin is obtained in 96.7% purity and 73.4% yield.

Table 1: Gradient data of the purification of epirubicine

Ret. time [min]	Flow Rate [mL/min]	400 mM NH_4HCOO pH 6 [%]	Water [%]	Isopropyl-alcohol [%]
0	0.25	30	70	0
29.5	0.25	30	70	0
29.5	0.25	30	10	60
67.5	0.25	30	10	60
67.5	0.50	30	10	60
120	0.50	30	10	60

Table 2: Analytical data of the fractionation of epirubicine and several known and unknown impurities:

fraction	Purity [%]	Yield [%]	Doxorubicin [%]	unknown RT 9,3 min [%]	unknown RT 13,0 min [%]	Sum [%] of these three impurities
I 3	4,67	0,74	0,57	-	2,62	3,19
I 4	37,21	2,46	6,23	6,81	22,69	35,73
I 5	67,9	4,86	3,43	3,83	9,19	16,45
I 6	82,07	7,56	3,85	2,32	3,94	10,11
I 7	90,78	9,57	1,37	1,47	2,38	5,22
I 8	93,27	11,14	1,05	1,29	1,74	4,08
I 9	95,22	12,44	0,89	0,94	1,17	3,00
I 10	96,83	13,35	0,87	0,58	0,56	2,01
I 11	98,01	14,24	0,67	0,13	0,15	0,95
I 12	98,69	14,91	0,2	0,05	0,08	0,33
I 13	97,44	7,36	0,06	0,07	0,1	0,23
I 14	86,53	0,56	0,17	0,43	0,83	1,43
I 15	79,75	0,82	0,05	0,42	1,05	1,52

RT: retention time

Sorbents similarly produced according to Example 1 having a molar ratio of the residues according to formula (I) to the amount of functional groups of the polymer of less than 0,3 or more than 0,6 are more than 50% deteriorated with respect to the purity and yield of the obtainable epirubicine.

Sorbents similarly produced according to Example 1 comprising more than 100 µmol/mL of residues according to formula (I) (ligand) showed a lower, but still acceptable purification capacity in good yields. Lowering the amount of ligand to less than 80 µmol/mL decreases the purification capacity and yield significantly. By using sorbents with an amount of more than 220 µmol/mL it was almost no retention of epirubicine could be observed. Tolerable values of purity and yield were only obtained with sorbents of values up to 190 µmol/mL.

Example 4: Purification of voglibose by using the sorbent produced in Example 2:

The crude mixture of voglibose and several impurities were separated using an Dionex HPLC system consisting of a four channel low-pressure gradient pump (LPG 580, LPG 680 or LPG 3400), auto sampler (Gina 50, ASI-100 or WPS-300), six-channel column switching valves (Besta), column oven and a diode-array uv detector (UVD 170U, UVD 340S or VWD 3400). The sorbent produced in Example 2 was filled in a 250x4 mm steel column. The mobile phase consisted solely of pure water. As indicated in Figure 2 the product fraction was taken after the two main impurities eluated around 17 to 19 minutes up to 99 minutes until the product peak reached baseline. The product fraction and the crude mixture were analyzed using LC-MS as shown in Figure 3a (product fraction with no impurities) and 3b (impurities). According to LC-MS the critical impurities were well depleted below the 0.047% of the standard mixture.

Sorbents similarly produced according to Example 2 having a molar ratio of the residues according to formula (I) to the amount of functional groups of the polymer of less than 0,3 or

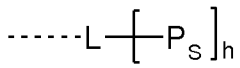
more than 0,6 are more than 40% deteriorated with respect to the purity and yield of the obtainable voglibose.

Sorbents similarly produced according to Example 2 comprising more than 100 µmol/mL of residues according to formula (I) (ligand) still showed an acceptable purification capacity in good yields. Lowering the amount of ligand to less than 80 µmol/mL decreases the purification capacity and yield significantly. By using sorbents with an amount of more than 220 µmol/mL it was almost not retention of voglibose was observed. Tolerable values of purification were only obtained with sorbents of values up to 190 µmol/mL.

Furthermore, sorbents with a ratio of residues according to formula (I) to residues according to formula (II) below 0,9 resulted in slightly decreased purity and yield, wherein a ratio below 0,75 still resulted in a lower but acceptable purity and yield, and a ratio below 0,5 was insufficient in this respect, as well as a ratio above 2. Ratios below 1,25 still resulted in acceptable purities and yields, but were more than 20 % deteriorated compared to the sorbent according to Example 2.

Claims:

1. Sorbent comprising a solid support material, the surface of which comprises a residue of the following general formula (I):



formula (I),

wherein the residue is attached via a covalent single bond represented by the dotted line in formula (I) to a functional group on the surface of either the bulk solid support material itself or of a polymer film on the surface of the solid support material, depending on whether the solid support material comprises a polymeric film or not; and

wherein the used symbols and parameters have the following meanings:

L is a (h+1)-valent aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms, wherein

one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S;
one or more CH-moieties in said groups may be substituted by N;
said groups may comprise one or more double bonds between two carbon atoms; and

one or more hydrogen atoms may be substituted by D, F, Cl or OH;

P_S represents independently at each occurrence either a deprotonizable group or an anionic group;

h is 1, 2 or 3.

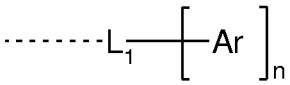
2. Sorbent according to claim 1, wherein L comprises at least a moiety
-C(O)-.

3. Sorbent according to claim 1 or 2, wherein
h is 1.

4. Sorbent according to any one of claims 1 to 3, wherein P_S is -COOH, $-SO_3H$, $-CONH_2$, $-CONHNH_2$, $-SO_2NH_2$, $-PO_3H_2$, $-PO(OH)(NH_2)$, -CO(NHOH), $-CO(NH(O-C_{1-4}-Alkyl))$, $-CSNH_2$, $-NHCONH_2$, $-N(OH)CONH_2$, $-NHCSNH_2$, $-CSNHNH_2$.

5. Sorbent according to claim 4, wherein P_S is -COOH.

6. Sorbent according to any one of claims 1 to 5 comprising a further residue of the following general formula (II):



formula (II),

wherein the residue is attached via a covalent single bond represented by the dotted line in formula (II) to a functional group on the surface of either the bulk solid support material itself or of a polymer film on the surface of the solid support material, depending on

whether the solid support materials comprises a polymer film or not; and

wherein the used symbols and indices have the following meanings:

L_1 is an (n+1)-valent linear aliphatic hydrocarbon group having 1 to 30 carbon atoms or branched or cyclic aliphatic hydrocarbon group having 3 to 30 carbon atoms;
wherein
one or more CH_2-moieties in said groups may be substituted by a CO, NH, O or S;
one or more CH-moieties in said groups may be substituted by N;
said groups may comprise one or more double bonds between two carbon atoms; and
one or more hydrogen atoms may be substituted by D, F, Cl or OH;

Ar represents independently at each occurrence a monovalent mono- or polycyclic aromatic ring system having 6 to 28 aromatic ring atoms or a monovalent mono- or polycyclic heteroaromatic ring system having 5 to 28 aromatic ring atoms, wherein one or more hydrogen atoms of the aromatic or heteroaromatic ring system may be substituted by D, F, Cl, OH, C_{1-6}-alkyl, C_{1-6}-alkoxy, NH_2, $-NO_2$, $-B(OH)_2$, -CN or -NC; and

n is an index representing the number of Ar-moieties bound to L_1 and is 1, 2 or 3.

7. Sorbent according to claim 6, wherein Ar is an aromatic ring system wherein one or more hydrogen atoms are substituted by F and/or -CN.

8. Sorbent according to any one of claims 1 to 7, wherein the surface of the solid support material is covered with a film of a polymer which comprises or consists of individual chains which are covalently crosslinked with each other, but which are not covalently bound to the surface of the solid support material.

9. Sorbent according to claim 8, wherein the polymer is a polyamine, a polyvinylamine, or a copolymer or a polymer blend comprising polyamine.

10. Use of a sorbent according to any one of claims 1 to 9 for the purification of organic molecules.

11. Use according to claim 10, wherein the organic molecules are pharmaceutically active compounds.

12. Use according to claim 10 or 11, wherein the organic compounds have a molecular weight in the range of from 500 to 200000 g/mol.

13. Use according to any one of claims 10 to 12, wherein the organic compounds are selected from the group consisting of epirubicine, voglibose and their derivatives, and endotoxines.