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(54) **Process for preparing a carrier for covalently binding biologically active substances**

(57) A process for the production of a support system for the covalent binding of biologically active material, comprising a support material, with a polymer-coated surface, wherein the polymer contains functional groups suitable for the covalent binding and the support material is coated with a mixture of a film-forming polymer dispersion and a non-film-forming dispersion, at least one of the two polymer dispersions possessing functional groups suitable for the covalent binding.

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**Process for preparing a carrier for binding
biologically active substances**

The invention relates to a process for preparing a carrier for binding biologically active substances, with covalent binding of said substances to the polymer-coated surface of a support material.

EP-B-71704 has proposed "large-surface systems for the fixing of substrates containing nucleophilic groups". The reactive units for the binding of the substrates which contain the nucleophilic systems here form part of a polymer latex which is produced by emulsion polymerization and which is constructed from 0.03 to 6 μm latex particles and where the polymer latex itself is aggregated to form a large-surface system and/or is fixed to a large-surface support material.

If the connection between the individual latex particles is not established by film formation, EP-B-71704 suggests that the linking of the particles to one another and to the support material can take place by covalent binding. Possibly the covalent linking of the latex particles can also be strengthened by the use of multifunctional nucleophiles. The addition of minor quantities of a soft, film-forming substance, e.g. latex particles with a low glass temperature, is also mentioned.

Precipitation by means of the biologically active units which are to be fixed is particularly preferred. In the aforementioned European patent the functional groups X are precisely defined as constituents of monomers $Z' - (R_n)-X$, where the groups X are capable of covalently reacting, in the physiologically effective pH-range (approximately pH 5.0 - 9.0) at temperatures below 40°C and in an aqueous medium, with the nucleophilic groups, in particular the amino-, hydroxy- and thiol groups of the biologically active materials

which are to be fixed.

DE-A-20 16 729 proposes a process for the production of insoluble enzymes in active form, wherein a copolymer is treated with an enzyme solution and cross-linking of the enzyme-polymer complex and/or neutralisation of the remaining, reactive chemical groups is carried out. The theory of DE-A-20 16 729 comprises the dissolution of any particle structure - where present from production - by dissolving the copolymers in solvents. This obviates the need for the existence of a particle structure in the film formation. To the extent that insoluble deposits or precipitates form the subject of the theory, these are not the direct result of the polymerization process, but follow-up products of an (external) cross-linking of the formed polymeric material which is more likely to reduce the accessibility.

The prior art relating to the immobilization of biologically active materials on polymeric support materials has been discussed in a large number of general articles (see "Encyclopaedia of Polymer Science and Engineering", Vol. 2, page 55 - 59, John Wiley 1985; "Characterization of Immobilized Biocatalysts", Ed. K. Buchholz, Dechema-Monographien No. 1724 - 1731, Vol. 84, Verlag Chemie 1984; Methods in Enzymology, Ed. W.B. Jokoby, Vol. 104, pp 3 - 369, Academic Press 1984; Biotechnology, Ed. H.J. Rehm & G. Reed, Vol. 7a, page 347 - 464, Verlag Chemie 1987).

In the last mentioned publication it is stated that, regardless of the large number of available immobilization techniques, no specific technique can claim to be universally applicable with an ideal result. If the immobilization leads to the entrapment of the biologically active units within the support matrix, impaired accessibility for the substrates is likely due to the diffusion resistance, an effect which distinctly influences inter alia the (apparent) Michaelis constant.

Therefore the classical "entrapment" is limited in its effects to low-molecular substrates and products in which the resistance to mass transfer is restricted. Internal diffusion effects naturally play a decisive role in the case of enzymes fixed inside a porous support system.

In "Biotechnology" (loc.cit. page 353) the advantages and disadvantages of porous and non-porous support materials are compared. In the case of non-porous support materials the advantage of few diffusion-related limitations is offset by the disadvantage of a small active surface and consequently low enzyme charging, the necessity to use small carrier particles and difficulties in handling, especially in continuous operation. In the case of porous support materials, although the large inner surface and high level of enzyme charging and the relative protection from external detrimental influences represent favourable factors, these can be contrasted with the diffusion problems associated with the large inner surface, the high costs inter alia connected with the indispensable monitoring of the cavity dimensions, and possibly liquid pressure problems which are associated with the tendency to gel formation.

Ideally the biologically active substrates should be fixed to a support material with the largest possible surface in such manner that as few as possible of the biologically active structures remain excluded from the reaction with the substrates and optimal access possibilities are provided for the substrate molecules.

The approach adopted in EP-B-71704 represents an approximation to this ideal, inasmuch as support materials with a large surface are made available which possess binding functions for fixing the biologically active material, in particular enzymes, antibodies etc. in the vicinity of the surface. An approach which is likewise of interest is adopted in US-A-4710525 which

describes redispersible polymer latices of core-shell construction which contain, in the shell, the functional groups for the covalent fixing of the biologically active materials.

Nevertheless the aim has continued to exist of making available more efficient polymeric support systems for the covalent fixing of biologically active materials, which ensure an optimal exploitation of the generally expensive biologically active materials.

Thus, according to one aspect, the present invention provides a process for the production of a carrier system capable of covalently binding biologically active substances, said process comprising coating a support material with a mixture of a film-forming polymer dispersion and a non-film-forming polymer dispersion, at least one of said film-forming polymer dispersion and said non-film-forming polymer dispersion comprising functional groups capable of covalent binding to biologically active substances.

There are also provided a carrier system prepared by such a process and the use of such a carrier system in the covalent binding of biologically active material.

Also provided is a carrier system capable of covalently binding biologically active materials comprising a support coated with a mixture of a film-forming polymer dispersion and a non-film-forming polymer dispersion.

Suitable support materials are preferably those that have been developed in accordance with the prior art. (See "Biotechnology", Vol. 7A, loc.cit. page 351 - 367; 411 - 412; "Dechema-Monographien", Vol. 84, loc. cit. page 49 - 72). Both inorganic and organic support materials are suitable, usually solid.

Examples of support materials of inorganic origin are: aluminium oxide, zirconium dioxide, magnesia, silicon dioxide, glass, minerals in various modifications, e.g. clays such as attapulgite, bentonite

and kieselguhr, pumice and the like, ceramic materials, sand, titanium dioxide, metals, such as for example ferromagnetic material both in the grafted and ungrafted state.

Also of importance are organic support materials, e.g. of natural origin, such as for example polysaccharides, (cellulose, dextrans, starch, agar, agarose, alginates, carragenates, chitin, chitosan) and proteins such as collagen, gelatin, albumin, silk and the like, as well as various modifications of carbon. Examples of synthetic polymeric support materials are polystyrene, polyacrylates and -amides, maleic acid anhydride polymers, vinyl- and allyl- polymers and polyamides.

As regards the preferred geometric configuration of the support materials, specific preferences have developed in the art, an example of which is the easy to handle spherical shape, for example with a diameter of 1 - 10 mm, in particular about 6 mm. In this context polystyrene spheres are particularly preferred.

However, coatable surfaces in the sense of the present invention can also comprise those of bodies which are not of spherical or a strictly regular shape. e.g. granules, sticks, and bodies developed with a view to obtaining a large surface constituent e.g. as catalyst supports, for example with a honeycomb structure and likewise vessel surfaces such as, for example, glass surfaces e.g. glass fibres. Also preferred are planar structures on a cellulose or a textile (e.g. silk) base, e.g. coated papers.

Generally, conventional support materials fulfil the requirement of being chemically inert (or activatable in a uniquely defined sense) under the fixing conditions. In the case of diagnostic applications, in the case of the present invention, it is preferable to use beads or spheres having a diameter of approximately 0.01 to 10 mm, preferably a bead range

of 0.1 to 0.6 mm, and the sizes of 0.01 - 10 mm, in particular the range of 6 - 7 mm, already used for diagnostic purposes. As regards surface area, support material areas of 0.03 mm² to 10 cm², preferably 10 mm² to 10 cm² are convenient. When planar support materials are employed, square or rectangular shapes may be used, but round shapes, for example filter plates known per se, may also be used. Irregular shapes are likewise usable.

A requirement which the support materials to be used in accordance with the present invention have in common with those of the prior art is that of mechanical stability, in particular under shearing stress. In the conventional operations such as filtration, stirring, shaking, and during transport and storage, the materials are to possess sufficient stability and are not to be subject to disturbing abrasion. Fixing in holders is possible in order to simplify handling.

The polymer dispersions, and indeed both the non-film-forming dispersion and the film-forming dispersion can be prepared in a manner known per se in accordance with the rules of emulsion polymerization. (See H. Rauch-Puntigam, Th. Völker, "Acrylic and Methacrylic Compounds", page 217 - 230, Springer-Verlag, 1967, Houben-Weyl, "Methods of Organic Chemistry", 4th Edition, Vol. 14/1, page 133 - 390, Georg Thieme-Verlag 1961). The practical implementation can correspond, for example, to the methods taught in DE-A-1804159, DE-A-1910488 and DE-A-1910532.

The desired size of the latex particles is determined in practice by the emulsifier concentration at the beginning of the polymerization. Generally the emulsifier concentration at the beginning of the emulsion polymerization ranges between 0.005 and 0.5 % by weight, relative to the complete polymerization batch. It is also possible to set the desired particle size by adding a defined quantity of a fine-particled seed latex. The size of the latex particles should

generally range between 0.03 and 6 μm , preferably 0.03 and 1 μm . Known anionic and non-ionic emulsifiers can be used, for example fatty alcohol sulphates and -sulphonates, -phosphates and -phosphonates, alkali salts of long-chain fatty acids, long-chain sarkosides and oxyethylated fatty alcohols, substituted phenols which can in part be sulphonated, and other emulsifiers used in emulsion polymerization (Houben-Weyl, "Methods of Organic Chemistry", Vol. XIV/I, loc.cit.).

The use of cationic surfactants is advisable only if these are derived from tertiary or quarternary ammonium salts. Polymerizable emulsifiers can also be used. The initiators can likewise consist of those generally used in emulsion polymerization (see J. Brandrup, E.H. Immergut, "Polymer Handbook", second Edition, J. Wiley & Sons; H. Rauch-Puntigam, Th. Völker, "Acrylic and Methacrylic Compounds", Springer-Verlag, 1967). Examples include peroxides, hydroperoxides, peroxy acids and azo compounds, for example potassium peroxidisulphate, hydrogen peroxide and others. The concentration of the initiators conveniently falls in the range of, for example, 0.01 to 1.0 % by weight, relative to the monomers. The solid content of the dispersion may range between 10 and 60 % by weight depending upon the particle size.

The polymer dispersions to be used in accordance with the invention may be constructed from the known monomers accessible to emulsion polymerization.

Examples are polymers based on

- acrylates and methacrylates
- styrene and styrene derivatives, e.g. alkyl styrene
- vinyl fatty acid esters such as vinyl acetate, vinyl propionate
- Vinyl halogen compounds such as, for example, vinyl chloride, tetrafluoroethylene
- vinylidene compounds

and mixed copolymers such as, for example, copolymers of

styrene with butadiene, polyvinylidene copolymers with vinyl acetate i.a. Generally the polymerization is radically initiated.

The molecular weight of the polymers (determined by gel permeation chromatography; see "Encyclopedia of Polymer Science and Engineering" Bikales, Overberger and Menges, 2nd Ed. Vol. 10, page 1 - 19; J. Wiley 1987) has proved to be non-critical.

Based on practical considerations the molecular weights of the polymers conveniently range between 10,000 and 2×10^6 , and preferably exceed 50,000.

A highly preferred constituent of at least one of the types of polymers which are used is a minimum of functional groups X which are suitable for covalent binding with the biologically active materials. Preferably the functional units X form part of the non-film-forming dispersions. They may constitute an activatable group (Group X') which requires an additional activating reagent Q, but preferably the functional group X is directly suitable for reaction with nucleophilic groups of the biologically active material, expediently under conditions which are biologically acceptable, i.e. which do not impair the biological activity in the final effect. Such conditions can be considered to comprise, for example, a physiologically effective pH-range of the aqueous medium e.g. the pH-range of 5.0 to 9.0, in particular buffer solutions, and also the absence of agents which impair the biological activity and a suitable temperature range which avoids the inactivation of the proteins, for example of less than 60°C, in particular less than 40°C.

Carboxylic groups arranged on the support material may be covalently linked to the amino groups of the biologically active materials in accordance with the methods of peptide synthesis, for example by the carbodiimide method. Amide functions can be activated by means of glutaraldehyde or by hydrazinolysis and

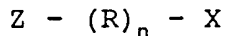
diazotization, the latter also applying to esters. Aromatic amino groups on the support material can also be activated by diazotization. Aliphatic amino groups may be covalently linked to the amino groups of the biologically active material for example by means of glutaraldehyde, with D-cyclopenta-dialdo-1,4-furanose or by means of thiophosgene. In the case of hydroxyl groups a reaction with halogenated triazines or with 1,1'-carbonyldiimidazole for activation is suitable and in the case of glycol configurations a reaction with bromocyanogen to form imidocarbonate is suitable (see "Biotechnology", Ed. H.J. Rehm & Reed, Vol. 7a, loc. cit.)

As already mentioned, group X preferably represents an activated function suitable for direct reaction with the nucleophilic groups of the biologically active materials.

Thus X preferably signifies a sulphonic acid halogenide group, a thioisocyanate group, an activated ester, a thiocarbonyldioxy-, carbonyl-imidoyldioxy-, haloethoxy-, haloacetoxy-, oxiran-, aziridine-, formyl-, formyl, keto-, acryloyl- or anhydride group. Suitable sulphonic acid halogenides are the chlorides and bromides, suitable haloacetoxy compounds are the fluoro-, chloro- and bromo- compounds, suitable ester components of the activated esters are those of hydroxylamine compounds, such as of N-hydroxysuccinimide or N-hydroxyphthalimide, of phenols (activated by electron attracting groups), such as halogenophenols such as trichlorophenol, or nitrophenols, and of heterocyclic lactams, such as pyridone.

Oxiran-, keto-, formyl-, sulphonic acid chloride-, thioisocyanate groups and activated carboxylic acid esters and carboxylic acid anhydrides are particularly preferred.

The monomers bearing the functional groups preferably correspond to the formula



wherein

X has the above described meaning

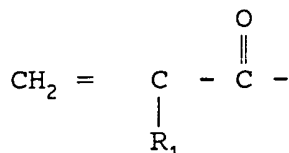
R is a chemically inert spacer between the functional unit and the polymerizable unit

n is 0 or 1 and

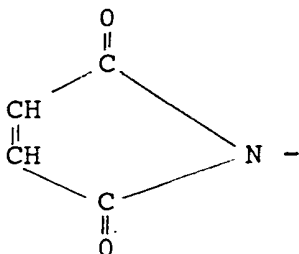
Z is a polymerizable unit.

The size and type of the spacer R are relatively non-critical. Typical examples of such spacers are, for example, alkylene groups of C₁ to C₂₀, preferably C₂ to C₁₂, wherein carbon atoms can if desired be replaced by ether bridges or by alkylene branches and/or substitution e.g. with a hydroxy function. Preferably, however, R is linear. Furthermore, other units originally (i.e. prior to incorporation) containing bifunctional groups, where linking via amide-, ester-, ether-, thioether-, urea-, urethane-, sulphonamide- and similar groups can take place both at the polymer end and at the functional end. Generally the spacer spaces the functional groups X from the polymer main chain by a distance in the range of 0.5 - 4 nm. The group R can be entirely omitted i.e. n can also have the value 0.

For Z, radically polymerizable units comprise, for example, vinyl groups, wherein Z, for example, represents

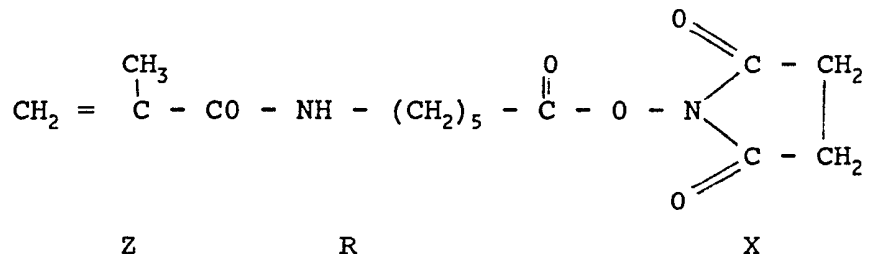


wherein R₁ is hydrogen or methyl or CH₂ - COOR₂, CH₂ - CONHR₂ or CH₂ - CON (R₂)₂, wherein R₂ is an alkyl residue containing 1 to 4 carbon atoms. Z can also derive from maleic acid:

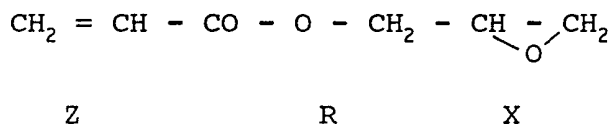


Maleic acid anhydride and itaconic acid anhydride also constitute suitable reactive and simultaneously polymerizable units.

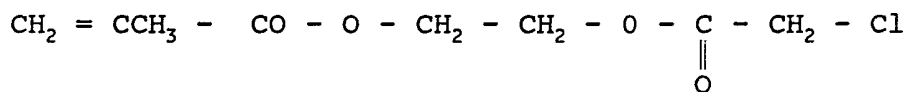
The following examples will serve to illustrate the schematic formula Z-R-X:



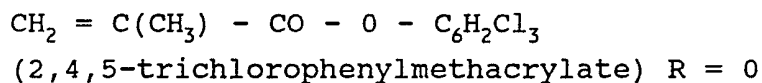
(polymerizable activatable esters with spacers)



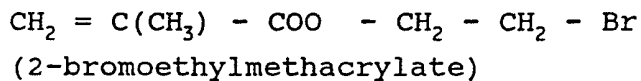
(glycidylacrylate)



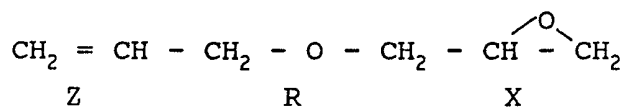
(2-(chloroacetoxy)-ethylmethacrylate)



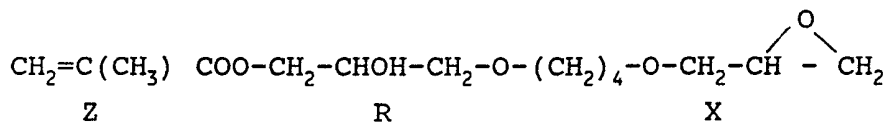
(2,4,5-trichlorophenylmethacrylate)



(2-bromoethylmethacrylate)

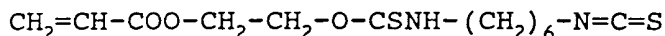


(allyl glycidylether)



(addition product of methacrylic acid to 1,4-butane

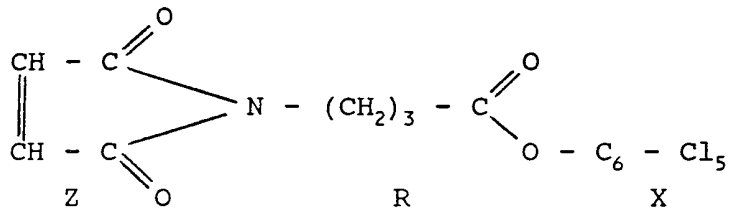
dioldiglycidylether)



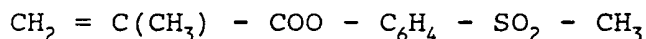
(addition product of acrylic acid-2-hydroxyethylester to 1,6-hexanediisothiocyanate)



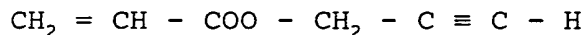
(chloroacetic acid vinyl ester)



(4-maleimido-butyric acid-pentachlorophenyl ester)

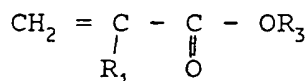


(4-methylsulphonylphenyl)-methacrylate)



(propargylacrylate)

Polymer dispersions of the acrylate type are conveniently constructed from (meth)acrylate monomers of the formula



wherein R_1 has the above defined meanings and wherein R_3 is an optionally branched alkyl group containing 1 - 18 carbon atoms, in particular 1 - 8 C-atoms or a cycloalkyl group containing 3 to 12 ring members, in particular a methyl-, ethyl-, propyl-, butyl, especially a butyl- or a 2-ethylhexyl group. (See H. Rauch-Puntigam, "Acrylic and Methacrylic Compounds", Springer Verlag 1967, page 217 - 230).

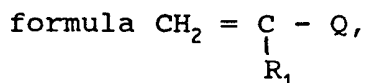
Suitable substantially hydrophobic comonomers are,

for example, dienes such as butadiene, chloropropene, isoprene. In addition, activated vinyl compounds, such as vinyl esters of fatty acids such as vinyl acetate and vinyl propionate, vinyl ethers, allyl ethers and allyl esters, acrolein, vinyl methylketone, heterocycles containing vinyl groups, such as vinyl imidazole, vinyl pyrrolidene, vinyl pyridine, vinyl carbazole and styrene and its derivatives, in particular alkylated derivatives such as α -methyl styrene, vinyl toluene and the like can be copolymerized, where it should be noted however, that the copolymers can enter into (undesired), group-related reactions with the biologically active materials to be fixed. From the standpoint of a possible hapten effect, for example, in specific cases it can be expedient to avoid entirely the use of monomers containing aromatics.

Generally it is the above mentioned (meth)acrylate monomer constituent which predominates in the polymers which form the non-film-forming polymer and film-forming polymer dispersions of the acrylate type. As a rule this constituent amounts to more than 50 % by weight and - if it represents non-functionalised polymer components - up to 99.9 % by weight, and preferably up to 99 % by weight.

At least one of the non-film forming and film-forming dispersions NFD and FD contains the monomers, already described in the foregoing, bearing functional groups X or activatable groups X'. The proportion of monomers bearing functional groups X in the polymers of the dispersions may preferably amount to 0.1 to 80 % by weight. Conveniently the proportion of (meth)acrylate monomers of the above formula amounts to 1 to 40 % by weight.

Apart from the functionalised monomers, the polymers may also contain other comonomers, e.g. distinctly hydrophilic monomers, in particular of the formula



wherein R_1 has the meanings given in the foregoing and Q signifies the residues $-CN$, $COOR_4$, wherein R_4 is hydrogen-, sodium-, potassium- or ammonium ions or Q signifies

$\begin{array}{c} O \\ || \\ C-NR_5R_6 \end{array}$, wherein R_5 and R_6 are hydrogen or an alkyl residue corresponding to R_3 or, including the nitrogen atom, form a 5- or 6-membered heterocycle possibly containing further nitrogen or oxygen, or signifies an optionally branched alkyl residue containing 2 to 8 carbon atoms which comprises at least one, preferably terminal, $-OH$ or $-NR_5R_6$ -group having the meanings given in the foregoing.

The proportion of hydrophilic monomers in the polymers, which can result in possibly improved bonding strength and improved suitability for specialised applications, amounts to - if present at all - 0.5 to 30 % by weight, preferably 1 to 20 % by weight (relative to the monomers used).

In addition to the above described monomer components, the polymers may also contain cross-linking monomers. "Cross-linking monomers" are to be understood, in the usual way, as monomers which contain two or more reactive double bonds in the molecule, such as for example diols or polyols esterified with acrylic acid or preferably methacrylic acid, and allyl compounds, such as for example allyl methacrylate, triallyl cyanurate and the like. Examples are ethylene glycol dimethacrylate, 1,4-butan-dioldimethacrylate, triglycoldimethacrylate, trimethylol propane trimethacrylate. The proportion of cross-linking agents is conveniently between 0 and 50 % by weight, preferably 2 to 20, in particular 5 - 20 % by weight, relative to the total quantity of monomers. The polymer content of the film-forming and non-film-forming dispersions is generally 10 to 60 % by weight.

The production of polystyrene dispersions is

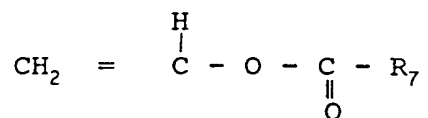
described in detail in Houben-Weyl, "Methods of Organic Chemistry", 4th Ed., Vol. XIV/1, published by Georg Thieme Verlag 1961, page 834 - 839. Suitable emulsifiers are, in particular, fat soaps, such as for example sodium oleate, also resin soaps and alkyl sulphonates and cationic emulsifiers, normally in quantities of 2 to 6 % by weight, relative to the quantity of water.

Suitable initiators are here again, in particular, water-soluble, e.g. of the peroxydisulphate salt type, also redox systems, usually in quantities of 0.01 to 0.2 % by weight, relative to the monomers. The conventionally used regulators e.g. sulphur regulators can be employed, normally in quantities of 0.05 to 2 % by weight, relative to the monomers. Advantageously a weakly acid medium (pH 3-6) is selected for the starting reaction with peroxydisulphates. In the polymerization it is advantageous to endeavour to exclude atmospheric oxygen, e.g. by working in a protective gas environment, such as nitrogen. The comonomers for styrene and the radically polymerizable styrene derivatives known per se may comprise the monomers known as "dispersions of the acrylate type", in which case the role of the (meth)acrylate monomers is assumed by styrene and/or its derivatives and vice versa.

Thus generally the proportion of styrene or styrene derivatives in the polymers will amount to more than 50 % by weight, and up to 100 % by weight. Copolymers of the styrene-butadiene type are also of particular interest. In the case of the styrene-butadiene type, on the basis of a 1 : 1 weight ratio, in a manner known per se, by varying the proportions it is possible to shift the spectrum of properties in the "harder" direction (higher styrene content) or "softer" direction (see e.g. Houben-Weyl, 4th Ed. loc.cit. Vol. XIV/1, page 147, 327).

Suitable monomers of the polyvinyl fatty acid type

are those corresponding to the formula



wherein R_7 is an alkyl residue containing 1 to 6 carbon atoms, in particular 1 to 2 carbon atoms i.e. vinyl acetate and -propionate. Suitable comonomers are, for example, optionally halogenated olefins such as ethylene, vinyl chloride, other vinyl esters such as vinyl phosphonic acid diester, (meth)acrylates of the aforementioned type (see "Dispersions of the Polyacrylate Type"), (meth)acrylonitrile and the like (see Houben-Weyl loc.cit. 4th Ed., Vol. 14/2, page 704, Vol. 14/1, page 911 - 918). The polymerization generally corresponds to the processes referred to in the foregoing, for example in respect of the emulsifiers, initiators and regulators. Advantageously the polymerization is in the pH-range of 4 - 5. Polyvinylidene copolymers are of particular interest.

Examples of monomers of the polyvinyl halogen type are, in particular, vinyl chloride and especially vinylidene chloride (Houben-Weyl, loc.cit. page 887, 891 - 905). Examples of acrylate components are acrylic acid methylester, -ethylester, -butylester, -octylester, -nonylester, -2-ethylhexylester, -3,5,5-trimethylhexylester and methacrylic acid methylester and methacrylic acid amide. Generally the proportion of comonomers of the acrylate type and/or of (meth)acrylonitrile, vinyl ester (or vinyl chloride) in the vinylidene copolymers amounts to 10 - 20 % by weight, whereby the softening temperature generally falls to a desired value.

The polymerization of the halogenated vinyl compounds also corresponds to the aforementioned processes. Suitable emulsifiers are, in particular,

alkali salts of fatty acids and alkyl- or acrylosulphonates, and non-ionogenic polyethylene oxide emulsifiers. Suitable initiators include conventional per-compounds, in particular redox initiators.

The film-forming dispersion is characterised in that during the course of drying i.e. during the vaporization of the liquid medium - generally an aqueous medium - the glass temperature T_g is exceeded. The glass temperature T_g can be most closely equated with the glass temperature of the dried polymer, as long as the polymer does not exhibit hydroplastic properties i.e. is softened by water as a result of its composition.

(The glass temperature T_g of a copolymer can be calculated from the contributions of the monomers. See R. Vieweg, F. Esser, "Synthetic Resin Handbook", Vd. IX, "Polymethacrylates", page 333 - 342, Carl Hanser Verlag 1975; H.F. Mark et al, Ed. Encyclopedia of Polymer Science and Technology Vol. 7, pp 531, 544, John Wiley 1987; T.G. Fox, Bull. Am. Phys. Soc. 1, 123 1956). Generally, however, knowledge of the minimum film formation temperature MFT is sufficient for the selection of suitable polymer dispersions and the monomers contained therein. In accordance with the understanding of the man skilled in the art (in accordance with which the MFT represents the determining characteristic for the film formation suitability of a polymer dispersion, and the value of which (in ° C) must be exceeded during the drying process), the MFT of the film-forming dispersion expediently is to be set at less than 60°C, preferably < 40°C, especially < 30°C.

The MFT may be determined in accordance with DIN 35 787. Film-forming dispersions based on (meth)acrylate dispersions which fulfil the above-mentioned conditions are particularly preferred (see Houben-Weyl, 4th Ed., Vol. XIV/1, loc.cit. page 1048).

The film-forming dispersion may be selected from the above recited classes of polymers, provided the

polymer satisfies the rules stated in the foregoing. In practice the glass temperature T_g of the polymers will not exceed the value of 30°C , i.e. these are relatively "soft" polymers. (Values for the glass temperatures T_g of homopolymers are given for example in Brandrup-Immergut, "Polymer Handbook", 2nd Ed., J. Wiley). By the copolymerization of appropriate monomers, a wide spectrum of latices with continuously stepped film hardnesses can be produced.

Special mention can be made of film-forming polymer dispersions of the following polymer composition: methacrylic acid esters of C_1 - C_4 -alcohols, in particular methylmethacrylate in proportions of 20 to 80 % by weight, in particular 20 - 60 % by weight, and acrylic acid esters of C_1 - C_4 -alcohols in proportions of 80 to 20 % by weight relative to the overall composition of the polymer.

The preferably aqueous, non-film-forming dispersion has the basic property that the freezing temperature of the polymers exceeds the film-forming temperature. The freezing temperature of the non-film forming polymer dispersion is preferably at least 30°C . In accordance with standard usage, the polymer of the non-film-forming dispersion can be referred to as "hard". This is conditional upon the predominance of so-called "hard" monomers, detectable from the softening points of the homopolymers (see Houben-Weyl, 4th Ed., loc.cit. Vol. XIV/1, page 1034). Polymethacrylic acid esters are known to be "harder" than polyacrylic acid esters. The "hardness" firstly decreases with an increasing quantity of the alcohol residues and then increases again, after the n-dodecyl in the methacrylester series and the n-octylester in the acrylester series. Methylmethacrylate and styrene and its derivatives can be considered as "hard" monomers.

As an alternative to the use of "hard" monomers, for example of the methylmethacrylate type or of the styrene type and its derivatives, the non-film-forming

dispersion can also be made in such manner that inherently "soft" monomers are combined with larger contents of cross-linking monomers, for example with a content of at least 5 % by weight and up to 50 % by weight. Such polymers do not form closed films.

Preferably the non-film-forming dispersions represent polymers containing > 60 % by weight "harder" monomers such as methylmethacrylate and styrene and its derivatives. The polymer content of the dispersions is generally conveniently in the range of 10 to 60 % by weight.

The non-film-forming dispersion generally contains particle sizes in the range of 0.03 to 5 μ , preferably 0.1 to 2 μ . The film-forming dispersion FD generally has particle sizes in the range of 0.02 to 5 μ , preferably 0.04 to 0.5 μ . The particle size is determined by photon correlation spectroscopy using the Nano-Sizer TDM manufactured by Coulter Electronics Ltd, Luton, Beds. A practical rule for dimensions in that the particles of the non-film-forming dispersion should each on average be a factor of 1.2 to 20 larger than those of the film-forming dispersion, preferably 1.5 to 10 times, especially 2 to 5 times larger.

The quantitative ratio of the two types of dispersion in the use according to the invention is advantageously 50 : 50 parts by weight to 99 parts by weight: 1 part by weight of non-film-forming to film-forming dispersion, in particular 60 parts by weight to 40 parts by weight to 95 parts by weight to 5 parts by weight, especially 65 parts by weight to 35 parts by weight to 90 parts by weight to 10 parts by weight, relative to the dry polymer. The following surprising finding was thereby arrived at: the value, derived from theoretical observations, of an effective upper limit of the film-forming polymer component in the mixture is approximately 26 vol. %. (In closest approximation vol. % and wt. % can be equated for this

observation).

With an increasing difference in the particle size, it is to be expected that the actually existing system will approximate the ideal form of the closest sphere packing of the non-film-forming dispersion particles, where the film-forming dispersion particles are located in the interstices. It would thus follow that with an increasing factor in the ratio of the particle size, the proportion of the film-forming component should be reduced to below the theoretically assumed limit of 26 vol. % in the interests of forming a porous material and in order to avoid the geometrically possible cavities becoming entirely filled. In reality, however, it has been proved that the above mentioned value of approximately 26 vol. % of FD-polymer can be drastically exceeded without manifestation of the exclusion effect, presumably because the actually existing system differs significantly from the ideal form of the closest sphere packing.

The carrier systems according to the invention for the covalent binding of biologically active material are produced by coating the carrier material with a mixture of the film-forming polymer dispersion and the non-film-forming polymer dispersion, preferably in the above stated ratios. The coating can be effected in a manner known per se, for example by spraying or dipping, preferably in a mixture, but individual application is also possible (sequential spraying).

For example, the application of the dispersion mixture by means of a spraying device has proved particularly advantageous. A particularly favourable embodiment comprises the spraying of the dispersion mixtures onto bead-like or sphere-like support material (see loc.cit.), advantageously in a coating drum with the simultaneous supply of air.

The coating of smaller particles necessitates particularly intensive drying in order to avoid adhesion

of the particles. Special devices with a high throughput of air, e.g. fluidized bed devices or coating apparatus comprising perforated drums are suitable for this purpose. In the coating process the process parameters, e.g. spraying speed, quantity of inlet air, inlet air parameters, are to be adapted to the respective properties of core material and dispersion mixture.

Based on a definition of "layer" as "the thickness of one monolayer", the following dimensioning limits have proved advantageous: approximately one layer to 1000 layers, preferably one layer to 100 layers. The ratio of the masses of the support body and coating can also be used as a rule of thumb, whereby a mass ratio of support material to coating (in the dried state) of 1 to 50 to 10,000 to 1 leads to utilizable results.

The biologically active materials, the fixing of which to solid carrier materials may be carried out in accordance with many different scientific and technical principles, can be assigned to a number of different classes. Thus, for example, it is possible to classify the said materials in accordance with chemical principles, e.g. in accordance with functional groups, such as amino acids, peptides and proteins, nucleosides, nucleotides and polynucleotides (saccharides) where - as again obvious - low-molecular and high-molecular types of compounds are to be differentiated.

Polymeric compounds, primarily proteins, lipoproteins, polysaccharides, (mucopolysaccharides), nucleic acids such as DNA and RNA and lipoids are of particular interest.

In many cases the type of the functional groups also governs the selection of the available fixing mechanisms, such as, for example, covalent, polar (ionic), complex-chemical, hydrophobic or by occlusion. Classification in accordance with biological effectiveness is also useful: it is thus possible, for example, to differentiate between biocatalysts such as

enzyme complexes, blood factors, hormones (messenger substances), immunologically active materials such as antigens and antibodies (e.g. monoclonal antibodies). Also accessible to fixing are morphologically (and generally also functionally) definable units, such as organelles, e.g. mitochondria, viruses, whole cells and cell constituents, cell hybrids, e.g. bacteria cells, eukaryotic cells and the like. Finally it is also possible to classify the immobilised materials in accordance with the given technical use. Thus, for example, in addition to the use of biocatalysts, use in the diagnostic field and in (affinity) chromatography are of interest.

The systems according to the invention are also outstandingly suitable for use in cell sorting and in depletion therapy. In this case a modification is employed, whereby, for example, a monoclonal antibody or another ligand specific to the receptor site in question is used.

The immobilised biocatalysts can be used, for example, for the production or conversion of very many different kinds of substrates, such as amino acids, peptides and enzymes, sugars, organic acids, antibiotics, steroids, nucleosides and nucleotides, lipids, terpenoids and organic key chemicals (see Ullmann, 5th Ed., Vol. 9A, loc.cit. page 389 - 390).

Examples of immunologically active materials are: micro-organisms, such as Gram-positive and Gram-negative bacteria, spirochaetes, mycoplasma, mycobacteria, vibrions, actinomycetales, protozoans such as intestinal protozoans, amoeba, flagellates, sporozoans, intestinal nematodes and tissue nematodes (worms), trematodes (schistosomes, leeches), cestodes, toxoplasma, and fungi, such as sporotrichum, cryptococcus, blastomycetes, histoplasma, coccidioides, candida, viruses and rickettsiae, such as canine hepatitis, Shope papilloma, influenza A + B, fowl pest, herpes simplex,

HIV, adeno-viruses, polyanes, Rous' sarcoma, vaccinia, polio-virus, measles, distemper, leukaemia, mumps, Newcastle disease, sendai, ECHO viruses, foot and mouth disease, psittacosis, rabies, ectromelia, tree viruses, also tissue antigens, enzymes such as pancreas chymotrypsinogen, procarboxypeptidase, glucose-oxidase, lactate dehydrogenase, uricase, amino acid oxidase, urease, asparaginase, proteases, blood cell antigens, blood group substances and other isoantigens, such as blood platelets, leucocytes, plasma proteins, milk proteins, saliva proteins, urine proteins, antibodies including auto-antibodies. Particular mention can be made of monoclonal antibodies which are directed against antigens, for example of the following type:

Antigen ClassesAntigen

Bacterial Tenanus-toxoid

H. influenza type b polysaccharide

Diphtheria toxin

Chlamydia trachomatis

M. leprae

Lipopolysaccharide/endotoxin

Pneumococci

LPS of P.aeruginosa

Exotoxin of P.aeruginosa

Streptococci Group A carbohydrate

Viral X31 influenza-virus-nucleoprotein

Measles virus

HSV glycoprotein D

Measles virus, nucleocapside

Cytomegaly virus

Influenza A viruses

Rubella virus antigens

HTLV I

Chickenpox-shingles

HBsAg

Auto-immune Double-stranded DNA
Islet cells (diabetes mellitus)
Myasthenia gravis, antiidiotypes
Thyrotropin receptor
Rheumatoid factor
Acetylcholine receptor
Thyroid
Sperm

Tumour Mamma-Ca
Prostate-Ca
Lung-Ca
Stomach-Ca
Melanoma
BD2 (human melanoma)
Glioma
Rectum-Ca
Leukaemia
Cervical-Ca

Blood/tissue Rhesus D
Blood group antigen A
HLA-A, B, C, DR
Intermediary filaments

Others Malaria
Forssman antigen
Sheep erythrocytes
Nitrophenol
Dinitrophenol
Trinitrophenol
Keyhole limpet hemocyanin (KLH)
Rheumatoid factor
Insulin

Particular mention can be given to the immunoglobulins of all classes, in particular the oligomer types, especially IgM, IgE and IgA.

The covalent fixing of the biologically active materials requires that the reactive groups X in the polymers of the film-forming and non-film-forming

dispersions should be intact and thus requires appropriately gentle production and handling. Gentle production of this kind is described, for example, in EP-A-71704.

The following examples will serve to explain the invention in a non-limiting manner.

EXAMPLES

Example 1

a) Production of a Seed Latex for Polymer Dispersions
Seed Latex A

In a 2 l. polymerization vessel, equipped with reflux condenser, mixer and thermometer

2.90 g methylmethacrylate
2.90 g isobutylmethacrylate
0.30 g glycol dimethacrylate
3.60 g ammonium peroxydisulphate
0.72 g sodium laurylsulphate

are added to 1.482 g double-distilled water and heated to 80°C. To this mixture a mixture of

171.00 g methylmethacrylate
171.00 g isobutylmethacrylate
18.00 g glycol dimethacrylate

is added at 80°C for two hours in drop form whilst stirring. The stirring is additionally continued at 80°C for a further two hours. Then the mixture is cooled to room temperature and filtered. A dispersion is obtained which is substantially free of coagulate and has a solid content of approximately 20 % by weight, a viscosity (determined in accordance with DIN 53 018) of 4 mPa s and an average particle radius of 120 nm (determined using the "Coulter nanosizer").

b) Production of a Seed Latex for Polymer Dispersions

In a Witts pot container the receiver was heated to 80°C whilst stirring, the initiator (ammonium peroxodisulphate or the sodium salt of 4,4'-azobis-(4-cyanovaleric acid)) was added, and then

the feed material was added in the form of an emulsion or monomer mixture within a specified period of time. 15 mins. after the end of the supply of the feed mixture the dispersion was cooled to room temperature and filtered.

Seed Latex B.

Vessel: 1478.0 g water
2.9 g isobutylmethacrylate
2.9 g methylmethacrylate
0.3 g glycoldimethacrylate
3.6 g ammonium peroxodisulphate

Feed Mixture: 168.1 g isobutylmethacrylate
(supplied
over a period 168.1 g methylmethacrylate
of 20 mins.) 17.7 g glycol dimethacrylate

Solid content: 21.1 % by weight; pH = 2.4; viscosity: 5 mPa s; particle diameter: 480 nm.

Seed Latex C

A procedure corresponding to that of Example 1b) was followed, except that:

Vessel: 1485.0 g water
0.72 g sodium laurylsulphate
3.6 g ammonium peroxodisulphate

Solid content: 21.4 % by weight; pH 2.3; viscosity: 5mPa s; particle diameter 270 nm.

Seed Latex D

Vessel: 427.0 g water
540.0 g seed latex B
9.0 g Titrisol solution pH 7
(Merck)
0.35 g 4,4'-azobis-(4-cyanovaleric
acid),
Na-salt.

Feed

Mixture: 585.0 g water
0.88 g sodium laurylsulphate
2.12g 4,4'-azobis-(4-cyanovaleric acid),
Na-salt
119.7 g isobutylmethacrylate
119.7 g methylmethacrylate
12.6 g glycol dimethacrylate

Solid content: 21.2 % by weight, pH = 6.2, viscosity: 5mPa s, particle diameter 730 nm.

Example 2

Production of a Film-Forming Polymer Dispersion comprising Epoxy Groups as Functional Groups X

In a 2 l polymerization vessel with reflux condenser, mixer and thermometer, a solution consisting of

7.50 g phosphate buffer, pH = 7.0
0.29 g sodium salt of 4,4'-azobis-(4-cyano)-valeric acid
0.48 g sodium laurylsulphate
150.00 g of a seed latex in accordance with Example 1 (= seed latex A)
275.00 g distilled water

was formed and heated to 80°C. To this solution an emulsion consisting of

210.00 g ethylacrylate
75.00 g methylmethacrylate
15.00 g glycidylmethacrylate
1.76 g sodium salts of
4,4'-azobis-(4-cyano)valeric acid

0.73 g sodium laurylsulphate and
933.00 g distilled water

was added in drop form, whilst stirring, for four hours at 80°C.

The dispersion is stirred for a further 15 minutes, allowed to cool to room temperature and filtered. A completely coagulate-free dispersion is obtained, having a solid content of approximately 20 % by weight, a pH-value of 7.0, a viscosity of 6 mPa s, and an average particle radius of approximately 240 nm.

Example 3

Production of a Non-Film-Forming Aqueous Polymer Dispersion Comprising Epoxy Groups as Functional Groups X.

A mixture comprising

7.50 g phosphate-buffer pH = 7.0
0.29 g sodium salt of 4,4'-azobis-(4-cyano)-valeric acid
30.00 g seed latex A in accordance with Example 1
and
340 g double-distilled water

is introduced into a polymerization vessel as described in Example 1, and heated to 80°C. To this mixture an emulsion comprising

180.00 g methylmethacrylate
105.00 g ethylacrylate
15.00 g glycidylmethacrylate
1.76 g sodium salt of
4,4'-azobis-(4-cyano)-valeric acid
0.73 g sodium laurylsulphate and
865.00 g double-distilled water

is added for four hours at 80°C. The dispersion is stirred for a further 15 minutes at 80°C, and then cooled to room temperature and filtered. A coagulate-free dispersion is obtained having a solid content of approximately 20 % by weight and a pH-value of 7.0, a viscosity of 6 mPa s and a particle radius of approximately 400 nm.

Example 4

Production of a Film-Forming Polymer Dispersion comprising Epoxy Groups as Functional Groups X

Vessel: 272.0 g water
 0.48 g sodium laurylsulphate
 7.5 g Titrisol solution pH 7
 (Merck)
 150.0 g seed latex C
 0.29 g 4,4'-azobis-(4-cyanovaleric
 acid)
 Na-salt

Feed

Mixture: 923.0 g water
(over a 0.73 g sodium laurylsulphate
period of 1.76 g 4,4'-azobis-(4-cyanovaleric
240 mins.) acid)
 Na-salt
 210.0 g ethylacrylate
 75.0 g methylmethacrylate
 15.0 glycidylmethacrylate

Solid content: 20.2 % by weight, pH = 7.3; viscosity 5 mPa s; particle diameter 560 nm, MFT: 5°C.

Example 5

Production of a Non-Film-Forming Polymer Dispersion
comprising Functional Groups X Containing Epoxy Groups.

The feed mixture comprises Emulsion 1 and Emulsion 2 in a ratio of 70 : 30, added consecutively in drops.

Vessel: 407.0 g water
9.0 g Titrisol solution, pH 7
(Merck)
36.0 g seed latex C
0.35 g 4,4'-azobis-(4-cyanovaleric
acid)
Na-salt

Emulsion 1: 726.0 g water
(over a 0.61 g sodium laurylsulphate
period of 1.48 g 4,4'-azobis-(4-cyanovaleric
168 mins.) acid)
Na-salt
163.8 methylmethacrylate
75.6 g ethylacrylate
12.6 g allyl methacrylate

Emulsion 2: 311.0 g water
(over a 0.27 g laurylsulphate
period of 0.64 g 4,4'-azobis-(4-cyanovaleric
72 mins.) acid)
Na-salt
64.8 ethylacrylate
43.2 glycidyl methacrylate

Solid content: 20.8 % by weight, pH: 7.3; viscosity: 5
mPa s; particle diameter: 760 nm, MFT > 50°C.

Example 6

Production of a Film-Forming Polymer Dispersion

Vessel: 560.0 g water
 1.12 g sodium laurylsulphate
 0.98 g 4,4'-azobis'(4'-cyanovaleric
acid)
 Na-salt

Feed

Mixture 860.0 g water
(over a 4.2g sodium laurylsulphate
period of 0.98 g 4,4'-azobis-(4-cyanovaleric
240 mins) acid)
 420.0 g ethylacrylate
 180.0 g methylmethacrylate

Solid content: 28.7 % by weight, pH= 6.7; viscosity: 6
mPa s; particle diameter: 82 nm; MFT: 8°C.

Example 7

Production of a Non-Film-Forming Polymer Dispersion

Vessel: 1411.0 g water
 37.5 g Titrisol solution, pH 7
 (Merck)
 1200.0 g seed latex D
 1.18 g 4,4'-azobis-(4-cyanovaleric
acid)
 Na-salt

After the addition of the initiator, firstly within a
period of 120 mins. an emulsion comprising

Feed

Mixture 1 1830.0 g water
 2.92 g sodium laurylsulphate
 5.29 g 4,4'-azobis-(4'-

cynovaleric acid)

Na-salt

313.5 g isobutylmethacrylate

313.5 g methylmethacrylate

33.0 g glycol dimethacrylate

is added in drops and then, within a period of 60 mins, simultaneously a) a monomer mixture comprising

126.0 g glycidyl methacrylate

102.0 g methylmethacrylate

12.0 g glycol dimethacrylate

and b) a solution comprising

Feed

Mixture 2

601.0 g water

60.0 g methacrylamide

1.77 g 4,4'-azobis-(4-cynovaleric acid)

Na-salt.

Solid content: 20.8 % by weight, pH = 7.7, viscosity : 5 mPa s, particle diameter: 1120 nm; MFT > 60°C.

Example 8

Production of a Non-Film-Forming Polymer Dispersion

The feed mixture comprises Emulsion 1 and Emulsion 2 in a ratio of 4 : 1, which were added consecutively in drops.

Vessel:

559.0 g water

0.42 g sodium laurylsulphate

0.98 g 4,4'-azobis-(4-cynovaleric acid)

Na-salt

Emulsion 1: 679.0 g water
(over a 3.36 g sodium laurylsulphate
period of 0.78 g 4,4'-azobis-(4-cyanovaleric
192 mins.) acid)
Na-salt
456.0 methymethacrylate
24.0 g allyl methacrylate

Emulsion 2: 177.0 g water
(over a 0.84 g sodium laurylsulphate
period of 0.2 g 4,4'-azobis-(4-cyanovaleric
48 mins) acid)
Na-salt
84.0 g ethylacrylate
36.0 g methymethacrylate

Solid content: 29.6 % by weight, pH: 6.7; viscosity 5
mPa s, particle size: 204 nm, MFT > 50°C.

Example 9

Production of a Film-Forming Polymer Dispersion

Vessel: 560.0 g water
1.12 g sodium laurylsulphate
0.98 g 4,4'-azobis-(4-cyanovaleric
acid)
Na-salt

Feed
Mixture: 860.0 g water
(over a 4.2 g sodium laurylsulphate
period of 0.98 g 4,4'-azobis-(4-cyanovaleric
240 mins.) acid)
Na-salt
420.0 g ethylacrylate
180.0 g methymethacrylate

Solid content: 28.7 % by weight, pH : 6.7, viscosity: 6 mPa s, particle diameter: 82 nm, MFT: 8°C.

Example 10

Production of a Film-Forming Polymer Dispersion

920 g water
29 g of an ethoxylated isononylphenol
(degree of ethoxylation 100)
346 g ethylacrylate
146 g methylmethacrylate and
6 g methacrylic acid

were introduced into a Witts pot container and the apparatus was flushed with N₂. The polymerization was initiated by the addition of 0.5 g ammonium peroxidisulphate, 0.7 g sodium pyrosulphite and 0.01 g iron-(II)-sulphate. When the temperature maximum was exceeded, the dispersion was cooled to 40°C and

100 g of the above mentioned emulsifier
346 g ethylacrylate
146 g methylmethacrylate and
6 g methacrylic acid

were added and the polymerization was re-initiated by the addition of 0.7 g sodium pyrosulphite and 0.5 g ammonium peroxidisulphate. When the temperature maximum was exceeded, the dispersion was cooled to room temperature, a pH of 7.0 was set using NaOH, and the dispersion was diluted to a solid content of 30% using water.

Solid content: 30%, particle diameter: 160 nm,
viscosity: 20 mPa s, MFT: 7°C

Example 11

Production of the Utilizable Mixtures from the Film-Forming Dispersion and the Non-Film-Forming Dispersion.

Utilizable mixtures are obtained by mixing the polymer dispersions of Examples 2 and 3 in ratios (stated in % by weight) which are given in the following Tables 1 - 7. 34 ml of the dispersion mixtures is diluted with deionized water to obtain a final volume of 200 ml.

Example 12

Coating of Polystyrene Beads with a Polymer Suspension

- a) The spraying unit is filled with 6 - 8 ml of the utilizable mixtures from Example 11.
- b) A coating pan (diameter 145 mm, two distributor plates) is charged with 150 polystyrene spheres (Spherotech spheres obtained from Spherotech-Vertriebs GmbH, Fulda, Federal Republic of Germany), with a diameter of 6.35 mm. The rotation of the pan commenced at 50 - 70 rpm, 5 - 7 ml of the utilizable mixture being sprayed onto the spheres while they were moving inside the coating pan. The spraying process was carried out at room temperature for approximately 30 minutes, during which time an unheated air jet (electrical air blower) was blown into the coating pan in order to vaporize the moisture.
- c) At the end of the coating step the coated beads are stored in a closed polystyrene flask at -15°C until the time of their use, for example for the immobilisation of antibodies.

Analogously, inorganic granulates, e.g. from

calcium carbonate, aluminium oxide, titanium dioxide, Aerosil® are used with similarly good results.

Example 13

Immobilisation of Monoclonal Antibodies on Polystyrene Beads Coated with the film forming/non-film-forming Dispersions

- a) 1000 μg of a monoclonal mouse antibody (directed against a human polypeptide hormone having a molecular weight of approximately 30,000) was dissolved in 20 ml of a 0.3 molar potassium phosphate buffer solution (pH = 8.0).
- b) This antibody preparation is applied to 100 coated beads, arranged in a beaker, in accordance with Example 12.
- c) This mixture is allowed to stand overnight, without movement, at 23°C.
- d) The aqueous constituent of this mixture is removed by vacuum suction and the remaining beads are washed three times with 80 ml PBS respectively (standard buffer, with common salt addition pH = 7.2). The washing process comprises five minutes of gentle shaking, followed by removal by suction of the aqueous phase, the beads remaining in the vessel which is used.
- e) After the third washing step 20 ml of a buffer solution containing 1 % by weight serum albumin in PBS (pH = 7.2) is applied to the beads. This mixture is allowed to stand overnight, without shaking, at 23°C.
- f) The washing is carried out as described under b).
- g) The charged beads are stored in a refrigerator at +5°C until they are used.

Example 14

Radio-immunometric Determination of the Antigen (Human Polypeptide-Hormone, Molecular Weight approx. 30,000).

- a) The polystyrene spheres obtained in accordance with Example 13 are introduced into test tubes with a cup-shaped base (9.6 mm diameter), with one bead per test tube.
- b) To this is added 100 μ l of a radioactive tracer (different monoclonal antibody which is directed against the above mentioned antigen and was labelled with ^{125}J of the order of approx. 50,000 counts per minute).
- c) Directly after the addition of the tracer, 100 μ l of the antigen is added in the concentrations stated in the following (see Tables).
- d) The mixture is shaken at room temperature (21°C) using an orbital shaking machine at 300 r.p.m. for a period of three hours.
- e) The aqueous constituent of the incubation batch is removed by vacuum suction.
- f) Each test tube, with its bead, is washed three times with respectively 1 ml buffer solution (2% by weight of cow serum albumin in 0.2 molar tris-buffer with a pH of 8.5 and 1.2 % by weight of $^{\text{R}}$ Tween-20-emulsifier)
- g) The test tubes are counted in a gamma-scintillation counter (type Multi Crystal Gamma Counter LB 2103).

The results obtained in this way are listed in Tables 1 - 7 (see below).

As can be seen from the values in Tables 1 - 7, the coating of the polystyrene spheres or beads, in accordance with the invention, with the mixture of film-forming and non-film-forming dispersions results in a substantial improvement, namely a reduction in the variation coefficient in parallel with a specific

increase (9585 cpm : 16636 cpm) in the range of the test results at the highest concentrations used.

In the case of the low concentrations of the analytical specimen, on the other hand, the difference between the coating exclusively with the film-forming dispersion compared with the mixture of the film-forming and the non-film-forming dispersion is not reflected in a distinct increase in the test sensitivity.

The best results are obtained from beads which are coated, in accordance with the invention, with the latex of a dispersion mixture containing 10 - 20 % by weight of the film-forming dispersion. Compared with uncoated polystyrene beads (which can be considered to represent the prior art), polystyrene beads coated with the mixture of the film-forming dispersion and the non-film-forming dispersion exhibit at least a four-fold increase in the measured counts per minute (cpm)-values in the lower and middle concentration band of the analytical specimen which even extends into the second highest concentration level. Even at the highest concentration level an increase in the measured cpm-values of a factor of approx. 2.5 is obtained. The advance in the technique of solid-state immunoassays achieved by the invention can thus be definitely proven.

TABLE 1: Results using uncoated polystyrene beads

cpm Measurements after 5 mins.	Average	Variation coefficient	Concentration Antigen (ng/ml)
0.6 0.0 9.3	3.3	157.8%	0
11.8 17.0 6.8	11.9	43.0%	0.016
23.2 30.7 13.2	22.4	39.3%	0.062
52.4 52.3 56.9	56.9	13.8%	0.218
213.7 205.6 193.1	204.2	5.1%	0.700
1299.8 750.0 738.8	929.6	43.5%	2.980
2891.3 2724.6	3089.9	16.1%	11.80
10872.2 11561.9 6322.9	9585.7	29.6%	50.80

TABLE 2 : Results using polystyrene beads which were coated only with the non-film-forming dispersion (in accordance with Example 3)

cpm Measurements after 5 min	Average	Variation coefficient	Concentration antigen (ng/ml)
23.6 22.4 29.3	28.8	21.3%	0
52.3 47.1 34.8	44.8	20.0%	0.016
93.5 87.9 88.9	90.1	3.4%	0.062
269.4 278.2 276.2	274.6	1.7%	0.218
889.6 853.6 904.0	882.4	3.0%	0.700
3227.2 3266.7 3233.4	3242.5	0.7%	2.980
10768.1 11230.9 11051.1	11016.4	2.2%	11.80
23203.6 23339.7 23258.4	23266.7	0.3%	50.80

TABLE 3: Results using polystyrene beads which were coated with a mixture of the non-film-forming dispersion (in accordance with Example 3) and the film-forming dispersion (in accordance with Example 2) in a ratio of 90 : 10 % by weight

cpm Measurements after 5 min	Average	Variation coefficient	Concentration Antigen (ng/ml)
19.7 23.6 28.6	24.0	18.7%	0
66.8 68.9 57.4	64.4	9.6%	0.016
133.6 124.2 117.0	125.0	6.7%	0.062
371.3 345.5 364.6	360.5	3.8%	0.218
1108.7 1151.4 1121.0	1127.4	2.0%	0.700
4165.3 4076.9 4203.4	4148.6	1.6%	2.980
13811.1 14083.3 14100.2	13998.1	1.2%	11.80
27782.0 28631.9 29383.4	28598.4	2.8%	50.80

TABLE 4: Results using polystyrene beads which were coated with a mixture of the non-film-forming and film-forming dispersions in a weight ratio of 80 : 20 (see Examples 2 & 3)

cpm Measurements after 5 min	Average	Variation coefficient	Concentration Antigen (ng/ml)
24.7 24.6 35.8	28.4	22.7%	0
51.4 58.7 56.8	55.7	6.8%	0.016
117.0 115.4 111.6	114.7	2.5%	0.062
350.3 348.8 336.3	345.2	2.3%	0.218
1054.3 1044.9 1124.7	1074.7	4.1%	0.700
3981.6 3904.3 3940.4	3941.7	1.0%	2.980
13249.4 12887.8 13074.8	13070.7	1.4%	11.80
27895.8 29123.3 28097.4	28372.2	2.4%	50.80

TABLE 5: Results using polystyrene beads which were coated with a mixture of 50% by weight respectively of film-forming and non-film-forming dispersions (see Examples 2 and 3)

cpm Measurements after 5 min	Average	Variation coefficient	Concentration Antigen (ng/ml)
78.6 28.9 32.6	46.7	46.7%	0
57.3 52.9 57.3	55.7	5.2%	0.016
99.8 104.6 109.8	104.8	4.8%	0.062
338.1 322.2 321.9	327.4	2.9%	0.218
982.4 1006.4 1048.2	1012.4	3.3%	0.700
3531.1 3741.1 3749.6	3673.7	3.4%	2.980
12408.6 12242.5 12463.8	12371.6	0.9%	11.80
26464.6 26530.6 27838.8	26944.0	2.9%	50.80

TABLE 6: Results using polystyrene beads which were coated with a mixture of film-forming and non-film-forming dispersions in a weight ratio of 80 : 20 (see Examples 2 and 3).

cpm Measurements after 5 min	Average	Variation coefficient	Concentration Antigen (ng/ml)
18.7 27.6 33.0	26.4	27.3%	0
47.6 54.8 56.3	52.9	8.9%	0.016
103.9 95.9 94.8	98.2	5.1%	0.062
304.7 294.0 303.7	300.8	2.0%	0.218
865.8 870.4 919.3	885.2	3.4%	0.700
3396.1 3231.9 3326.0	3317.7	2.5%	2.980
11132.8 10865.5 10899.1	10965.4	1.4%	11.80
22130.6 22364.9 22313.9	22269.8	0.6%	50.80

TABLE 7: Results using polystyrene beads which were coated exclusively with film-forming dispersion in accordance with Example 2.

cpm Measurements after 5 min	Average	Variation coefficient	Concentration Antigen (ng/ml)
41.4 27.6 39.9	36.3	20.9%	0
42.9 49.8 47.6	46.8	7.6%	0.016
90.9 75.1 68.8	78.3	14.6%	0.062
241.8 243.6 229.5	238.3	3.3%	0.218
698.9 663.8 757.8	706.9	6.8%	0.700
2747.1 2700.0 2543.3	2663.4	4.1%	2.980
8310.1 8138.1 7925.8	8124.4	2.4%	11.80
17829.9 15204.7 16635.9	16556.0	8.0%	50.80

Example 15

Coating of the Inert Carrier Material in the Form of Polymer Beads.

700 g of PMMA bead polymer with an average particle size of 310 μm is coated in a fluid bed device (Uniglatt, manufacturer Glatt) with the following dispersion mixture:

Non-film-forming dispersion in accordance with

Example 7 1225 g

and film-forming dispersion in accordance with

Example 10 525 g

(diluted to 20% dry substance) _____

1750 g

Ratio core/shell = 2 : 1

Process Conditions:

Spraying pressure	:1.8 bar
Inlet air temperature	:40°C
Outlet air temperature	:23 - 25°C
Spraying speed	: 8.75 g/min

The obtained product is dried for 24 hours in a vacuum. It is free-flowing and after a particle size analysis using a vibrating sieve, 81.4% (by weight) of the yield falls in the range of 0.3 - 0.6 mm.

Activity after the binding of trypsin: BAEE : 0.7 U/g

Casein : 0.3 U/g

Example 16

Coating of the Inert Carrier Material in the Form of Polymer Beads.

900 g of PMMA bead polymer with an average particle size of 310 μm is coated in a fluid bed device (Uniglatt, manufacturer Glatt) with the following

dispersion mixture:

Non-film-forming dispersion in accordance with Example 7	3150 g
and film-forming dispersion in accordance with Example 4	<u>1350 g</u>
	4500 g

Ratio core/shell = 1 : 1

Process Conditions:

Spraying pressure	: 1.8 bar
Inlet air temperature	: 40 - 50°C
Outlet air temperature	: 19 - 23°C
Spraying speed	: 11.84 g/min.

The obtained product is dried for 24 hours in a vacuum.
It is free-flowing and 85.8% by weight of the yield
falls in the granule size range of 0.3 to 0.6 mm.

Activity after the binding of trypsin: BAEE : 3.9 U/g

Casein: 1.04 U/g

Example 17

Binding of Trypsin to the Product from Example 16

500 mg of trypsin (bovine Art. No. 24 579, 40 v/mg; E. Merck, D-6100 Darmstadt) is dissolved in 10 ml 1 M potassium-phosphate buffer (pH 7.5) and added to 5 g of the product according to Example 16.

The mixture is shaken gently for 10 secs. and allowed to stand for 72 hours at +23°C. Then it is washed seven times with in each case 10 volumes deionised water and three times with in each case 10 volumes 0.1 M phosphate buffer (contains 500 ppm p-hydroxybenzoic acid for preservation and 2% 2-propanol). After washing (in a filter under vacuum) a moisture yield of 5.6 g is obtained.

Activity in relation to BAEE : 3.2 U/g

Casein : 0.36 U/g

No loss of activity compared with the prior art was observed.

Activity Determination.

Activity in relation to Casein (High-Molecular Substrate).

Substrate

350 ml deionised water and 32 ml 0.5 M NaOH are added to 20 g Hammarstein's casein (Art. No. 2242, E. Merck, D-6100 Darmstadt) and stirred at 60°C until the casein is dissolved. After cooling to room temperature the pH-value is set at pH 8.0 by the addition of 0.1 M HCl. Then the volume is filled with deionised water to 500 ml. (It should be noted that because of the aggregation of casein the solution is always somewhat cloudy).

Determination

20 ml substrate solution and 2 g of the product in accordance with Example 17 are stirred at 37°C, pH 8.0 in a thermo-stabilized reaction vessel with a pH-stab.-unit. At the same time the quantity of hydrolyzed substrate is recorded over time in respect of the consumption of 0.01 N NaOH.

After 10 mins. of incubation, the product is collected in a glass filter (porosity No. 2) and the same product is incubated for a further 10 mins. The declaration of the activity is based on the 4th cycle.

Activity in relation to N-benzoyl-L-arginine ethylesterhydrochloride (BAEE, low-molecular substrate).

Substrate

1% BAEEE solution (Art. No. 1672; E. Merck, D-6100 Darmstadt) is dissolved in 0.05 M potassium-phosphate buffer (pH 7.5).

Incubation

20 ml substrate solution and 2 g of the product in accordance with Example 17 (weighed moisture) are titrated at 37°C and a pH of 7.5 with 0.1 M NaOH. Incubation time: 10 min. The declaration of the activity was based on the 4th cycle.

Example 18

Coating of the Inert Carrier Material in the Form of Polymer Beads.

900 g of PMMA bead polymer with an average particle diameter of 310 μm is coated in a fluidized bed device (Uniglatt, manufacturer Glatt) with 1.575 g of the dispersion from Example 5.

Ratio of core/shell : 3 : 1

Process conditions:

Spraying pressure	: 1.8 bar
Inlet air temperature	: 40 - 45°C
Outlet air temperature	: 20 - 23°C
Spraying speed	: 8.75 g/min.

The obtained product is dried for 24 hours in a vacuum. It is free-flowing and 62.2% (by weight) of the yield falls in the granule size range of 0.3 - 0.6 mm. Activity after the binding of trypsin: BAEE : 0.3 U/g

Casein : 0.9 U/g

Example 19

Coating of the Inert Carrier Material in the Form of Polymer Beads.

775 g of PMMA bead polymer (PLEXIDON^R M 449) with an average particle diameter of 100 μm is coated in a fluidized bed device (Uniglatt, manufacturer Glatt) with the following dispersion mixture:

Dispersion from Example 6: 1,803.33 g
Dispersion from Example 8: 775.00 g
2,583.33 g

Ratio core/shell : 1 : 1

Process conditions:

Spraying pressure : 2 bar
Inlet air temperature : 40 - 45°C
Outlet air temperature : 22 - 25°C
Spraying speed : 10.5 g/min
Drying : 24 hours in a vacuum

The obtained product is free-flowing and white.
The average particle diameter is 189 μm .

Example 20

Film-forming and non-film-forming dispersions were prepared analogously to Examples 2 and 3, except that glycidyl methacrylate was replaced by respectively equal quantities by weight of each of the following monomers:

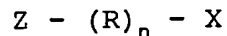
- a) methacrylic acid anhydride
- b) acrolein
- c) 2-(acetoacetoxy)-ethylmethacrylate
- d) acrylamidoglycolic acid methyl ester
- e) 2-(chloroacetoxy)-ethylmethacrylate
- f) 2-chloroethyl methacrylate

The thus-obtained dispersions were, according to the invention, comparable with those of Examples 2 and 3. They were likewise suitable for the preparation of carrier materials for biologically active systems analogously to Examples 11 and 12 and produced similar results.

CLAIMS:

1. A process for the production of a carrier system capable of covalently binding biologically active substances, said process comprising coating a support material with a mixture of a film-forming polymer dispersion and a non-film-forming polymer dispersion, at least one of said film-forming polymer dispersion and said non-film-forming polymer dispersion comprising functional groups capable of covalent binding to biologically active substances.
2. A process as claimed in Claim 1, wherein said support material is an inorganic support material.
3. A process as claimed in Claim 1, wherein said support material is an organic support material.
4. A process as claimed in any one of the preceding claims wherein said support material is in the form of spherical particles.
5. A process as claimed in Claim 4 wherein said spherical particles are spheres having a diameter in the range of 0.01 to 10 mm.
6. A process as claimed in either one of Claims 2 and 3, wherein said support material comprises planar structures on a cellulose or textile base or glass fibres.
7. A process as claimed in any one of the preceding claims wherein said polymer dispersions are selected from the group comprising polyacrylates, polystyrene and polymers of alkyl styrene, polyvinyl esters, polyvinyl halogen compounds and copolymers thereof.

8. A process as claimed in any one of the preceding claims wherein said polymer dispersions have a content of monomers bearing functional groups in accordance with the formula



wherein X signifies a sulphonic acid halogenide-, thioisocyanate-, thiocarbonyldioxy-, carbonyl-imidoxyldioxy-, haloethoxy-, haloacetoxyl-, oxiran-, aziridin-, formyl-, keto-, acryloyl-, anhydride- or activated ester group;

R is a chemically inert spacer;

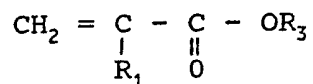
n is 0 or 1; and

Z is a polymerizable unit.

9. A process as claimed in any one of the preceding claims, wherein said non-film-forming polymer comprises functional groups capable of covalent binding to biologically active material.

10. A process as claimed in any one of the preceding claims wherein the content of functional monomers in the polymer amounts to 0.1 to 80 % by weight, based on the total quantity of monomers.

11. A process as claimed in any one of the preceding claims wherein the polymer dispersions comprise polyacrylates comprising, to at least 50 % by weight based on the total quantity of monomers, monomers in accordance with the formula



wherein R₁ is hydrogen or methyl or a group CH₂-COOR₂,

wherein R_2 signifies an alkyl group containing 1 to 4 carbon atoms and

R_3 is an optionally branched alkyl group containing 1 to 18 carbon atoms or a cycloalkyl group containing 3 to 12 ring members.

12. A process as claimed in any one of the preceding claims, wherein said film-forming dispersion has a minimum film forming temperature (in accordance with DIN 53 787) of less than 60°C.

13. A process as claimed in any one of the preceding claims, wherein said film-forming dispersion has a minimum film-forming temperature (in accordance with DIN 53 787) of less than 40°C.

14. A process as claimed in any one of the preceding claims, wherein the freezing temperature of the polymer of the non-film-forming dispersion is higher than the film forming temperature.

15. A process as claimed in any one of the preceding claims, wherein the freezing temperature of said non-film-forming dispersion is at least 30°C.

16. A process as claimed in any one of the preceding claims, wherein the particles of the film-forming dispersion have an average particle size in the range of 0.02 to 5 μm .

17. A process as claimed in any one of the preceding claims, wherein the particles of the non-film-forming dispersion have an average particle size in the range of 0.03 to 5 μm .

18. A process as claimed in any one of the preceding

claims, wherein the particles of the non-film-forming dispersion are on average a factor of 1.2 to 20 larger than those of the film-forming dispersion.

19. A process as claimed in any one of the preceding claims, wherein the ratio of said non-film-forming dispersion to said film-forming dispersion is 50 parts by weight : 50 parts by weight to 99 parts by weight : 1 part by weight.

20. A process as claimed in any one of the preceding claims, wherein said support material is coated by dipping or spraying with said film-forming polymer dispersion and said non-film-forming polymer dispersion.

21. A process as claimed in Claim 20, wherein a mixture of the polymer dispersions is used for said dipping or spraying.

22. A process as claimed in claim 1 substantially as herein defined and with reference to the Examples.

23. A carrier system capable of covalently binding biologically active material prepared by a process as claimed in any one of claims 1 to 22.

24. A carrier system capable of covalently binding biologically active materials comprising a support coated with a mixture of a film-forming polymer dispersion and a non-film-forming polymer dispersion.

25. The use of a support system as defined in claim 23 in the covalent binding of biologically active material.

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Examiner's report to the Comptroller under
Section 17 (The Search Report)

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Relevant Technical fields

(i) UK CI (Edition K) B2E (EM) C3H (HH1)

(ii) Int CI (Edition 5) C07K, B32B

Search Examiner

A DONLAN

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI

Date of Search

12.12.91

Documents considered relevant following a search in respect of claims

1-25

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
	NONE	



Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

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