PATENT SPECIFICATION (11) 1 560 166

1 560 166

(21) Application No. 51534/77 (22) Filed 12 Dec. 1977 (19)

(31) Convention Application No. 2656317 (32) Filed 11 Dec. 1976 in

(33) Fed. Rep. of Germany (DE)

(44) Complete Specification Published 30 Jan. 1980

(51) INT. CL. [3] B01J 13/02
A61K 9/50
B01D 13/00

(52) Index at Acceptance
B1X 6AX 6B5 6BX 6DX 6FX 6HX
A5B 802 805 806 822 837 838 G M
C3H M

(54) LOADED CELLS

(71) We, KERNFORSCHUNGSANLAGE JÜLICH GESELLSCHAFT MIT BESHCHRÄNKTER HAFTUNG, of Postfach 1913, 5170 Julich, Federal Republic of Germany, a Body Corporate organised according to the laws of the Federal Republic of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The invention relates to so-called loaded cells, that is biological cells having a substance artificially incorporated within the cell interior inside the semi-permeable cell membrane. The substance incorporated in the cells can be intended for chemical or physical interaction with material dissolved in a solution into which the cells are introduced: the cell membranes thus function as containers. The term "loaded cells" expresses the point that the cells concerned have had some substance loaded into them which differs from the natural cell content. Alternative designations for loaded cells are "membrane vesicles", "ghost cells" or "membrane envelopes".

Loaded cells are prepared from natural cells, which may be human or animal cells. Loaded cells can be prepared from cells which naturally occur as individual cells in a physiological solution, for example erythrocytes, lymphocytes, thrombocytes or leucocytes. They can also be prepared from cells which - as for example liver cells - are arranged in tissues as associations of cells cohering to one another, since cells which are associated together in a tissue can be dissociated by biochemical or biophysical measures so as to obtain cells which are capable of being suspended in a solution.

The preparation of loaded cells, and in particular the step of enclosing substances in them, exploits a property of the membrane of living cells, namely that a limited permeability increase is capable of healing. On healing, the membrane of the loaded cells regains the semipermeable property of the membrane of the original cells.

Generally, for the preparation of loaded cells naturally occurring cells are suspended in a solution containing the substance to be enclosed in the cells and the permeability of the cell membranes is increased so that substances can pass into and out of the cells. Some of the substance to be enclosed passes through the cell membranes into the cells and at the same time the original cell contents largely escape to the surrounding solution. The membranes are subsequently allowed or caused to heal and thereby some of the substance which it is desired to enclose becomes trapped in the cells. The loaded cells can then be stored in a solution having an osmolarity comparable with that of their contents.

The increase in the permeability of the cells can be caused by exposing the cells to a solution of lower osmolarity than that of the natural cell contents (i.e. osmotic pressure causes the permeability of the membranes to increase) and in this case healing can be induced by restoring the osmolarity of the solution to an osmolarity comparable with that of the natural cell contents. Alternatively, a temporary increase in permeability can be caused by exposing the cells to the action of an electric field.

Processes for the preparation of loaded cells are described in our U.K. patent specifications 1474238, 1474418 and 1481480. The first of these relates to the enclosing of complexing agents in the cells, the second to the enclosing of enzymes. The processes described in both of these two specifications bring about the increase of the permeability of the cell membranes by exposing them to a solution of lower osmolarity. The third of these three specifications described a process in which the increase in permeability is brought about by the action of an

electric field.

Since the cell membrane is regenerated on healing and its semipermeable property is regained, it is possible to bring the substance enclosed in the loaded cells into interaction with material present in a physiological solution outside the loaded cells without the enclosed substances being liberated into the physiological solution. The loaded cells are introduced into a physiological solution containing the material and this diffuses through the semipermeable membrane of the loaded cells. It is for example possible in this way to use the enzyme invertase enclosed in loaded cells to convert sucrose present in a physiological solution into glucose and fructose, since both the sucrose and glucose and fructose can pass through the cell membrane whereas the enzyme invertase remains enclosed in the loaded cells. As another example, it is possible to load cells with urease, and then inject the loaded cells into the blood stream of a human body to destroy urea in the blood. The urease is not liberated from the loaded cells, but the urea can diffuse into them through the cell membranes, and is destroyed inside them.

However, if a quantity of the known loaded cells are introduced into a solution they will in general become distributed freely throughout the solution, and the interaction of the substances enclosed in the loaded cells with material in the solution outside the cell membrane will extend to the whole of that solution. For instance, if loaded cells formed from erythrocytes are injected into the bloodstream, the loaded cells would distribute themselves indiscriminately over the entire bloodstream, which fact has the result that the effect of the substance(s) enclosed in the loaded cells also extends to the whole of the bloodstream.

The present invention broadly resides in including particles of magnetic material in the cells so that the cells - and thus the action of whatever substance(s) is enclosed in them - can be concentrated at a preferred location. In one aspect this invention provides loaded cells having enclosed therein both a substance intended to undergo chemical or physical interaction, and particles of magnetic material whose diameter lies in the range 1 to 20 nanometres whereby an agglomeration of the loaded cells may be held at a desired location in a solution by an external magnetic field.

In another aspect of the invention there is provided a process for the preparation of a quantity of loaded cells comprising increasing the permeability of the cell membranes of natural cells suspended in a solution, allowing a substance or substances which are to be enclosed to pass from a solution containing them into the cells through the membranes thereof while their permeability is increased, and allowing or inducing healing of the cell membranes while the cells remain in a solution containing the said substances so that the substances become enclosed in the cells, separating the cells from the solution containing the said substances and suspending them in a solution of comparable osmolarity to that of the contents of the loaded cells, wherein there is provided in the solution containing the substance or substances to be enclosed both a substance intended to undergo chemical or physical interaction, and particles of magnetic material whose diameter lies in the range 1 to 20 nanometres, whereby an agglomeration of the loaded cells formed by the process may be held at a desired location in a solution by an external magnetic field.

When the loaded cells are introduced into a solution a magnetic field can be applied to retain them at a desired point or region in the solution. The solution may be the blood flowing in the arteries or veins of a human or animal body, and by means of the invention a medicament enclosed in the loaded cells may be brought to action at or in a specific organ of the body.

If, for example, ferrimagnetic, ferromagnetic or paramagnetic compounds such as cobalt ferrite or nickel ferrite or magnetite or ferritine, are enclosed in the loaded cells, then it is possible in simple manner, through a magnetic field applied at a preferred point of the human body, to hold fast at this point the loaded cells present in the bloodstream of a body and therefore to bring the substances - which may be pharmacological compounds, or other substances, such as radionuclides - into action preferentially at this point. For the production of a sufficient field gradient of the magnetic field a metal piece can be brought to the point concerned. Where appropriate this can be coated with a physiological plastics material.

It may be that the substance intended to undergo chemical or physical interaction at the desired location in the solution would act upon the cell membrane so as to lead to premature destruction of the latter - which would result in uncontrolled liberation of the contents of the loaded cells possibly a distribution of this substance in the physiological solution. Preferably then the cells have additionally enclosed therein a sufficient quantity of a second substance which forms hydrogen bonds or covalent bonds with the first said substance and reduces its action on the cell membranes as to prevent or delay i.e. the action of the first said substance on the cell membranes, destruction of the cell membranes, and liberation of the loaded cells' contents.

Suitably both substances and the magnetic particles are provided together in the solution from which the substances pass into the cells while their membranes have increased permea-

bility.

The inclusion of substances to prevent or delay destruction of the cell membranes may be employed either when the substance intended for the desired interaction should undergo this interaction while it remains enclosed in the loaded cells, or when the substance is intended to be liberated from the cells to undergo interaction, but it is desired to prevent premature liberation.

As an example, of the enclosure of a substance to delay destruction of the cell membranes, if there is enclosed in loaded cells not only the substance 6-fluorouracil, known as an agent for combating cancer, and the magnetic particles, but also for example the protein albumin or a sugar, e.g. sucrose, it can be achieved that the membranes of the loaded cells remain stable for about twice as long. It is of course also possible, through suitable dosage of the said second substance (which delays the destruction) to arrange that the loaded cells remain intact for only a short time.

A particularly valuable application of such loaded cells is their use for the treatment of tumours. They can for example be used to deliver methotrexate, which belongs to the folic acid antagonists which today, besides alkylating agents, are classed with the most effective substances for the treatment of formations of neoplasms (tumours). For this purpose methotrexate is enclosed in loaded cells together with the magnetic particles and substance delaying the destruction of the cell membrane. The loaded cells are then injected into the bloodstream of the body and held at the point of the tumour by an external magnetic field. The methotrexate is liberated after predetermined time and develops its effect at the point of the tumour. In this way the methotrexate is, as is advantageous, prevented from being distributed in the entire blood circulation, and thus from developing its action everywhere in the body with concomitant attacking of healthy tissue. This disadvantage could not be prevented in the hitherto usual application of methotrexate in which methotrexate is injected directly into the bloodstream. The use of such loaded cells also achieves the result that a smaller dose than hitherto necessary for the treatment of tumours need be introduced into the body or that a smaller number of injections need be given than hitherto necessary which contributes to reducing or avoiding negative side-effects during the use of this agent.

In a further and advantageous form of the invention there is enclosed in the cells not only the magnetic particles and the substance intended to undergo chemical or physical interaction but also where this substance would not if enclosed on its own, cause destruction of the cell membrane or would do so only at a relatively slower rate, there is additionally enclosed a sufficient quantity of a second substance causing relatively faster destruction of the cell membranes as to cause destruction of the cell membranes and liberation of the loaded cells' contents after a predetermined time. Suitably both substances and the magnetic particles are provided together in the solution from which the substances pass into the cells while the permeability of their membranes is increased.

This enables the loaded cells to be used to convey substances which have to be liberated from the loaded cells to undergo the desired chemical or physical interaction, but which are compatible with the cell membrane and would not cause its destruction, or would not do so soon enough. If substance(s) are also enclosed which cause the destruction of the cell membrane, as for example proteolytic enzymes and lipid-degrading substances, such as pronase, phosphilipase and trypsin, it is for example possible, after injection of loaded cells into the bloodstream of an animal or human body, to accumulate, through holding the loaded cells fast at a preferred point in the bloodstream, specific substances enclosed in the loaded cells, as for example tetracyclin, to which ferritine is added, and to liberate the substance into the bloodstream after a predetermined time. In this way, a particularly high effectiveness of the substance used is achieved. It is of course possible to inject into the bloodstream a mixture of loaded cells with varying doses of the active substance which destroys the cell membrane in order thus to control in predetermined manner the course of the liberation in the body of the substance which the loaded cells are being used to convey.

In cases where cells are to be loaded with a substance which is intended eventually to be liberated to undergo interaction outside the cells, and this substance is one which if enclosed alone would interact with the cell membrane to destroy it, it is preferable that there is loaded into the cells not only this substance and the magnetic particles, but also a second substance which forms hydrogen bonds or covalent bonds with the first said substance and reduces its action on the cell membranes so as to prevent or delay destruction of the cell membranes by the first said substance, and in addition a sufficient quantity of a further substance causing destruction of the cell membranes as to cause relatively faster destruction than is caused by the first and second substances in combination and bring about destruction of the cell membranes and liberation of the loaded cells' contents after a predetermined time.

In this way an uncontrolled liberation of the first substance can be avoided, and yet the desired eventual liberation can be achieved. All three substances and the magnetic particles can be provided together in a common solution from which they pass into the cells while the

cell membranes have increased permeability.

In a very advantageous further development of the invention the said substance is intended to undergo chemical or physical interaction after liberation from the cells and is in the form of particles coated with lipids - so that the coated particles are liposomes, - the coated particles having a diameter in the range 5 to 20 nanometres. The particles of the substance can be coated with the liposomes before being introduced into the solution from which they pass into the cells.

Since the liposomes, after their liberation out of the loaded cells, immediately pass into the tissue adjoining the bloodstream, it is possible to bring specific substances into action in the interior of organs of a body, which interior is not reached by blood capillaries. For this purpose it is merely necessary to initially hold the loaded cells in the bloodstream of the organ through action of a magnetic field applied to the body from outside and then liberate the substance(s) coated with lipids through destruction of the cell membrane.

In the carrying out of the process according to the invention the permeability increase may, depending on requirements, optionally be effected though action of osmotic pressure or through action of an electric field.

When, as in our U.K. patent specifications 1474238 and 1474418 the permeability increase is to take place through action of osmotic pressure, the following procedure is suitable:

The cells which are to be loaded are added to a cell-compatible solution which may be an aqueous solution containing at least 0.5 mmole/litre of magnesium ions and/or calcium ions as well as potassium ions and whose osmolarity is so low vis-a-vis the osmolarity of the cell content that the resultant osmotic pressure in the cells causes the permeability increase of the cell membrane but without destroying the latter. If erythrocytes are used for the preparation of loaded cells, a suitable osmolarity difference is approximately a factor of 15. Suitable quantities of the substances to be enclosed in the loaded cells are also contained in the cell-compatible solution or these substances are now added to it.

An exchange of substances takes place between the cell-compatible solution and the cell content has through the cell membrane, which now has an increased permeability, and the loaded cells which form have a content which practically corresponds to the cell-compatible solution. Following this the osmolarity of the cell-compatible solution is increased to substantially the osmolarity of the original cell content through addition of osmotically active substances, such as calcium ions, potassium ions and sodium ions. By osmotically active substances are here meant substances which have a reflexion coefficient of about 0.8 but which nevertheless, since they are in general contained in a cell-compatible solution, build up a sufficient osmotic pressure. After a residence time in which the cell membranes heal substantially completely, the loaded cells thus formed are separated from the cell-compatible solution and the thus prepared quantity of loaded cells is put into an isotonic physiological liquid for storage. If erythrocytes are used it is expedient, in order substantially completely to heal changes of the cell membrane which were caused during the permeability increase, to leave the cells for about 5 minutes at 0°C and then to warm them up for about 30 to 60 minutes to body temperature.

When, as in our U.K. patent specification 1481480, the permeability increase is to be effected through action of an electric field, a suitable procedure is as follows:

The cells which are to be loaded are introduced into a liquid which expediently has a temperature lying between 0°C and 25°C, conducts electric current and forms a cell-compatible electrolyte solution. Following this, the electrolyte solution containing the cells is exposed to an electric field with a strength of 10^3 to 10^5 V/cm until the permeability of the cell membranes is increased to such an extent that molecules with a radius of at least 0.5 nanometres pass through the cell membranes. It is expedient for the electrolyte solution to be passed through the focus of an electric field. The permeability increase effected is, for example in the case of the application of the process to erythrocytes, detectable by the decoloration of the electrolyte liquid as a result of the haemoglobin emerging from the cells' interior and by the decoloration of the erythrocytes. If the substances which are to be enclosed in the loaded cells are already contained in the cell-compatible electrolyte solution, they enter the cells from the cell-compatible solution during an exchange of substances between the solution and the cell content after the permeability increase. It is, however, also possible, after the permeability increase but before the healing of the cell membranes, to add the cells to a cell-compatible solution whose osmolarity substantially corresponds to the osmolarity of the cell content of the original cells, and which contains the substances which are to be enclosed in the loaded cells. In this cell-compatible solution an exchange takes place between the substances in solution and the cell content. After a residence time in which the cell membranes heal substantially completely, the loaded cells which are formed are separated from the cell-compatible solution and the thus prepared quantity of loaded cells is put into an isotonic physiological liquid for storage. In the case of erythrocytes, it is expedient to

prepare the loaded cells in a potassium chloride solution and then when the loaded cells have been formed, to transfer them to an isotonic sodium chloride solution which, in its ion concentration and osmolarity, corresponds to blood serum.

Example 1

Ferrite particles first were prepared according to the method of W.J. Schnehle and V.D. Deetschreak stated in J. Appl. Phys. 32, 2355 (1961). 1 mole of cobalt chloride and 2 moles of iron (III) chloride were dissolved in 2 litres of hot distilled water and added, with stirring, to 1 litre of a boiling 8-molar sodium hydroxide solution. The cobalt ferrite formed was then washed with water until neutrality was achieved and, thereafter, large particles were filtered off. The ferrite particles were then coated with a silicone film in order to avoid a subsequent haemolysis or the destruction of the loaded cells after the enclosure of the ferrite particles in the cells. For this the suspension containing the ferrite particles was shaken with silicone oil of the trade name AR5. After separation of the silicone oil by centrifuging, the ferrite particles were suspended, in a weight ratio of 1: 10, in a solution of the following composition: 105 mM KCl; 20 mM NaCl; 4 mM $MgCl_2$; 7.6 mM Na_2HPO_4; 2.4 mM NaH_2PO_4 and 10 mM glucose.

For the forming of the loaded cells, erythrocytes, independently of the preparation of the ferrite particles, were suspended in a solution of the aforesaid composition in the ratio of about 1 part by volume of erythrocytes to 10 parts by volume of the solution. The pH value of the solution was 7.2.

Of the thus formed suspension containing the erythrocytes, 10 ml were exposed to an electric field strength of 12 KV/cm for 40 μsec. at 0°C in an apparatus suitable for this purpose. About 1 minute after application of the electric field, which was followed by haemolysis, methotrexate in the ratio of 5 mmoles per litre of solution and 1 ml of the suspension containing the ferrite particles were added. After the haemolysis, which lasted for about 5 minutes, the solution was kept to 0°C for a further 5 minutes in order to achieve a compensation between the medium present in the cell interior with the external solution which contained the methotrexate. Following this, the temperature of the solution was increased to 37°C in order to accelerate the healing of the changes in the membranes, which changes were caused by the electric field. The process of substantially complete healing was complete after about 20 minutes. In order to recover the cells loaded with the ferrite particles from the suspension, this suspension was then centrifuged several times for 2 minutes at an acceleration of 1000g; in each case, the cell layer lying on the unenclosed particles was removed.

The loaded cells obtained were then suspended in a volume ratio of 1: 10 in a physiological solution which had the following composition:
138.6 mM NaCl; 12.3 mM Na_2HPO_4; 2.7 mM NaH_2PO_4.
The pH value of the solution was 7.4.

The suspension so obtained was placed in a test-tube with a diameter of 10 mm and this test-tube was brought between the pole shoes of a U-shaped permanent magnet. As could be established by means of a microscope, the loaded cells accumulated at the test-tube walls adjoining the pole shoes.

During a storage of the loaded cells at about 4°C, after about one day 90%, after 2 days 87%, after 4 days 65% and after 7 days 53% of the loaded cells were detected in the cell sediment.

Example 2

Loaded cells were prepared as described in Example 1 but, instead of the solution containing the ferrite particles, 1 ml of a 10% strength isotonic ferritine solution was added to the solution containing the erythrocytes. After preparation of the loaded cells which were formed, the enclosed ferritine was evidenced by means of an electron microscope.

Example 3

Loaded cells were formed as described in Example 1 but, in addition to the methotrexate and the ferrite particles, 0.1% by volume of albumin was added to the solution in which the erythrocytes were exposed to the electric field for the purposes of permeability increase and enclosure of the substances.

The loaded cells could be stored at a temperature of about 4°C for a longer period than the loaded cells formed in Example 1. After 7 days, 85% of the loaded cells were evidenced in the cell sediment.

Example 4

(This Example is outside the scope of the appended claims, but is included to illustrate the effect of enclosing pronase). Loaded cells were prepared as in Example 1. However, the addition of methotrexate and ferrite particles after application of the electric field did not take place. Instead, before the application of the electric field, sucrose and pronase P were added to the solution to which the erythrocytes were added. These were added in such a dosage that this solution contained 10 mM sucrose and 0.01 mg of pronase per 100 ml of

solution. The sucrose was labelled with the radionuclide C 14.

In order to follow the effect of the pronase P enclosed in the loaded cells, the loaded cells were stored in a physiological solution of the composition stated in Example 1, centrifuged after 20 hours and the amount of intact loaded cells was determined through measurement of the radioactivity contained in the solution and in the still intact loaded cells. Compared with loaded cells which had been prepared without the enclosure of pronase P, the number of still intact loaded cells after 20 hours was only 11%.

Example 5

(This Example is also outside the scope of the appended claims, but is included to illustrate the effect of enclosing phospholipase). Loaded cells were prepared as described in Example 1. Again however, the addition of methotrexate and ferrite particles after the application of the electric field did not take place. Instead, before the application of the electric field, there were added tritium labelled methotrexate, albumin and phospholipase C. The proportion of methotrexate in the solution was 5 mM, the proportion of albumin was 0.1% by volume and the proportion of phospholipase C was 0.01 mg per 100 ml of solution.

After formation of the loaded cells, the effect of the phospholipase C enclosed in the cells was determined through measurement of the radioactivity as in Example 4. After 20 hours, the quantity of intact loaded cells present were only 17% - compared with loaded cells which had been prepared without enclosure of phospholipase C.

Example 6

For the preparation of loaded cells through action of osmotic pressure, erythrocytes were suspended in a volume ratio of 1 : 1 in isotonic, phosphate-buffered NaCl solution of the following composition:

138.6 mM NaCl; 12.3 mM Na_2HPO_4; 2.7 mM NaH_2PO_4.

The pH value of the solution was 7.4.

To increase the permeability of the membranes of the cells, 1 ml of the thus formed suspension was added, with stirring, to 10 ml of a solution which contained 5 mM tritium labelled methotrexate, 4mM $MgSO_4$ and 50 mM sucrose. Following this, a further 1 ml of a solution was added which contained 4 mM $MgSO_4$, 50 mM sucrose and ferrite particles in the weight ratio of 1 : 10. The solution so formed was left at 0°C for 5 minutes.

Following this, the osmolarity was restored to that of the original solution by adding an appropriate amount of a 2-molar KCl solution. The solution was then left for a further 5 minutes at 0°C and then the temperature was increased to 37°C for 20 minutes in order to accelerate the substantially complete healing of the membranes. After the loaded cells thus formed had been centrifuged out of the solution, the cells were incubated in an isotonic, phosphate-buffered NaCl solution of the aforesaid composition.

The loaded cells thus formed contained - with reference to the unit of volume - practically the same amount of methotrexate as the external medium, namely 98% of the methotrexate concentration present in the external medium.

The effect of the ferrite particles enclosed in the loaded cells was demonstrated as described in Example 1.

Attention is directed to the claims of our co-pending application 51329/77 (Serial No. 1560165).

WHAT WE CLAIM IS:-

1. Loaded cells having enclosed therein both a substance intended to undergo chemical or physical interaction, and particles of magnetic material whose diameter lies in the range 1 to 20 nanometres whereby an agglomeration of the loaded cells may be held at desired location in a solution by an external magnetic field.

2. Loaded cells according to claim 1 wherein the said substance would, if enclosed on its own, cause destruction of the cell membranes, the cells having additionally enclosed therein a sufficient quantity of a second substance which forms hydrogen bonds or covalent bonds with the first said substance and reduces its action on the cell membranes as to prevent or delay destruction of the cell membranes, and liberation of the loaded cells' contents.

3. Loaded cells according to claim 2 having additionally enclosed therein a sufficient quantity of a further substance causing destruction of the cell membranes as to cause relatively faster destruction than is caused by the first and second substances in combination and bring about destruction of the cell membranes and liberation of the loaded cells' contents after a predetermined time.

4. Loaded cells according to claim 1 wherein the said substance would not, if enclosed on its own, cause destruction of the cell membrane or would do so only at a relatively slower rate, the cells having additionally enclosed therein a sufficient quantity of a second substance causing relatively faster destruction of the cell membranes as to cause destruction of the cell membranes and liberation of the loaded cells' contents after a predetermined time.

5. Loaded cells according to any one of the preceding claims wherein the said substance is intended to undergo chemical or physical interaction after liberation from the cells and is in

the form of particles coated with lipids, the coated particles having a diameter in the range 5 to 20 nanometres.

6. Loaded cells according to any one of the preceding claims wherein the particles of magnetic material are coated with silicone oil.

7. Loaded cells according to any one of the preceding claims wherein the magnetic particles are of ferromagnetic or ferrimagnetic material.

8. Loaded cells according to any one of the preceding claims formed from natural cells of animal or human origin.

9. Loaded cells according to any one of the preceding claims substantially as herein described in any one of Examples 1, 2, 3 or 6.

10. A process for the preparation of a quantity of loaded cells comprising increasing the permeability of the cell membranes of natural cells suspended in a solution, allowing a substance or substances which are to be enclosed to pass from a solution containing them into the cells through the membranes thereof while their permeability is increased, and allowing or inducing healing of the cell membranes while the cells remain in a solution containing the said substances so that the substances become enclosed in the cells, separating the cells from the solution containing the said substances and suspending them in a solution of comparable osmolarity to that of the contents of the loaded cells, wherein there is provided in the solution containing the substance or substances to be enclosed both a substance intended to undergo chemical or physical interaction, and particles of magnetic material whose diameter lies in the range 1 to 20 nanometres, whereby an agglomeration of the loaded cells formed by the process may be held at a desired location in a solution by an external magnetic field.

11. A process according to claim 10 wherein the said substance intended to undergo interaction would, if enclosed on its own, cause destruction of the cell membranes, and there is additionally provided in the said solution containing the said substance or substances to be enclosed, a sufficient quantity of a substance which forms hydrogen bonds or covalent bonds with the first said substance and reduces its action on the cell membranes as to prevent or delay destruction of the cell membranes at least for a predetermined time after enclosure of the substances and separation of the loaded cells from the solution containing the substances.

12. A process according to claim 11 wherein there is additionally provided in the said solution containing the said substance or substances to be enclosed a sufficient quantity of a further substance causing destruction of the cell membranes as to cause relatively faster destruction than is caused by the first and second substances in combination and bring about destruction of the cell membranes and liberation of the loaded cells' contents after a predetermined time after enclosure of the magnetic particles and all three said substances and separation of the loaded cells from the said solution.

13. A process according to claim 10 wherein the said substance intended to undergo interaction would not, if enclosed on its own, cause destruction of the cell membranes or would do so at a relatively slower rate, and there is additionally provided in the said solution containing the substance or substances to be enclosed a sufficient quantity of a second substance causing relatively faster destruction of the cell membranes as to cause destruction of the cell membranes after a predetermined time after enclosure of the magnetic particles and both said substances and separation of the loaded cells from the said solution.

14. A process according to any one of claims 9 to 13 wherein the said substance is intended to undergo chemical or physical interaction after being liberated from the cells and is introduced into the said solution containing the substance or substances to be enclosed in the form of particles coated with lipids, the coated particles having a diameter in the range 5 to 20 nanometres.

15. A process according to any one of claims 10 to 14 wherein the particles of magnetic material are coated with silicone oil before they are introduced into the said solution.

16. A process according to any one of claims 10 to 15 wherein increasing the permeability of the cell membranes of natural cells is effected by exposing the cells to a solution of lower osmolarity than the natural cell contents.

17. A process according to any one of claims 10 to 16 wherein increasing the permeability of the cell membranes of natural cells is effected by exposing the cells to an electric field.

18. A process according to any one of the preceding claims wherein the magnetic particles are of ferromagnetic or ferrimagnetic material.

19. A process according to any one of claims 10 to 18 wherein the natural cells are of animal or human origin.

20. A process for the preparation of a quantity of loaded cells, according to any one of claims 10 to 19 substantially as herein described with reference to any one of Examples 1, 2, 3 or 6.

21. A quantity of loaded cells prepared by the process of any one of claims 10 to 20.

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Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1979.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from
which copies may be obtained.