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(54) CARRIER MATERIAL FOR ENZYMES CONSISTING OF AN INORGANIC POROUS MATERIAL AND A POLYMER, AND A METHOD FOR ITS PREPARATION

(71) We, ČESKOSLOVENSKÁ AKADEMIE VĚD, a Czechoslovak Corporation, of No, 3 Národní, Praha 1, Czechoslovakia, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to a carrier comprising an inorganic porous material and a polymer containing in the polymeric molecule epoxy groups, and to a method for its preparation. The form of the system is completely dependent on the form in which the inorganic material used is present.

At present, the reactivity of the epoxy groups of monomers is made use of mainly in the preparation of insoluble resins, when an inorganic material, glass fibres in this case, is incorporated in the polymeric network under comparatively exacting polymerization conditions and with addition of another monomer or crosslinking agent. Monomers containing epoxy groups are in this case some kind of "binding" compound for the material formed, which consequently is completely inactive.

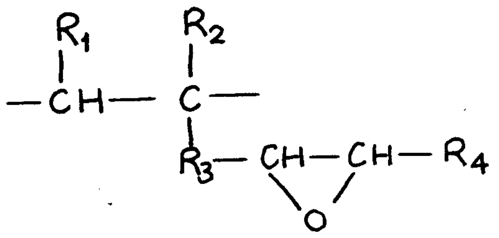
The use of inorganic materials as carriers is restricted by the low reactivity of the inorganic surface. Activations carried out with aminoalkylsilanes remove this disadvantage. Free amino groups of aminoalkylsilane can then be employed in bonding active compounds (e.g. enzymes), which however reduces the possibilities of bonding only to known reactions of the amino group. Such procedure of activation of the surface of inorganic materials does not lead to any improvement in the stability of inorganic materials in certain pH regions.

The carriers comprising an inorganic porous material and a reactive polymer combine good properties of the inorganic material with the high reactivity of the polymeric coating. Advantages of such known carriers consist in their high mechanical stability, shape variability, noncompressibility within a wide range of hydrostatic pressures, abrasion resistance, resistance against microorganisms, shape stability against solvents and temperature, and last but not least stability within a wide range of pH, so that there is no need to use other, more expensive coatings.

The variable porosity of the known carrier removes the disadvantage of high diffusion resistivities against mass transfer, which is an obstacle to the uses of inorganic materials in reactors.

An undeniable advantage of the carrier according to invention is not only the combining of inorganic porous materials with a polymer containing epoxy groups, but also an ample possibility of modifying the carrier by chemical reactions of the epoxy group. This may be employed in bonding enzymes.

The invention relates to a carrier consisting of an inorganic porous material and 0.001—95% by weight based on the weight of the carrier of bonded or unextractably sorbed reactive polymer of the general formula I:



I,

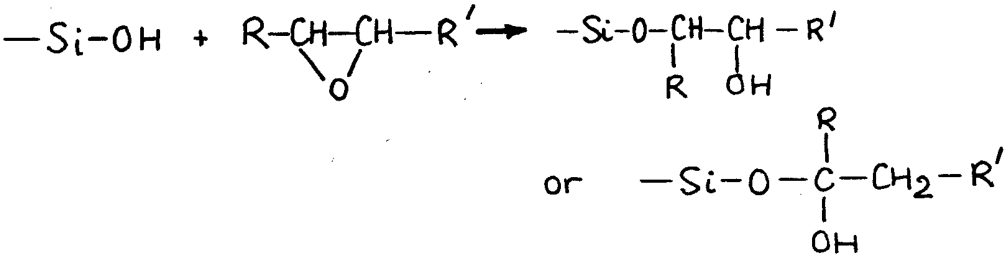
where R_1, R_2, R_4 respectively are hydrogen atom, alkyl with 1—5 carbon atoms, or aryl, and R_3 is a direct bond or is the group —COO—$(CH_2)_n$—, where n is an integer. The value of n may be 1 to 6. The term "reactive" in this context means that the polymer due to the presence of free epoxy groups or other functional groups derived therefrom is capable of directly or indirectly bonding enzymes.

A three-dimensional carrier may be prepared by coating the inorganic porous material with a monomer, which is capable of polymerization to form a polymer of the general formula I, the monomer is caused to polymerize to form such a polymer under the conditions of solution polymerization, while the soluble polymer, which is not bound on the surface of the inorganic material, is removed by extraction.

The three-dimensional carrier may also be prepared according to the invention by depositing a solution of a polymer of general formula I and with a degree of polymerization lower than 10^3 on the inorganic porous material, heating the two materials in contact with each other, and separating the unbound polymer by extraction. The monomer or reagent may be deposited at reduced pressure.

Glass and silicagel are advantageously used as the inorganic porous material.

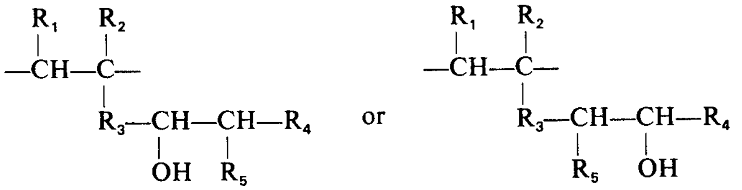
The method for preparation of the said carriers consists in the deposition of the monomer on the inorganic material, at reduced pressure is required. The establishment of sorption equilibrium is probably accompanied by a reaction between, for example, the SiOH groups of the surface of the inorganic material and the epoxy groups of the monomer according to the scheme:



The possibility of formation of such a bond was confirmed by a reaction with 3,4-epoxybut-1-ene, which can be bonded on the inorganic material without any possibility of washing it out or removing it by drying in vacuo.

A suspension of porous inorganic material with bonded reaction product of the epoxy group is polymerized after the establishment of sorption equilibrium, in order to obtain a suspension of inorganic material in the polymer solution. The whole process can be carried out in one reaction step, without separation of the inorganic porous material with the bonded reaction product of the epoxy group of the monomer. By using various amounts of solvent in the polymerization, it is possible to obtain various polymer contents in the inorganic material, and thus also various contents of the epoxy groups in the carrier, and eventually various specific surfaces and various pore diameters.

On a three-dimensional porous carrier thus prepared, owing to the high reactivity of the epoxy groups of the polymeric component of the carrier, it is possible to change functional groups according to need by reaction with various reagents. In this way a three-dimensional carrier is obtained, consisting of an inorganic porous material and 0.001 to 95% by weight based on the weight of the carrier of bonded or unextractably sorbed reactive polymer of the general formula II:



II,

where R_1, R_2, R_4 respectively are hydrogen atom, alkyl with 1—5 atoms of carbon, or aryl, R_3 is a direct bond or the group —COO—$(CH_2)_n$— and R_5 is amino group, hydroxy group, carbonyl, —NH—$(CH_2)_n$—NH_2, —NH—$(CH_2)_n$—N=CH—$(CH_2)_m$—CHO, —NH-arylene-NH_2, —NH-arylene-N_2Cl, —NH—$(CH_2)_n$—NCS, —NH—$(CH_2)_n$—NCO, —NH—$(CH_2)_n$—CON_3, —NH—$C_3N_3Cl_2$, —O—$C_3N_3Cl_2$, or —NH—$(CH_2)_n$—NH—$C_3N_3ClOCH_3$, where m and n are integers. In these formulae, the value of n and m may independently be 1 to 6.

Preparation of said carrier according to the invention consists in that a three-dimensional carrier composed of inorganic porous material and of a polymer of the general formula I is subjected to treatment with ammonia, amines and dials or phosgene, thiophosgene, diazotization solutions, compounds which oxidize or isomerize the epoxy group of the polymeric component of the carrier, (with advantage with periodic acid and its salts or boron trifluoride etherate), or solutions which hydrolyze the epoxy groups, (with advantage dilute acids, esters or hydrazides of amino acids, carbodiimides, substituted sym.triazines).

The procedure used in the preparation of said carrier consists in that a carrier composed of inorganic porous material and a polymer of the general formula I is subjected to treatment with a suitable reagent under such conditions that the epoxy group of the polymeric component of the carrier could react with the reagent. In this way it is possible to replace the epoxy group with the aminoalkyl or aminoaryl group by reaction with amines, aldehyde group, by oxidation or isomerization of the epoxy group, to obtain diol by hydrolysis and to employ a number of methods to bind enzymes.

The bonding of enzymes onto a carrier may also be effected by direct reaction between the enzyme and the epoxy groups of the carriers with a polymeric component of the general formula I. Here, the polymeric chain to a certain extent plays the role of a "spacer". The invention is further described in the following illustrative examples. The given contents of the epoxy groups have been calculated as per cent of the theoretical content based on the polymeric material.

Example 1

Onto 100 g of glass microporous crushed material (prepared according to Czechoslovak Patent No. 130475), grain size 0.1—0.3 mm, 180 ml of 2,3-epoxypropyl methacrylate was deposited by distillation in a vacuum of 26.66 Pa. The suspension was left to stand at ambient temperature for 100 h and then polymerized after dilution with a solution of 1.5 g α,α'-azo-bisisobutyronitrile in various amounts of dry 1,4-dioxane (cf. Table) at 70°C for 60 min. On cooling, the glass was separated, washed with a large amount of 1,4-dioxane, and dried.

TABLE

dilution (per monomer	1:2	1:4	1:8	1:12	1:16	1:30
% polymer	19.29	16.43	12.04	10.60	8.16	5.75
% epoxy groups	57.3	54.5	47.3	43.7	46.2	38.5

Example 2

Onto 3 g of porous glass CPG-10, 200—400 mesh, with various pore diameters, 10 ml of 2,3-epoxypropyl methacrylate was deposited by distillation in a vacuum of 26.66 Pa. After standing at ambient temperature for 100 h, the suspension of glass in the monomer was diluted with a solution of 0.8 g AIBN in 20 ml 1,4-dioxan and polymerized at 70°C for 60 min. On cooling, the glass was filtered by suction, washed with a large amount of 1,4-dioxan and dried. The polymer contents in glasses cf. Table.

TABLE

Glass CPG-10					
specific surface area (m²/g)	213.4	160.4	56.9		12.6
pore diameter (Å)	74.5	176	493	977	2023
% polymer	33.6	27.9	13.7	8.4	3.3
% epoxy groups	60.7	58.4	55.9	53.6	49.2

Example 3

Onto 5 g of silicagel, 10 ml of 2,3-epoxypropyl acrylate was deposited by distillation in a vacuum of 26.66 Pa, and the suspension was left to stand at 10°C for 72 h. The polymerization was carried out after dilution of the suspension with a solution of 0.075 g AIBN in 50 ml of dried distilled 1,4-dioxan at 70°C for 20 min. Further treatment as in Example 1,2. The carrier contains 7.03% of polymer with 47.5% of epoxy groups.

Example 4

Onto 2 g of glass microporous crushed material (prepared according to Czechoslovak Patent No. 132 896), a solution of 1 g of poly(2,3-epoxypropyl methacrylate) in 5 ml of dry 1,4-dioxan was poured. Microporous glass with 1.6% of polymer was obtained after heating the suspension for 50 h, separation, and washing of the sample with 1,4-dioxan.

Example 5

Onto 1 g of glass prepared according to Czechoslovak Patent No 130 475 and onto 1 g of glasses CPG-10, pore diameters 176 Å and 493 Å, 6 ml of 3,4-epoxybut-1-ene was deposited in vacuo. After standing at ambient temperature for 100 h, the sample was polymerized in the usual manner and the solid compound was separated and thoroughly washed with 1,4-dioxan, acetone, and ether. On drying for analysis in a vacuum of 13.33 Pa at 100°C, the content of polymer in the Czechoslovak glass was 10.26% and in CPG-10 glasses 14.17% (glass with the pore diameter 176 A) and 6.39% (glass with the pore diameter 493 A).

Example 6

Onto 0.5 g of glass, pore diameter 493 Å, with bonded 6.39% of the reaction product of 3,4-epoxybut-1-ene, 2 ml of 2,3-epoxypropyl methacrylate was deposited by distillation in vacuo. After dilution with a solution of 0.015 g AIBN in 5 ml of 1,4-dioxan, the suspension was polymerized in an inert atmosphere at 70°C for 60 min. The glass was filtered by suction, thoroughly washed with 1,4-dioxan and dried for analysis in a vacuum of 13.33 Pa at 100°C. The glass thus modified contains 9.77% of polymer, in which the assessed content of epoxy groups was 29.8%.

Example 7

Onto 1 g of silicagel, pore diameter 300 Å, 4ml of 2,3-epoxypropyl crotonate was deposited by distillation at a pressure of 13.33 Pa, and the suspension was left at ambient temperature for 90 h. After dilution with a solution of 0.035 g AIBN in 10 ml of 1,4-dioxan, the mixture was heated to 65°C and left at this temperature for 50 min. After separation of the solid phase and thorough washing with 1,4-dioxan and benzene and drying for analysis, the carrier was found to contain 11.7% of polymer with 62.4% theor. of epoxy groups.

Example 8

Onto 1 g of CPG glass, pore diameter 74.5 Å, 4 ml of 2,3-epoxybutyl acrylate was deposited by distillation at a pressure of 40 Pa. After standing for 120 h at 15°C, the suspension was diluted with a solution of 0.04 g AIBN in 20 ml of 1,4-dioxan and polymerized by heating to 70°C for 15 min. The amount of polymer ascertained after separation of the solid phase, thorough washing with 1,4-dioxan, and drying for analysis was 12.9%, and the content of epoxy groups was 59.8% theor.

Example 9

Onto 1 g of CPG-10 glass, pore diameter 368 Å, 3 ml of 3,4-epoxybutyl methacrylate was deposited by distillation at 20 Pa, and the suspension was left at ambient temperature for 100 h. After dilution with a solution of 0.025 g AIBN in 7 ml of 1,4-dioxan, the suspension was polymerized by heating to 70°C for 60 min.

After separation and washing with 1,4-dioxan and drying for analysis, 15.7% of polymer was ascertained containing 47.7% theor. of epoxy groups.

Example 10

1 g of glasses CPG-10 with various pore diameters and various contents of poly(2,3-epoxypropyl methacrylate)—cf. Table—was placed into a solution of 0.1 g of chymotrypsin in 10 ml of borate buffer, pH 9.0, cooled to 4°C. After standing at 4°C for 60 h, the glasses were filtered by suction, washed with ca. 1 l of icy water and with icy borate buffer until the activity had disappeared in the filtrate. After washing with icy water to neutral reaction, the activity was determined using N-acetyl-L-tyrosine ethyl ester in a Tris buffer, pH 8.2.

TABLE

Pore diameter of glass (Å)	Amount of polymer (%)	Activity units (min) 1 g		
176	27.9	707	652	773
493	12.9	639	590	631
977	9.5	320	323	362
2023	3.4	192	198	265

Example 11

1 g of silicagel, modified with poly(2,3-epoxypropyl methacrylate), was treated similarly to glasses in the preceding Example. The activities obtained are given in the Table.

TABLE

Pore diameter of silicagel (Å)	Amount of polymer (%)	Activity units (min) 1 g
95	21.2	477
300	8.6	282

Example 12

Onto 1 g of porous glass or silicagel with bonded poly (2,3-epoxypropyl acrylate), penicillinacylase was bonded in a phosphate buffer, pH 7.5, by standing at 38°C for 10 days. After thorough washing of the sample—until the activity in the filtrate had disappeared—activities were measured in a buffer pH 7.5 using benzylpenicillin. The activities obtained, pore diameters of glass or silicagel and the amount of polymer bonded on the inorganic material are given in the Table.

TABLE

Pore diameter (Å)	Amount of polymer (%)	Activity units/(min) 1 g	
176	27.9	47	CPG—10 glasses
493	12.9	34	
977	9.5	33	
2023	3.4	18	
95	21.2	38	silicagel
300	8.5	18	

Example 13

Onto 1 g of glasses CPG-10 with bonded poly(2,3-epoxypropyl methacrylate)—pore diameters and amounts of the polymer of Table—cooled to –30°C, liquid ammonia was poured, and the glasses were left to stand in a sealed ampoule at ambient temperature for 15 h. After that, ammonia was evaporated and the glasses were dried for analysis. The nitrogen content in the samples corresponds to a 98% conversion of epoxy groups.

Aminated porous carrier was covered with a 2% aqueous solution of glutaraldehyde and left at ambient temperature for 6 h. After separation of the solid phase, the carrier was washed with water, covered with 2.5 ml of a solution of 0.2 chymotrypsin in a phosphate buffer pH 7.5, cooled to 4°C and left to stand 8 h at this temperature. After separation of the solid phase, the samples were washed similarly to example 12. The activity of the bonded enzyme measured similarly to Example 9 was ascertained after lyophilization of the samples.

TABLE

Pore diameter of glass (Å)	Amount of polymer (%)	Activity units (min) 1 g
176	28.4	448
493	6.1	267

The activity measured for silicagel with the pore diameter 95 A and with bonded 21.2% of poly(2,3-epoxypropyl methacrylate) after modification described above of the epoxy ring of the polymeric coating was 505 units (min) 1 g.

Example 14

2 g of CPG-10 glasses, pore diameter 176 Å, with bonded 23.8% of poly(2,3-epoxypropyl methacrylate) were heated with an aqueous solution of 0.8 g of periodic acid (150 ml) to 60°C for eight hours. The carrier thus modified contains 72% theor. of aldehyde groups onto which enzyme can be bonded by leaving the suspension of the carrier to stand for 12 h in a solution of 0.2 g of chymotrypsin in a phosphate buffer of pH 7.5 cooled to 4°C. After separation of the solid phase and washing of the sample until the activity in the filtrate had disappeared, the porous carrier with bonded enzyme was washed with a 20% solution of saccharose and then lyophilized. Activity (measured as in Example 9) was 552 unites (min) 1 g.

Example 15

1 g of silicagel with bonded 21.2% of poly(2,3-epoxypropyl acrylate) was oxidized with periodic acid similarly to the porous carrier in Example 14.

The porous carrier thus obtained with 59% theor. of aldehyde groups was poured into 4 ml of a phosphate buffer pH 7.2 and mildly evacuated. After that, 0.1 g penicillinacylase was added and the suspension thus obtained was left to stand at room temperature for 120 h. Activity of the sample lyophilized as in Example 14 was measured similarly to Example 11 and amounted to 29 units (min) 1 g.

Example 16

Onto 1 g of glasses CPG-10—a pore diameter and amount of bonded poly(2,3-epoxypropyl methacrylate) are given in the Table—ca.4 ml of ethyl ester of ε-aminocaproic acid were deposited by distillation in a vacuum of 26.66 Pa. The suspension was heated to 80°C for 10 h. The solid phase was separated, thoroughly washed with ethanol and acetone. The nitrogen content assessed after drying the sample for analysis corresponds to 94% conversion of the epoxy groups.

The porous carrier thus modified was sealed into an ampoule with 15% aqueous solution of hydrazine hydrate and heated to 70°C for one hour. After separation and thorough washing of the solid compound, the hydrazide formed on the carrier was proved by means of trinitrobenzenesulphonic acid.

The carrier containing hydrazide groups was covered with 3 ml of 10% hydrochloric acid while cooling to 0°C. The solution was left to stand for 10 min, and a solution of 0.1 g of sodium nitrite in 1 ml water was then added to it, slowly and dropwise and with cooling. The cooled suspension was stirred for half an hour, the carrier was separated, washed while cooling with icy water and mixed into a solution of 0.14 g chymotrypsin in 2 ml of 0.5 M sodium bicarbonate. The suspension cooled to 4°C was left to stand 3 h with occasional stirring. After that, the solid phase was removed by filtration with suction, washed with icy water and then slowly with a 4°C 0.5 aqueous solution of sodium chloride buffered to pH 3.4 with citrate buffer until the activity had disappeared in the filtrate. After washing with water to neutral reaction to chloride ions, the activity was ascertained similarly to Example 9.

TABLE

Pore diameter of glasses (Å)	Amount of polymer (%)	Activity units (min) 1 g
176	28.4	218
493	6.1	418

Example 17

10 g silicagel with the pore diameter 95 Å and with bonded 8.5% poly(2,3-epoxypropyl methacrylate) was covered with a ca. double volume of 0.1 N sulphuric acid and heated in a sealed ampoule to 80°C for 5 h. After that, the carrier was filtered off by suction, washed to neutral reaction with water and dried

at reduced pressure—13.33—at 100°C for 12 h. The content of organic matter was 8.3%. No epoxy groups could be proved on the carrier thus modified.

Example 18

1 g of CPG-10 glass, pore diameter 368 Å, and 15.7% of poly(3,4-epoxybutyl methacrylate) bonded was heated with 5 ml ethylenediamine to 70°C for 8 h. After separation, washing and drying for analysis, the nitrogen content determined on the glass corresponded to a 79.8% conversion of the epoxy groups.

The porous glass thus modified was heated for 15 h in 10 ml of a 10% solution of thiophosgene in dry chloroform. After filtration by suction and washing with dry chloroform, the glass was freed from the excess chloroform by sucking air on the fritted disc.

The carrier was suspended into a 1% solution of trypsin in 0.05 M carbonate buffer, pH 9.0, cooled to 2°C. After standing at 4°C for 5 h, the carrier was separated with cooling, washed with an icy carbonate buffer until the activity had disappeared in the filtrate, and then washed again with about one litre of icy water to neutral reaction. The activity of bonded trypsin measured colorimetrically to benzoylarginine-p-nitroanilide in a phosphate buffer at pH 7.8 was 85 units (min) 1 g.

Example 19

1 g of CPG-10 glass, pore diameter 977 Å, and 9.5% of bonded poly(2,3-epoxypropyl acrylate) was heated with 5 ml of a 25% solution of 1,6-diaminohexane in 1,4-dioxan to 70°C for 8 h. After separation and washing of the solid phase, the analysis revealed a 65.2% conversion of the epoxy groups.

The carrier thus modified was suspended into a solution of 0.2 ml triethyl amine and 0.4 g 1,3-dichloro-5-methoxytriazine in 10 ml benzene, and the suspension was heated to 50°C for 5 h. After separation the carrier was washed with benzene and dried for analysis. The conversion of the amino groups calculated from the chlorine content was 73.1% theor.

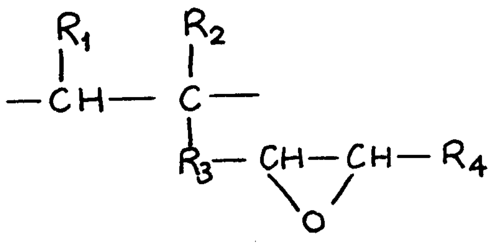
Example 20

1 g of porous glass CPG-10, pore diameter 2023 Å, with bonded 3.4% poly(2,3-epoxypropyl crotonate) was sealed at a reduced pressure of 3.3 kPa and with cooling with a 30% 1,4-dioxan solution of p-phenylenediamine, and heated to 80°C for 12 h. The solid phase was separated, the carrier was thoroughly washed, and dried. The conversion of the epoxy groups as revealed by analysis was 27.5%. Thus, reactivity of the epoxy groups not only makes possible direct bonding of enzymes in a simple way, but also allows, by using a suitable modification, to adjust the basic porous carrier to the type of compound to be bonded, so that it is possible to bond onto a carrier with the polymeric component of the general formula I or II not only trypsin, chymotrypsin, or penicillinacylase, but also proteolytic enzymes, hydrolases, amylases, dehydrogenases, kinases, oxidases, deaminases, amidases, including lactate dehydrogenase, creatine, phosphokinase, papain, ribonuclease, alkali phosphatase, amyloglucosidase, dextranase, glucosooxidase, penicillinamidase, β-galactosidase, ficin, pepsin, carboxypeptidase, streptokinase, urease, amylase, invertase, mglycosidase, maltase, zymase, catalase, pectolase, proteases, tyrosinase, cellulase, carboxylase, pectinase, aldehydase, chloinesterase, cozymase and adenase.

The bonding of an enzyme is accomplished by acting upon the three-dimensional carrier with a solution of the enzyme.

WHAT WE CLAIM IS:—

1. A carrier consisting of a porous inorganic material and 0.001—95% by weight based on the weight of the carrier of bonded or unextractably sorbed reactive polymer of the general formula I

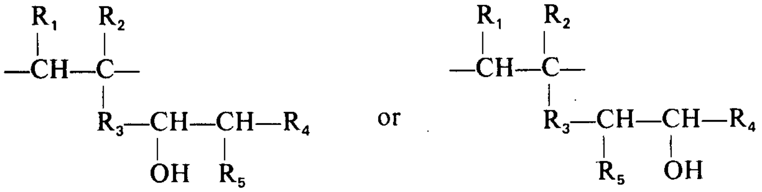


wherein R_1, R_2, R_4 respectively are hydrogen, alkyl with 1—5 carbon atoms, or aryl, and R_3 is a direct bond or is the group —COO—$(CH_2)_n$—, where n is an integer.

2. Method for producing a carrier as claimed in Claim 1, wherein an inorganic porous material is coated with a monomer which is capable of polymerization to form a polymer of the general formula I, the monomer is caused to polymerize to form such a polymer under the conditions of solution polymerization, and the soluble polymer not bound onto the surface of said inorganic material is removed by extraction.

3. Method for producing a carrier as claimed in Claim 1, wherein the inorganic porous material is coated with a solution of polymer of the general formula I and with the polymerization degree lower than 10^3, the two are heated in contact, and the polymer not bound to the carrier is separated by extraction.

4. A porous carrier consisting of a porous inorganic material and 0.001—95% by weight based on the weight of the carrier of bonded or unextractably sorbed reactive polymer of the general formula II



$$\begin{matrix} R_1 & R_2 & & & \\ | & | & & & \\ -CH- & C- & & & \\ & | & & & \\ & R_3- & CH- & CH- & R_4 \\ & & | & | & \\ & & OH & R_5 & \end{matrix} \quad \text{or} \quad \begin{matrix} R_1 & R_2 & & & \\ | & | & & & \\ -CH- & C- & & & \\ & | & & & \\ & R_3- & CH- & CH- & R_4 \\ & & | & | & \\ & & R_5 & OH & \end{matrix} \qquad \text{II,}$$

wherein R_1, R_2, R_4 respectively are hydrogen, alkyl with 1—5 carbon atoms, or aryl, R_3 is a direct bond or is the group —COO—$(CH_2)_n$—, and R_5 is amino group, hydroxyl, carbonyl, —NH—$(CH_2)_n$—NH_2, —NH—$(CH_2)_n$—N=CH—$(CH_2)_m$—CHO, —NH-arylene-NH_2, —NH-arylene-N_2Cl, —NH—$(CH_2)_n$—NCS, —NH—$(CH_2)_n$—NCO, —NH—$(CH_2)_n$—CON_3, —NH—$C_3N_3Cl_2$, —O—$C_3N_3Cl_2$, or —NH—$(CH_2)_n$—NH—$C_3N_3ClOCH_3$, where m and n are integers.

5. A carrier as claimed in Claim 1 and 4, wherein the inorganic material has been chosen from glass and silicalgel.

6. Method for producing a porous carrier as claimed in Claim 4, wherein a porous carrier as claimed in Claim 1 is treated with ammonia, amine and dials or with phosgene, thiophosgene, diazotization solutions, compounds oxidizing or izomerizing the epoxy group of the polymeric component of the carrier, with solutions hydrolyzing the epoxy groups.

7. A method as claimed in Claim 6, wherein the compound oxidizing or izomerizing the epoxy group of the polymeric component of the carrier is selected from periodic acid and its salts and boron trifluoride etherate.

8. A method as claimed in Claim 6, wherein the solution hydrolyzing the epoxy group is selected from dilute solutions of acids, esters or hydrazides of amino acids, carbodiimides or substituted sym.triazines.

9. Method as claimed in Claims 2 and 6, 7 or 8, wherein the monomer or the reagent is deposited at reduced pressure.

10. Method for bonding enzymes, wherein the porous carrier as claimed in Claim 1 is treated with a solution of an enzyme.

11. Method for bonding enzymes, wherein the porous carrier as claimed in Claim 4 is treated with a solution of an enzyme.

12. A carrier as claimed in Claim 1 or 4, substantially as hereinbefore described with reference to the accompanying Examples.

13. A method for producing a porous carrier, substantially as hereinbefore described with reference to the accompanying Examples.

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