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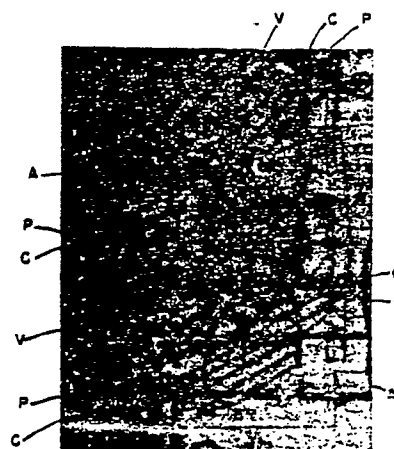
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54 **Intravascularly-administrable, magnetically-localizable biodegradable carrier and process for its preparation.**

57 The intravascularly-administrable, magnetically-localizable biodegradable carrier comprises microspheres (A) formed from an amino acid polymer matrix (C) with magnetic particles (P) embedded therein. The microspheres can be used for intra-arterial administration and capillary level localization and/or release of therapeutic and diagnostic agents, thereby obtaining much more precise targeting of the agents than has heretofore been possible.

Also a process is provided for incorporating water-soluble therapeutic agents in albumin microspheres. This process is particularly advantageous where the therapeutic agent is heatsensitive. All steps of the process can be carried out at relatively low temperatures, such as ambient room temperature. The process may be applied to the preparation of the above mentioned intravascularly-administrable, magnetically-responsive microspheres.

FIG. 1



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INTRAVASCULARLY-ADMINISTRABLE, MAGNETICALLY-LOCALIZABLE
BIODEGRADABLE CARRIER AND PROCESS FOR ITS PREPARATION

Specific delivery of chemotherapeutic agents to desired target sites with a minimum of systemic side effects constitutes one of the ongoing challenges of chemotherapy. Chemical approaches to such "targeting" depend on biochemical differences between cells, but such differences are more quantitative than qualitative. Thus the drugs administered have activity in areas of the body where activity is not desired. An alternative to
10 chemically mediated targeting is the entrapment of a chemotherapeutic agent in a carrier which can mechanically effect distribution of the drug.

In 1974, Kramer proposed albumin microspheres as vehicles for achieving specificity in drug delivery. J. Pharm. Sci., 63, 1646-1647 (Oct. 1974). Mercaptopurine was dissolved in the aqueous albumin prior to conversion to microspheres, and this water-soluble drug was shown to be entrapped in the microspheres. Kramer proposed that sonication could be used to produce smaller and more uniform size particles. Kramer did
20 suggest that intravenous administration of the microspheres might result in preferential uptake in such tissues as liver or bone marrow due to non-specific phagocytosis with a possible reduction in required total doses and thereby less systemic side effects. This would still be far short of the desired objective since only diseases of the reticulo-endothelial system would be affected. Local compartmentalization of water soluble chemotherapeutic agents at desired target sites, if it could be achieved, would permit administration of much lower doses by largely eliminating systemic dilution of the drug. In
30 addition, many of the adverse side effects that are often the

result of systemic distribution could be eliminated. Unfortunately, prior to the present invention, no system of administration has been provided which can effectively deliver therapeutic agents intravascularly to a selected site. Such a system also has value for administration of diagnostic agents.

Freeman et al proposed in 1960 that magnetic iron particles might be used as a means for transporting radiation or some healing chemical to a particular spot in the body, the particles being magnetically directed. J. App. Phys., Supp. 10 Vol. 31, 404S-405S (May 1960). It was proposed that the iron particles could be alloyed with the proper choice of radioactive element, or that they could be coated with an absorbed layer of a therapeutic agent. Later Meyers et al suggested the use of carbonyl iron particles as vehicles for site specific delivery of chemotherapeutic agents. Amer. J. Roentg., 90, 1068-1077 (Nov. 1963). Magnetic iron particles of 1 to 3 microns in diameter were shown to be localized in the vessels or gastrointestinal tract of dogs with a magnetic field of approximately 5,000 gauss. It appeared that some of the particles had been 20 pulled through the artery into the tissues by the magnetic field. However, the surface properties of magnetic particles, such as carbonyl iron, lead to irreversible intravascular clumping upon exposure to a magnetic field unless they are coated with electronegative polymer such as albumin. Nakamura et al; J. App. Phys., 42, 1320-1324 (1971). Alksne et al, Surgery, 60, 212 (1966) employed carbonyl iron microspheres to occlude intracranial aneurysms in both animals and humans, thereby utilizing the "clumping" phenomenon for a therapeutic purpose. In 1973, Mosso et al reported an improved method for 30 clumping particles by the use of ferromagnetic silicon which

they used for selective vascular occlusion and subsequent necrosis of tumors in humans Ann. Surg., 178:5, 663 (1973).

The prior art does not provide a solution to the problem of how accurate magnetic direction of intravascularly administered magnetic particles can be obtained, nor to the equally difficult problem of how sufficient loading of the chemotherapeutic agent per particle can be obtained.

Microcapsules containing magnetic particles are disclosed in United States patent 2,971,916. Microcapsules of 3 to 150 microns in diameter are formed by coacervation, the capsules having walls of hardened organic colloid material enclosing an oily liquid containing a dispersion of magnetic powder. No medical application is suggested, the capsules being indicated as useful for imprinting of data on record sheets.

In 1970, Zolle et al reported the preparation of metabolizable radioactive human serum albumin microspheres. Int. J. Appl. Radiat., 21, 155-167 (1970). The microspheres were prepared by dispersing droplets of a 25% solution of albumin in heated cottonseed oil with continuous stirring. Solidified microspheres were obtained after heating for 75 minutes at temperatures from 118 to 165°C. The microspheres were separated from the oil by centrifugation, washed free of oil with diethyl ether and dried in air. A similar procedure is described in Zolle United States Patent 3,937,668 for incorporating precipitated drugs and other substances in albumin microspheres. According to the Zolle patent, it is necessary to heat the oil in which the microspheres are formed to a temperature above 100°C to evaporate water and to form the



spheric albumin particles having the precipitate encapsulated therein. Scheffel et al prepared albumin microspheres for study of the reticuloendothelial system using a modification of the Zolle procedure. Aqueous albumin was homogenized at room temperature with a small quantity of the oil, and the resulting emulsion was dispersed in a body of oil heated to 175-185°C with continuous stirring. On cooling, diethyl ether was added, the microcapsules recovered by centrifugation, washed with diethyl ether, and dried. Scheffel et al J. Nucl. Med., 13, 498-503 (1972).

Hardening of such microcapsules by techniques other than denaturation of the protein are known, and include particularly treatment of the microcapsules with aqueous formaldehyde as a hardening agent. See Madan et al, J. Pharm. Sci., 65, 1476 (Oct. 1976), and United States patents 2,800,457 and 3,265,629.

The intravascularly-administrable, magnetically-localizable biodegradable carrier of the present invention comprises microspheres formed from an amino acid polymer matrix, with magnetic particles embedded therein. For example, albumin can be used as the matrix material and magnetite (Fe_3O_4) as the magnetic particles. The microspheres have a number average size of less than 1.5 microns and the magnetic particles have an average size of not over 1,000 Angstroms. The microspheres may contain from 5 to 350 parts by weight of the magnetic particles per 100 parts of the amino acid polymer. The therapeutic or diagnostic agent, which may be a water-soluble chemotherapeutic agent, is dissolved or dispersed in the matrix material during the formation of the microspheres.

For effective magnetic control, the microspheres will be introduced into an artery upstream of the capillary bed where they are to be localized, the selected capillary bed being associated with the target site. It is therefore of critical importance that the microspheres have a degree of magnetic responsiveness which permit them to pass through the arteries without significant holdup under the applied magnetic field while being immobilized and retained in the capillaries. The present invention achieves this objective by utilizing the difference in flow rates of the blood in the larger arteries and in the capillaries. In addition, the albumin surface prevents clump formation, thus allowing relatively normal blood perfusion at the area of retention. Prior to the present invention it had not been recognized or demonstrated that such discrimination in magnetic responsiveness could be obtained.

With respect to the circulatory system, mean flow velocity may be defined as the volume of blood flow through an artery, capillary, or vein divided by the cross-sectional area of the vessel. In large arteries, the velocity is of the order of 30 cm/sec, while in smaller arteries it may range from about 10 to 20 cm/sec. In veins, the flow velocity is of the order of 15 cm/sec. In contrast to the flow rates in veins and arteries, the blood flow rate in capillaries is of the order of 0.05 cm/sec. By means of a standardized test apparatus (discussed hereinafter), it was demonstrated that with an ordinary permanent bipolar magnet producing a field of 8,000 gauss the difference in arterial and capillary flow rates could be used to achieve carrier retention at the desired flow of 0.05 cm/sec while permitting passage at higher flow rates. This permits



the magnetic field to be applied at the time of the intra-arterial administration, assuring that the microspheres will be caught in the target capillary bed without at the same time immobilizing any substantial amount of the administered microspheres in the larger arteries. The microspheres carrying the therapeutic or diagnostic agent will therefore pass rapidly through the artery into which they are administered to the target capillary bed where they will be caught and retained, thereby effectively concentrating the agent at the target site.

10 While being retained in the capillary bed, if desired, the applied magnetic field can be increased in strength, causing the microspheres of 0.5-1.5 microns to be drawn through the capillary walls into the tissue, and thereby retained at the target site after the magnetic field is removed. Alternatively, the applied magnetic field can hold the microcapsules at the capillary site until proteolytic enzyme action dissolves the amino acid polymer sufficiently to release the therapeutic or diagnostic agent. Further, the release rate can readily be controlled by hardening techniques to be described below.

20 With microspheres prepared in accordance with the present invention, at least 90% of the microspheres will be immobilized by a magnetic induction of 8,000 gauss when an aqueous suspension of the microspheres is pumped at a rate of 0.05 cm/sec. through a conduit of 0.168 cm internal diameter, but not over 10% of the microspheres will be immobilized by the same magnetic induction when pumped through the conduit at a flow rate of 10 cm/sec. or greater. The details with respect to this standardized test procedure are set out subsequently.

30 The microspheres may contain the magnetic particles uniformly distributed throughout the matrix material. However,

it has been discovered in connection with the present invention that greater magnetic responsiveness is obtained when the magnetic particles are concentrated in the peripheral portions of the microspheres. Such microspheres are therefore preferred though microspheres with distributed iron are not excluded. The same size microspheres can thereby contain less of the magnetic particles and relatively more of the matrix material, which is the carrier for the therapeutic or diagnostic agent. In effect, therefore, the microspheres can be more highly loaded with the active agent.

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In the accompanying drawings, FIG. 1 is an electron photomicrograph of the preferred form of the carrier in which the magnetic particles are concentrated in the peripheral portions of the microspheres;

FIG. 2 is an illustration of a test apparatus which can be used to test the magnetic responsiveness of the microspheres; and

FIG. 3 is a fragmentary enlarged view of a portion of the apparatus of FIG. 2 wherein the microspheres are subject to a standardized magnetic field.

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The matrix material for forming the microspheres is an amino acid polymer. Such polymers are biodegradable by proteolytic enzyme action. Usable amino acid polymers include natural amino acids (proteins) and synthetic amino acid polymers. The preferred polymer is albumin, which may be animal or human albumin, but is preferably human serum albumin (HSA). Other water-soluble proteins such as hemoglobin can be substituted for albumin, the preference being for human hemoglobin. The albumin matrix material may be modified by using it in combination with a minor proportion of other biodegradable amino

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acid polymers. For example, from 0 to 25 parts by weight (dry basis) of hemoglobin (preferably human hemoglobin) or a synthetic amino acid polymer can be combined with 75-100 parts of albumin. Usable synthetic amino acid polymers include poly-L-lysine and poly-L-glutamic acid. For example, a poly-L-lysine or poly-L-glutamic acid in the molecular weight range of 20,000-50,000 can be used alone or in combination with another polymer such as albumin. However, since human serum albumin is a nearly ideal material for the purpose of the present invention, there is no necessity to use other comparable amino acid polymers. At the same time, however, such amino acid polymers are within the scope of this invention.

The magnetic particles include ferri- and ferro-magnetic compounds, such as magnetic iron oxides. The preferred magnetic particles are the black oxide of iron, magnetite (Fe_3O_4). Carbonyl iron of appropriate size can be used instead of the Fe_3O_4 .

It is essential that the magnetic particles be in an ultra-fine state of subdivision. The magnetic particles should have an average size of not over 1,000 Angstroms, and preferably not over 300 Angstroms. The optimum size range for use in microcapsules of less than 1.5 microns average diameter (preferably less than 1.2 microns) is from about 50 to 250 Angstroms.

Techniques are known for producing such extremely small size magnetic particles. These include fine grinding, vacuum deposition, and chemical precipitation. Fine grinding in a ball mill can be used to produce a colloidal suspension of magnetic particles. (Commercially, fine powders or suspensions of Fe_3O_4 are available from Ferrofluidics Corporation, Burlington,

Massachusetts.) The size range of the particles is from 100 to 200 Angstroms. Aqueous base suspensions of the Fe_3O_4 particles with or without a surfactant can be used, but it is preferred to employ surfactant-free magnetic particles, such as Fe_3O_4 in a dispersed homogeneous suspension or in a dry powder form.

The carrier of this invention can be used for administering a wide variety of therapeutic or diagnostic agents. The agent may be incorporated in the amino acid polymer as a powder, or if water-soluble, in the form of a water solution. The carrier of this invention is believed to be of particular value for administering water-soluble chemotherapeutic agents, such as anti-cancer agents whose use is now limited because of adverse side effects. Heat-labile therapeutic agents can be used such as natural products since the microcapsules can be prepared at temperatures where the therapeutic agent is stable.

In practicing the present invention, from 5 to 150 parts by weight of the magnetic particles can be employed per 100 parts of the amino acid polymer. This will result in microspheres containing corresponding proportions of the matrix material and magnetic particles. The preferred amount of magnetic material is from 10 to 150 parts by weight per 100 parts of the amino acid polymer. The amount of the therapeutic or diagnostic agent can vary over a wide range, depending on the purpose for which the microspheres are to be used. However, in general, for water-soluble chemotherapeutic agents, from 1 to 20 parts by weight of the agent can be incorporated per 100 parts by weight of the matrix material. It will be understood, however, that the relative proportions of the therapeutic or diagnostic agent to the matrix material are not critical.

In preparing the microspheres, an aqueous solution or dispersion of the matrix material is prepared, which can be formed into microspheres. The amount of matrix material to be used will usually be within the range from 5 to 50 parts by weight of the matrix material per 100 parts of water. With albumin and similar matrix materials preferred proportions are from 20 to 30 parts per 100 parts of water. Where a water-soluble therapeutic agent is being incorporated, it may be dissolved in the water of the matrix material solution, either before or after preparing the matrix solution.

The aqueous solution of the matrix material containing the therapeutic or diagnostic agent, either dissolved or in particulate form, is emulsified with an oil, which is preferably a vegetable oil, such as cottonseed oil, peanut oil, or the like. Other oils or suitable non-polar solvents for forming water-in-oil emulsions can be used. The aqueous phase at the time of addition of the oil will also contain the magnetic particles, which were previously added to the aqueous solution of the matrix material and dispersed therein. The proportions of the aqueous phase to the oil phase can conveniently range from about 1 to 5 parts by weight of the aqueous phase per 100 parts of the oil phase. This provides separation of the oil droplets, and prevents coalescence of the droplets in forming the microspheres. The water-in-oil emulsion is then treated to reduce the size of the dispersed droplets such as to an average size of below 3.0 microns. Procedures such as homogenization, or sonication, or both can be used. The resulting emulsion should be as homogeneous as possible. Where the microspheres are being prepared for intravascular administration, the completed emulsion should contain dispersed water

droplets of an average size of less than 1.5 microns and preferably of an average size of less than 1.2 microns, corresponding to the desired size of the microspheres. If desired, an emulsifying agent may be used, but one is not needed and preferably is not used.

In one procedure, the emulsion is then added to a larger body of oil, which is preferably the same oil used to form the emulsion. In practice, cottonseed oil has been found to give good results. To promote the separation of the water droplets, the emulsion can be added in small increments to the oil bath, such as by dropwise addition. Preferably, also, the addition is accompanied by rapid stirring of the oil into which the emulsion is being introduced.

Alternatively, the aqueous albumin may be emulsified with the whole body of oil, such as by gradual or incremental addition of the oil to the albumin solution, using the droplet dispersion procedures referred to above.

Where the therapeutic or diagnostic agent contained in the emulsion is not heat sensitive, the oil bath into which the emulsion is introduced can be heated to a temperature at which the matrix material, such as albumin, is partially denatured and hardened. For maximum hardening, temperatures in excess of 100°C can be used, such as temperatures ranging from about 125 to 175°C. A lesser degree of hardening and denaturation can be obtained at temperatures within the range from 50 to 100°C. Where heat-hardening is employed, no chemical treatment is needed to harden the microspheres.

For incorporation of water-soluble heat-labile chemotherapeutic agents in the microspheres, it has been found that the process can be carried out at essentially room temperature

The body of oil into which the emulsion is introduced can be maintained at a temperature at which there is no inactivation of the chemotherapeutic agent, such as a temperature in the range of 1 to 45°C. Usually, it will not be necessary to either heat or cool the body of oil, using an essentially ambient temperature, such as a temperature ranging from about 20 to 30°C.

It has been found that although there is no heat-denaturation of the matrix material, such as albumin, the microspheres after introduction into the oil bath will maintain morphology and integrity as separate microspheres in a non-water miscible organic solvent, such as diethyl ether, ligroin, benzene, hexane, petroleum ether, and the like. The oil may be removed by washing with the organic solvent, such as diethyl ether, and the microspheres suspended in the organic solvent for further processing. The organic solvent can be removed by centrifugation and/or evaporation, and the resulting microcapsules dried, preferably by lyophilization. The resulting product has a relatively rapid drug release rate in water or serum, but the lyophilized microspheres if not subjected to proteolytic enzyme action will continue to retain and release a water soluble agent over periods up to 48 hours.

Where a slower release rate is desired, and particularly where greater resistance to proteolytic enzyme degradation is needed, the microspheres after being formed and before drying can be treated with a cross-linking agent to increase their stability and decrease the drug release rate from the microspheres. Hardening of amino acid materials such as albumin can be accomplished, as is known in the art, by treatment with

a glyoxal or aldehyde. Specific reagents include dimethyl glyoxal, glyoxal, diphenyl glyoxal, formaldehyde, 2,3-butanedione, and similar aldehydes. The glyoxal or aldehyde is preferably soluble in the organic solvent used to wash the microcapsules free of oil. For example, the organic solvent can contain a concentration of 0.2% to 20% by weight of the cross-linking agent, and may be contacted with the microspheres after or during the removal of the oil for from 5 to 120 minutes, depending on the degree of cross-linking desired. In
10 general, the greater the amount of cross-linking, the slower will be the release rate for the water-soluble chemotherapeutic agent.

After completion of the cross-linking step, the microspheres can be washed free of excess cross-linking agent with a suitable organic solvent, as described above, such as diethyl ether, the residual solvent evaporated, and the microspheres dried, such as by lyophilization.

Formaldehyde is a particularly desirable cross-linking agent, but is generally available commercially only as
20 a water solution, such solutions contain from 4 to 37% by weight formaldehyde together with a small amount of methanol as a stabilizer. Although formaldehyde is preferentially water soluble, it can be transferred to an organic solvent, such as the solvents described above, by adding a salt to the water solution. Ammonium sulfate can be used for this purpose at a concentration in the aqueous formaldehyde of about 60 to 80% by weight. The organic solvent containing the transferred formaldehyde can then be used for treating the microspheres to cross-link the matrix material.

The process of the present invention can be used for encapsulating any water-soluble therapeutic agent. As indicated, however, one process is preferably applied to therapeutic agents which are heat-sensitive, and which would be damaged by prolonged heating, particularly heating at temperatures above 100°C. With many of such therapeutic agents, however, partial inactivation may occur at temperatures in the range of 50 to 100°C, and at those temperatures, at least partial denaturation of the albumin could be expected. Therefore, the process of the present invention is preferably carried out at a temperature in the range of 1 to 45°C, such as 20 to 30°C, or essentially ambient room temperature. The kinds of therapeutic agents which may be encapsulated include enzymes, chemotherapeutic agents, immunological adjuvants, and various natural products. Such water-soluble, heat-labile therapeutic agents include the following:

Enzymes

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|------------------|-----------|
| trypsinogen | insulin |
| chymotrypsinogen | glucagon |
| plasminogen | coumarin |
| streptokinase | heparin |
| adenyl cyclase | histamine |

Chemotherapeutic Agents

- | | |
|---|---|
| tetracycline | isoniazid |
| aminoglycosides | rifampin |
| penicillin group of drugs, + cephalosporins | water-soluble alkylating agents in Ca therapy |
| sulfonamide drugs | water-soluble antimetabolites |
| chloramphenicol sodium succinate | antinomycin D |
| erythromycin | mithramycin |
| vancomycin | daunomycin, adriamycin |
| lincomycin | bleomycin |
| clindamycin | vinblastine |
| nystatin | vincristine |
| amphotericin B | L-asparaginase |
| amantidine | procarbazine |
| idoxuridine | imidazole carboxamide |
| p-amino salycilic acid | |

Immunological Adjuvants

concanavalin A
BCG
levamisole

Natural Products

prostaglandins, PGE₁, PGE₂
cyclic nucleotides
TAF antagonists
water-soluble hormones
lymphocyte inhibitors
lymphocyte stimulatory products

As will be discussed in further detail and illustrated by the following examples, the carrier microspheres prepared in accordance with this invention are capable of being immobilized at the rate of blood flow in capillaries while not being retained in the arteries to which they are introduced under the same magnetic field, the difference in magnetic responsiveness or retention being due to the difference in blood flow rates between arterial and capillary flow. For the purposes of the present invention, at least 90% of the microspheres should be immobilized by a magnetic induction of 8,000 gauss when an aqueous suspension of the microspheres is pumped at a rate of 0.05 cm/sec. through a conduit of 0.168 cm internal diameter. However, not over 10% of the microspheres should be immobilized by the same magnetic induction when pumped through the same conduit at a flow rate of 10 cm/sec. or greater. Preferably, at least 90% of the microspheres are immobilized by the described procedure at a flow rate of 0.05 cm/sec. but not over 5% of the microspheres are immobilized at the flow rate of 10 cm/sec. As will be described and further illustrated in the examples, for test purposes the magnetic induction is applied by a bipolar magnet with its poles equidistant from the centerline of the tube through which the suspension is being pumped,

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and the 8,000 gauss field is referenced to a plane intersecting the tube at right angles to the direction of flow and extending for at least 10 cm in the direction of flow.

For further details, reference should be made to the following non-limitive examples.

Example I

125 mg human serum albumin (HSA), 10 mg bulk purified adriamycin HCl, and 36 mg Fe_3O_4 powder (200 Å average particle size) was placed in a 50 ml beaker and dissolved and suspended respectively in 0.5 ml distilled water. For experimental purposes, the albumin may be trace labeled with 0.1 mg ^{125}I -bovine-serum-albumin. The suspension was stirred well to evenly disperse the Fe_3O_4 in the albumin-adriamycin solution, but no surfactant was employed to aid the dispersion. Next 30 ml of cottonseed oil was added to the suspension forming a water-in-oil emulsion, which was then stirred well to disperse the aqueous phase into the oil.

The resultant emulsion was homogenized by sonication (Branson Sonifier Model 185) at 100 watts for one minute at 4°C. Next, the homogenate was added dropwise into 100 ml of cottonseed oil at 25°C being constantly stirred at 1800 RPM for 10 minutes to fully disperse the emulsion.

The oil was then removed by washing 4 times in 60 ml diethyl ether anhydrous and centrifuged at 2000 x g for 30 minutes. After the fourth wash the oil free microspheres were then hardened by a formaldehyde 1% w/v solution in 100 ml ether (8 mg microspheres/ml ether-formaldehyde solution). The ether-formaldehyde solution was prepared by transferring aqueous formaldehyde to the ether phase by shaking a 1:5 (37% aqueous formaldehyde: ether) solution in the presence of saturating

ammonium sulfate. The amount of formaldehyde transferred at this ratio was determined in a separate study using tritium labeled formaldehyde (1.5 mCi/1.5 mg) as a trace label in the 37% aqueous solution. The hardening was accomplished by dispersing the washed microspheres in the formaldehyde/ether and stirring at 100 RPM for the desired time (5 min to 2 hrs), depending on the extent of hardening desired. After hardening was terminated, the formaldehyde cross-linking reagent was removed by centrifugation in ether, four times. Any remaining ether was allowed to evaporate and the resultant material was further processed by lyophilization, and then stored at 4°C.

The microcapsule product contained approximately by weight 21% Fe₃O₄, 73% albumin, and 6% adriamycin. Examination by immersion fixation-transmission electron microscopy confirmed that the microcapsules were generally spherical in shape and of an average size of about 1 micron. The appearance of the microcapsules is shown in Fig. 1 (Transmission E.M. Mag. = X28,000).

The fixation and processing procedures used for the electron microscopy were as follows:

The microspheres were placed in paraformaldehyde-glutaraldehyde for 0-2 hrs. They were then washed in cacodylate buffer, dehydrated in a graded series of alcohols and embedded in Epon 812. Thin sections were stained with uranyl acetate followed by lead citrate. Thick sections were stained with toluidine blue.

Refereing to Fig. 1, it will be noted that there are a few aberrant microspheres (A) which are non-spherical.

However, the general uniformity of the microspheres with respect to both shape and size distribution is evident. Some

of the microspheres appear to contain relatively large vacuoles (V), but most appear to have substantially solid albumin matrices (C).

The magnetic iron particles (P) of Fe_3O_4 are concentrated in the peripheral portions of the microspheres. No particle-dispersing surfactant was used in preparing these microcapsules. When the Fe_3O_4 is incorporated in the microcapsules in the form of an aqueous suspension containing the surfactant, the magnetic particles tend to disperse throughout
10 the microcapsules relatively uniformly.

Example II

The procedure for preparing the microspheres was identical to that of Example I except that 135 mg Fe_3O_4 was used instead of the 36 mg of Example I. The microcapsule product contained approximately by dry weight 50% Fe_3O_4 , 4% adriamycin, and 46% albumin.

Example III

Microcapsules were prepared by the identical procedure of Example I, using approximately the same amount of
20 Fe_3O_4 as in Example II. The Fe_3O_4 was in the form of an aqueous suspension containing a surfactant, aqueous base Ferrofluidics Fe_3O_4 Catalog No. A-01, 400 gauss saturation (Ferrofluidics Corporation, Burlington, Massachusetts). 0.3 ml of the A-01 product was added, containing approximately 130-140 mg Fe_3O_4 . The average Fe_3O_4 particle size was in the range of 150-200 Angstroms. The microcapsular product contained approximately by dry weight 51% Fe_3O_4 , 4% adriamycin, and 45% albumin.

Example IV

The procedure of Example I was followed except that the homogenate was added to 100 ml of preheated oil (135°C) for 10 minutes. Washing is as described previously, but the aldehyde hardening is omitted. The rest of the procedure is the same.

Example V

A. Same procedure as Example I except microspheres are not hardened by a cross-linking agent or by heat. The oil bath is at a temperature of 20-25°C. After the oil has been washed away with diethyl ether anhydrous 4 times, the spheres are air dried, then lyophilized and stored at 4°C.

Stability of the resultant microspheres was tested as follows: First, 5 microliters of ¹²⁵I-bovine serum albumin (New England Nuclear, 1.51 mCi/mg) was added in the initial homogenate to trace label the microspheres. An aliquot of the resultant microspheres was then suspended and sonicated for 2 minutes in 0.154 M NaCl-0.1% Tween 80 and incubated at 37°C for 24 and 48 hours. After this period of time, the suspension was centrifuged at 2000 x g for 10 minutes and the supernatant and pellet were counted in a gamma counter. The number of counts obtained in the supernatant (after subtracting free label) divided by the total number of counts was regarded as the percentage breakdown of the carrier (non-pelleting). Only 16% of the microspheres had deteriorated after 24 hrs. and 37% after 48 hrs. With formaldehyde or heat-hardening less than or equal to 3% deterioration occurs in 48 Hrs.

B. Same procedure as Example I except 3,400 units of urokinase was added to the 125 mg of HSA omitting the Fe₃O₄ and adriamycin. No cross-linking was done in this experiment.

Two mg of the resultant microspheres were placed into 16 12x75 mm tubes for duplicate time course of 0, 15, 30, 60 minutes; 2, 4 and 6 hours. At the appropriate time, microspheres were suspended in sodium barbital buffer (0.05M) and left at room temperature. Finally at zero time all tubes were centrifuged at 3,500 RPM (1900 x g) for 15 minutes at 4°C and 25 microliters of the supernatants were pipetted into appropriate wells on fibrin-agar plates. Plates were then read 4 and 6 hours later for fibrinolysis (i.e. diameters).

10 It was found that 60% of maximum lysis was seen after 10 minutes on fibrin-agar plate.

Example VI

Microspheres can be prepared by the procedure of Example I omitting the magnetic iron (Fe_3O_4), and incorporating other water-soluble therapeutic agents, such as any of those referred to above, instead of the adriamycin.

Other Examples

As a variation of the procedure of Example I, 2,3-butanedione (5% v/v in anhydrous ether) or butyraldehyde (10%
20 v/v in anhydrous ether) is employed as a cross-linking agent, the contact time ranging from 5 minutes to 2 hours. The product is recovered and dried as described in Example I.

As a further variation of the procedure of Example I, 20 mg of poly-L-lysine or polyglutamic acid is combined with the 125 mg of human serum albumin. The rest of the procedure is identical. In another modification, hemoglobin is substituted on an equal weight basis for the albumin.

Determination of Magnetic Responsiveness

The magnetic responsiveness of the microspheres under
30 varying liquid flow rates was studied under standardized con-

ditions, using the apparatus of Fig. 2. The apparatus includes a container 10 providing a reservoir containing an aqueous fluid. Normal saline was used. A pick-up tube 12 connects the reservoir through a three-way valve 13 with a syringe 14, the plunger 15 of which is driven by the pusher block 16 of a variable speed syringe pump 17. (The syringe pump was Model 314, manufactured by Sage Instruments Division, Orion Research Incorporated, Cambridge, Massachusetts.) The on-off switch is indicated at 18 and the variable speed selector at 19. Since the construction and operation of such syringe pumps are well known in the art, it will not be necessary to describe the pump mechanism herein.

Using the 50 cc syringe employed for the tests, the syringe pump had a low and high setting range which permitted the flow rate through the measurement tube of the apparatus to be varied over the range from 0 to 10 cm/sec. with a measurement tube internal diameter of 0.168 cm. During the discharge of the liquid from syringe 14, plunger 15 moves in the direction of the arrows as shown in Fig. 2, the liquid flowing through outlet nipple 20, connecting tube section 21, and valve 13 to supply conduit 22, which connects through on-off valve 23 to sample injector 24. As shown, injector 24 includes an injection chamber 25 of downwardly-converging cross-section, the lower end of which communicates with the liquid-flow passage 26. The upper end of chamber 25 is closed by a rubber diaphragm stopper 27 through which the needle of a hypodermic injection syringe 28 can be inserted.

A guide tube 29 is attached to a nipple extension at the lower end of injector passage 26. Inserted within guide tube 29 is a removable and replaceable measurement tube 30. A

series of such tubes are used. The measurement tube fits snugly within the guide tube so that the flow of liquid is through the measurement tube. Preferably, the guide tube is formed of relatively rigid material, such as plastic or glass, and the measurement tubes are formed of flexible plastic tubing such as polyethylene. For the purposes of the standardized measurements, which define the magnetic responsiveness of the microcapsules under different liquid flow conditions in accordance with the present invention, the internal diameter of the guide tube should be 0.168 cm. (cross-section 0.0222 cm²).

Intermediately between the upper and lower ends of guide tube 29 and measurement tube 30, there is located a bipolar magnet designated generally by the number 31. As indicated, the poles of magnet 31 should be equidistant from the centerline of the tubes 29, 30. An adjustable gap permanent U-magnet can be used capable of generating a magnetic induction in the range of 7500-8500 gauss with a pole spacing permitting straddling of the guide tube. (In the tests described herein, the magnet was Model No. 70,810 Adjustable Gap Permanent Magnet, Edmund Scientific Co., Barrington, N. J.) In Fig. 2 the complete magnet is not illustrated, but only the adjustable pole shoes 31a and 31b. By varying the spacing between the inner ends of the pole shoes the magnetic induction can be selectively varied. The pole shoes were adjusted to a separation of approximately 3/16 inches and until a magnetic induction of 8,000 gauss was obtained. The measured 8,000 gauss magnetic field for standardization purposes is referenced to a plane intersecting the measurement tube 30 at right angles to the direction of flow through the tube. Measurement of the magnetic induction was made with Bell Model 600 Gauss meter

(4) Turn valve 13 to connect syringe 14 with tube 22, and open valve 23.

(5) Start pump 17 to fill tube 22, injector 24 (except for a small air space below diaphragm 27), the upper end of guide tube 29, and measurement tube 30.

(6) Stop pump 17 and close valve 23.

(7) Inject bolus of microspheres to be tested with hypodermic syringe into injection chamber 28 of injector 24. A 0.1 ml. bolus was used, but this can be varied.

10 (8) Open valve 23 and start pump 17, the rate of travel of pusher 16 having been selected in relation to the 50 cc size of syringe 14 to give a selected uniform flow rate through the 0.168 cm I.D. sample tube (e.g. 0.05 cm/sec, etc.).

(9) The flow of the water carrier (normal saline) aspirates the microspheres from the injection chamber 25 into the liquid stream passing between the poles of the magnet 31.

20 (10) The flow is continued until all of the magnetic material has been removed from the injection chamber and has either collected within the section of the measurement tube subject to the magnetic field, or has passed with the flowing liquid into the first collection vessel 33. The retained or immobilized microspheres are indicated in Fig. 3.

(11) For each sample, the test can be repeated at different flow rates such as 0.05 cm/sec and 10 cm/sec to determine the difference in magnetic retention. The non-retained fraction collected first in container 33 includes the microspheres that are not immobilized by the magnetic field at the particular flow rate.

30 (12) The syringe pump is turned off, valve 23 closed, and the lower end of sample tube 30 is clamped or otherwise closed.

using a transverse probe (F. W. Bell, Inc., Columbus, Ohio). These measurements indicated that the 8,000 gauss field was substantially uniform between the opposed parallel ends of the magnet shoes 31a, 31b, as represented by the distance x. The extent of the magnetic field along the direction of flow is not critical, providing the flow-direction of the field is sufficient to permit the magnetic force to act on the microcapsules. For purpose of standardization, it is specified that the reference 8,000 gauss field should extend for at least 10
10 cm in the direction of flow (the distance "x"). To avoid any inaccuracy due to acceleration effects, the upper end of the measurement tube 30 should extend above the area of the magnetic field, such as by the distance "y". This distance is not critical, but in practice it was found that a 20 cm extension of the tube was satisfactory. However, greater or lesser extensions can be used.

Conveniently, a clamp may be used for closing the projecting lower end of measurement tube 30 after the completion of a measurement run, such as hemostat clamp 32. It
20 will be understood that a series of the measurement tubes 30 will be used, as well as a series of sample collection containers 33, 34.

In using the apparatus of Fig. 2, the following steps are followed:

- (1) Insert measurement tube 30 in guide tube 29, as shown in Fig. 2.
- (2) With valve 23 in the off position, turn valve 13 to connect tube 12 to the syringe 14 using a 50 cc syringe.
- (3) Fill the syringe with the fluid from the reservoir, plunger 15 and pusher 16 moving to their outermost positions.
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(13) The sample tube is withdrawn, and the liquid is drained into a second sample collection vessel 34. To eliminate possible error due to microspheres remaining within the tubing 30 after draining, it is cut up and added to the liquid, as indicated in Fig. 2.

(14) The microsphere content of the two samples is measured conveniently by using trace labeled microspheres (e.g. ^{125}I -albumin) and a gamma counter. (See Example I.)

In the test results reported below, a bolus of 0.1 ml of the magnetic microspheres (1 mg/ml) trace labeled with ^{125}I -albumin (4×10^4 CPM/mg microspheres) was employed, the carrier liquid being 0.9% saline containing 0.1% Tween 80. The injection was 20 cm upstream of the zone of the magnetic field. The counting of the non-retained and retained fractions was made with a Packard Model 578 well-type gamma counter. The amount retained was then calculated as a percent of the total (retained fraction plus non-retained fraction). Reproduceability was found to be $\pm 2\%$ with at least two determinations of each measured value. In the operation of the test apparatus, it will be understood that the liquid flow should be laminar and uniform.

The comparison of trace-labeled samples of microspheres, prepared as described in Examples I and II, is summarized below in Table A. The data demonstrates that magnetic retention varies reproducibly with flow rate, and shows that over the range from 0.05 to 10 cm/sec that the retention can be varied from substantially complete to substantially no retention. For the microspheres containing 21% Fe_3O_4 and flowing at 0.05 cm/sec, 99% were retained, but only 0.2% at 9.8 cm/sec.

The microspheres containing 50% Fe₃O₄ (dry weight basis) were 99% retained at 0.05 cm/sec but only 8-10.7% retained at flow rates of 6.60-9.80 cm/sec.

Table B as set out below represents a comparison of magnetic responsiveness of the microspheres prepared in Example II with those of Example III. The microspheres are of the same size (average 1 micron diameter) and contain approximately the same amount of magnetic iron (50-51%) but the magnetic particles are differently distributed. With peripheral type distribution greater magnetic responsiveness is obtained. This makes it possible to use a lesser proportion of the magnetic particles in relation to the matrix material, permitting larger amounts of a therapeutic agent to be incorporated with the matrix material in the same size microspheres.

TABLE A

Retention of Microspheres
at 8,000 Gauss

Velocity (cm/sec)	% Retention	
	21% Fe ₃ O ₄	50% Fe ₃ O ₄
0.05	97.0	99.0
0.20	85.3	98.0
0.50	66.5	94.6
0.70	56.2	91.9
1.00	40.1	78.3
1.60	24.2	66.0
2.30	19.2	51.0
3.30	9.6	40.1
5.00	6.1	16.3
6.60	5.3	8.0
9.80	0.2	10.7

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TABLE B

Comparison Magnetic Responsiveness
of Microspheres with Peripheral
and Dispersed Magnetic Material

Velocity (cm/sec)	% Retention	
	Dispersed	Peripheral
0.05	99.0	99.3
0.20	98.0	99.5
0.50	94.6	95.4
0.70	91.9	89.9
1.00	78.3	88.1
1.60	66.0	83.1
2.30	51.0	71.0
3.30	40.1	58.0
5.00	16.3	35.6
6.60	8.0	29.1
9.80	10.7	10.4

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In Vivo Tests

Microspheres containing approximately 21% Fe₃O₄ were prepared as described in Example I using trace labeled albumin.

The microspheres were tested in vivo. The model chosen, based on ease of manipulation and access, was the ventral caudal artery in the tail of the rat. The animals used were 400 gram female retired breeder rats. The artery was partially exposed at the base of the tail and a polyethylene catheter presoaked in a 0.6% heparin 1000 in saline solution was inserted caudally 4 cm. A permanent bipolar magnet with a field strength of 8000 Oe was placed 7 cm caudally from the point of insertion of the catheter. Varying amounts of microspheres, suspended in 0.1% Tween 80 in 0.9% NaCl, were infused by a constant flow syringe pump (Sage, Model 341) at 0.06 ml/min which corresponds to the blood flow rate of this artery previously determined. After infusion the catheter was removed and the magnet was retained in position for thirty minutes. A transcutaneous Doppler apparatus (Parks Electronics Lab, Model 881-A) was used to verify resumption of blood flow following

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removal of the catheter from the artery. After the thirty minute period, the rat was sacrificed via intracardiac injection of saturated KCl and the organs were removed and counted for ^{125}I activity in a Packard (Model 578) gamma counter. The tail was cut into four equal sections, and each section was counted individually. Results of this study are shown in Table C. Multiple animals for each field strength were used to determine the average ^{125}I -magnetic-microsphere body distribution.

10 In a modification of the foregoing procedure, the animals were sacrificed 24 hours after removing the tail from the magnetic field. Once again, 50% of the injected counts were found at the target site. This phenomenon, as well as the carrier distribution in the skin, suggests the possibility that the carrier is lodging in the vascular endothelium or possibly traversing the vascular basement membrane into interstitial tissue due to the magnetic force applied. This phenomenon would be extremely desirable as the microspheres would act as extra vascular depots releasing the drug at a fixed rate at a
20 desired target site.

 An additional animal model was chosen to illustrate carrier localization. In this study the carrier was localized to the lungs of BDF_1 female mice. A modification of the above procedure for introduction of the carrier consisted of tail vein injection rather than catheterization, and the use of a unipolar magnet instead of a bipolar to generate the field. Thirty minutes following injection, the mouse was sacrificed and the organs counted for ^{125}I activity as described above. In the experimental animals, 45-50% of the total counts in-

jected were found in the lung as compared to 6-12% of the
counts found in control animals lungs.

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TABLE C

Average Percent Distribution Of
¹²⁵I-Magnetic Microspheres

MAGNETIC FIELD	TAIL SECTION				ORGAN				
	1	2	3	4	Liver	Spleen	Kidney	Lung	Heart
GAUSS	1	2	3	4	Liver	Spleen	Kidney	Lung	Heart
No field	0	0	0	0	80	10	1	10	0
4000	0	0	3	0	85	7	1	5	0
6000	0	0	19	-	57	8	1	12	0
8000	0	1	50	0	39	4	1	7	0

CLAIMS

1. An intravascularly-administrable, magnetically-localizable biodegradable carrier, comprising microspheres formed from an amino acid polymer matrix with magnetic particles embedded therein, said microspheres having an average size of less than 1.5 microns and said magnetic particles having an average size of not over 1,000 Angstroms, said microspheres containing from 5 to 350 parts by weight of said magnetic particles per 100 parts of said amino acid polymer, at least 90% of said microspheres being immobilizable by a magnetic induction of 8,000 gauss when an aqueous suspension of said microspheres is pumped at a rate of 0.05 centimeters per second through a conduit of 0.168 centimeter internal diameter but not over 10% of said microspheres being immobilizable by said magnetic induction when pumped through said conduit at a flow rate of 10 centimeters per second, said magnetic induction being applied by a bipolar magnet with its poles equidistant from the centerline of said tube, said 8,000 gauss being referenced to a plane intersecting said tube at right angles to the direction of flow and extending for at least 10 centimeters in the direction of flow.

2. The carrier of claim 1 in which said magnetic particles are concentrated in the peripheral portions of said microspheres.

3. The carrier of claim 1 in which said microspheres contain from 10 to 150 parts by weight of said magnetic material per 100 parts of said amino acid polymer.

4. The carrier of claim 3 in which said magnetic material is Fe_3O_4 and said amino acid polymer is albumin.

5. The carrier of claim 1 in which said microspheres have an average size of less than 1.2 microns, said magnetic particles have an average size of not over 300 Angstroms, said microspheres containing from 10 to 150 parts by weight of said magnetic particles per 100 parts of said amino acid polymer, and at least 95% of said microspheres are immobilized by the magnetic induction of 8,000 gauss as further indicated by claim 1.

10 6. The carrier of claim 5 in which said magnetic particles are concentrated in the peripheral portions of said microspheres.

7. The carrier of claim 5 in which said magnetic material is Fe_3O_4 and said amino acid polymer is albumin.

8. The process of incorporating a water-soluble therapeutic agent in albumin microspheres, comprising the process in which all steps thereof are carried out at a temperature within the range from 1 to 45°C, including the steps of preparing an aqueous albumin solution of the said therapeutic agent, said solution containing from 5 to 50 parts by
20 weight of albumin per 100 parts of water and from 1 to 20 parts by weight of said therapeutic agent per 100 parts of albumin, emulsifying said solution with a vegetable oil to form a water-in-oil emulsion containing dispersed droplets of the albumin solution, removing the oil by washing the dispersed droplets with an oil-soluble water-immiscible organic solvent, and recovering the resulting microspheres in a portion of said organic solvent.

9. The process of claim 8 in which said solution contains from 20 to 30 parts by weight of albumin per 100
30 parts of water.

10. The process of claim 8 in which said microspheres in said organic solvent are further treated by adding a hardening agent selected from aldehydes and glyoxals to said solvent which is soluble therein.

11. The process of claim 10 in which said hardening agent is formaldehyde, which is added to said organic solvent by transference from an aqueous solution thereof.

12. The process of incorporating a water-soluble heat-sensitive therapeutic agent in intravascularly-admin-
10 istrable magnetically responsive microspheres, comprising the process in which all steps are carried out at a temperature in the range of 1 to 45°C, including the steps of preparing an aqueous albumin solution of said therapeutic agent, said solution containing from 5 to 50 parts by weight of albumin per 100 parts of water and from 1 to 20 parts by weight of said therapeutic agent per 100 parts of albumin, said solution also containing from 5 to 350 parts by weight of magnetic
20 particles per 100 parts of said albumin, said magnetic particles having an average size of not over 1,000 Angstroms, emulsifying said solution with a vegetable oil to form a water-in-oil emulsion containing dispersed droplets of the albumin solution, reducing the size of the dispersed droplets to an average size of less than 1.5 microns, removing the oil by washing dispersed droplets with an oil-soluble water-immiscible organic solvent, and recovering the resulting microspheres in a portion of said organic solvent.

13. The process of claim 12 in which said solution contains from 20 to 30 parts by weight of albumin per 100 parts of water.

14. The process of claim 12 in which said micro-
 spheres in said organic solvent are further treated by adding a
 hardening agent selected from aldehydes and glyoxals to said
 solvent which is soluble therein.

15. The process of claim 14 in which said hardening
 agent is formaldehyde, which is added to said organic solvent
 by transference from an aqueous solution thereof.

16. The process of incorporating a water-soluble
 heat-sensitive therapeutic agent in albumin microspheres,
 10 comprising the process in which all steps thereof are carried
 out at a temperature of from about 20 to 30°C, including the
 steps of preparing an aqueous solution of human serum albumin
 containing from 5 to 50 parts by weight of albumin per 100
 parts of water, said solution also containing from 1 to 20
 parts by weight of said therapeutic agent per 100 parts of
 albumin, emulsifying said solution with a vegetable oil to
 form a water-in-oil emulsion containing dispersed droplets
 of the albumin solution, reducing the size of the dispersed
 droplets of said solution to an average size of less than
 20 3 microns, introducing said emulsion into a body of vegetable
 oil to further disperse said droplets, removing the oil by
 washing the dispersed droplets with an oil-soluble water-
 immiscible organic solvent, and recovering the resulting
 microspheres in a portion of said organic solvent.

17. The process of claim 16 in which said solution
 contains from 20 to 30 parts by weight of said human serum
 albumin per 100 parts of water.

18. The process of claim 16, in which said micro-
 spheres in said organic solvent are further treated by adding a

hardening agent selected from aldehydes and glyoxals to said solvent which is soluble therein.

19. The process of claim 18 in which said hardening agent is formaldehyde, which is added to said organic solvent by transference from an aqueous solution thereof.

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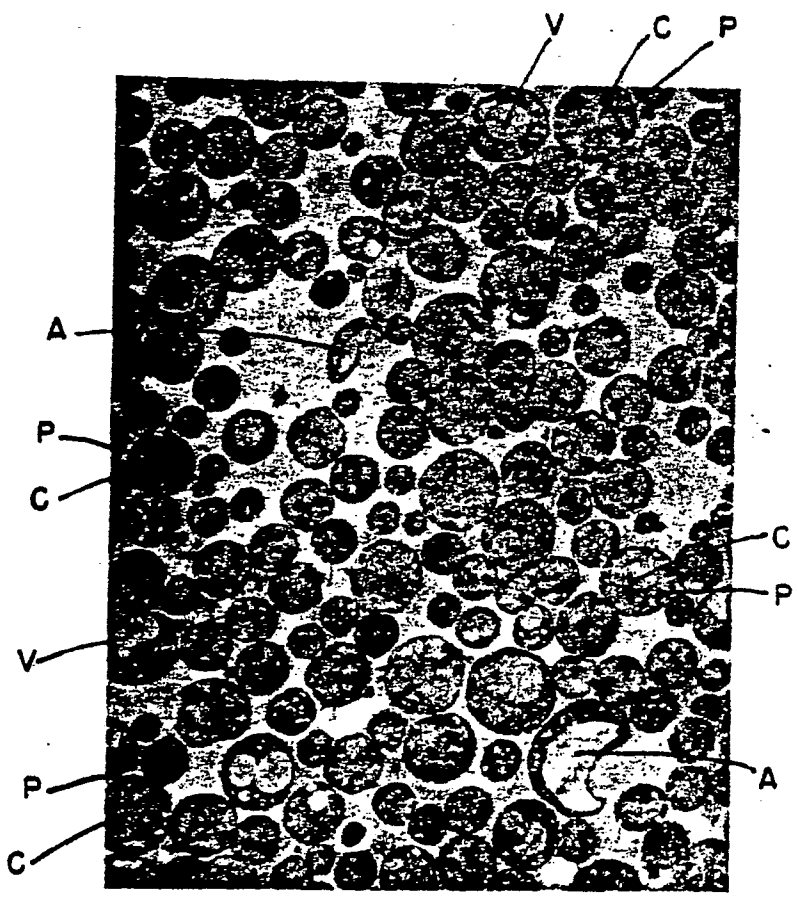
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FIG. 1



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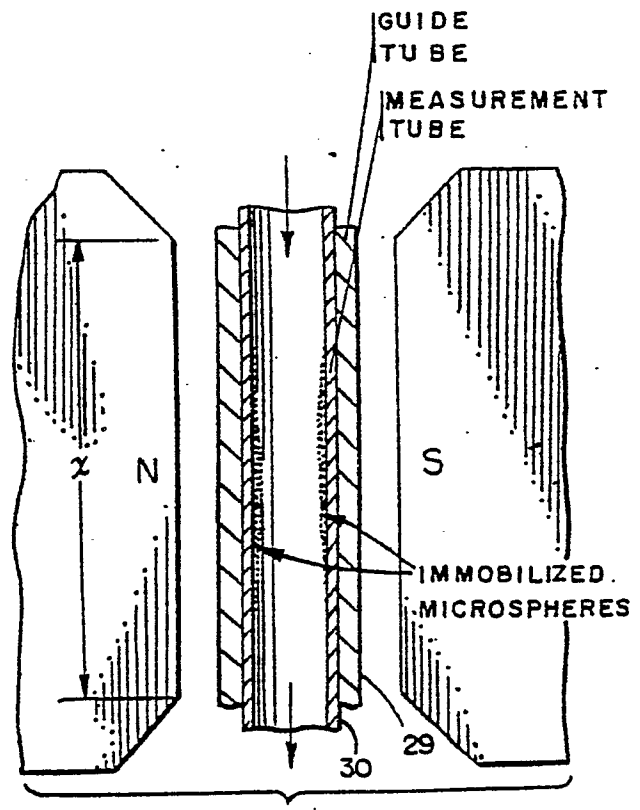
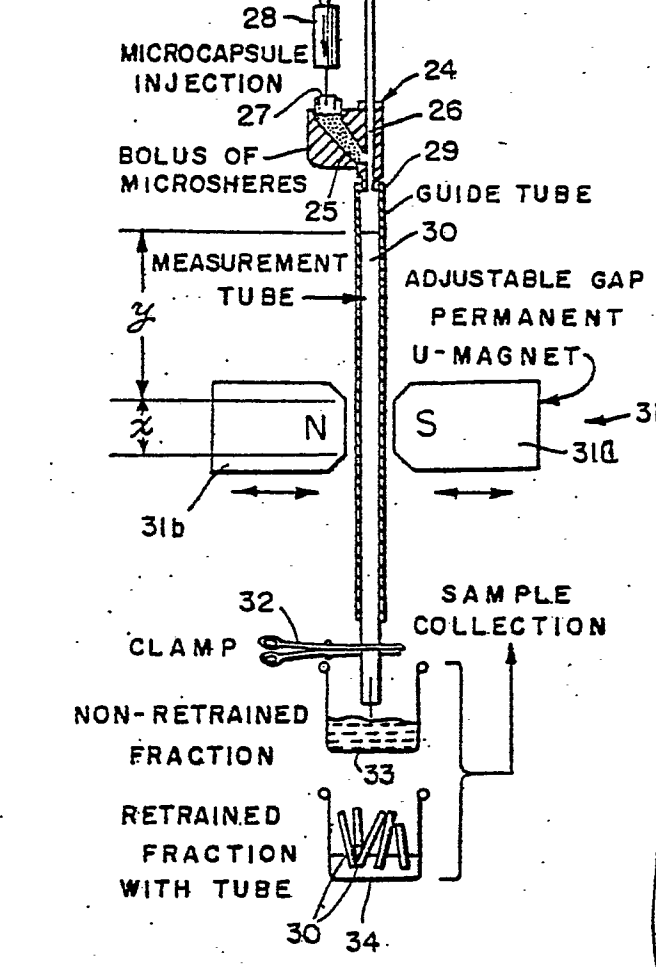
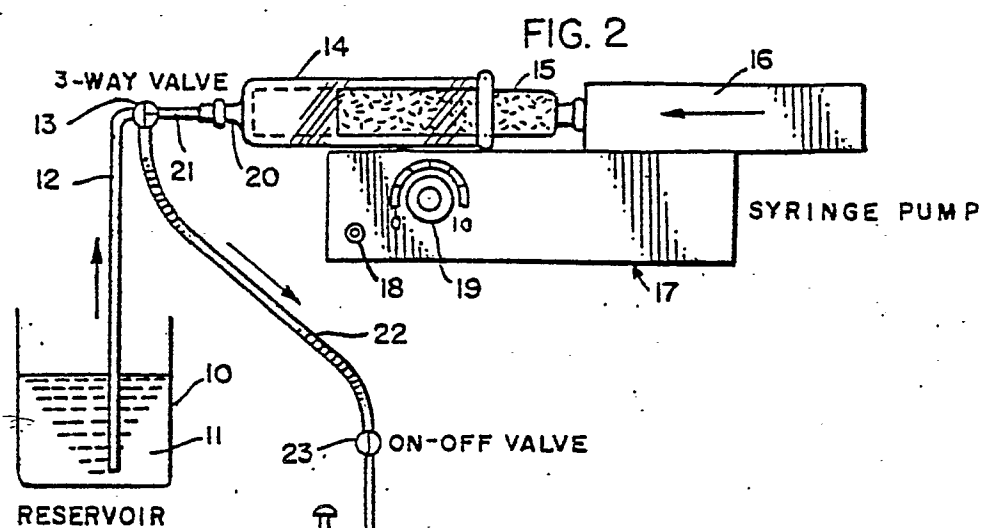


FIG. 3



DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
	<p><u>US - A - 3 474 777</u> (FIGGE et al.)</p> <p>* Column 2, line 33 - column 4, line 12; the claims *</p> <p>---</p> <p>CHEMICAL ABSTRACTS, vol. 80, no. 5, March 11, 1974. Columbus, Ohio, USA TAKAI et al: "Coated magnetic powder".</p> <p>& <u>JP - A - 73 24246</u> (TDK ELECTRONICS)</p> <p>* Page 298, abstract no. 52392a *</p> <p>---</p>	<p>1,2,4, 5,6</p> <p>1,2,4, 5,6</p>	<p>A 61 K 9/50 A 61 K 9/52 A 61 K 9/00</p>
			TECHNICAL FIELDS SEARCHED (Int.Cl. ³)
			<p>A 61 K 9/50 A 61 K 9/52 A 61 K 9/00</p>
D	<p><u>US - A - 3 937 668</u> (ZOLLE)</p> <p>* Column 1, line 6 - column 6, line 61; the claims *</p> <p>---</p>	1-19	
	<p><u>GB - A - 929 401</u> (UPJOHN)</p> <p>* Page 1, lines 11-43; page 2, lines 73-83; page 3, lines 17-30; page 5, lines 18-26 *</p> <p>---</p>	1-19	
A	<p><u>FR - A - 2 326 934</u> (MINNESOTA MINING)</p>	1	
A	<p><u>FR - A - 1 468 601</u> (NATIONAL CASH)</p>	1	
A	<p><u>FR - A - 1 351 358</u> (NATIONAL CASH)</p>	1	
A	<p><u>US - A - 3 725 113</u> (CHANG)</p>	1	
A	<p><u>US - A - 3 190 837</u> (BRYNKO et al.)</p>	1	
A	<p><u>US - A - 3 057 344</u> (ABELLA et al.)</p>	1 ./.	
<p>X The present search report has been drawn up for all claims</p>			<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention E: conflicting application D: document cited in the application L: citation for other reasons</p> <p>&: member of the same patent family. corresponding document</p>
Place of search	Date of completion of the search	Examiner	
The Hague	3-11-1978	JONAS	





DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	<u>US - A - 2 971 916</u> (SCHLEICHER et al.)	1	
A	<u>US - A - 2 671 451</u> (BOLGER)	1	
A	<u>NL - A - 280 825</u> (GEVAERT)	1	
A	<u>NL - A - 280 826</u> (GEVAERT)	1	
-----			TECHNICAL FIELDS SEARCHED (Int. Cl. ³)