Flow scheme for the production by sol synthesis & spray drying:

Production of a pure physical mixture of the educts

Addition of an ethanol/water-mixture = start of the reaction to an alkaline-catalyzed sol synthesis

homogenous transparent sol

addition of magnetic pigment

sol with pigment dispersion suitable for spraying

spray drying process (variable pressure, temperature, flow rate, nozzle geometry)

Xerogel-magnetic core-particles
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Xerogel-magnetic core-particles
Figure 2

Flow scheme for the preparation processing of the premature particles

Precondensation by temperature treatment (4h/200°C) in air for the stabilisation of the surface

↓

Final condensation and sintering under inert atmosphere (N₂) through treatment at high temperatures (1h, 750°C) => pigment cores surrounded by a weakly subcooled melt

↓

Rapid cooling => magnetic pigment core-glass coated-particle = MGP

↓

Temperature treatment in the air (4h, 200°C) = Oxidation of the glass shell

↓

Preparation of the non-aggregating fraction by the use of a sieve

↓

Bottling of the MGP powder, then 4h/200°C temperature treatment

↓

Cooling to approximately 80°C, Closing of the bottle with foil and screw top

↓

Storage of the MGP powder ==> Suspension in R-OH (e.g. isopropanol)
Technical data:

- Diameter of the drying chamber: 0.75 m
- Height of the drying chamber: 2.5 m
- Evaporation power (water): 1-2 l/h
MAGNETIC GLASS PARTICLES, METHOD FOR THEIR PREPARATION AND USES THEREOF

[0001] This invention relates to magnetic particles having a glass surface and are substantially spherical. This invention also relates to methods for making them, as well as to suspensions thereof and their uses for the purification of biological material in particular in automated processes.

[0002] Many biological materials, especially nucleic acids, present special challenges in terms of isolating them from their natural environment. On the one hand, they are often present in very small concentrations and, on the other hand, they are often found in the presence of many other solid and dissolved substances that make them difficult to isolate or to measure, in particular in biospecific assays.

[0003] Biospecific binding assays allow of the detection of specific analytes, e.g. nucleic acids, or specific analyte properties and play a major role in the field of diagnostics and bioanalytics. Examples thereof are hybridisation assays, immuno assays and receptor-ligand assays.

[0004] Hybridisation assays make use of the specific base-pairing for the molecular detection of nucleic acid analytes e.g. RNA and DNA. Hence, oligonucleotide probes with a length of 18 to 20 nucleotides may enable the specific recognition of a selected sequence in the human genome. Another assay which makes use of the selective binding of two oligonucleotide primers is the polymerase chain reaction (PCR) described in U.S. Pat. No. 4,683,195. This method makes use of the selective amplification of a specific nucleic acid region to detectable levels by a thermostable polymerase in the presence of deoxynucleotides triphosphates in several cycles.

[0005] Nucleic acids are comparatively complex analytes which have normally to be extracted from complex mixtures before they can be used in a probe-based assay.

[0006] There are several methods for the extraction of nucleic acids:

- sequence-dependent or biospecific methods as e.g. affinity chromatography
- hybridisation to immobilised probes on beads
- sequence-independent or physico-chemical methods as e.g.: liquid-liquid extraction with e.g. phenol-chloroform
- precipitation with e.g. pure ethanol
- extraction with filter paper
- extraction with micelle-forming agents as cetyl-trimethyl-ammonium-bromide
- binding to immobilised, intercalating dyes, e.g. acridine derivatives
- adsorption to silica gel or diatomic earths
- adsorption to magnetic glass particles (MGP) or organo silane particles under chaotropic conditions

[0007] Many procedures and materials for isolating nucleic acids from their natural environment have been proposed in recent years by the use of their binding behavior to glass surfaces. In Proc. Natl. Acad. USA 76, 615-619 (1979), for instance, a procedure for binding nucleic acids in agarose gels in the presence of sodium iodide in ground flint glass is proposed.

[0009] The purification of plasmid DNA from bacteria on glass dust in the presence of sodium perchlorate is described in Anal. Biochem. 121, 382-387 (1982).

[0010] In DE-A 37 34 442, the isolation of single-stranded M13 phage DNA on glass fiber filters by precipitating phage particles using acetic acid and lysis of the phage particles with perchlorate is described. The nucleic acids bound to the glass fiber filters are washed and then eluted with a menthol-containing buffer in Tris/EDTA buffer.

[0021] A similar procedure for purifying DNA from lambda phages is described in Anal. Biochem. 175, 190-201 (1988).

[0022] The procedure known from the prior art entails the selective binding of nucleic acids to glass surfaces in chaotropic salt solutions and separating the nucleic acids from contaminants such as agarose, proteins or cell residue. To separate the glass particles from the contaminants according to the prior art, the particles are either centrifuged or fluids are drawn through glass fiber filters. This is a limiting step, however, that prevents the procedure from being used to process large quantities of samples.

[0023] It has been demonstrated that magnetic particles covered with a glass surface offer considerable advantages for isolating biological materials. If the magnetic particles have not been brought in contact with a magnetic field, gravity is the only force that can cause them to sediment out. They can be resuspended by shaking the solution. The sedimentation procedure that does not utilize a magnetic field proceeds more slowly than the immobilization of biological materials on the surface of the particles. This is especially true for nucleic acids. The magnetic particles can be easily collected at a specific location in the sample fluid by means of a magnet. The fluid is then separated from the particles and, therefore, from the immobilized biological materials.

[0024] The use of magnetic particles to immobilize nucleic acids after precipitation by adding salt and ethanol is described in Anal. Biochem. 201, 166-169 (1992) and PCT GB 91/00212. In this procedure, the nucleic acids are agglutinated along with the magnetic particles. The agglutinate is separated from the original solvent by applying a magnetic field and performing a wash step. After one wash step, the nucleic acids are dissolved in a Tris buffer. This procedure has a disadvantage, however, in that the precipitation is not selective for nucleic acids. Rather, a variety of solid and dissolved substances are agglutinated as well. As a result, this procedure can no be used to remove significant quantities of any inhibitors of specific enzymatic reactions that may be present.

[0025] Magnetic, porous glass is also available on the market that contains magnetic particles in a porous, particular glass matrix and is covered with a layer containing streptavidin. This product can be used to isolate biological materials, e.g., proteins or nucleic acids, if they are modified.
in a complex preparation step so that they bind covalently to biotin. Magnetizable particular adsorbents proved to be very efficient and suitable for automatic sample preparation. Ferrimagnetic and ferromagnetic as well as superparamagnetic pigments may be used for this purpose.

[0026] Particles, according to the expert, are solid materials having a small diameter. Particles like these are often also referred to as pigments.

[0027] Those materials are referred to as magnetic that are drawn to a magnet, i.e., ferromagnetic or superparamagnetic materials, for instance. Superparamagnetism is seen as advantageous and preferable in the state of the art (e.g. U.S. Pat. No. 5,928,958; U.S. Pat. No. 5,925,573; EP 757 106). The glass or organosilane surfaces are often functionalised in order to be used for biospecific capture reactions, e.g. U.S. Pat. No. 5,928,958, U.S. Pat. No. 5,898,071, U.S. Pat. No. 5,925,573, EP 937 497, U.S. Pat. No. 4,554,088 or U.S. Pat. No. 4,910,148. Alternatively, glass or organosilane surfaces may be treated with various solvents or salts to modify their hydrophilicity and/or electropositivity, e.g. U.S. Pat. No. 5,438,127.

[0028] However, the underivatized silanol groups of the glass or the silane surface may be used for the adsorption with pure physico-chemical forces under suitable reaction conditions as described in DE 195 20 964, DE 195 37 985, WO 96/41840, WO 96/41811, EP 757 106 or U.S. Pat. No. 5,520,899. Typically, magnetic cores or magnetic core aggregates are covered with a glass surface which is formed by an acid- or base-catalyzed sol-gel-process. These particles are called core-shell particles. The glass shell then has a typical layer thickness (see e.g. DE 195 20 964) wherein the size and shape of the pigment, which may contain a non-magnetic support as e.g. mica in addition to the magnetic metal oxide, determines size and form of the produced particle (see e.g. DE 195 37 985 and corresponding WO 96/41811). To obtain a high surface activity, glass material with a high porosity is used (see e.g. EP 757 106; WO 99/26005). Further, composite magnetic particles are described, e.g. silicate-covered ferric oxide covered with an inorganic silica matrix from silica particles (EP 757 106) or mixtures of glass and silica gel (WO95/06652).

[0029] The problem to be solved by the present invention can be seen as providing magnetic glass particles with improved properties for sample preparation and for biological assays, in particular for automated processes.

[0030] The deficiencies of the magnetic glass particles in the state of the art are overcome by the findings of the present invention.

[0031] It is an object of the invention to provide a composition of magnetic glass particles. The magnetic glass particles (MGP s) according to the present invention are a solid dispersion of small magnetic cores in glass. The MGPs are comparatively small and are substantially spherical. The non-magnetic fine content of a composition of the MGPs is very low because of the method of their preparation. This has the effect that suspensions of the MGPs sediment slowly and can therefore be advantageously used for processes in molecular biology which can be automated. In one embodiment of the invention compositions and suspensions of the MGPs according to the present invention are provided. In another embodiment of the invention a method for the composition of the MGPs is provided. In still another embodiment of the invention a method for the purification of DNA or RNA is provided in which the MGPs according to the present invention are used.

[0032] It is a object of the present invention to provide a composition of magnetic glass particles which are substantially spherical and have a small diameter and contain at least one magnetic object with a diameter between 5 and 500 nm. This has surprising consequences on the sedimentation kinetics, quantified by the half time values t_1/2, which is the time span until 50% of the particles have sedimented from a specific volume element (see Example 6). The half-life period for the sedimentation of a 3 mg/ml weight-per-volume suspension of the composition in isopropanol is more than 3 min, preferably 4 min, more preferably 6 min. However the most preferred values for the half-life period is more than 10 min or even 20 min. Smaller and closer to the ideal sphere, the longer the MGPs will be suspended. This may be explained by the fact that the closer the form will resemble an ideal sphere, the lower the possibility that there or more particles will stick together and build up aggregates which may sediment more rapidly. These data are shown in Example 6 and high resolution scanning electron microscopical images can be seen in FIG. 4 to FIG. 10. This has the advantage that for automated processes the required mixing intensity and mixing frequency of the storage conditions containing the MGP suspension is reduced as the repetitive dosage of a specific MGP suspension volume from a surplus volume sucked into a syringe is easier (more precise delivery with regard to mass of MGP).

[0033] The MGPs according to the present invention are glass droplets in which some small non-aggregating magnetic objects are dispersed. Those objects that are referred to as magnetic are drawn to a magnet, i.e., ferromagnetic or superparamagnetic materials, for instance. Preferred are ferromagnetic materials, in particular if they have not yet been premagnetized. Premagnetization in this context is understood to mean bringing in contact with a magnet, which increases the remanence. Preferred magnetic materials are iron or iron oxide as e.g. magnetite (Fe_3O_4) or Fe_2O._3, preferably γ-Fe_2O._3. In principle, barium ferrite, nickel, cobalt, Al—Ni—Fe—Co alloys or other ferri- or ferromagnetic could be used. The magnetic objects may be e.g. a magnetic pigment. The size of the magnetic objects is in the nanoscale range, i.e. according to the present invention the diameter is between 5 to 500 nm, preferably between 10 to 200 nm, most preferably between 15 to 50 nm. Suitable magnetic pigments are manufactured by the company CERAC which have a mean diameter of 25 nm and consist of γ-Fe_2O._3 (BET-surface 50 m^2/g, CERAC: P.O. Box 1178, Milwaukee, Wisc. 53201-1178 USA; Article-No. I-2012). The magnetic glass particles according to the present invention are further characterized by the fact that the MGPs have a particle diameter between 0.5 μm and 5 μm, preferably between 1 μm to 2 μm as determined by high resolution scanning electron microscopy, whereas the magnetic objects have a diameter between 5 to 500 nm, preferably between 10 to 200 nm, most preferably in the range of 15 to 50 nm as said above. Hence, the MGPs of the present invention are further characterized by a diameter ratio of magnetic pigment core to magnetic glass particle of less than 1 to 10 as determined by high resolution scanning electron microscopy. Because of these diameter ratios as well as the absence
of any inert carrier that would determine shape and size of the particles, the geometry of the MGPs and the number of incorporated magnetic objects, are determined by the conditions of manufacturing. The MGPs according to the present invention are microporous but have highly-struc-
tured and therefore relatively large surface with more than 6 m²/g. Preferably, the magnetic glass particles according to the present invention have a surface area in the range of 5 to 100 m²/g, preferably 5 to 90 m²/g, more preferably in the range of 10 to 50 m²/g, most preferably in the range of 15 to 30 m²/g. This surface is approximately double the size of the particles described in DE 195 37 965. This can be determined by the Brauener-Emett-Teller-method using an automated commercial apparatus (see Example 4). For a discussion of this method, familiarly called the BET method, see S. Brauener. The Adsorption of Gases and Vapors, Vol. 1, Princeton University Press, 1943. For example, the sample EJ0096.5R-01 which is of preferential interest (see Example 1 and Table 1 to Table 3 for a summary of the production parameters) has a BET-surface of 26.8525 m²/g, a microarea of 2.3058 m²/g and an average pore diameter of 24.9132 nm. This means that the pore surface is less than 10% of the total surface and that the magnetic glass particle is microporous.

[0034] A pore is understood to be a recess in the outer surface of the particle. The surface reaches so far into the particle that a perpendicular line drawn in the recess on the surface cuts the particle at least once in the direction of the adjacent environment of the particle. In addition, pores reach into the particle to a depth that is greater than one radius of the pore.

[0035] The slower sedimentation kinetics, larger surface and the aggregation-inhibiting spherical form manifest themselves in the better function performance and extraction of nucleic acid diagnosis (see Example 3, 5 and 7) when compared to the German patent applications DE 198 54 973.3 or DE 198 55 259.9. This criterion can be quantified by a shift of the threshold cycles in so-called TaqMan® assays, the signal-to-noise ratio and of the statistically validated lower detection limit. The methods for this assay are disclosed in WO92/02638 and the corresponding US patents U.S. Pat. No. 5,210,015, U.S. Pat. No. 5,804,375, U.S. Pat. No. 5,487,972. Radioactivity experiments (see Example 5.2) showed that the binding behavior with regard to DNA and RNA was the same when compared to reference material known in the state of the art. Surprisingly, the production parameters had an influence on the performance in the radioactivity experiments. A further advantage of the MGP-type of the present invention is that no tensions in the glass layer can lead to fissure during the drying process and corresponding damages in the glass shell because of the inner structure (solid dispersion of small magnetic cores in a glass drop). This can be investigated by image-producing methods (see Example 3).

[0036] Another embodiment of the present invention is a suspension of magnetic particles. It is obvious for the person skilled in the art to produce a suspension by adding a liquid to a composition of the MGPs and mix the suspension to homogeneity. A liquid according to the present invention may comprise any liquid which does not affect the stability of the magnetic particles and may be used to produce a homogenous suspension. Preferably liquids are used which are suitable for processes in molecular biology, in particular desoxyribonucleic acid (DNA) or ribonucleic acid (RNA) purification processes which make use of the binding of these substances to glass particles under certain conditions. Preferred liquids comprise alcohols or any mixtures thereof with water or ketones. Alcohols shall include according to the invention preferably primary, secondary or tertiary alco-
hol-gels of the general formula R—OH where the R stands for the general formula —(—CH2)—CH3 with n>0. However, other alcohols can also be used if they are suitable for molecular biology purposes as e.g. glycerol. Particularly suitable are the alcohols isopropanol, ethanol or mixtures thereof with water, preferably a mixture of 80 volume parts of isopropanol with 20 volume parts of water (. In another embodiment of the invention the liquid comprises ketones (as e.g. acetone. In a preferred embodiment of the invention these suspensions contain between 5 to 60 mg/ml MGPs. In another embodiment of the invention the MGPs are sus-
pended in aqueous buffered solutions which may optionally contain a chaotropic agent in a concentration of between 2 and 8 mol/l, and preferably between 4 and 6 mol/l. Cha-
otropic salts can be sodium iodide, sodium perchlorate, guanidinium thiocyanate, guanidinium isothiocyanate or guanidinium hydrochloride. Other compounds are also pos-
sible. A chaotropic agent according to the present invention will be any chemical substance which will disturb the ordered structure of liquid water and will have the effect that DNA or RNA will bind to the MGPs according to the present invention if this agent is present in the DNA or RNA containing solution. It is obvious for the artisan to produce suitable aqueous buffered solutions. Buffer systems which may be used for molecular biology purposes may be found e.g. in Sambrook et al. (1989), Molecular Cloning, Cold Spring Harbor University Press, New York, N.Y., USA. Preferred buffer substances are Tris-hydroxymethylaminomethane (TRIS), phosphoric acid, N-(2-Hydroxyethyl)pyperazine-N’-(2-
ethanesulfonic acid) (HEPES), salts thereof or other suitable substances. Additionally, substances may be present which modify the ionic strength of the solution as e.g. NaCl, KCl or CaCl2, or which are metal cation complexes such as e.g. ethylene-diamine-tetra-acetic acid (EDTA) or the salts thereof. Biological material known to the expert in the field may also be present. In another embodiment of the invention the suspension of MGPs may additionally contain DNA or RNA optionally in a mixture with proteins, fatty acids, carbohydrates and other material from biological origin. In another embodiment of the invention the liquid may contain a mixture of one or more constituents selected from the group of alcohols, ketones, aqueous buffered solutions, chaotropic agents, substances which modify the ionic strength of the solution, complexing agents, biological material, DNA or RNA all with the features as described above.

[0037] In another embodiment of the invention a tube or reaction vessel containing the suspension according to the invention is provided. The tube can be made of plastics but it may also be part of a larger structure, e.g. part of a microtitreplate in 96- or 384-well-format. In still another embodiment of the invention a storage container is provided which contains a composition of magnetic glass particles or suspensions thereof. In another embodiment of the invention a kit of parts is provided which comprises a storage con-
tainer containing the magnetic glass particles or a suspen-
sion thereof according to the present invention. The kit may be used for the purification of DNA or RNA. Such kits known in the art further comprise plastics ware which may
be used during the purification procedure as e.g. microtitre-plates in the 96 or 384 well format or just ordinary reaction tubes manufactured e.g. by Eppendorf, Hamburg, Germany. The kit may further comprise a washing solution which is suitable for the washing step of the magnetic glass particles when DNA or RNA is bound thereto. Often the washing solution is provided as a stock solution which has to be diluted before the use. The kit may further comprise an eluent, i.e. a solution or a buffer (e.g. TE, 10 mM Tris, 1 mM EDTA, pH 8.0) or pure water to elute the DNA or RNA bound to the magnetic glass particles. Further, additional reagents may be present which can be used for the purification process of a nucleic acid, i.e. DNA or RNA. In one embodiment of the invention the kit of parts according to the present invention is used for the purification of a nucleic acid.

[0038] In one embodiment of the invention the composition of MGPS may be used to produce a suspension as already described.

[0039] In another embodiment of the invention the suspensions according to the present invention may be used for the purification of nucleic acids, i.e. RNA or DNA, from complex mixtures with other biological substances containing them. Thereby also mixtures of different nucleic acids may be purified, even mixtures containing a nucleic acid of interest in low abundance. The purification effect results from the behavior of DNA or RNA to bind to magnetic glass particles under certain conditions e.g. in the presence of certain concentration of a chaotropic agent. Preferably, the MGPS with the bound DNA or RNA are washed afterwards at least once, preferably with a mixture of 70 volume parts ethanol with 30 volume parts water ("70:30 Ethanol"). Afterwards, the conditions are reversed, e.g. the concentration of the chaotropic agent is decreased, to elute the DNA or RNA bound to the MGPS particle. Preferably, this is done by the pelleting of the magnetic glass particles, e.g. by gravity force or by the use of a magnet, and resuspending in a solution with no or only a low amount of chaotropic agent. Alternatively, the solution can be diluted with a solution with no or only a low amount of chaotropic agent. The purified DNA or RNA can now be used for other reactions.

[0040] It is one object of the invention to provide a production method for the MGPS according to the present invention. A glass according to the present invention is understood to be an amorphous material that contains silicon. Glass can contain other materials such as B2O3 (0-30%), Al2O3 (0-20%), CaO (0-20%), BaO (0-10%), K2O (0-20%), Na2O (0-20%), MgO (0-18%), PbO (0-15%). Glass can also contain a smaller percentage (0-5%) of a number of other oxides such as MnO, TiO2, As2O3, Fe2O3, CuO, CoO, etc.


\[
\begin{align*}
\text{NaOH} & + \text{B}_2\text{O}_3 + \text{Al(OH)}_3 + \text{Si(OH)}_4 & \rightarrow & \text{Na}_2\text{B}_2\text{O}_3\text{Al}_2\text{O}_3\text{SiO}_2 \text{Glass} \\
\text{OH} & \rightarrow & \text{H}_2\text{O}
\end{align*}
\]

[0042] Water is added to begin the hydrolysis process of the starting components. The reaction proceeds relatively quickly because the alkali ions have a catalytic effect on the speed of hydrolysis of the silicate acid ester. Once the gel is formed it can be dried and densified (or condensed) by means of a thermal process to form glass.

[0043] In one embodiment of the invention the glass matrix will be produced by acid or base catalyzed sol-gel synthesis as shown schematically in FIG. 1 and FIG. 2 and described in detail in Example 1. Here, use is made of colloidal systems wherein at first the solid constituents are dispersed in the liquid phase (sol) and after the processing they are interconnected like a honeycomb pattern (gel). The composition of the glass (code EJ) as calculated from the quantity of the educts was 70.67 Mol % SiO2, 14.33 Mol % B2O3, 5.00 Mol % Al2O3, 4.00 Mol % K2O, 2.00 Mol % CaO, 4.00 Mol % MgO, 4.00 Mol % ZnO. The composition of the glass (code R) was 74 Mol % SiO2, 15 Mol % B2O3, 5.00 Mol % Al2O3, 4.00 Mol % K2O, 2.00 Mol % CaO. The composition of the glass (code RP) was 73.61 Mol % SiO2, 14.93 Mol % B2O3, 5.21 Mol % Al2O3, 4.17 Mol % K2O, 2.08 Mol % CaO.

[0044] The reaction may be described as follows:

[0045] either acid-catalyzed, e.g.:

\[
\begin{align*}
\text{BCl}_3 + \text{H}_2\text{O} & \rightarrow \text{B(OH)}_3 + 3\text{H}^+ + \text{Cl}^- \rightarrow \text{H}_3\text{BO}_3 \text{Cl}^- \\
\text{AlCl}_3 + \text{H}_2\text{O} & \rightarrow \text{Al(OH)}_3 + 3\text{H}^+ + \text{Cl}^- \rightarrow \text{H}_3\text{AlCl}_3 \text{OH}^- \\
\text{SiCl}_4 + \text{H}_2\text{O} & \rightarrow \text{Si(OH)}_4 + 4\text{H}^+ \rightarrow \text{H}_4\text{SiO}_4 \\
\text{Na}^{+}\text{NO}_3^- + \text{H}_2\text{O} & \rightarrow \text{Na}^{+}\text{OH}^- + \text{H}^+ + \text{NO}_3^- \\
\text{K}^{+}\text{OOCCH}_3 + \text{H}_2\text{O} & \rightarrow \text{K}^{+} \text{OH}^- + \text{H}^+ + \text{OOCCH}_3 \\
\text{K}^{+}\text{OCH}_2\text{CH}_3\text{H}_2\text{O} & \rightarrow \text{K}^{+} \text{OH}^- + \text{H}^+ + \text{HOCCH}_3 \\
\text{Na}^{+}\text{OCH}_2\text{CH}_3\text{H}_2\text{O} & \rightarrow \text{Na}^{+} \text{OH}^- + \text{H}^+ + \text{HOCH}_3 \\
\text{Al}^{3+}\text{OCH}_2\text{CH}_3\text{CH}_2\text{H}_2\text{O} & \rightarrow \text{Al(OH)}_3 + 3\text{H}^+ + \text{HOCCH}_3 \\
\text{B(OCH}_2\text{CH}_3\text{H}_2\text{O} & \rightarrow \text{B(OH)}_3 + 3\text{H}^+ + \text{HOCH}_3 \\
\end{align*}
\]

[0051] or alkaline-catalyzed, e.g.

[0052] \[ \text{K}^{+}\text{OCH}_2\text{CH}_3\text{H}_2\text{O} \rightarrow \text{K}^{+} \text{OH}^- + \text{H}^+ + \text{HOCH}_3 \]

[0053] \[ \text{Na}^{+}\text{OCH}_2\text{CH}_3\text{H}_2\text{O} \rightarrow \text{Na}^{+} \text{OH}^- + \text{H}^+ + \text{HOCH}_3 \]

[0054] \[ \text{Al}^{3+}\text{OCH}_2\text{CH}_3\text{CH}_2\text{H}_2\text{O} \rightarrow \text{Al(OH)}_3 + 3\text{H}^+ + \text{HOCCH}_3 \]

[0055] \[ \text{B(OCH}_2\text{CH}_3\text{H}_2\text{O} \rightarrow \text{B(OH)}_3 + 3\text{H}^+ + \text{HOCH}_3 \]

[0056] The diverse hydroxides condensate to the corresponding oxides which form a three-dimensional network,
the amorphous glass matrix of SiO₂/B₂O₃/Al₂O₃ with metal ions occupying interstitial sites. In addition to the above-captioned alkaline and alkaline earth metal ions, transition metal ions as e.g. Zn²⁺ and Zr²⁺ may be incorporated in the matrix as network modifying agents.

\[
\text{Si-O-Si} \rightarrow \text{Si}^{2+} \text{O}^{2-} (\text{Zn}^{2+}) \rightarrow \text{Si-O-Si}
\]

In another embodiment of the invention, the glass may be produced with methods known in the state of the art by the melting of the raw material SiO₂ and of carbonates of the alkali or alkaline earth metals Na₂CO₃, K₂CO₃ or CaCO₃.

The reaction may be described as follows:

\[
\begin{align*}
\text{Na}_2\text{CO}_3 + \text{SiO}_2 & \rightarrow \text{Na}_2\text{SiO}_3 + \text{CO}_2 \\
\text{K}_2\text{CO}_3 + \text{SiO}_2 & \rightarrow \text{K}_2\text{SiO}_3 + \text{CO}_2 \\
\text{CaCO}_3 + \text{SiO}_2 & \rightarrow \text{CaSiO}_3 + \text{CO}_2
\end{align*}
\]

However in most cases, there is no pure silicate matrix but a borate- aluminate-silicate-matrix i.e. regarding the network building constituent a part of SiO₂ is substituted by B₂O₃ and Al₂O₃.

The sol:to: pigment ratio has a considerable effect on the yield of magnetic particles provided by this invention. It is essential for the process that the sol can still be pumped and sprayed which is in the skill of artisan.

To create a powder, the slurry is preferably sprayed through a two-fluid nozzle as described in FIG. 1 and in Example 1.3. Suitable spray-drying systems are produced by Nüblerosa Molecularzüsätzung, Lädisch GmbH & Co. KG, Konstanz, Germany, e.g. the “Labor-Zürsätzungstocker (Typ LTK)” or by Büchi A G, Uster, Switzerland, e.g. the Mini Spray Dryer (Type B-191).

Because of the diameter ratios of magnetic cores to the glass shell are less than 1 to 10, preferably between 1:10 and 1:1000, the geometry and the number of incorporated magnetic cores or of their inert carriers do not determine shape and size of the particles but the conditions of manufacturing, in particular the conditions during spray drying. In other words, the choice of pressure, inlet temperature, outlet temperature and flow rate during the spray drying procedure are the degrees of freedom which will determine the size distribution, the shape of the glass drops and thereby will modify the MGPs. When the spray pressure is increased, the size distribution will shift into the sub-μm-range. The decreased temperature of the spray drying process will lead to slower evaporation of the solvent and thereby the form of the MGPs will come closer to an ideal sphere, i.e. the ratio of the radii in x- and z-plane will become approximately 1. The ratio of the radii will vary between 0.8 and 1.2, preferably between 0.9 and 1.1.

In a preferred embodiment of the invention the nozzles are heated. The inlet temperature is between 120° C. and 300° C., preferably between 170° C. and 230° C. or 150° C. and 230° C., most preferably between 150° C. and 200° C. or 190° C. and 210° C. or at 200° C. or slightly less. The outlet temperature depends on the boiling point of the sol and thereby on the solvent and may be above, equal or slightly under, i.e. less than 10° C., the boiling point of the solvent. When ethanol is used as solvent, it is between 50° C. and 300° C., preferably 70° C. and 150° C., most preferably between 80° C. and 110° C. The optimal temperature is between 90° C. to 100° C. The nozzle pressure is more than 3 bar, preferably it is regulated to 4 to 6 bar. The artisan will appreciate the fact that the exact parameters will depend on the spray-drying system used. However, he can transfer the teachings of the present invention to any other spray-drying and find out the parameters by taking the disclosures of this invention into account. Formula as described in Masters: Spray Drying Handbook, Fifth Edition, John Wiley & Sons, 1991, New York can lead him the way to find out which parameters have to be chosen for another setting. Preferably, he will question the manuals of his spray-drying system or contact the technical service of the spray-drying system manufacturer.

To optimize the yield, the densification or sinter temperature should be as high as possible, i.e. slightly below the melting range. If it is too high, however, the particles will stick together and form agglomerates that must be sieved out. If too low, the MGPs will not be optimally densified. Additional treatment of the particles at too high temperature will result in a loss of magnetic properties. Too high temperatures should therefore be omitted. The exact temperatures depend on the glass composition but may be between 400° C. to 1200° C. In the case of the EJ glass composition the sinter temperature is between 720° C. and 770° C., preferably around 750° C. It is in the skill of the artisan to find out the temperatures for each glass composition when taking the teachings of the present invention into account. According to the present invention, the spray-dried MGP powder may be further processed as described in FIG. 2 and as described in Example 1.4. Preferably, the powder is heated for 1 hour to 200° C., optionally cooled to room temperature and heated to 750° C. (densification or sinter temperature) in a nitrogen atmosphere with a heating rate of 1 K/min and is held at that temperature for 1 hour. Then the furnace is cooled to 150° C. and heated again to 200° C. for one hour in air. After the cooling to room temperature, the powder is transferred to a sieve (50 µm) and sieved for 30 min. The sieved sample is bottled and sterilized at 200° C. for 4 h and then cooled to 80° C. Then the glass vessels are taken from the oven, covered with sterile foil and closed.

Surprisingly, the magnetic particles provided by the invention are especially suited for isolating biological materials from samples. In addition, the core material is a natural resource and therefore causes little ecological concern. Moreover, the particles according to the invention are inexpensive and easy to manufacture.

Another object of the invention is a procedure for isolating a biological material by bringing a sample containing the biological material in a liquid in contact with the
magnetic particles according to the invention under conditions in which the biological material binds to the particle surface, and separating the biological material from the liquid. According to the invention the term “in a liquid” means that liquid may be added to the sample before the magnetic particles are added. However, it shall also comprise the situation when the sample itself has a low viscosity and is itself a liquid so that no additional liquid or buffer may have to be added to the sample for the sample preparation which may be done by adding solid agents before the magnetic glass particles are added. The order of reagent addition may be varied according to process requirements.

[0070] Biological materials are understood to mean materials with a particular or a molecular basis. They include, in particular, cells such as viruses or bacteria, as well as isolated cells from multicellular organisms as e.g. human and animal cells such as leukocytes, and immunologically active low and high molecular chemical compounds such as haptens, antigens, antibodies and nucleic acids. Nucleic acids such as DNA or RNA are especially preferred. In one embodiment of the invention mixtures of specific nucleic acids are purified, in which the target nucleic acid(s) may be a minor component in terms of concentration (or may be present in low abundance). According to the present invention, a target nucleic acid shall be the nucleic acid of interest, i.e. a nucleic acid which shall be investigated as its presence is indicative of a certain condition or disease of a human or animal. For example, the presence of a viral sequence (e.g. from Hepatitis B Virus, Hepatitis C Virus or Human immodificity virus) indicates that the respective individual is infected by the specific virus. Then, this viral sequence would be the target sequence. Other target sequences are sequences which are indicative of a predisposition of an individual to a certain disease as e.g. an inherited disease as sickle cell anemia or to certain types of cancer. This examples should be illustrative of the invention but should not be delimiting.

[0071] Samples according to the invention include clinical samples such as blood, serum, oral rinses, urine, cerebral fluid, sputum, stool, biopsy specimens and bone marrow samples. The sample can also be of a type used for environmental analysis, food analysis or molecular biology research, e.g., from bacterial cultures, plas e lysates and products of amplification procedures such as the PCR.

[0072] The procedure described can be used to isolate native or modified biological material. Native biological material is understood to be material, the structure of which was not irreversibly changed compared with the naturally-occurring biological materials. This does not mean that other components of the sample can not be modified, however. Modified biological materials include materials that do not occur in nature, e.g., nucleic acids that are modified by attaching to them groups that are reactive, detectable or capable of immobilization. An example of this is biotinylated nucleic acids. In certain cases the sample can be used without pretreatment in the isolation procedure according to the invention. In many cases, however, the sample should be lysed using an appropriate method, releasing the biological material contained in the sample. Procedures for lysing samples are known by the expert and can be chemical, enzymatic or physical in nature. A combination of these procedures is applicable as well. For instance, lysis can be performed using ultrasound, high pressure, by shear forces, using alkali, detergents or chaotropic saline solutions, or by means of proteinases or lipases. With regard for the lysis procedure to obtain nucleic acids, special reference is made to Sambrook et al.: Molecular Cloning, A Laboratory Manual, 2nd Addition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y. and Ausubel et al.: Current Protocols in Molecular Biology 1987, J. Wiley and Sons, NY.

[0073] In addition to the biological material to be isolated, the sample can also contain other components in a liquid such as cell residue, proteins, salts and other substances that are not to be isolated. This sample, which preferably contains the biological material in native form, is brought in contact with the particles under conditions in which the target biological material binds to the particle surface. The conditions for this depend on the type of biological material involved, but are basically known. They also depend on the method by which the biological material is bound to the surface. If immunological interactions are utilized for the binding, for instance, conditions must be selected that are suitable for the formation of immunocomplexes. If modified nucleic acids are used, the binding can take place via the groups of nucleic acids that represent the modification, e.g., biotin via binding with streptavidin-coated surfaces. With nucleic acids in particular, however, a direct binding of nucleic acids to glass is preferred because among other reasons the nucleic acids do not have to be modified and even native nucleic acids can be bound. The procedure for binding native nucleic acids to glass particles can be analogous to the procedure described in the prior art. It is preferably performed in the presence of chaotropic salts with a concentration of between 2 and 8 mol/l, and preferably between 4 and 6 mol/l. Chaotropic salts can be sodium iodide, sodium perchlorate, guanidium thiocyanate, guanidinium isothiocyanate or guanidinium hydrochloride. Other compounds are also possible.

[0074] To bring the sample in contact with the particles, the sample is mixed with the particles and incubated for a period of time sufficient for the binding to occur. Experts are usually familiar with the duration of the incubation step from procedures for performing treatment with non-magnetic particles. This step can be optimized by determining the quantity of immobilized biological material on the surface at different points in time. Incubation times between 10 seconds and 50 minutes can be appropriate for nucleic acids.

[0075] Depending on the size and type of magnetic particles, the particles either separate out of the fluid during the incubation period itself or the suspension remains intact for a longer period of time. If the particles are very small and unmagnetized, the suspension remains intact for a longer period of time. If the particles are of larger size, the particles slowly separate out of the fluid during the incubation period.

[0076] Immobilization is preferably not performed via precipitation by lowering the solubility of the materials to be immobilized. Rather, immobilization is based on biospecific interactions (capture molecules) or adsorption. This largely prevents contaminants from being non-specifically included.

[0077] After incubation, the biological material is separated from the liquid. This is achieved in general by separating the material bound to the magnetic particles by applying a magnetic field. For instance, the magnetic par-
articles can be pulled to the wall of the vessel in which incubation was performed. The liquid containing the sample contents that were not bound to the magnetic particles can then be removed. The removal procedure used depends on the type of vessel in which incubation was performed. Suitable steps include removing the liquid via pipetting or aspiration.

[0078] The magnetic particles can then be purified one or more times using a wash solution, if desired. A wash solution is used that does not cause the biological material to be released from the particle surface but that washes away the undesired contaminants as thoroughly as possible. This wash step preferably takes place by incubating the wash solution with the particles. The particles are preferable resuspended during this step, e.g., by means of shaking or applying a magnetic field that is not identical to the first magnetic field. The contaminated wash solution is preferably separated just like the sample in the step described above for binding the biological material.

[0079] After the last wash step, the magnetic particles can be dried briefly in a vacuum, or the fluid can be allowed to evaporate. A pretreatment step using acetone may also be performed. If desired, the biological material purified in this manner can be separated from the magnetic particles. This step also depends on the manner in which the biological material was bound to the magnetic particles. If the biological material is native nucleic acids and the magnetic particles are glass-coated particles, the nucleic acids can be removed from the particles according to the invention using an elution buffer having a low salt content. Buffers of this nature are known from DE 3724442 and Analytical Biochemistry 175, 196-201 (1988). The elution buffers with a low salt content are in particular buffers with a content of less than 0.2 mol/l. In an especially preferred embodiment, the elution buffer contains Tris. In another special embodiment, the elution buffer is demineralized water.

[0080] In yet another embodiment, the purification and isolation procedure described is performed after the cells (e.g., viral particles or prokaryotic or eukaryotic cells) are separated immunomagnetically from a bodily fluid or tissue. In this step, the sample is incubated, e.g., while shaking, with magnetic particles to which an antibody against an antigen on the cell is immobilized. These particles can be particles according to the invention or commercially available particles (e.g., MACS Microbeads from Miltenyi Biotech GmbH, Bergisch Gladbach, Germany). After a magnetic field is applied, one or more wash steps are performed using a saline solution. Particles are obtained to which the desired cells are bound. The bound cells are then resuspended in a saline buffer. In a preferred embodiment, this saline buffer is a chonotropic saline solution so that the nucleic acids contained in the cell are released from the cells.

[0081] An especially advantageous procedure for isolating nucleic acids from samples containing cells is achieved by combining the isolation of cells described above with the isolation of nucleic acids also described above, on the magnetic particles according to the invention. The advantage of this embodiment is its potential simplicity (single-tube method), high sensitivity (especially important in medical microbiology and oncology), and the ease with which it can be automated.

[0082] The biological materials isolated using the procedure according to the invention can now be used further as necessary. For instance, they can be used as a substrate for various enzymatic reactions. When nucleic acids are involved, they can be used for sequencing, radioactive or non-radioactive labelling, amplification of one or more of the sequences they contain, transcription, hybridization with labelled probe nucleic acids, translation or ligation. An advantage of the procedure according to the invention is that it is very easy to separate the biological material from the fluid. In the prior art, a centrifugation step was used to separate the glass particles from contaminants, or, when the biological material is bound to glass fiber filters, the fluid is drawn through the filters. This is a limiting step that makes it difficult to process large quantities of sample. The biological material can now be separated effectively using the particles according to the invention. In particular, inhibitors for certain enzymatic reactions can be removed to a large extent according to the invention.

[0083] In the preferred embodiment, the particles according to the invention are added to the lysis mixture. After a suitable period of time for adsorption to take place—which can be optimized by mechanical agitation—the particles are separated from the surrounding fluid that contains additional cell components that are not to be detected.

[0084] This is performed preferably by applying a magnetic field by placing a magnet against the vessel wall.

[0085] To remove any contaminants that may still be present, a wash step is preferably performed with a fluid that does not cause the nucleic acids to be determined to be released from the glass surface. An elution buffer having reagent conditions under which the nucleic acids separate from the glass surface is added to remove the nucleic acids from the glass surface. These conditions are low salt conditions in particular. Depending on the intended further use of the nucleic acids, the fluid can now be separated from the particles and processed further. This separation step is preferably performed via application of a magnetic field so that the particles are separated from the eluate.

[0086] A preferred embodiment of the present invention is to use the MGPs of the present invention in automatable methods as e.g. described in WO 99/16781. Automatable methods means that the steps of the method are suitable to be carried out with an apparatus or machine capable of operating with little or no external control or influence by a human being. Automatable method means that the steps of the method are carried out with an apparatus or machine capable of operating with little or no external control or influence by a human being. Only the preparation steps for the method may have to be done by hand, e.g. the storage containers have to be filled up and put into place, the choice of the samples has to be done by a human being and further steps known to the expert in the field, e.g. the operation of the controlling computer. The apparatus or machine may e.g. add automatically liquids, mix the samples or carry out incubation steps at specific temperatures. Typically, such a machine or apparatus is a robot controlled by a computer which carries out a program in which the single steps and commands are specified. Preferred automatic methods are those embodiments carried out in a high-throughput format which means that the methods and the used machine or apparatus are optimized for a high-throughput of samples in a short time.

[0087] In another embodiment the MGPs according to the present invention are used in semi-automated process which
means that some reaction steps may have to be done manually. In a preferred embodiment of the invention, a suspension containing MGP's according to the present invention is taken from a storage container and partial volumes are added to different reaction vessels. Reaction vessels may be reaction tubes made from plastics eventually in microtitre plate format contain 96 or 384 or more wells where a reaction can be carried out. However, these vessels may be made from other material e.g. from steel.

[0088] A preferred embodiment of the invention are purification methods followed by a detection step or purification methods followed by an amplification and detection step: The target nucleic or nucleic acid or nucleic acids of interest may be contained in a matrix of non-target nucleic acids, and may even be a minor component in said mixture of specific nucleic acids. Suitable DNA detection methods are known to the expert in the field and are described in standard textbooks as Sambrook et al.: Molecular Cloning, A Laboratory Manual, 2nd Addition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y. and Ausubel et al.: Current Protocols in Molecular Biology 1987, J. Wiley and Sons, NY. There may be also further purification steps before the DNA detection step is carried out as e.g. a precipitation step. The detection methods may include but are not limited to the binding or intercalating of specific dyes as ethidiumbromide which intercalates into the double-stranded DNA and changes its fluorescence thereafter. The purified DNA may also be separated by electrophoretic methods optionally after a restriction digest and visualized thereafter. There are also probe-based assays which make use of a oligonucleotide hybridisation to specific sequences and following detection of the hybrid. It is also possible to sequence the DNA after further steps known to the expert in the field. Newer methods apply a diversity of DNA sequences to a silicon chip to which specific probes are bound and yield a signal when a complementary sequences bind.

[0089] Preferred methods according to the invention are amplification methods as the ligase chain reaction and the polymerase chain reaction which specifically amplify target sequences to detectable amounts. Particularly preferred detection methods are the TaqMan® method disclosed in WO92/02638 and the corresponding US patents U.S. Pat. No. 5,210,015, U.S. Pat. No. 5,804,375, U.S. Pat. No. 5,487,972. This method makes use of the exonuclease activity of a polymerase to generate a signal. In detail, the nucleic acid is detected by a process comprising contacting the sample with an oligonucleotide containing a sequence complementary to a region of the target nucleic acid and a labeled oligonucleotide containing a sequence complementary to a second region of the same target nucleic acid sequence strand, but not including the nucleic acid sequence defined by the first oligonucleotide, to create a mixture of duplexes during hybridization conditions, wherein the duplexes comprise the target nucleic acid annealed to the first oligonucleotide and to the labeled oligonucleotide such that the 3'-end of the first oligonucleotide is adjacent to the 5'-end of the labeled oligonucleotide. Then this mixture is treated with a template-dependent nucleic acid polymerase having a 5' to 3' nucleic acid activity under conditions sufficient to extend a nucleic acid from the labeled nucleic acid of the polymerase to cleave the annealed, labeled oligonucleotide and release labeled fragments; and the signal generated by the hydrolysis of the labeled oligonucleotide is detected and/or measured. TaqMan® technology eliminates the need for a solid phase bound reaction complex to be formed and made detectable.

[0090] In more general terms, a procedure for the purification of biological material followed by a detection step is disclosed wherein the amplification and/or detection reaction is a homogeneous solution-phase multiplex assay for the simultaneous detection of multiple targets (see examples 7.2).

[0091] In another embodiment of the invention, the described purification procedure is combined with a amplification procedure using one of the methods described in the following, preferably the use of blocking oligonucleotides. A problem often associated with amplification especially of low amounts of target nucleic acid is the activity of thermostable polymerases at lower temperatures (room temperature up to 40°C). At this temperature primer oligonucleotides often bind unspecifically to each other or to background nucleic acid and may be extended by the polymerase. This entails a decrease of reaction components as well as to a higher level of background signal and consequently to a decreased sensitivity. It may also lead to false-positive results. In order to avoid unspecific activity of polymerases several approaches have been described, like “hot start”-PCR (Chou et al., 1992, Nucl. Acid Res 20, 1717-1723), covalently modified polymerases (e.g. AmpliTaq Gold, Perkin Elmer) or antibodies (Salcie et al., J. Immunol. Methods, 172, 147-163, 1994) and oligonucleotides (Beg and Jayasena, JMB 264, 268-278, 1996; U.S. Pat. No. 5,763,173; U.S. Pat. No. 5,693,302). According to the present invention blocking oligonucleotides shall be oligonucleotides which are able to block the active center of the polymerases up to the said temperature. These oligonucleotides may be e.g. an aptamer as described in Example 7.2.2.1.3. In another embodiment of the invention aptamers (see e.g. in Example 7.2.2.1.3) and/or modified primers alone (see e.g. in Example 7.2.2.1.3) can be used in an amplification reaction and the detection methods connected thereto. This has advantageous effects and can be considered to be an invention on its own which provides superior results. In a preferred embodiment of the invention the 3'-terminal nucleobase, preferably an adenin is modified with a p-(butyl)-benzyl-residue. Further modifications, including those at the 3'-1-position are described in EP 866 071 A2 which is incorporated herein by reference.

[0092] In still another embodiment of the invention for the purification of biological material followed by a detection step is disclosed wherein for at least 5 cycles of the polymerase chain reaction, the annealing temperature is less than 8°C, preferably less than 3°C, above the dissociation temperature of the polymerase-aptamer complex.

[0093] The following examples serve to illustrate the embodiments of the invention.

FIGURE LEGENDS

[0094] FIG. 1: Flow scheme for the production by sol synthesis & spray drying:

[0095] FIG. 2: Flow scheme for the preparation of the raw MGP's

[0096] FIG. 3: Schematic depiction of the spray dryer manufactured by Nubillosa. The details are described in the text under Example 1.3
FIG. 4: High resolution scanning electron microscopic image of MGP with Merck pigment BM

FIG. 5: High resolution scanning electron microscopic image of MGP with CERAC pigment

FIG. 6: High resolution scanning electron microscopic image of MGP with Merck pigment MMB

FIG. 7: High resolution scanning electron microscopic image of MGP with Streum pigment

FIG. 8: High resolution scanning electron microscopic image of MGP with BASF pigment FA

FIG. 9: High resolution scanning electron microscopic image of MGP with BASF pigment CE-HQ

FIG. 10: High resolution scanning electron microscopic image of MGP with BASF pigment CE-SU

FIG. 11: Influence of the magnetic core pigment or the spray dryer on the RNA isolated (spray dryer: Büchi (B) or Nublissos (N))

FIG. 12: Influence of the parameter “Spray pressure” on the isolation of DNA or RNA

FIG. 13: Influence of different MGP production parameters on the isolation of RNA (spray pressure, L=air, N=nitrogen), inlet temperature (E) or outlet temperature (A)

FIGS. 14a. b: Sedimentation behaviour of different MGFs in different suspension media

FIG. 15: HREM-image of sample EJ00965.5R-01; MGP with CERAC pigment

FIG. 16: HREM image of EJ01005.5R-01, sprayed at low pressure and high temperature, consisting mainly of deformed µ-scale particles

EXAMPLE 1

Production of the Magnetic Glass Particles

Classically, raw materials like SiO₂, and alkaline or alkaline earth carbonates (Na₂CO₃, K₂CO₃, CaCO₃) are molten together

Type of reaction:

[0112] Na₂CO₃ + SiO₂ → Na₂SiO₃ (Na₂O·SiO₂) + CO₂↑

[0113] K₂CO₃ + SiO₂ → K₂SiO₃ (K₂O·SiO₂) + CO₂↑

[0114] CaCO₃ + SiO₂ → CaSiO₃ (CaO·SiO₂) + CO₂↑

[0115] In most cases, however, a composite matrix of silicate, borate and aluminate is used, i.e. pertinent to the matrix constituents, SiO₂ is partially replaced by B₂O₃ and Al₂O₃.

[0116] Alternatively, glass can be synthesized via sol-gel reaction.

Type of reaction:

[0117] either acid-catalyzed sol-gel reaction, e.g.

B₄Cl₃ + 3H₂O → B(OH)₃ + 3HCl

Al₂Cl₆ + 3H₂O → Al(OH)₃ + 3HCl

Si₄Cl₆ + 4H₂O → Si(OH)₄ + 4HCl

[0118] Na⁺NO₃⁺ + H₂O → NaOH⁻ + H⁺NO₃⁻

K⁺⁺OOCCH₃ + H₂O → K⁺OH⁻ + H⁺OOCCH₃

or base-catalyzed, as in our case, e.g.

K⁺⁺OCH₂CH₃ + H₂O → K⁺OH⁻ + HOCH₂CH₃

Na⁺⁺OCH₃ + H₂O → Na⁺OH⁻ + HOCH₃

Na⁺⁺OCH₂CH₃ + H₂O → Na⁺OH⁻ + HOCH₂CH₃

Advantage: alcohols are easily evaporated during spray-drying; ideally no recrystallisation of salts on the surface.

Thus, alcoholates are turned into hydroxides, which yield by way of elimination of water the corresponding oxides. These, then, form a 3-dimensional, amorphous glass matrix consisting of SiO₂/B₂O₃/Al₂O₃, into which certain metal oxide ingredients are embedded as matrix bond separators, e.g.

[0129] For the experiments described here, the glass component was produced via base-catalyzed sol-gel synthesis. The glass composition used in all experiments was (unless stated otherwise) as follows:

70.67 Mol % SiO₂, 14.33 Mol % B₂O₃, 5.00 Mol % Al₂O₃, 4.00 Mol % K₂O, 2.00 Mol % CaO, 4.00 Mol % ZnO

(calculated from the mass of particular educts brought to reaction)

[0132] 1.1 Description of the Investigated Magnetic Pigments

[0133] Different types of magnetic pigments were investigated and are summarized in Table 1.

[0134] 1.2 Production of the Coating Sol (EJ-Composition)

[0135] The educts are added in the following order and amount into the heatable stirring vessel:

Tetraethoxysilane (TEOS) 10700 ml
Triethoxyborane (TEB) 3305 ml
K-methanolate 25% (W/V) in Methanol 1601 ml
Ethanol 11292 ml
Aluminiumisopropanolate 1385 g
Calcium 54.4 g
Zincacetate 498 g

[0136] The vessel is closed thereafter. The sol is heated to 70° C. and stirred overnight (15 h). The temperature is regulated via a thermosensor which dips into the liquid.

[0137] Then the sol is heated to 90° C. and the alcohol/water mixture (Ethanol: 3781 mL, H₂O: 1512 mL) is added at a rate of 5000 mL/h. The vessel is cooled to 20° C. after the complete addition of the said mixture.

[0138] The lid is opened and 10249 g magnetic pigment (CERAC) is added under vigorous stirring. The prepared sol is transferred into the spray dryer via a hose.

[0139] 1.3 Spray Drying

[0140] The inlet temperature of the spray dryer is regulated to 200° C. The nozzle pressure is regulated to 6 bar and the nozzle is cooled for 3 min with ethanol. Then the hose is connected to the outlet of the glass container and the pigment-containing sol is pumped at a rate of 110 mL/min to the two fluid nozzle (diameter of the opening: 2 mm; supplier: Nubilosa, Type 1B1VV1S1) of the spray dryer via a ultrasound device (200 W). The spray drying system is shown schematically in FIG. 3. The particles which are formed in the first two minutes are discarded. After the complete spraying of the sol the container under the cyclone (AVO, see FIG. 3) with the particles will be taken and the particles will be further treated as described in the next steps.

[0141] The pigment containing sol is stirred in the stirring vessel to prevent sedimentation of the suspended particles. The sol is transferred from the vessel to the nozzle using a pump (SP). Nitrogen heated by an electric heater (EWI) is used as drying gas. The coated pigment is transferred from the drying chamber (I) to the cyclone (ZY). The dried powder can be removed from the container (AVO) under the cyclone. Very fine particles are removed by a filter (SF) from the nitrogen. The spray dryer is used with overpressure to prevent an air intake into the dryer. The gas flow is produced by gas blower (AV).

[0142] 1.4 Further Processing of the Spray-Dried Powder

[0143] The powder is transferred into a ceramics bowl and heated in an furnace to 200° C. at a heating rate of 1 K/min. Then temperature is held at 200° C. for one hour and the oven is cooled to room temperature afterwards. The bowl is transferred to an atmosphere furnace with a volume of 27 l which is rinsed with 60 l nitrogen per hour. The furnace is heated to 750° C. with a heating rate of 1 K/min and is held at that temperature for 1 hour. Then the furnace is cooled to 150° C. and rinsed with 60 l air per hour. The furnace is heated to 200° C. with a heating rate of 2 K/min and is held at that temperature for 1 hour and is cooled to temperature afterwards. The powder is transferred to a sieve (50 μm) and sieved for 30 min. Afterwards, the sieved sample is bottled in glass containers, which are sterilized unclosed in an oven which is heated to 200° C. with a heating rate of 1 K/min, held at that temperature for 4 h and then cooled to 80° C. Then the glass vessels are taken from the furnace, covered with sterile foil and closed with a lid.

[0144] A summary of the important process parameters is presented in Table 2 and Table 3, the produced individual samples with some data are provided in Table 3. Each sample has a specific code. The first two letters describe the used glass chemistry (see below) and the next four numbers encode the production process (see Table 2). The number after the describes the used pigment (see Table 1). The letter R means that the fine content has not been removed, whereas E means that the fine content has been removed with ethanol. The last two numbers describe the number of the lot. The composition of the glass (code EJ) as calculated from the quantity of the educts was 70.67 Mol % SiO₂, 14.33 Mol % B₂O₃, 5.00 Mol % Al₂O₃, 4.00 Mol % K₂O, 2.00 Mol % CaO, 4.00 Mol % ZnO. The composition of the glass (code RN) was 74 Mol % SiO₂, 15 Mol % B₂O₃, 5.00 Mol % Al₂O₃, 4.00 Mol % K₂O, 2.00 Mol % CaO. The composition of the glass (code EP) was 73.61 Mol % SiO₂, 14.93 Mol % B₂O₃, 5.21 Mol % Al₂O₃, 4.17 Mol % K₂O, 2.08 Mol % CaO.

2 EXAMPLE 2

High Resolution Scanning Electron Microscopy

[0145] To obtain information about the surface, size and form of the MGP, we carried out investigations with a high resolution scanning electron microscope manufactured by the company JEOL (JSM). The samples were spread onto the sample holder with an electrically conducting double-sided adhesive and sputtered with gold for 36 s with a current of 30 mA. For the imaging of the surface, the emitted secondary electrons (topography) as well as the back-scattered electrons (order number contrast) were viewed. The used primary electron voltage was 10 kV (secondary electrons) or 25 kV (back-scattered electrons).

[0146] The FIG. 4 shows MGPs with mica covered with ferric oxide as magnet pigment (HM). The fissure in the glass shell can be clearly seen which stem from the spray drying of the particles when the layer starts to shrink on a non-shrinking substrate (drying fissures). Further more a greater number of spherical particles can be seen which do not contain one of the bigger magnet particles (10-60 μm). These spheres without magnetic core (non-magnetic fine content) cannot be removed in a magnetic field, may be transferred into the PCR reaction and will interfere with this reaction.

[0147] The particles with CERAC pigment (see FIG. 5) do not have fissure, as the magnet pigment (23 nm average particle size) does not cause tension forces in the layer so that no fissures are created during the spray drying process. Furthermore, the small CERAC-particles are also incorporated in the fine content so that there are no non-magnetic particles contained therein. The particles are also substantially smaller than the particles with mica which can be observed in these figures with the same magnification. MGPs with the other magnetic pigments are shown at the same magnification in FIG. 6 to FIG. 10.

3 EXAMPLE 3

Physical Investigations of the MGPs

[0148] To allow an evaluation of the MGPs before the actual functional tests, in addition the HiREM investigations further physical measurements were done with the MGPs.
was interesting to learn about the iron solubility in water, the magnetic force, the fine content and the density. In the following the experiments are described and the results presented.

[0149] 3.1 Extinction Measurements

[0150] To test whether the fines have been removed, 1000 mg of the temperature-treated and sieved powder, are weighed into a 50 ml centrifugation tube (Sarstedt), 40 ml water added and dispersed by shaking. Then, the tube is dipped into an ultrasonic bath (Sonorex (RK 102H; 120/240W) with the total filling height and is put into a magnetic separator (Roche Diagnostics GmbH RD Art.Nr. 1858 025). After 3.5 min magnetic separation, liquid is taken from the vessel with a pasteur glass pipette in the height of the 15 ml mark at the opposite side of the magnet and filled into a 5 mm quartz cuvette (Type 110-QS, Hellma). The extinction of the supernatant is measured in an UV-VIS-NIR spectrometer (Hitachi U-3000) against a corresponding cuvette filled with deionized water. The measuring range was 200 to 1100 nm in steps of 1 nm to investigate this wavelength range for the absorption bands of eventual impurities. The extinction at a wavelength of 280 m and 400 nm was taken as a reference.

[0151] 3.2 Density Measurements

[0152] For the density measurements a gas pyknometer (AccuPyc 1330, Fa. Micromeritics) is used. Helium 6.0 is used as gas. The device is calibrated with the supplied standard (steel beads with known volume). Further, the sample is dried for at least 1 hour at 150°C and then filled into the measurement container and weighed. After the measuring container is put into the gas pyknometer, 10 washing cycles and 5 measuring cycles are used to determine the average value and the standard deviation.

[0153] 3.3 Determination of the Iron Solubility

[0154] The iron solubility of the coated and temperature-treated samples is determined with Inductive Coupled Plasma—Atomic Emission Spectroscopy (IUPAC, company ISA). Therefor, 1 g of the sample is transferred into a 50 ml polypropylene-tube, filled up with distilled water and kept at a temperature of 60°C for 20 hours. Then, the samples are filtrated with a 0.2 µm syringe filter and the filtrate is measured. Four single determinations are carried out at a wavelength of 259,940 nm and an average value is computed therefrom.

[0155] 3.4 Measurement of the Magnetic Force

[0156] For the measurement of the magnetic force a PP-weighing tube (Company Licefa, Art. Nr. V2-3) completely filled with MSGs is weighed. With the help of a stencil, this sample container is placed in the middle of LDPE tube (Company Kartell, TS 735) burdened with brass so that the lid of the tube can be still closed. The vessel is positioned in the middle of a balance (detection accuracy 0.1 g) with the help of another stencil. After the equalization of the balance, a plastic cap which contains an cylinder-shaped magnet (diameter: 30 mm; height: 113.5 mm; material: samarium-cobalt 2/17) is positioned above the scale.

[0157] Thereby, the MSGs in the scale are attracted against to the gravity thereby decreasing their weight. After the adjustment of the equilibrium (1.5 min) the weight loss of the sample is determined and the value is normalized for 250 mg MGP.

[0158] 3.5 Results

[0159] The results of the physical characterization of the MSGs with EJ composition and different pigments are summarized in Table 3. It is noticeable that the extinction values are very low when CERAC pigment is used. This can be explained by the lack of non-magnetic fine content because the small CERAC pigment (23 nm) can be incorporated into each magnetic glass particle. The density of the CERAC-MGPs is not very high. This has a positive influence on the sedimentation velocity. The magnetic force is subjected to high fluctuations, however, it is positively influenced by the use of CERAC-pigments. The CERAC-MGPs have a comparably high leak of iron to the water. However, even ten-fold higher values did not show any effect on the PCR process.

[0160] In summary, it can be said that the physical properties do not have any significant differences between the different pigments but the CERAC-MGPs have a very low fine content.

4 EXAMPLE 4

Influence of the Magnetic Core Pigment and of the Spray Dryer on the BET Surface

[0161] The surfaces were determined using a device from Micromeritics (Type ASAP 2400). The measurement was performed at liquid nitrogen temperature. Nitrogen 5.0 was used as measuring gas and Helium 4.6 was used as inert gas. Typically, 5 g of sample was used for one measurement.

[0162] The surface of the MGPs plays an obvious role for the isolation of DNA and RNA. The higher the surface the more DNA can be bound by the same mass of MGPs. It is also possible to use less MGPs and obtain the same performance. This has the effect that the intergrain volume is less which means that less alcohol may be introduced as contaminant into the PCR reaction. The data for the measured BET-surfaces for some samples are summarized in Table 4. When the surfaces of the samples produced on the big spray dryer are compared, it should be noted that the samples with small pigments (BASF FA, STREM and CERAC) have relatively high surfaces, whereas the big Merck pigments have small surfaces. This may be caused by the fact that big magnetic particles cause big MGPs whereas the small magnetic particles are incorporated into the spherical droplets during the spray drying process and have therefore similar surfaces under similar spraying conditions. The BASF CE-SU particles have a size distribution in between the small (BASF FA, STREM and CERAC) and the big Merck pigments. In contrast to the Merck particles, they do not possess a structured surface so that these particles have a very smooth glass surface. This results into a smaller surface. A higher pressure was used with the smaller spray dryer and the nozzle of this spray dryer is remarkably smaller than that of the bigger spray dryer. This causes smaller particles whereby the surface increases remarkably. This is in good agreement with the results of Example 5.2.1

[0163] Any influence of the magnetic pigment or the spraying pressure on the results of further physical investigations cannot be recognized except the above-mentioned influence on the fissure formation and the fine content. However, only the measurements of the surface show a
direct connection to the results of the functional investigations. Other physical investigation results do not show this direct correlation.

5 EXAMPLE 5

Binding Studies via Radio Tracing

[0164] There are several methods for the evaluation of MGP5s with regard to their suitability for the extraction of nucleic acids. The determination of the extinction at 260 nm before and after the purification is not very sensitive and does not resemble the situation when small amounts of target nucleic acid are extracted from clinical material. The results of functional evaluation methods which rely upon a method for the amplification of nucleic acids, as, e.g., by PCR, RT-PCR, NASBA or anything similar, are often not enough convincing. Furthermore, these methods, which are suitable for the determination of a small number of copies of the target genome, are susceptible to disturbances by substances from the sample material or from the sample preparation process. Radioactive binding studies are suitable a analytical method which makes it possible to analyze the sample preparation process step by step. The performance data are no absolute data but are always relative to the performance of a reference particle

[0165] 5.1 Experimental Protocol

[0166] At first, radioactively labelled DNA or RNA is synthesized enzymatically in a PCR or in vitro transcription process in the presence of 32P-marked deoxynucleoside or nucleoside-triphosphates. Then, the marked DNA or RNA is separated from the free nucleoside-triphosphates, the content determined and a defined dilution is prepared.

[0167] A small amount of marked DNA or RNA is added to each sample before the examination. During sample preparation, all nucleic acids bind to the MGP5s in the presence of chaotropic agents. The MGP5s are pelleted by the exertion of magnetic forces and the supernatants can be discarded. The pellet is washed and the bound nucleic acids are eluted at elevated temperature by the reversal of the reaction conditions, i.e. by the addition of a low salt elution buffer. After the binding and an optionally after the washing step, an aliquot of the particle supernatant is spotted onto a filter. The eluate as well as the in water dispersed MGP5 are spotted onto a filter as well and dried thereafter. At last, the filters are measured in a Beta.Counter and for each sample preparation a distribution is calculated.

[0168] 5.1.1 Preparation of Radioactively labelled DNA

[0169] 5.1.1.1 A.1.1. Reagents

[0170] Expand High Fidelity PCR System (Roche Molecular Biochemicals Cat. No. 732641)

[0171] dNTP Mix (Roche Molecular Biochemicals Cat. No. 1277049)

[0172] Deoxycytidine 5'-alpha-P32 Triphosphate dCTP 3000 Ci/mmol (Amersham Cat. No. PB 10205)

[0173] lambda-DNA (Roche Cat. No. 1029053) concentration 1 mg/ml

[0174] Radiotracer primer 1 (SEQ ID NO 1) concentration 5.3 OD260/ml

[0175] Radiotracer primer 2 (SEQ ID NO 2) concentration 5.2 OD260/ml

[0176] QIA Quick PCR Purification Kit (Qiagen Cat. No. 28104)

[0177] 5.1.1.2 Reaction

[0178] 29.51 μl double distilled water

[0179] 5 μl Expand High Fidelity Buffer

[0180] 2.5 μl dNTP Mix (1:10) diluted with double distilled water

[0181] 1 μl Radiotracer primer 1 (SEQ ID NO 1)

[0182] 1 μl Radiotracer primer 2 (SEQ ID NO 2)

[0183] 0.3 μl 32P-dCTP

[0184] 10 μl lambda-DNA

[0185] 0.75 μl Expand High Fidelity Enzyme Mix

[0186] 5.1.1.3 Amplification

[0187] 2 min. 94°C.

[0188] 10 cycles (10 sec. 94°C/30 sec. 60°C/60 sec. 72°C C.)

[0189] 20 cycles (10 sec. 94°C/30 sec. 60°C/60 sec. 72°C C.+10 sec. 72°C extension per cycle)

[0190] 7 min 72°C.

[0191] 4°C.

[0192] 5.1.1.4 Purification

[0193] according to the QIA Quick PCR Purification Protocol (Qiagen)

[0194] 5.1.1.5 Dilution

[0195] Dilution of DNA 1:10 in double distilled water and measurement in the Beta-Counter

[0196] 5.1.2 A.2. Preparation of Radioactively Labelled RNA

[0197] 5.1.2.1—Reagents

[0198] SP6/T7 Transcription Kit (Roche Molecular Biochemicals Cat. No. 999644)

[0199] Uridine 5’-alpha-P32 Triphosphate UTP 3000 Ci/mmol (Amersham Cat. No. PB 10203)

[0200] Plasmid pBKHB10S, linearized with EcoRI at 100 μg/ml

[0201] High Pure RNA Isolation Kit (Roche Molecular Biochemicals Cat. No. 1826665)

[0202] 5.1.2.2—Reaction

[0203] 2 μl 10× buffer

[0204] 3 μl NTP buffer (AGC)

[0205] 1 μl UTP (1:50 with double distilled water)

[0206] 5 μl 32P-UTP

[0207] 7 μl linearized plasmid
[0208] 1 μl RNAse Inhibitor (Roche Molecular Biochemicals Cat. No. 802808)
[0209] 1 nT7 RNA-Polymerase
[0210] 5.1.2.3 Transcription and DNase-Digest
[0211] 20 min incubation at 37° C.
[0212] Addition of 2 μl DNase, RNase free
[0213] 10 min incubation at 37° C.
[0214] Addition of 178 μl double distilled water
[0215] 5.1.2.4—Purification
[0216] according to the High Pure RNA Isolation Protocol (Roche Molecular Biochemicals)
[0217] 5.1.2.5—Dilution:
[0218] Dilution of RNA 1:30 in double distilled water and measurement at the Beta-Counter
[0219] 5.1.3—Radioactive Sample Preparation
[0220] 5.1.3.1—Reagents
[0221] negative plasma
[0222] Proteinase K concentration 20 mg/ml (e.g. Roche Id.Nr. 1942387)
[0223] Poly-A-RNA (e.g. Roche Id.Nr. 108626) concentration 1 mg/ml; diluted 1:1000 (volume/volume ratio) with lysis buffer
[0224] lysis buffer (50 mM Tris pH 7.0, 15% (v/v) Polydocanol, SM Guanidiniumisothiocyanate, 1 mM DTT)
[0225] MGP (with glass composition EJ and different core pigments (BM, MMB, CERAC, STREM, BASF-FA, BASF-CE)) suspended in isopropanol at 60 mg/ml or at 6 mg/ml
[0226] Washing buffer (20 mM Tris pH 7.5, 20 mM NaCl, 70% (v/v) Ethanol)
[0227] Elution solution: double distilled water
[0228] Auxiliary material: Whatman GF/D Filter
[0229] 5.1.3.2—Reaction (1.5 ml protocol)
[0230] 80 μl Proteinase K
[0231] add 410 μl negative plasma and mix
[0232] add 500 μl lysis buffer and mix
[0233] add 10 μl radioactive labelled DNA or RNA and mix
[0234] 10 min incubation at room temperature under shaking
[0235] add 500 μl MGP suspension (concentration 6 mg/ml) and mix
[0236] 20 min. incubation at room temperature under shaking
[0237] separation for 2 min in Magnetic separator (Dynal)
[0238] discard supernatant and spot 300 μl onto a filter
[0239] add 750 μl wash buffer, vortex, 2 min separation
[0240] discard supernatant, eventually spot 375 μl supernatant onto a filter
[0241] repeat washing procedure twice
[0242] add 100 μl elution solution, incubate 5 min. at 80° C. in a thermostirer
[0243] separate 2 min in a magnet holder, spot supernatant onto a filter
[0244] add 100 μl elution solution, resuspend and spot MGP's onto a filter
[0245] dry filters for 60 min at 75° C. in a drying oven
[0246] transfer filter into scintillation tubes, add 5 ml scintillation solution and measure in the beta-counter
[0247] 5.1.3.3—Experimental Protocol (1 ml protocol)
[0248] 25 μl Proteinase K
[0249] add 415 μl negative plasma and mix
[0250] add 500 μl lysis buffer and mix
[0251] add 10 μl radioactive labelled DNA or RNA and mix
[0252] 5 min incubation at room temperature under shaking
[0253] add 50 μl MGP suspension (concentration 60 mg/ml) and mix
[0254] 20 min. incubation at room temperature under shaking
[0255] separation for 2 min in the magnetic separator (Dynal)
[0256] discard supernatant and spot 300 μl onto a filter
[0257] add 700 μl wash buffer, vortex, 2 min separation
[0258] discard supernatant, eventually spot 350 μl supernatant onto a filter
[0259] repeat washing procedure twice
[0260] add 120 μl elution solution, incubate 10 min. at 80° C. in a thermostirer
[0261] separate 2 min in a magnet holder, spot supernatant onto a filter
[0262] add 100 μl elution solution, resuspend and spot MGP's onto a filter
[0263] dry filters for 60 min at 75° C. in a drying oven
[0264] transfer filter into scintillation tubes, add 5 ml scintillation solution and measure in the beta-counter
5.2 Results of the Radiotracing Experiments:

5.2.1 Influence of the Magnetic Core Pigment or the Spray Dryer on the RNA Isolation

Different MGP-types, which were produced using Li glass chemistry and various cores in the nano- or micrometerange (MMB, CERAC, etc.) on different spray dryers (Büchi or Nubilosa), were characterized with regard to their behaviour at the nucleic acid extraction from virus-negative pool material with the radiotracing method according to Example 5.1.3.2. The RNA parameter proved to be the most sensitive in the course of the studies and was therefore chosen as parameter. FIG. 11 shows BASF-CE is not suitable as core material because only small amounts of the bound RNA can be found in the eluate. The EJ/CERAC particles which were sprayed in the Büchi system at 6 bar showed the performance of the reference EJ/MBB which were sprayed in the Nubilosa system.

5.2.2 Influence of the Parameter Spray Pressure on the Isolation of DNA or RNA

Different MGP types produced with the Nubilosa system with different spraying pressures are compared according to 5.1.3.3. The reference particles are EJ/MBB MGP. The DNA and RNA binding properties are investigated. Whereas the DNA parameter shows no dependence from the spraying pressure, the RNA parameter shows significant performance differences. The performance of the EJ/CERAC particles produced with 1.5 bar spraying pressure with the Nubilosa system is lower than that of the reference. There is a lower performance in the series in dependence of the spraying pressure which varied from 1.5 to 3.4 bar (30%). Particles which are sprayed with 4.3 bar reached 90% of the performance of the particles which are sprayed at 1.5 bar (see FIG. 12).

The experiments prove the direct connection between the process parameters for the production of the MGP and of the performance in the test. It should be noted that an increase in the spraying pressure leads to a lower test performance but, beginning from a certain spraying pressure, the particle properties are improved leading to a better performance.

5.2.3 Influence of Different MGP Production Parameters on the Isolation of RNA and DNA

These experiments should lead to the result whether a variation of the spraying pressure leads to MGP with even better performance.

Therefor, MGP are produced on the Nubilosa system at a pressure of 1.5 bar, 4.3 bar and 6 bar using nitrogen as spraying gas.

In order to obtain a careful drying process, inlet and outlet temperature of the spray dryer are additionally decreased. The influence of these factors on the RNA isolation is investigated according to 5.1.3.3. using EJ/MBB MGP as reference.

The results (see FIG. 13) show the surprising effect of a dramatic performance reduction which is caused by the decrease of the spraying temperature and could not be foreseen. It was possible to reach the performance of the reference quality by the increase of the spraying pressure. Concomitant with the superior suspension stability, these particles are therefore superior to the reference.

6 EXAMPLE 6

Sedimentation Analytics of the Magnetic Glass Particles

6.1 Experimental Protocol

An Uvikon Spectrophotometer Model 930 produced by Kontron Instruments is used for the evaluation of the sedimentation behaviour of the magnetic glass particles. This spectrophotometer is modified to allow variable adjustment of the cuvette along a scale bar. For the measurements the cuvette is set at a position in which the measuring beam with a wavelength of 650 nm traverses the cuvette at a position which is 1/2 of the filling height (scale position 7.5). Macro cuvettes made from polystyrene are used which have a volume of 4 ml and a path length of 1 cm. Prior to measurements, calibration is made in a one beam procedure against pure suspension medium. The MGP samples are dispersed to homogeneity in suspension medium, typically at a mass/volume ratio of 3 mg/ml, and measured immediately. The change of the extinction with time is monitored continuously at the said wavelength of 650 nm.

The physical quantity used for the comparison of the sedimentation velocity of different samples is the half life \( t_{1/2} \) in a particular suspension medium. This is the period of time until the extinction of the suspension in the upper third of the cuvette is half the value at the beginning of the measurement. The decline of extinction is caused by the sedimentation of the particles and the concomitant clearance of the upper third of test volume.

The above-described device further allows the installation of a magnet beneath the cuvette. Thereby the velocity of the magnetic separation can be determined.

6.2 Results of the Sedimentation Analytics

6.2.1 Experiment 1:

Several MGP-types with different cores in the range of nano- or micrometers and produced with EJ glass chemistry are investigated spectrophotometrically for their sedimentation and separation behaviour, i.e. with and without magnet under the cuvette. Mass/volume is 6 mg/ml in this case. The suspension medium is a 1:1 mixture of isopropanol and lysis buffer. The results are summarized in SEQARABIC. The particles from the CERAC type show a clear advantage in the stability of the suspension but no disadvantages for the magnetic separation. (See also FIG. 14).

6.2.2 Experiment 2:

The sedimentation behaviour of different MGP from the type CERAC produced with EJ glass chemistry are investigated in pure isopropanol.

Important Production Parameters

EJ0100.5R-01-1.5 bar spraying pressure, 230° C. inlet temperature, Nubilosa system

EJ0108.5R-01-4.3 bar spraying pressure, 230° C. inlet temperature, Nubilosa system
7 EXAMPLE 7

Functional Test with PCR

7.1 Heterogeneous Functional Assays: Amplification with PCR on a Perkin Elmer GeneAmp 9600BD and Detection with a Biospecific Binding Test with a Chemiluminescent-Marked Detection Probe on a Modified Elesys1010®

7.1.1 General Considerations

Virus particles in the sample (e.g. plasma) are lysed in the presence of protease and high concentrations of chaotropic salt such as well as detergent. Then, the particulate adsorbent (=MGP) is added for physico-chemical adsorption of released nucleic acids onto the glass surface, followed by magnetic separation and washing of the loaded beads, i.e. bound/free separation. Finally, dissociation of bound nucleic acids from the beads (=elution) is conducted under reversed reaction conditions relative to adsorption, i.e. with low salt buffer or even distilled water. An aliquot of eluate is then mixed with an aliquot of PCR mastermix to start amplification of the nucleic acids recovered in the eluate. This reaction is characterized by the equation

\[ N_f = N_0 \times (1+E)^n \],

with

- \( N_f \): number of molecules at the end of the polymerase chain reaction
- \( N_0 \): number of molecules at the start of the polymerase chain reaction
- \( E \): efficiency of amplification
- \( n \): number of reaction cycles (typically 20–35)

After PCR with at least one biotinylated primer, an aliquot of biotin-tagged amplification is mixed with hybridization buffer and detection probe. After incubation, streptavidin-coated beads are added, followed by another incubation. Finally, the beads are washed, signal buffer is added and the chemiluminescent signal intensity measured, which is correlated with the mass of amplified nucleic acid bound and, thus, the viral load of the plasma sample.

7.1.2 Experimental Protocol

7.1.2.1 Reagents

- Proteinase K, liquid in glycerol/Ca-acetate, 20 mg/ml
- Poly-A-RNA, 1 mg/ml, use level is a 1:1000 dilution in lysis buffer
- lysis buffer, consisting of:
  - Tris-buffer, 50 mmol/l, pH 7.0 (1.0 & extractor protocol, respectively) or 4.0 (1.5 ml protocol)
  - Polydocanol, 15% (v/v) (1.0 & extractor protocol, respectively) or Triton X-100 20% (v/v) (1.5 ml protocol)
- Guanidinium-isothiocyanate, 5 mol/l
- 1 mmol/l DTT
- Magnetic glass particles (MGP) either of the type EJ/BM, EJ/MMB or EJ/CERAC suspended in isopropanol (99.8% purity) at 60 mg/ml
- wash buffer consisting of
  - Tris-Puffer, 20 mmol/l, pH 7.5
  - 60% Ethanol/aq. (1.0 & extractor protocol, respectively) or 70% (1.5 ml protocol)
- NaCl, 20 mmol/l
- Eluent=double distilled water
- Amplification/Mastermix:
  - PCR-buffer, consisting of
  - buffer medium
  - RT-PCR (Human immunodeficiency virus (HIV), Hepatitis C Virus (HCV)): 250 mmol/l Bicine/KOH pH 8.2, 557 mmol/l K-Acetat, 40% Glyceral
  - HBV-PCR (Hepatitis B virus (HBV)): 20 mmol/l Tris-HCl pH 8.3, 100 mmol/l KCl, 0.012% Brij 35 (=2×-concentrate)
  - Metal cations, MgCl₂, (HBV-PCR) 3 mmol/l or MnCl₂ (RT-PCR) 2.5 mmol/l (HCV) and 1.25 mmol/l (HIV)
  - Deoxyribonucleoside-triphosphate (dATP, dGTP, dCTP, dTTP, dUTP)
  - Analyte-specific forward-primer
  - Analyte-specific reverse-primer
  - Uracil-N-glycosidase (UNG)
  - DNA-polymerase (Taq- or Tth-polymerase for HIV or HCV/HCV, respectively)
  - H₂O-de-mineralised (molecular biology grade) for volume adjustment ad 100 µl
- Stop-Reagent for UNG After the Amplification

7.1.2.2 Detection

- Hybridisation buffer (e.g. Roche Id.Nr. 1930273)
- chemiluminescence-marked detection probes
[0332] HCV (Roche BMO 28.140336), working stock= 8.0 mmol/l

[0333] HIV (Roche BMO 28.540948), working stock= 7.8 mmol/l

[0334] HBV (Roche BMO 28.540917), working stock= 9.2 mmol/l

[0335] SA-covered ECL-Beads (e.g. Roche Id.Nr. 186594-301)

[0336] denaturation solution (e.g. Roche Id.Nr. 1930257)

[0337] ProCell (e.g. Roche Id.Nr. 1717685)

[0338] CleanCell (e.g. Roche Id.Nr. 1717642)

[0339] 7.1.2.1.3 Primers and Probes

[0340] Primer:

<table>
<thead>
<tr>
<th>Primer</th>
<th>SEQ ID No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-forward</td>
<td>3</td>
</tr>
<tr>
<td>HIV-reverse</td>
<td>4</td>
</tr>
<tr>
<td>HCV-forward</td>
<td>5</td>
</tr>
<tr>
<td>HCV-reverse</td>
<td>6</td>
</tr>
<tr>
<td>HBV-forward</td>
<td>7</td>
</tr>
<tr>
<td>HBV-reverse</td>
<td>8</td>
</tr>
<tr>
<td>Detection probes</td>
<td>9</td>
</tr>
<tr>
<td>HIV:</td>
<td>10</td>
</tr>
<tr>
<td>HCV:</td>
<td>11</td>
</tr>
</tbody>
</table>

[0341] 7.1.2.2 Reaction Conditions & Test Procedure

[0342] Sample Preparation:

[0343] 1.0 ml Manual Protocol; => Used for Experiment 1 See Example 7.3.1

[0344] add 25 µl of proteinase K to 425 µl of plasma sample, vortex,

[0345] add 500 µl of lysis buffer, incubate 5 min at room temperature with mechanical agitation (1300 rpm in Eppendorf mixer),

[0346] add 50 µl of MGP suspended in isopropanol (concentration 60 mg/ml), vortex, and incubate 20 min at room temperature on a roller mixer,

[0347] magnetic separation using a Dynal magnetic separator (2 min);

[0348] remove unbound fraction through aspiration, and add 700 µl wash buffer, vortex, separate, aspirate;

[0349] repeat wash procedure another 4 times,

[0350] add 120 µl of DEPC-water, vortex, and elute 10 min at 80ºC in an Eppendorf Thermomixer at 1300 rpm with cups uncapped,

[0351] separate beads from aqueous supernatant (=eluate) with Dynal magnetic separator (2 min),

[0352] freeze eluate until further use.

[0353] Automated Protocol on In-House-Built Extractor; => Used for Experiment 2 and 3 (See Example 7.3.2 and Example 7.3.3)

[0354] Basically the same procedure and reagent set as above. Additionally, an amoured RNA internal control (IC, see below) is spiked into each and every sample, the extraction process is thermostated at ca. 40ºC, and volumes are adjusted to 2 ml total volume of lysis mix, i.e.

[0355] 50 µl IC

[0356] 50 µl proteinase K

[0357] 850 µl sample

[0358] 1000 µl lysis buffer

[0359] 100 µl MGP suspension

[0360] 2200 µl wash buffer for 5 wash steps each

[0361] 125 µl eluent

[0362] 1.5 ml Manual Protocol; => Used for Experiment 4 (See Example 7.3.4)

[0363] add 80 µl of proteinase K to 420 µl of plasma sample, vortex,

[0364] add 500 µl of lysis buffer, incubate 10 min at room temperature with mechanical agitation (1300 rpm in Eppendorf mixer),

[0365] add 500 µl of MGP suspended in isopropanol (concentration 60 mg/ml), vortex, and incubate 20 min at room temperature on a roller mixer,

[0366] magnetic separation using a Dynal magnetic separator (2 min);

[0367] remove unbound fraction through aspiration, and add 700 µl wash buffer, vortex, separate, aspirate;

[0368] repeat wash procedure another 4 times,

[0369] add 100 µl of DEPC-water, close the cups, vortex, and elute 15 min at 80ºC in an Eppendorf Thermomixer at 1300 rpm,

[0370] separate beads from aqueous supernatant (=eluate) with Dynal magnetic separator (2 min),

[0371] freeze eluate until further use.

amplification

<table>
<thead>
<tr>
<th>DNA-Mastemix</th>
<th>2×</th>
<th>1×-5×</th>
<th>0.05 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂</td>
<td>25 mM</td>
<td>1300 rpm</td>
<td></td>
</tr>
<tr>
<td>Primer SEQ ID NO 7</td>
<td>5 µM</td>
<td>0.2 µM/µl</td>
<td></td>
</tr>
<tr>
<td>Primer SEQ ID NO 8</td>
<td>5 µM</td>
<td>0.4 µM/µl</td>
<td></td>
</tr>
<tr>
<td>UNG</td>
<td>1 U/µl</td>
<td>2 U/µl</td>
<td></td>
</tr>
<tr>
<td>HCV:</td>
<td>11</td>
<td>50 µl</td>
<td></td>
</tr>
</tbody>
</table>

[0354] end concentration in PCR/µl

<table>
<thead>
<tr>
<th>Amplification stock solution</th>
<th>end concentration in PCR/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (PCR-Grade)</td>
<td>4 µl</td>
</tr>
<tr>
<td>DNA-Mastemix</td>
<td>2×</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>25 mM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 7</td>
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</tr>
<tr>
<td>Primer SEQ ID NO 8</td>
<td>5 µM</td>
</tr>
<tr>
<td>UNG</td>
<td>1 U/µl</td>
</tr>
<tr>
<td>HCV:</td>
<td>11</td>
</tr>
</tbody>
</table>

[0354] HCV: eluate volume for PCR = 20 µl
amplification

total reaction volume = 100 µl for all assays

stock solution end concentration in PCR/µl

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV:</strong></td>
<td></td>
</tr>
<tr>
<td>dilute volume for PCR</td>
<td>40 µl</td>
</tr>
<tr>
<td>HIV stock solution</td>
<td></td>
</tr>
<tr>
<td>Water (PCR-Grade)</td>
<td>20 µl</td>
</tr>
<tr>
<td>Bicine-Buffer RT 5x</td>
<td>5 µl</td>
</tr>
<tr>
<td>MoGAc</td>
<td>25 mM</td>
</tr>
<tr>
<td>dNTP/Mix (dUTP)</td>
<td>0.2 mM/2 µl</td>
</tr>
<tr>
<td>dATP/dCTP/dGTP</td>
<td>10 mM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 3</td>
<td>0.2 mM/4 µl</td>
</tr>
<tr>
<td>Primer SEQ ID NO 4</td>
<td>5 µl</td>
</tr>
<tr>
<td>UNG</td>
<td>U/µl</td>
</tr>
<tr>
<td>Tlb-Polymerase</td>
<td>5.5 U/µl</td>
</tr>
</tbody>
</table>

After PCR, UNG is blocked through the addition of laurylsarcosine concentration 1% use level.

Thermocycling profiles are as follows:

**HBV:**
- UNG-step: 3x 10 min 37° C.
- PCR: 35x 20 sec 92° C.
- 30 sec 55° C.
- 40 sec 72° C.

**HCV:**
- UNG-step: 3x 10 min 37° C.
- reverse transcriptase step: 3x 30 min 60° C.
- denaturation: 1 min 95° C.
- PCR: 2x 10 sec 95° C.
- 20 sec 60° C.
- 3x 15 sec 90° C.
- 20 sec 60° C.
- follow-up incubation: 7 min 72° C.

**HIV:**
- UNG-step: 3x 10 min 37° C.
- reverse transcriptase step: 3x 30 min 60° C.
- PCR: 4x 10 sec 95° C.
- 10 sec 55° C.
- 10 sec 95° C.
- 10 sec 72° C.
- 3x 10 sec 95° C.
- 10 sec 60° C.
- 10 sec 72° C.

**Detection on modified Elucsys 1010 R**

<table>
<thead>
<tr>
<th>reagent</th>
<th>volume (µl)</th>
<th>incubation time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR product</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>denaturation solution</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>detection probe</td>
<td>320</td>
<td>30</td>
</tr>
<tr>
<td>SA-beads</td>
<td>35</td>
<td>10</td>
</tr>
</tbody>
</table>

7.2 Homogeneous Functional Assays: 5'-Nuclease Assay-Technology on a Cobas Taqman®

7.2.1 General Considerations

Lysis of viral particles as well as adsorption, purification and elution of nucleic acids extracted from the sample are conducted as described above. Amplification of target molecules is described by the equation \( N_f = N_0 \times (1 + E)^g \), with:

- \( N_f \): number of molecules at the start of the polymerase chain reaction
- \( N_0 \): number of molecules at the end of polymerase chain reaction
- \( E \): efficiency of amplification

With 5'-nuclease technology, however, amplification and detection reactions, respectively, are closely interwoven, and occur in solution phase, i.e. without solid phase immobilisation and corresponding washing steps (homogeneous PCR). To this end, detection probes with 2 particular chemical modification are added to the PCR mastermix. One of these modifications is a fluorogenic reporter group (R, for instance a derivative of 6-carboxy-fluorescein) covalently attached to the backbone of the probe, the other one is a dye (for instance a polymethine-cyanine derivative) capable of absorbing the fluorescent light of the reporter and to quench it (quencher). The quencher is typically attached to the probe backbone at the 5'-end, whereas the reporter is located within the oligo sequence, spaced from the quencher by a number of nucleotide building blocks. These probes bind to the target nucleic acid (sense or anti-sense strand) close to the 3'-end of a primer (reverse or forward). As soon as primer has annealed to the target DNA, polymerase gets bound to the primer-target hybrid, elongation starts. Due to the 5'-nuclease activity of the enzyme, simultaneously with copy strand synthesis the probe is cleaved as soon as the polymerase reaches the probe binding site, reporter and quencher get separated and the fluorescent signal becomes measurable. This process is repeated with every cycle, and more and more fluorescent reporter is accumulated in solution until reagent depletion at the end of the reaction. So, in a signal-over-time plot, sigmoidal growth curves are generated. The bigger No, the earlier the signal curve rises above noise level.

The point on the time axis, where the fluorescent signal can be significantly distinguished from background signal is called threshold cycle (ct). ct is a measure of assay sensitivity: the smaller the ct-value, the more sensitive is the assay. ct values can be calculated by means of different mathematical operations, for example cut-off approaches (average background signal intensity multiplied by a constant factor yields a cut-off signal intensity to discard negative from positive) or approaches where the location of the maximum of the first differentiation of the signal-over-time curve (i.e. the steepness profile), or the location of the maximum of the second differentiation of the time axis, are computed and defined as ct.

This amplification/detection technology allows for real-time monitoring of PCR and for closed-tube processing as well, i.e. in contrast to standard procedures, the PCR tubes remain closed after pipetting eluate and mastermix which effectively reduces contamination risks.

Moreover, if one uses primer and probe sets for different viral species (and, thus, different analyte molecules and target sequences) combined in one mastermix, it is possible to perform multiple amplification/detection reactions simultaneously, depending on the viral load of the individual under inspection. This is the basis for so-called multiplex assays.

What is more, due to the generic nature of MGP based sample preparation, all nucleic acids present in the sample are extracted by way of physico-chemical adsorption to the particle surface, independent of sequence characteristics. This will also include human DNA (hDNA), released from disrupted blood cells, e.g. leucocytes, the titers of which...
may differ widely according to the physiological or pathological status of the individual blood sample donor. For instance, in cases of autoimmune diseases like SLE, hDNA levels may be elevated substantially. So, hDNA and one or more pathogenic nucleic acids present in a given sample constitute a mixture of various nucleic acids of different sequence specificities which are co-extracted. They constitute a matrix of polynucleotides that are extracted without discrimination, followed by sequence-specific amplification and detection of target nucleic acids via interaction with specific primers and probes under appropriate reaction conditions.

**[0385]** Given a pathological level of 4000 ng/ml hDNA in plasma in case of SLE, and a low viral load of 50 copies of viral genomic RNA per ml of plasma, and 10,000 nucleotides per genomic RNA. With ca. 325 dalton (1 dalton = 1.66x10⁻²⁴ g) per nucleotide, 50 copies or 500,000 nucleotides of target RNA make ca. 2.7x10⁻⁴ ng per ml of plasma, resulting in a relative abundance of target: non-target nucleic acid of approximately 1:10¹⁴ to 1:12!

**[0386]** hat is more, in order to monitor the whole process, an artificial nucleic acid construct may be added to the samples, preferably packaged (armored) in a modified virus particle, which is co-extracted and co-amplified with the natural target. This internal control (IC) features a unique probe binding region for an IC detection probe, which differs from target-specific probes by a different reporter group with distinguishable emission characteristics. Thus, IC signal can be discerned from target signal, and as the IC is known to be present in the sample, it functions as a monitoring agent. So, multi-labeling expands the utility of multiplex assay technology. The internal control (IC) is described in WO98/00547.

**[0387]** 7.2.2 Experimental Protocol

**[0388]** 7.2.2.1 Reagents

**[0389]** 7.2.2.1.1 Sample Preparation

**[0390]** See above (Example 7.1.2->automated protocol on in-house-built extractor)

**[0391]** 7.2.2.1.2 5'-Nuclease-Assay, Reactants & Reaction Conditions

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Concentration Addition of stock solution</th>
<th>Final concentration in the test (µl/reaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo(OAc)₄</td>
<td>50 mM</td>
<td>3 mM</td>
</tr>
<tr>
<td>dNTPs</td>
<td>100 mM</td>
<td>300 µM</td>
</tr>
<tr>
<td>DEPC-H₂O</td>
<td>100 µM</td>
<td>100 µM</td>
</tr>
<tr>
<td>Glycerol</td>
<td>80%</td>
<td>5,64% in total</td>
</tr>
<tr>
<td>K H₂O pH 7.5</td>
<td>2 M</td>
<td>100 mM</td>
</tr>
<tr>
<td>Tricine pH 8.3</td>
<td>1 M</td>
<td>50 mM</td>
</tr>
<tr>
<td>DMSO</td>
<td>80%</td>
<td>5,96%</td>
</tr>
<tr>
<td>Primer SEQ ID NO 12</td>
<td>50 µM</td>
<td>150 nM</td>
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<tr>
<td>Primer SEQ ID NO 13</td>
<td>50 µM</td>
<td>150 nM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 14</td>
<td>50 µM</td>
<td>400 nM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 15</td>
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<td>150 nM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 16</td>
<td>50 µM</td>
<td>150 nM</td>
</tr>
<tr>
<td>Primer SEQ ID NO 17</td>
<td>50 µM</td>
<td>150 nM</td>
</tr>
</tbody>
</table>

**[0392]** The common multiplex thermocycling profile for all test parameters is as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Condition</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG-step denaturation</td>
<td>45°C - 10 min</td>
<td></td>
</tr>
<tr>
<td>reverse transcription</td>
<td>94°C - 30 sec</td>
<td></td>
</tr>
<tr>
<td>PCR</td>
<td>58°C - 30 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91°C - 15 sec/52°C - 50 sec</td>
<td></td>
</tr>
</tbody>
</table>

**[0393]** Termology of the Chemical Derivations:

**[0394]** Some of the oligonucleotides are derivatised with Cy5 which is a Pentamethine-di-indocarbocyanine coupled to alkylphosphatidyl-linker (Pharmacia Biotech Cy5-N-ethyl-phosphoramidite) and which functions as quencher (Q); λ₀ₑₓ=630 nm, λₑₘₐₓ=665 nm. Some of the oligonucleotides are derivatised with FAM which is 6-carboxy-fluorescein coupled to a 2-(aminocyclohexyl)propane-1,3-diol-linker (Biogenex CX-FAM-phosphoramidite) and which functions as s reporter for targets (R); λ₀ₑₓ=485 nm, λₑₘₐₓ=515 nm. Some of the oligonucleotides are derivatised with which is hexachloro-6-carboxy-fluorescein coupled to a 2-(aminocyclohexyl)propane-1,3-diol-linker (Biogenex CX-HEX-phosphoramidite) and which functions as reporter for the internal control (R); λ₀ₑₓ=530 nm, λₑₘₐₓ=585 nm

**[0395]** 7.2.2.1.4 Reaction Conditions

**[0396]** The reaction conditions are described in the results of the experiments (see Example 7.3).
7.2.2.1.5 Other Materials

- Virus-negative plasma (O-Matrix, single plasma or Plasma-Pool), e.g. citrate-plasma or EDTA-plasma
- Virus-positive plasma or virus-containing culture supernatants, which can be used to adjust certain virus titers by mixing with O-matrix in appropriate ratios.
- Alternatively, in vitro-transcripts with the target-sequences to be investigated

7.2.2.1.5 Other Materials

- Human placental DNA (e.g. genomic, Sigma cat. no. D4642; b. fragmented, Sigma cat.no. D3287), added to the samples to simulate pathological conditions which lead to the enhanced release of intracellular substances and thus to enhanced levels of DNA/RNA in the blood, e.g. auto immune disease as SLE or hemolysis.

7.3 Results of the Functional Tests

7.3.1 Experiment 1

Example 7.2. The results are summarized in Table 10 and show the potential of the of the EJ006.5R-01-type particles to obtain higher sensitivity as exemplified by the earlier threshold cycle or higher hit rate irrespective of the particular assay.

7.3.4 Experiment 4

Example 7.2. Several MGP preparations of the type EJ/MMB and EJ/CERAC are functionally compared using the method as described in Example 7.1 wherein the adsorption incubation is conducted with or without shaking. Thereby, the sedimentation velocity becomes decisive, i.e. the higher the sedimentation velocity the higher the number of particles which are removed from the interaction with the analyte in the forming precipitate. In contrast, if the sedimentation velocity is smaller then time span is longer in which anlyte molecules from the liquid may adsorb to the surface of the adsorbent. The results are summarized in Table 11 (EJ004.7.2R was used as a reference) show that without mechanical agitation the loss of performance is highest (>40%) with the MMB-type (differs considerably from the characteristics of this invention), and least, or practically absent, with the CERAC-type (most close to the characteristics of the invention within this series).

7.4 Experiment 7.2. To be able to assess the functionality of the different types of MGP thoroughly, a performance index was calculated for each type in the following way:

- The eluate of several batches for each type of MGP and each titer level, respectively, are pooled into 1 eluate pool
- Out of each eluate pool, 3 amplifications were performed for each assay (HIV, HCV, HBV)
- Each amplificate was measured in singlicate on the modified Ekesys 1010®
- Signals are averaged for each type of MGP, assay, and titer level
- Signal-to-noise (S/N) factors are calculated for each type of MGP and assay, respectively, i.e. low titer (weakly positive) over O-matrix (negative), and elevated titer over O-matrix
- S/N factors are normalized to the reference type of MGP at the time
- Calculation of the sum of normalized S/N factors for each type of MGP, cumulated over all assays and titer levels; S/N factors for the low titer range are weighted 2-fold relative to those for the high titer level so as to pronounce sensitivity aspects
- The resulting sum is assigned performance index for each type of MGP investigated

7.3.5 Experiment 5

Different types of MGP were used to extract HCV in vitro transcripts which were diluted to various titer levels in diluent containing 10 mM Tris, pH 8.0, 1 mM EDTA, 20 μg/ml poly-A-RNA and 0.05% NaN₃, and spiked into pooled plasma. Human background DNA (hBG-DNA) was spiked at 0 or 4000 ng/ml into the sample, respectively. Surprisingly, it is possible to reduce interference by hBG-DNA by way of bead selection, as demonstrated by the data presented in Table 12.
7.3.6 Experiment 6

Various types of MGP were used to process HCV positive plasma samples. Human background DNA (hBG-DNA) was spiked at 0 or 4000 ng/ml into the sample, respectively. Again, a significant impact of bead size and bead geometry is being observed. MGP preparation E30096.5R, which represents the MGP quality according to the present invention the most, shows very clearcut advantages both in terms of minimal shift of ct values, and reducing loss of specific signal generation (S—N), as indicated by the data presented in Table 13.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Company</th>
<th>Abbreviation</th>
<th>Description</th>
<th>Code (see Example)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck</td>
<td>BM</td>
<td>No pigment</td>
<td>0</td>
</tr>
<tr>
<td>Daarmstadt, Germany</td>
<td>MMB</td>
<td>Merck MicroMatte Black</td>
<td>2</td>
</tr>
<tr>
<td>BASF</td>
<td>BASPA</td>
<td>BASF (iron oxide FA28-41)</td>
<td>4</td>
</tr>
<tr>
<td>Ludwigshafen, Germany</td>
<td></td>
<td>Needles in the nanoscale range made from γ-Fe₃O₅, length approximately 200–400 nm, very high tendency to aggregate to particles in the μ-scale because of the form and large highly structured surface</td>
<td>5</td>
</tr>
<tr>
<td>Cera, Milwaukee, WI, USA</td>
<td>CERAC</td>
<td>CERAC  γ-Fe₃O₅ (Catalogue number I-2012, distributed by Chemco Chemieprodukte GmbH in Germany) γ-Fe₃O₅-microbeads in the nanoscale (porous oxide), diameter = 23 nm, non-aggregating</td>
<td>7</td>
</tr>
<tr>
<td>Stem, Chemicals, Newburyport, MA, USA</td>
<td>STREM</td>
<td>STREM Fe₃O₅ &gt; 95% (Catalogue-No: 93-2916) Microbeads in the nanoscale range made from precipitated Fe₃O₅, diameter = 200–500 nm, strong tendency to aggregate to particles in the μ-range</td>
<td>8</td>
</tr>
<tr>
<td>BASF</td>
<td>BASFCE</td>
<td>Beads in the μ-scale made from metallic iron</td>
<td>9</td>
</tr>
<tr>
<td>Ludwigshafen, Germany</td>
<td></td>
<td>BASF Carbonyl iron powder HQ: 10% &lt; 1.04 μm, 50% &lt; 1.91 μm, 90% &lt; 3.71 μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BASF Carbonyl iron powder SU: 10% &lt; 0.43 μm, 50% &lt; 0.93 μm, 90% &lt; 1.81 μm</td>
<td></td>
</tr>
</tbody>
</table>

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Code</th>
<th>MI TEOS</th>
<th>Water (ml/100 ml TEOS)</th>
<th>Alcohol (ml/100 ml TEOS)</th>
<th>Ageing (h)</th>
<th>Spray pressure (bar)</th>
<th>Nozzle</th>
<th>Inlet temperature (°C)</th>
<th>Temperature in air before FA (°C)</th>
<th>Temperature in N₂ after FA (°C)</th>
<th>Temperature in O₂ after FA (°C)</th>
<th>Temperature treatment in bottle under air (°C)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>EIOH 50</td>
<td>1</td>
<td>1.5</td>
<td>A</td>
<td>230</td>
<td>250</td>
<td>675</td>
<td>300</td>
<td>300</td>
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<tr>
<td>0009</td>
<td>1750</td>
<td>13.33</td>
<td>EIOH 50</td>
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<td>A</td>
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<td>675</td>
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<td>A</td>
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<td>200</td>
<td>675</td>
<td>300</td>
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<td>191</td>
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<td>750</td>
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<td>200</td>
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<td>A</td>
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<td>200</td>
<td>750</td>
<td>200</td>
</tr>
<tr>
<td>Sample</td>
<td>Outlet temperature (°C)</td>
<td>Density (g/cm³)</td>
<td>Extinction (280 nm)</td>
<td>Extinction (400 nm)</td>
<td>Iron solubility (mg/l)</td>
<td>Magnetic force (g/250 mg)</td>
<td></td>
<td></td>
<td></td>
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</tr>
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</tr>
<tr>
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</tr>
<tr>
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### [0434]

**SEQARABICTABLE 6**

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<td>1.39</td>
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### [0435]

**TABLE 7**

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</thead>
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### [0436]

**SEQARABICTABLE 8**

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<td>E0108.5</td>
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#### Counting statistics: Which type scores better?

- **"standard samples"**
  - MMB: 47.28
  - CERAC: 54.1

- **"development variants"**
  - MMB: 47.28
  - CERAC: 54.1

#### Total: best type per sample * assay

- MMB: 54.1
- CERAC: 54.1

### [0437]

**TABLE 9**

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<th>Description</th>
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<td>E0096.5R</td>
<td>6.0 bar spraying pressure, 150° C. inlet temperature, Büchi-laboratory equipment. This preparation approximates the characteristics according to the invention very well, see FIG. 15.</td>
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<td>E0111.5R</td>
<td>1.5 bar spraying pressure, 230° C. inlet temperature, Nebirolas-laboratory equipment</td>
</tr>
<tr>
<td>E0119.5R</td>
<td>1.5 bar spraying pressure, 230° C. inlet temperature, Nebirolas-laboratory equipment, changed glass composition without Zn-acetate, K-methanoate substituted by K-acetate, with identical stoichiometry</td>
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<tr>
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<td>4.3 bar spraying pressure, 230° C. inlet temperature, Nebirolas-laboratory equipment</td>
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### [0438]

**SEQARABICTABLE 10**

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<th>MGP lot</th>
<th>MGP lot</th>
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<tr>
<td>ct-calculation via maximum of first differentiation</td>
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</tr>
<tr>
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<td>44.1(6/6)</td>
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<td>75%</td>
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SEQARABICTABLE 10-continued

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calculation via cut-off formula

calculation via maximum of first differentiation

<table>
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<tr>
<th>Parameter</th>
<th>Titer</th>
<th>MGP lot</th>
<th>MGP lot</th>
<th>MGP lot</th>
<th>MGP lot</th>
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<tbody>
<tr>
<td></td>
<td>NK</td>
<td>AVG</td>
<td>AVG</td>
<td>AVG</td>
<td>AVG</td>
</tr>
<tr>
<td>HIV</td>
<td>30</td>
<td>43.2(6/8)</td>
<td>43.6(3/8)</td>
<td>43.9(3/7)</td>
<td>43.9(1/7)</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>42.7(4/7)</td>
<td>42.6(4/7)</td>
<td>42.8(7/7)</td>
<td>42.2(2/7)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>42.4(5/7)</td>
<td>42.0</td>
<td>41.7(6/6)</td>
<td>42.6(8/6)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>41.8(5/6)</td>
<td>41.0(5/5)</td>
<td>41.2(6/6)</td>
<td>41.3(6/6)</td>
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<tr>
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<td>600</td>
<td>41.6(7/7)</td>
<td>40.9(6/6)</td>
<td>40.8(5/5)</td>
<td>41.5</td>
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<tr>
<td>hit rate</td>
<td>79%</td>
<td>76%</td>
<td>87%</td>
<td>64.0%</td>
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calculation via cut-off formula

<table>
<thead>
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<th>Parameter</th>
<th>Titer</th>
<th>MGP lot</th>
<th>MGP lot</th>
<th>MGP lot</th>
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<td>NK</td>
<td>AVG</td>
<td>AVG</td>
<td>AVG</td>
<td>AVG</td>
</tr>
<tr>
<td>HIV</td>
<td>30</td>
<td>44.3(6/8)</td>
<td>47.3(2/8)</td>
<td>46.8(1/7)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>50.3(3/7)</td>
<td>54.1(1/7)</td>
<td>51.4(4/7)</td>
<td>—</td>
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<tr>
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<td>47.4(5/7)</td>
<td>43.5(5/8)</td>
<td>45.5(5/7)</td>
<td>52.2(8/8)</td>
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<tr>
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<td>300</td>
<td>42.2(8/5)</td>
<td>44.8(5/5)</td>
<td>41.6(4/6)</td>
<td>46.5(6/9)</td>
</tr>
<tr>
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<td>41.2(7/7)</td>
<td>41.3(5/5)</td>
<td>41.1(5/5)</td>
<td>42.5</td>
</tr>
<tr>
<td>hit rate</td>
<td>62%</td>
<td>53%</td>
<td>59%</td>
<td>44%</td>
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</table>

[0440]

TABLE 12

<table>
<thead>
<tr>
<th>HCV-Titer (I/V:RNA)</th>
<th>ΔHΔNA</th>
<th>Act</th>
<th>AS-N</th>
</tr>
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<tbody>
<tr>
<td>RN009.1E = big platelets</td>
<td>1000 cp/PCR</td>
<td>4000 ng/ml</td>
<td>+2</td>
</tr>
<tr>
<td>(5-60 µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EJ0100.2R = deformed balls</td>
<td></td>
<td></td>
<td>−1</td>
</tr>
<tr>
<td>(ca. 1-2 µ-range)</td>
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<td></td>
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</tr>
<tr>
<td>EJ0108.5R = deformed balls*</td>
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<td></td>
<td>±0</td>
</tr>
<tr>
<td>(ca. 1-4 µ-range)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*high-pressure variant, see sections 7.2.4.2. & 7.3.1, respectively.

Note: (+Act = less sensitive (i.e. bias towards false negative results); (-Act = more sensitive (bias towards false positive results)?)

(-AS-N = less specific signal generation = negative interference

[0441]

TABLE 13

<table>
<thead>
<tr>
<th>HCV-Titer (extracted genomic RNA)</th>
<th>ΔHΔNA</th>
<th>Act</th>
<th>AS-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>EJ0096.5R = design goal*</td>
<td>200 IU/ml sample</td>
<td>4000 ng/ml</td>
<td>−0.95</td>
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<tr>
<td></td>
<td></td>
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<tr>
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</tr>
<tr>
<td>EJ0127.5R = standard</td>
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<td>−3.08</td>
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<td></td>
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<tr>
<td>EJ0129.5R = p-variant</td>
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<td>−2.30</td>
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<td></td>
<td></td>
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<tr>
<td>EJ0130.5R = p-variant</td>
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<td></td>
<td>−2.62</td>
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<tr>
<td></td>
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<tr>
<td>EJ0131.5R = p-variant</td>
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<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*0.5-5 µ-range; ±50% sub-polymers; big spherical aureas (27 m²/g BET); perfectly spherical beads
p-variant = spray-dried at elevated pressure (see section 7.3.1).

[0439]

SEQARABICTABLE 11

<table>
<thead>
<tr>
<th>MGP type</th>
<th>HIV 1</th>
<th>HCV</th>
<th>ratio of performance indices</th>
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<td></td>
<td>60 sgu/</td>
<td>600 sgu/</td>
<td>480 sgu/</td>
</tr>
<tr>
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<td>neg</td>
<td>neg</td>
<td>neg</td>
</tr>
<tr>
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<td>100</td>
<td>100</td>
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<td>55</td>
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<td>44</td>
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<td>EJ0096.7R</td>
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<td>47</td>
<td>18</td>
</tr>
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<td>EJ0097.4R</td>
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<td>RN045.5R</td>
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<td>RN046.7R</td>
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<td>EJ0047.2R (reference)</td>
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<td>24</td>
<td>52</td>
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<td>EJ0095.5R</td>
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<tr>
<td>EJ0096.7R</td>
<td>34</td>
<td>23</td>
<td>38</td>
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<tr>
<td>EJ0097.4R</td>
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<td>RN045.5R</td>
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<td>RN046.7R</td>
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<td>RN048.5R</td>
<td>55</td>
<td>113</td>
<td>25</td>
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Performance index

Cumulated/weighted/normalized

Without/with shaking

900 | — |
SEQUENCE LISTING

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<220> FEATURE:
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<221> NAME/KEY: modified_base
<222> LOCATION: (46)
<223> OTHER INFORMATION: derivatisation with a 3’-terminal phosphate group
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1. A composition of magnetic glass particles wherein
the magnetic glass particles comprise at least one magnetic object with a mean diameter between 5 to 500 nm.
2. The composition of claim 1 wherein
the magnetic object has a mean diameter between 10 to 200 nm, preferably between 15 to 50 nm.
3. The composition of claim 1 wherein
the half-life period for the sedimentation of a 3 mg/ml weight-per-volume suspension of the composition in isopropanol is more than 3 min.
4. The composition of claim 1 wherein
the half-life period is more than 6 min.
5. The composition of claim 1 wherein
the magnetic object has a mean diameter of 23 nm.
6. The composition of claim 1 wherein
the diameter ratio of the magnetic body to the glass shell is less than 1 to 10.
7. The composition of claim 1 wherein
the magnetic glass particles have a mean diameter between 0.5 μm and 5 μm.
8. The composition of claim 7 wherein
the magnetic glass particles have a mean diameter between 1 μm to 2 μm.
9. The composition of claim 1 wherein
the magnetic glass particles are microporous.
10. The composition of claim 1 wherein
the pore surface of the magnetic glass particles is less than 10% of the total surface.
11. The composition of claim 1 wherein
the magnetic object is superparamagnetic.
12. The composition of claim 1 wherein
the magnetic object is ferrimagnetic or ferromagnetic.
13. The composition of claim 1 wherein
the magnetic object comprises iron or iron oxide.
14. The composition of claim 13 wherein
the iron oxide is Fe₃O₄ or γ-Fe₂O₃.
15. The composition of claim 1 wherein
the magnetic glass particles have a surface area of more than 4 m²/g.
16. The composition of claim 15 wherein
the magnetic glass particles have a surface area between 5 to 100 m²/g, preferably in the range of 10 to 50 m²/g, most preferably in the range of 15 to 30 m²/g.
17. The composition of claim 1 wherein
the magnetic glass particles are substantially spherical.
18. A suspension comprising a composition of magnetic glass particles according to claim 1 in a liquid.
19. The suspension of claim 18 wherein
the liquid comprises an alcohol.
20. The composition of claim 19 wherein
the alcohol is isopropanol or ethanol.
21. The composition of claim 19 wherein
the liquid is 70% (V/V) ethanol.
22. The suspension of claim 18 wherein
the liquid is a buffered aqueous solution.
23. The suspension of claim 22 wherein
the solution is buffered by Tris-hydroxymethylamine, phosphate, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) or a salt thereof.
24. The suspension of claim 22 wherein
the solution further contains substances modifying the ionic strength or metal-complexing agents.
25. The suspension of claim 22 wherein
the suspension additionally contains DNA or RNA.
26. The suspension of claim 22 wherein
the suspension contains additionally DNA or RNA or both in a mixture with one or more compounds selected from the group of proteins, fatty acids, carbohydrates or any other biological material.
27. The suspension of claim 18 wherein
    the suspension additionally contains a chaotropic agent.
28. The suspension of claim 27 wherein
    the chaotropic agent is selected from the group consisting of sodium iodite, sodium perchlorate, guanidinium thiocyanate, guanidinium isothiocyanate or guanidinium hydrochloride.
29. The suspension of claim 27 wherein
    the chaotropic agent is present in a concentration between 2 and 8 mol/l, and preferably between 4 and 6 mol/l.
30. The suspension of claim 18 wherein
    the suspension contains 5 to 60 mg/ml of a composition of magnetic glass particles wherein
    the magnetic glass particles comprise at least one magnetic object with a mean diameter between 5 to 500 nm.
31. A tube containing the composition of claim 1.
32. A storage container containing the composition of claim 1.
33. A kit of parts comprising the tube of claim 1 or the storage container of claim 1.
34. The kit of parts of claim 1 further comprising a washing solution or an eluent.
35. The kit of parts of claim 1 further comprising reagents suitable for the purification of a nucleic acid.
36. A tube containing the suspension of claim 18.
37. A storage container containing the suspension of claim 18.
38. A kit of parts comprising the tube of claim 36 or the storage container of claim 37.
39. A method for the production of a composition of magnetic glass particles comprising the steps of
    a) suspending magnetic objects with a diameter between 5 and 500 nm in a sol,
    b) spray-drying the suspension in a two-nozzle spray-drier, and
    c) sintering the spray-dried powder.
40. The method of claim 39 wherein
    the inlet temperature of the two-nozzle spray-drier is between 120° C. and 500° C., the outlet temperature is chosen according to the boiling point of the sol and the spray pressure is at least 3 bar.
41. The method of claim 39 wherein
    the inlet temperature of the two-nozzle spray-drier is between 170° C. and 230° C., preferably between 190° C. and 210° C.
42. The method of claim 39 wherein
    the inlet temperature of the two-nozzle spray-drier is 200° C.
43. The method of claim 39 wherein
    the spray pressure is between 4 and 6 bar.
44. The method of claim 39 wherein
    the sol contains ethanol as solvent.
45. The method of claim 44 wherein
    the outlet temperature is between 50° C. and 300° C.
46. The method of claim 44 wherein
    the outlet temperature is between 90° C. and 100° C.
47. The method of claim 39 wherein
    the sinter temperature is between 400° C. to 1200° C., preferably between 720° C. and 770° C.
48. The magnetic glass particles produced by the method of claim 1.
49. A procedure for isolating a biological material comprising
    a) contacting a sample that contains the biological material in a liquid with a composition of magnetic glass particles wherein the magnetic glass particles comprise at least one magnetic object with a mean diameter between 5 to 500 nm under conditions in which the biological material binds directly to the surface of the magnetic glass particles, and
    b) separating the biological material from the liquid.
50. The procedure of claim 49 wherein the biological material is a nucleic acid.
51. The procedure of claim 49 wherein the biological material contains a mixture of nucleic acids, wherein the target nucleic acid or target nucleic acids is/are present in low abundance.
52. The procedure of claim 49 wherein
    the separation is accomplished with the aid of a magnet.
53. The procedure of claim 49 wherein
    the magnetic particles are not premagnetized when brought into contact with the sample.
54. The procedure of claim 49 wherein the procedure is automatized.
55. The procedure of claim 49 wherein
    the procedure is in a high-throughput format.
56. The procedure of claim 49 wherein
    during the procedure a suspension of magnetic glass particles, wherein the magnetic glass particles comprise at least one magnetic object with a mean diameter between 5 to 500 nm, is taken from a storage container and partial volumes of the suspension are added to different reaction vessels.
57. The procedure of claim 49 wherein
the nucleic acid is detected after the purification.
58. The procedure of claim 49 wherein the nucleic acid is
detected after an amplification step.
59. The procedure of claim 57
wherein
the nucleic acid is detected by a process comprising:
a) contacting the sample with an oligonucleotide
comprising a sequence complementary to a region
of the target nucleic acid and a labeled oligonucle-
otide comprising a sequence complementary to a
second region of the same target nucleic acid
sequence strand, but not including the nucleic acid
sequence defined by the first oligonucleotide, to
create a mixture of duplexes during hybridization
conditions, wherein the duplexes comprise the
target nucleic acid annealed to the first oligonucle-
otide and to the labeled oligonucleotide such that
the 3'-end of the first oligonucleotide is adjacent to
the 5'-end of the labeled oligonucleotide;
b) maintaining the mixture of step a) with a template-
dependent nucleic acid polymerase having a 5' to
3' nuclease activity under conditions sufficient to
permit the 5' to 3' nuclease activity of the poly-
merase to cleave the annealed, labeled oligonucle-
otide and release labeled fragments; and
c) detecting and/or measuring the signal generated
by the hydrolysis of the labeled oligonucleotide.
60. The procedure of claim 59
wherein
the detection is carried out in the presence of a blocking
oligonucleotide.
61. The procedure of claim 60
wherein
the blocking oligonucleotide is an aptamer.
62. The procedure of claim 61
wherein
the aptamer has the sequence SEQ ID NO: 23.
63. The procedure of claim 60
wherein the amplification and detection reaction is carried
out in a homogeneous solution-phase multiplex assay
format for the simultaneous detection of multiple tar-
gets.
64. The procedure of claim 60
wherein for at least 5 cycles of the polymerase chain
reaction, the annealing temperature is less than 8° C.,
preferably less than 5° C. above the dissociation tem-
perature of the polymerase-aptamer complex.
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