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(54) Preparation for the diagnosis of the transport function of salpinx

(57) A diagnostic preparation for investigation of the transport function of female salpinx, consists of a suspension of biocompatible, biodegradable, and, if it is desired, labelled particles in physiologic saline or other vehicle commonly used for parentheral applications in medicine, e.g., in the isotonic solution of sodium chloride or infusion solution of dextran, wherein the particles have a spherical shape with diameter in the region 10 to 600 μm, are formed from a soft hydrophilic gel based on a polymer selected from crosslinked water-soluble nontoxic and biodegradable inert polysaccharides and physiologically inert water-soluble and biodegradable poly(amino acids), polypeptides, and their derivatives, which may contain physiologically active substances, e.g., poly(amino acid) and/or hormons, and retain their shape and size in the environment of peritoneal cavity and uterotubal tract for at least 80 hours with the subsequent degradation to nontoxic product within 5 to 60 days.

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SPECIFICATION

Preparation for the Diagnostics of the Transport Function of Salpinx and a Method for Preparation Thereof

The invention pertains to a preparation for the diagnostics of the transport function of salpinx and to a method for preparation of the said preparation.

The inability of salpinges to transport an ovum, i.e. a blastocyst from the ovarian region into the lumen of uterus, is the frequent reason of the female infertility estimated to one third of all infertile marriages. The reason may be anatomic, e.g., the totally impervious salpinges, or functional, when the salpinx is permeable but the transport does not occur. This functional tubal sterility (FTS) is usually classified among so called idiopathic sterilities, the frequency of which is estimated to 15% of female infertility. The reasons may be various, as an inborn hypoplasia, disorder of muscular activity, disorder of ovum pick-up, but probably the most often reason is the persistent spasm of isthmic sphincter, which narrows the lumen of this section to 150 μm, while the blastocyst has diameter of 380 to 400 μm.

While a surgical treatment is indicated for anatomic defects, the disorders of transport are reparable by conservative procedures. A reliable differencial diagnostics is therefore needed.

The recent diagnostic procedures employ the ascendent or descendent methods. In the ascendent methods, the salpinx is filled by application of a liquid or gas (CO_2) into the uterotubal tract. In laparoscopy, the penetration of a suitably coloured liquid is observed and/or a resistance against the introduced medium is recorded. It was also described a method utilizing the active transport of radioactive microspheres from human albumine (diam. 10—35 μm; labelled with ^{99}Tc; S. C. Stone et al., Fertil. Steril. *43*, 757 (1985)), which were introduced into vaginal fornix and to the inlet of cervical channel. Hysterosalpinographs were recorded during 60 min by means of scintigraphy. The disadvantages consists in the possible penetration of the applied medium through the region of spasm which prevents from the transport of blasocyst and the positive result is false. The change in pressure (resistance) enables to indicate the spasm during the application under pressure (cineinsufflation), but the spasm may be also induced as a consequence of irritation and the results may be false negative.

In the descendent methods, a diagnostic medium of particulate character is applied into the region of ovarys or into the Douglas' space and its penetration into the lumen of uterus is followed. Chinese ink (D. v. Ott, 1925; Ztbl. Gynekol. Nr. 10,546), starch grains (A. Decker and M. Decker, 1954; Obstct. Gynecol. *4*, 35), or colloidal radioactive gold (^{198}Au) (A. Stabile and F. E. Leborgne, 1958; Int. J. Fertil. *3*, 139) are used for this purpose. In the experiments with animals (rabbit), also models of ovum were used in the form of spherical particles of crosslinked dextran (Sephadex) (H. B. Croxatto, C. Vogel, J. Vasquez; J. Reprod. Fert. *33*, 337 (1973)) or polystyrene (M. J. K. Harper et al., 1960; J. Reprod. Fert. *1*, 249), which can be labelled with radioactive iodine ^{125}I (C. J. Pauerstein and B. J. Hodgson, 1976; Am. J. Obstet. Gynecol. *124*, 840).

The above methods are disadvantageous in the clinical application because the particles (Chinese ink, colloidal gold, starch grains) are too small so that they pass through the isthmic region even in the case of persistent spasm. The models of ovum used in the experiments with animals differ from the natural transported objects by physical properties and the character of surface, on the one hand, and are objected from the standpoint of safety of patient (esp. polystyrene), on the other.

We have found now, that biocompatible spherical particles may be prepared which correspond to the properties of human blastocyst by their size, shape, mechanical properties, and partly also by biochemical properties, so that they keep their properties in the environment of uterotubal tract for at least 20 hours and then undergo the biodegradation to soluble nontoxic products. We have also found, that if the particles are applied in a suitable way, they are actively transported through the salpinx into uterus, provided that the transport function of salpinx is not damaged. This fact can be utilized in the diagnostics of transport function of human salpinx.

An object of the invention is a diagnostic preparation for investigation of the transport function of salpinx, which consists of a suspension of biocompatible, biodegradable, and, if it is desired, labelled particles in physiologic saline, or other vehicle commonly used for parenteral applications in medicine, e.g. in the isotonic solution of sodium chloride or infusion solution of dextran, whereas the particles have have a spherical shape with diameter in the region 10 to 600 μm, are formed from a soft hydrophilic gel based on a polymer selected from the group comprising additionally crosslinked water-soluble and biodegradable inert polysaccharides and physiologically inert, water-soluble and biodegradable poly(amino acids), polypeptides, and their derivatives, which may contain physiologically active substances, e.g., poly(aminoacids) and/or hormons, and retain their shape and size in the environment of peritoneal cavity and uterotubal tract for at least 80 hours with the subsequent degradation to nontoxic products within 5 to 60 days.

The hydrophilic gel particles are made from a covalently cross-linked water-soluble biodegradable polymer, which is chosen from the group comprising physiologically inert and biodegradable polysaccharides, poly(amino acids), polypeptides, and their derivatives. The suitable polysaccharides contain glucose bound prevailingly through 1—4 glycoside bonds, for example, starch, glycogen, amylose, and their biocompatible derivatives, e.g. hydroxyethyl starch. The suitable poly(aminoacids) are namely the polymers derived from glutamic acids aspartic acid, e.g., poly(N - hydroxyethyl) - L - asparagine, poly(N - hydroxyethyl) - L - glutamine, and the copolymers derived from them and containing other amino acids,

e.g. the copolymers with L-lysine, L-alanine, and L-valine. The suitable polypeptides are soluble derivatives of collagen and elastin, e.g., gelatine.

The crosslinking agents according to the invention were selected from the group of agents comprising halogenoepoxides, e.g., 2,3 - epoxy - 1 - chloropropane or 2,3 - epoxy - 1 - brompropane, or from the group comprising diisocyanates, e.g., 1,6-hexanediisocyanate and dialdehydes, e.g., glutaraldehyde.

The spherical particles according to the invention may contain substances which enable their identification in biological material. Such compounds according to the invention are chromophoric or fluorophoric compounds covalently bound to the polymeric material of particles, e.g., acylated derivatives of fluoresceine, rhodamine, acridine, and the like, or the microparticles of an inert pigment, e.g., Chinese ink, or magnetic materials, e.g., oxides of iron. The spherical particles of the diagnostic preparation according to the invention may contain substances which influence their biological properties. These substances may be bound to the polymeric material of particles through a covalent or non-covalent bond and are, for example, poly(amino acids) or polypeptides, as choriogonadotropic hormons and/or substances isolated from follicular liquor.

An object of the invention is also a method for preparation of the diagnostic preparation, which consists in dispergation of an aqueous solution of the physiologically inert biodegradable polymer selected from the group comprising polysaccharides, poly(amino acids), polypeptides, and their derivatives, which may contain an aminoacyl derivative of a fluorochrome or dispersed pigment or magnetic particles in the amount of 0.1 to 10 wt.%, in a medium of nonpolar water-immiscible solvent containing 0.1 to 10 wt.% of polymer with the degree of polymerization 50 to 500, which contains 80 to 100 mol.% of γ-alkyl-L-glutamate units and 0 to 20 mol.% of L-glutamic acid units, as a suspension stabilizer under the formation of polymeric spherical particles with diameter in the chosen region 10 to 600 μm, which are transferred into a gel state by the action of bifunctional crosslinking agents. The nonpolar solvent suitable as a continuous phase of the dispersion is selected from the group of solvents comprising halogenated hydrocarbons, e.g., dichloromethane, dichloroethane, chloroform, cyclic hydrocarbons, e.g., benzene, toluene, and their mixtures. The obtained spherical particles are washed with an alkaline aqueous solution, e.g., with the solution containing 1 to 10 wt.% of sodium hydroxide, in order to saponify γ-alkyl esters of glutamic acid and to decompose the contingent unreacted reactive groups of bifunctional crosslinking agents and the products of their reaction, and the washed particles are dispersed in a suitable solution selected from the group of solutions for parenteral application comprising isotonic solution of sodium chloride, Ringer's isotonic solution, infusion solution of dextran, and the like, and sterilized by heat. The resulting spherical particles are chemically activated for binding of biologically active peptides, for example, hormons, by methods common in the immobilization of peptides and proteins on polymeric carriers.

The spherical particles prepared according to the invention are further characterized by the fact, that bonds of the polymer forming the gel are cleaved by the action of enzymes present in uterotubal tract and thus the gel microspheres are gradually dissolved. In this way, they are cleaved 1,4-glycoside bonds of the polysaccharide forming the gel, e.g., by the action of enzymes, 1,4-glycosidases, above all by α-amylase, the presence of which in a tubal liquor is generally known, with the formation of soluble products containing glucose, glucose oligomers, and their derivatives, which may be either metabolised or excreted. The rate of particle degradation by α-amylase may be controlled, in the procedure according to the invention, by the degree of conversion of the crosslinking reaction, i.e. by the degree of chemical modification and the degree of crosslinking. The degree of crosslinking depends on the concentration ratio of the bifunctional crosslinking agent and the polymer, on temperature, and on the time of reaction. The increased concentration of bifunctional agent and/or extension of polymerization time leads to the increased degree of crosslinking and to the higher resistance of particles towards biodegradation. In this way, the period of time, for which the particle keeps its shape and size and the time of its persistence in the organism may be controlled in a sufficiently broad region. Similarly, the degree of conversion of the crosslinking reaction of poly(amino acids) and polypeptides, e.g., the concentration ratio of a bifunctional agent, as 1,6-hexanediisocyanate, and gelatine, controls the rate of degradation of the formed gel with proteolytic enzymes, for example, cathepsins, elastase, plasmin, pepsin, etc., which ability to catalyze hydrolysis of the above mentioned poly(amino acids) and polypeptides is generally known.

An advantage of the preparation according to the invention consists in enabling a diagnostic descendent procedure with particles which approach, by their shape, mechanical, and partly biochemical properties, the properties of human ovum as much as possible. Another advantage is that the particles detained in body are degraded in a relatively short time to harmless products, whereas the rate of degradation may be influenced within broad limits by the degree of crosslinking of the polymer. It is also advantageous, that the particles can be easily found and identified in an uteral irrigation, so that the probability of false negative or false positive findings is low and the method does not require special apparatuses. The advantage of the preparation is eventually its easy sterilization and long life and that it may carry biologically active components affecting the transport of ovum through the salpinx.

EXAMPLE 1

A solution obtained by mixing 7.0 g of soluble starch (mol. wt. 25,000—35,000), 12 ml of distilled water, and 1.2 g of sodium hydroxide (NaOH) was filtered and deaerated in vacuum and 15 ml of this solution was dispersed in 35 ml of 1,2-dichloroethane containing 0.070 g of poly(γ - benzyl - L - glutamate) in a reaction

cylindric vessel equipped with a stirrer and heating jacket. Temperature 40°C was kept and revolutions of the stirrer were set in such a way that the average size of obtained particles was 120 to 150 μm. Then, 4.0 g of 1 - chloro - 2,3 - epoxypropane (epichlorohydrine) was added and the emulsion was allowed to react under constant stirring at 40°C for 11 hours. The suspension of particles was then washed by decantation in 300 ml of a cold dichloroethane—dioxane (1:1) mixture and twice in 300 ml of dioxane, and distributed into 4 fractions: a) smaller than 40 μm, b) 40 to 120 μm, c) 120 to 240 μm, d) larger than 240 μm by sieving in dioxane through screens with mesh size 40 μm, 120 μm and 240 μm. The particles of individual fractions were sedimented, the sediment was dispersed by shaking in 2% sodium hydroxide (NaOH), allowed with occasional shaking for 16 hours at ambient temperature, and then washed with water and physiologic saline (0.154 M NaCl). The average size of particles dispersed in the isotonic solution of sodium chloride (NaCl) was determined by optical microscopy. The values 38±12 μm, 84±16 μm, 205±34 μm and 340±62 μm were obtained for the fractions a, b, c, and d, respectively. The content of particles in individual fractions after drying, expressed in percent of the total amount was a) 3%, b) 20%, c) 65%, d) 12%.

EXAMPLE 2

In a three-neck reaction flask provided with a stirrer, it was dissolved 3.47 g of 6-aminofluoresceine in 120 ml of acetonitrile. The mixture was cooled in a bath to −10°C and 3.78 g of 3-chloropropionyl chloride along with 4.04 g of triethylamine were gradually added during 30 minutes. The reaction was carried out for 2 hours alltogether at −10°C and the mixture was then allowed at ambient temperature overnight. Acetonitrile was evaporated from the mixture and the residue was dissolved in 300 ml of methanol which contained 17 g of ammonia. The solution of ammonia was allowed to rest at ambient temperature for 3 days. The excess of ammonia was then removed by distillation with methanol, 200 ml of water was added to the residue, the present ammonium chloride (NH_4Cl) was decomposed by addition of sodium hydroxide (NaOH) and the resulting ammonia was distilled off again. The product was obtained after neutralization to pH 6 as a precipitate, which was repeatedly crystallized from water. Yield: 2,75 g of 6 - (3 - aminopropionamido)fluorescein. For $C_{23}H_{18}O_6N_2 \cdot 2H_2O$ calculated C 60.79, H 4.84, N 6.169; found C 60.34, H 4.62, N 6.09%.

20 mg of 6-(3-aminopropionamido)fluoresceine and 7.0 g of soluble starch were dissolved in 12 ml of water containing 1.2 g of sodium hydroxide (NaOH); 15 ml of this solution was filtered, degased, and dispersed in 35 ml of 1,2-dichloroethane containing 70 mg of poly(γ-benzyl glutamate). The reaction with 4.0 g of 1-chloro-2,3-epoxypropane (epichlorohydrine) was carried out at 40°C for 11 hours in the same way as in example 1 and the prepared particles were distributed by sieving in dioxane analogously as in example 1. The fraction passing through the screen with mesh size 240 μm and retained by the screen with mesh size 120 μm was dispersed in 2% solution of sodium hydroxide (NaOH) for 16 hours, when all remaining reactive groups were decomposed, washed with water and the isotonic solution of sodium chloride (NaCl), and then extracted with the isotonic solution of NaCl at pH 7 and 120°C for 40 min in an autoclave. The particles were then washed with the sterile isotonic solution and under aseptic conditions filled into sterile serum ampouls and again sterilized in the autoclave at 120°C for 40 min. They were stored as a sterile suspension in the isotonic solution.

The particles had a regular spherical shape with the size 140 to 280 μm and its average value 195±36 μm. At the excitation with blue light (λ=430 nm), they exhibit a brilliant fluorescence in the yellow-green region 515 to 530 nm. The fluorescent label is stable and is not washed out until the gel degradation does not occur.

EXAMPLE 3

Fluorescently labelled microspheres differing in the duration of reaction with 1 - chloro - 2,3 - epoxypropane were prepared at the same ratio of reacting components and under the same reaction conditions as in example 2, with the distinction that the reaction was stopped in individual samples by dilution of the suspension with a cold mixture of dioxane and 1,2-dichloroethane (1:1) after 7, 9, 11, 13, and 18 hours of reaction at 40°C, respectively. The samples of particles were dispersed in the amount corresponding to 10 mg of dry substance in 2 ml of physiologic saline with pH 7.4, which contained 115 U/ml of pancreatic α-amylase, and incubated at 37°C under moderate shaking. Time required for the complete dissolution of particles was followed in a fluorescent microscope. The stability of particles in the medium containing α-amylase increases with the extended reaction time with epichlorohydrine; see Tab. 1.

TABLE 1

Sample	Reaction Time (h)	Time Required for the Complete Dissolution
1	7	3—6 min
2	9	12—18 min
3	11	40—70 min
4	13	3—4 h
5	18	more than 24 h

EXAMPLE 4

The samples of fluorescently labelled microspheres differing in the duration of reaction with 1 - chloro - 2,3 - epoxypropane were prepared by the same procedure as in example 3; 0.4 ml of the sterile suspension of microspheres containing 40 mg of the dry gel was applied to rats with live weight ranging from 180 to 200 g through the superficially disinfected abdomenal wall into peritoneal cavity. The rats were killed in suitable time intervals (3 rats in each time interval and per each microsphere sample), the abdominal cavity was rinsed with physiologic saline, and the sediment of irrigation as well as the surface of abdominal cavity were investigated for the presence of fluorescent microspheres. Table 2 surveys the time course of the degradation of microspheres in the abdominal cavity of rats in vivo.

TABLE 2
Evaluation of the Degradation Process of Microspheres in Abdominal Cavity

Sample	Reaction Time with 1-chloro-2,3-epoxypropane	Average Diameter of Particles	Description of Finding
1	7	240 μm	The integral particles disappear from the peritoneal cavity before 6 hours. The soluble fluorescent material can be detected in urine and urinary bladder
2	9	210 μm	Sporadic integral particles are present in the irrigation of perit. cavity after 24 h together with the soluble fluorescent material from urine and urinary tract
3	11	162 μm	After 72 h since application, most particles in the irrigation are without visible changes in size and shape; some particles have traces of degradation. After 6 days since application, particles are found in a progressive stage of degradation; plentyful macrophages containing phagocytosed fluorescent material occur in cytoplasm. The soluble fluorescent material is excreted with urine. On the 21st day since application, complete disappearance of the particles from perit. cavity, disappearance of the fluorescent material from cytoplasm of leucocytes present in perit. irrigation
4	18	180 μm	On the 21st day after application; most of the particles in the sediment of irrigation without signs of degradation. Some particles have an injured surface—dissolving circumference. On the 40th day after application: the particles present in irrigation show a progressive stage of degradation.

EXAMPLE 5

The microspheres were prepared in the same way as in example 2. To 5 ml of suspension containing 200 mg of gel in water, it was added 43 mg of sodium periodate ($NaIO_4$) and the suspension was agitated by shaking in darkness at 22°C for 2 hours. The microspheres were washed on filter with distilled water until the reaction on iodine after addition of potassium iodide dissappeared, then washed with sterile physiologic saline, and redispersed in 5 ml of the solution containing 5,000 I.U. of human chorionic genadotropin (Sigma) and adjusted with 0.1 mol l^{-1} phosphate buffer to pH 7.2. The suspension was agitated by shaking at 4°C for 4 hours and then washed with sterile physiologic saline. The washed particles were divided into three fractions and incubated a) in the phosphate buffer at pH 7.4, b) in the phosphate buffer at pH 6.2, c) in the phosphate buffer at pH 6.2 with addition of 1.5 U/ml of α-amylase. The released chorionic gonadotropin was determined in the solution by radio-immuno assay. The rate of release of gonadotropin into medium increased in the sequence a) b) c).

EXAMPLE 6

Into 10 ml of black Chinese ink, it was added 4 g of gelatine and allowed to swell overnight. The gelatine was then dissolved by heating on a water bath and the solution was added into 40 ml of 1,2-dichloroethane containing 80 mg of poly(γ - methyl - L - glutamate), which was saponified to 8 mol.%, and heated to 45°C.

The revolutions of stirrer were set as in example 1 so that the average particle size was 120 to 150 µm. Then, 5 ml of 1,6 - hexanediisocyanate was slowly added into the reaction vessel, the stirring was continued for further 4 hours and at ambient temperature for further 18 hours. Further processing of particles was carried out as in example 1. The particles were washed with physiologic saline until the reaction on amines disappeared.

EXAMPLE 7

The spherical particles were prepared by the procedure described in example 1 with the distinction that 1.5 g of iron trioxide (Fe_2O_3) with particle size below 1 µm was added into the solution of starch and thoroughly dispersed in this solution.

EXAMPLE 8

The microspheres prepared by the procedure according to example 2 were dispersed in the isotonic solution of sodium chloride and sterilized in autoclave at 120°C for 30 min. The sterile suspension in the amount of 1 ml containing 12 mg of microspheres with diameter 150—250 µm was applied into the retrouterinal space of investigated women. The microspheres transported through salpinx were identifed in the samplings of uterinal and cervical secreta under a binocular magnifier according to a yellow-green fluorescence at illumination with mercury lamp (HBO lamp). The examinated set comprised 86 female patients with idiopathic sterility, where the investigations of tubaly transport was indicated for the purpose of therapy. The set consists of patients who were previously examinated on the patency of salpinx either by chromopertunation in laparoscopy or by hysterosalpingography and the salpinges were diagnosticated as permeable for liquid in all cases. With the application of microspheres, the undamaged transport function of salpinx was proved in the given set in 38 cases, i.e. 44%. In this group, the microspheres were unambiguously proved in the samplings of cervical and uterinal secreta during 38 to 56 hours after application irrespective of the period of ovulation cycle.

CLAIMS

1. Diagnostic preparation for investigation of the transport function of female salpinx, wherein the said preparation consists of a suspension of biocompatible, biodegradable, and, if it is desirable, labelled particles in physiologic saline or other vehicle commonly used for parenteral applications in medicine, e.g., in the isotonic solution of sodium chloride or infusion solution of dextran, whereas the particles have a spherical shape with diameter in the region 10 to 600 µm, are formed from a soft hydrophilic gel based on a polymer selected from the group comprising additionally crosslinked water-soluble nontoxic and biodegradable polysaccharides poly(amino acids), polypeptides, and their derivatives, which may contain physiologically active substances, e.g., poly(amino acids) and/or hormons, and retain their shape and size in the environment of peritoneal cavity and uterotubal tract for at least 80 hours with the subsequent degradation to nontoxic products within 5 to 60 days.

2. The diagnostic preparation according to Claim 1, wherein the particles of soft hydrophilic gel are formed from the additionally crosslinked water-soluble nontoxic and biodegradable polymers selected from the group of physiologically inert polysaccharides, for example, polysaccharides containing glucose bound through 1—4 glycoside bonds, e.g., starch, amylose, amylopectin, glycogen, and their biocompatible derivatives, e.g., hydroxyethyl starch.

3. The diagnostic preparation according to Claim 1, wherein the particles of soft hydrophilic gel are formed from a nontoxic biodegradable polymer selected from the group comprising physiologically inert water-soluble and biodegradable poly(amino acids) and polypeptides and their derivatives, for example, poly(N - hydroxyalkyl) - L - asparagine, poly(N - hydroxyalkyl) - L - glutamine, and copolymers derived from them and containing other amino acids, e.g., L-lysine, L-alanine, or N-valine, soluble derivatives of collagen and elastin, e.g., gelatine, which are additionally crosslinked.

4. The diagnostic preparation according to Claims 1 through 3, wherein the spherical particles contain chromophoric or fluophoric groups covalently bound to the polymer making the material of particles, for example, derivatives of acylaminofluoresceine, rhodamine, acridine, and the like, or contain microparticles of a pigment, for example, Chinese ink, or magnetic materials, for example, oxides of iron.

5. The diagnostic preparation according to Claims 1 through 4, wherein poly(amino acids) and/or hormons, for example, chorionic gonadotropic hormon, are bound in the spherical particles.

6. The diagnostic preparation according to Claims 1 through 5, wherein the spherical biodegradable particles are dispersed in a vehicle applicable in parenteral medicine, which contains also chromophoric or contrast substances, for example, indigo carmine, methylene blue, and/or macromolecular derivatives of these compounds, for example, their conjugates with dextran.

7. Method for preparation of the diagnostic preparation according to Claims 1 through 6, wherein an aqueous solution of a physiologically inert, biodegradable polymer, which is selected from the group comprising polysaccharides, poly(amino acids), polypeptides, and their derivatives, which may contain an aminoacyl derivative of fluorochrome, for example, 6 - (ω - aminoacylamido)fluoresceine, and/or dispersed pigment or magnetic particles in the amount of 0.1 to 10 wt.%, is dispersed in a medium of nonpolar water-immiscible solvent, for example, dichloromethane, dichloroethane, chloroform, benzene, toluene, and their mixtures, which contains 0.1 to 10 wt.% of the polymer with degree of polymerization 50

to 500 containing 80—100 mol.% of γ - alkyl - L - glutamate units and 0—20 mol.% of L-glutamic acid as a suspension stabilizer, under formation of polymeric spherical particles with diameter 10 to 600 μm, which are transferred into a gel state by action of bifunctional crosslinking agents, washed in baths including alkaline aqueous solutions, for example aqueous solution of sodium hydroxide, dispersed in a suitable solution selected from the group comprising solutions for parenteral application, for example, isotonic solution of sodium chloride and Ringer's physiologic solution, and the resulting suspension is sterilized by heat.

8. The method for preparation of the diagnostic preparation according to Claim 7, wherein the obtained spherical particles are chemically activated for binding of biologically active peptides, for example, hormons, by the common methods for immobilization of peptides and proteins on polymeric carriers.

9. Diagnostic preparation as claimed in claim 1 substantially as described in any of the examples disclosed herein.

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