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(54) Title: A METHOD FOR ENTRAPMENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND THE USE THEREOF

(57) Abstract

A pharmaceutical formulation is described, which is made of a prefabricated microspher/particle, combined with a pharmaceutically acceptable polymer which delays the release of the entrapped biologically active substance. Also described is the use of the formulation for the biologically active substance.

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A METHOD FOR ENTRAPPMENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND THE USE THEREOF

BACKGROUND:

This invention relates to a way of produce, use and/or utilize a pharmaceutical formulation for biologically active substances. Such formulations may be used within human and veterinary medicin and in the agricultural areas.

Examples of this kind of formulations are slow release systems for drugs, targeting of drugs or the use as contrast agents. More directly this invention relates however, to a method for fabrication and use of slow release systems for a biologically active substances using a process which allows entrappment of biologically active substances within polymers which are biodegradable and biocompatible.

A drug, within the scope of the present invention, is defined in its broadest sense, such as a biologically active substance having effects and/or is used within human and/or veterinary medicin as well as wihin agricultural areas.

Within the medical areas one can divide the biologically active substances after there area of use.

Substances for the use within the respiratory tract; cough reducing (e.g. noscapine) or opiates (e.g. ethylmorphine). Mucusmembrane affectors (e.g. ephedrin, terbutalin and theophylline).

Heart and bloodvessel agents; glycosides, such as digoxin, kinidin, lidocain, procainamide.

Beta-blocking agents such as alprenolol or metoprolol.

Other groups includes alfa-blocking agents (e.g. phentolamine), beta-stimulaters (e.g. bamethan), alkylnitrates, calciumantagonists (e.g. nifedipin), nicotinic acid derivatives, adrenergics (e.g. adrenalin) sympaticus moderaters (e.g. guanetidin) ganglie blockers (e.g. trimetafan), hydrazine derivatives, tiazide derivatives, bensen-sulfonamide derivatives, bumetamide, furoseamide, etacrynic acid, spironolacton.

Varix treatment (e.g. polidokanol), cholesterol synthesis blockers (e.g. clofibrate).

Antihistamins (e.g. prometazin, terbutalin) at allergic disorders.

Spasmolytic substances; papeverinderivatives, anticholinergic (e.g. atropin), cholinergic substances (e.g. karbacol).

Drugs for tumour diseases; vitamines (e.g. B-12 or folic acid), alkylating

cytostatic drugs (e.g. cyclofosfamid), antibiotics (e.g. daunomycin, bleomycin), mitos blockers (e.g. vinblastin), cisplatinum, nitrosurea derivatives, estramustin, steroidderivatives, cimetidin, ranitidin.
Chemotherapeutic and antibiotic substances, sulfonamides, penicillines, cephalosporins, tetracyclines, aminoglycosides, macrolides, aminosalicylicacid derivatives, iso-nicotinic acid derivatives, iodine.
Malaria drugs (e.g. clorokin).
Substances against fungus infections (e.g. griseofulvin).
Vitamins.
Proteins and peptides, digestion enzymes, coagulation factors (e.g. factor VIII), immunglobulins, vaccines, hormones (e.g. oxytocin), corticotropins, thyrotropin, growth hormon, anti-diuretic hormon (or DDAVP), glucocorticoids, mineralcorticoids, androgens, oestrogens, thyroid hormones, insulin, calcitonin, glucagon, sulfonureids, enkefalins.
Immunstimulating substances (e.g. interferons, interleukins).
Psychopharmacological drugs; barbituric acid derivatives, piperindindion derivatives, propandiol derivatives, bensodiazepin derivatives, fentiazin derivatives, tioxantan derivatives, butyrofenon derivatives, tricyclic thymoleptic drugs, coffein, antihistaminic substances.
Antiepileptic drugs (e.g. derivatives of hydantoin).
Muscle relaxation substances (e.g. kinin, curare).
Prostaglandins, nicotinamid.
Anticholinergic and anestetic drugs; morfin derivatives, fenylpiperidin derivatives, diphenylpropylamine derivatives, salicylic acid derivatives, bensotriazin derivatives, anilides, indol-acetic acid derivatives, fenyl-acetic acid derivatives, naftyl-acetic acid derivatives, ergotamine derivatives, serotonin antagonists, clonidin, lidocain derivatives.
For the person skilled in the art it is obvious that these substances are not by any means limited to the use within the areas mentioned above, the substances can be, and are used for other purposes or indications than the ones described above.
In agricultural areas substances that are used as herbicides or stimulators on crop may be used. Also substances that have an affect on various parasites are included (e.g. pesticides).
Within the pharmaceutical industry there are at present several methods described for entrappment of hydrophobic substances.
A hydrophobic substance is characterized by beeing preferentially solvable in a hydrophobic solvent. This means that the solvent has a capability of dissolving various fatty substances, such as fatty acids, oils or the like. Hydrophilic refers to similar solvation capability but for

water soluble substances.
However, still there are no acceptable methods for preparation of slow release formulations for hydrophilic substances, due to the technical difficulties, in the manufactoring of such formulations.
In principle there are two basic principles to prepare a pharmaceutical formulation for a biologically active substance: entrappment into, or covalent coupling to a matrix. In the case of entrappment you take advantage of the characteristics of the formulation and the biologically active substance have respectively, in order to create association phenomena, resulting in a stable preparation. Of great importance working with formulations within the areas mentioned above, is that the formulation in it self, will not create toxic metabolites. Having a choice with these aspects in mind you are mainly directed towards using materials that are made of endogenous substances or polymerized in biocompatible way.
One type of polymers that has attracted large interest during the last years are the use of polymerized hydroxicarboxylic acids. An example of a monomer, which can be used for this type of polymisationer is lactic acid: polymerized into poly-lactic acid (PLA), often polymerized together with glycolic acid. This co-polymer is namned as PLGA (poly-lactic-glycolic acid). Microspheres prepared of PLGA are relativly stable in a physiological enviroment due to the hydrophobic interactions between the hydrophobic PLGA polymer. Another polymer showing the same characteristics is poly-capronic acid.
The great intrest for this type of polymers, in particular in there use in the preparation of microspheres, is reflected in the patent litterature, where a large number of applications and patents dealing with variants of preparations procedures and/or use is described.
The use and the interest for polymers made of PLGA is partly based upon the fact that the monomer is an endogenous substance and partly that the monomers are bonded to each other by ester bonds. These esterbonds are slowly hydrolized in contact with water whereby the original monomer is reformed.
The hydrophobic interactions within the polymer are utilized when the polymer is used as matrix. Since PLGA is a hydrophobic polymer it will adsorb hydrophobic substances.
The hydrophobic interactions are only slowly broken up, preferentially this is seen in connection with hydrolysis of the esterbonds in the polymers, appearing in a hydrophilic enviroment such as a human body.
Another type of monomers that can be used for the purpose of this invention, due to their biocompatability, are polymers of oxaloacetate.

citrate, isocitrate, oxalosuccinate, ketoglutarate, succinate, fumarate, malate or a derivative of these.

Another type of acceptable polymer that can be used within the scope of this invention is a graft polymer between PLA/PLGA and carbohydrates. This type of polymers are described in Swedish patent application 8601563-3.

Another example of polymers which already have found use within this area and could be used in connection with this invention are the poly-anhydrides (e.g. poly-bis(p-carboxyphenoxy)alkane anhydride), poly-ethylenevinyl acetate, poly-orthoesters, poly-vinyl alcohol, poly-vinyl acetate, poly-vinyl chloride, acrylic polymers, poly-amino acids, poly-urethane, poly-silanes. Furthermore, there are a number of combinations and derivatives of these that can be used.

As mentioned above, the main use of PLGA is for hydrophobic low molecular weight biologically active substances. However, there is a great interest from the pharmaceutical industry to be able to produce matrix systems for hydrophilic substances. This has however, proven to be far more difficult than for hydrophobic substances.

The main reason for the difficulty in preparing a hydrophilic matrix system that can retard a hydrophilic substance in the hydrophilic environment such as a human body, is the following; to be able to dissolve a hydrophilic substance you need a hydrophilic solvent. Thus, by preparing a hydrophilic matrix system, this will be rapidly dissolved in a hydrophilic enviroment and the entrapped hydrophilic substance will rapidly be released.

Alternatively, if you prepare a hydrophobic matrix system this can be dissolved in a hydrophobic enviroment and only very slowly or not at all in a hydrophilic environment.

By the reasoning above it is obvious that there is a great deal of interest from the pharmaceutical industry for pharmaceutical formulations for hydrophilic drugs. In particular, it would be advantagous if one could prepare a formulation for entrappment, since the so formed formulation would contain the biologically active substance without having to manipulate the molecular structure of the substance. When, and if, such a formulation is obtained, documentation regarding toxicity, metabolism and elimination routes of the biologically active substance would already be at hand due to previous registration and approval.

As an example it may be worth mentioning that there is a great interest in preparing microspheres to be used for entrappment of X-ray contrast agents. The main interest is in preparing a microsphere

having a diameter of 2 µm which can be injected into the bloodstream. Such a microsphere should thus be useable as a specific contrast agent for the reticuloendothelial system (RES). Of particular interest would be to use the microspheres as a contrast agent for the liver.

Another great interest for the type of formulations, as described in this invention, is if one could prepare a microsphere with entrapped biologically active substances having a size allowing inhalation, e.g. as a spray. The use of such a preparation containing e.g. terbutalin or theofylline, is primarely in the treatment of asthma. Another substance awoking a new and special interest is nicotinic acid, since it has been shown it has effects at tumour treatment.

Proteins are also incorporated into this group of particularly interesting substances since this group of substances have high demands on the preparation methodology in order to protect the three dimensionell structure.

Methods for preparation of known formulations, as discussed in this invention is mainly based on the following principles: phase evaporation, precipitation or spraydrying. As an example of phase evaporation may be mentioned that PLGA microspheres are primarely prepared according to this technology (US 4,389,330). Another example of polymers used for the preparation of microspheres are carbohydrate polymers, e.g. starch, which also are considered to have fulfilled all the necessary demands in order to be used as a carrier of biologically active substances.

Precipitation systems (crystallization) is described in PCT/SE83/-00268 where the polymer beeing described is starch. Polymerization systems is also described for the preparation of starch microspheres (SE 7407461-8). Also complexes and solutions are described (Swedish appl. 8501094.0) as useful formulations within this area. However, starch is to hydrophilic to be used for hydrophilic low molecular weight substances.

However, the invention is not restricted to the use within the methodologies mentioned above for slow release, the expert in the field may easily adopt the methodology for the use in other areas where there is a need for a matrix system according to the present invention.

DESCRIPTION OF THE INVENTION.

One possibility for the preparation of a formulation, which is not described earlier, and which is described in this invention, involves

the use of an already existing matrix, formed as a sphere or a particle, this sphere/particle containing the biologically active substance only, the sphere or particle substequently beeing covered by a surface of a polymer which does not have the same solubility parameters as the biologically active substance. In certain cases it might be difficulties in preparing a sphere/particle only using the biologically active substance, in such a case there is the possibility of mixing the biologically active substance with a protective colloid. Protective colloids that can be used are preferentially substances such as carbohydrates, and the methodology for such a preparation procedure for a sphere/particle is preferentially done according to PCT/SE83/00268.

Of speciall interest, as shown in this invention, is the possibility of preparing a sphere/particle of a hydrophilic biologically active substance, subsequently covered by a surface of a hydrophobic polymer. A hydrophobic polymer gives, in this speciall case as a result, that upon suspending the formulation in water, the water will have great difficulties in penetrating the hydrophobic surface and dissolve the entrapped hydrophilic substance.

The invention shows that by using this technology, which involves covering a hydrophilic matrix with a hydrophobic polymer in a process where water is absent, a retardation of the release of the biologically active substance after suspension of the formulation in a physiological enviroment is achieved. The expert in the field can thus easily adopt and use the described invention for the preparation of formulations which all shows a slow release effect of the entrapped biologically active substance.

The methodology involves in the first step production of a aphere/particle of a biologically active substance according to the basic technology described in PCT/SE83/00268. There is also the possibility to use a microsphere of crossslinked starch as described in Swedish applications 7407461 or 7900737-3, to which one or more biologically active substances has been associated. These dried and water free speres made of only biologically active substance or a biologically active substance entrapped, or otherwise associated to a hydrophilic matrix, is suspended into a hydrophobic solvent having the ability to function as a solvent of a hydrophobic polymer but not to the biologically active substance. The hydrophobic polymer is dissolved into the hydrophobic solvent, this solution is added to another solvent, into which said polymer is insoluble, whereafter the first solvent is removed.

Of importance in the use of the invention for e.g. contrast agents or by inhalation, is that the formulation my be obtained as a suspension in physiological enviroment without having the microspheres aggregated.

This may be accomplished using known technology, such as adsorbation of detergents on the surface of the microspheres before these are dried.

The invention in based upon the unexpected result that the hydrophobic polymer was shown to cover the surface of the hydrophilic microsphere, resulting in a sphere, to the extent that a high retardation of the release of the biologically active substance was obtained after suspension of the formulation in water.

At the process of covering of the hydrophilic microsphere there is also the possibility to adsorb the hydrophobic polymer using succesivly higher molecular weight of the polymer. By this process the low molecular weight polymers will be able to penetrate further into the hydrophilic structure, whereby is given the possibility to prepare the formulation with various release rates of the biologically active substance. In particular, this methodology has shown to be useful by adsorption of the hydrophobic polymer using sonication.

The following example is to be considered as illustrating but not limiting, since the basic technologies used are well known and can easily be modified by an expert in the field.

EXAMPLE 1.

4 gram of polycaprolacton was dissolved in 15 ml of chloroform, whereafter 1.78 gram of Metrizamid® in the form of microspheres, having a mean diameter of 1 µm, was suspended. The suspension was spray dried using a Büchi 190 spray drier. The dried product was subsjected to elementary analysis of the iodine. The product was shown to contain 12% metrizamid. The product was suspended in water and the iodine content was determined after 1 hour and was shown to have been reduced to 4.2%. If microspheres of metrizamid (unprocessed) are suspended in water, the metrizamid is immediately dissolved and the solubility is, according to the manufactorer, unlimited.

CLAIMS

1.
A method for the preparation of an injectible formulation **characterized by,** that one or several biologically active substances first has been prepared in the form of microspheres/particles, eventually together with a protective colloid, whereafter this microsphere/particle is covered by a surface of a polymer that does not show the solubility characteristics of the biologically active substance.

2.
A formulation according to claim 1, **characterizedby,** that the microsphere/particle, containing only the biologically active substance, is prepared by crystallization.

3.
A formulation according to claim 1, **characterizedby,** that the protective colloid is a carbohydrate, chosen from the group of starch, glycogen, pullullan, maltodextrin, glucose or a derivative of these.

4.
A formulation according to claim 1-3, **characterizedby,** that the hydrophobic and biocompatible polymer used at the covering of the surface of the micropshere/particle is made from hydroxi-carboxylic acids.

5.
A formulation according to claim 1-4, **characterizedby**, that the hydrophobic polymer is from the group polymers containing lactic acid, glycolic acid, hydroxi-lactonic acid or a graft polymer of these.

6.
A formulation according to claim 1-5, **characterizedby**, that the hydrophobic polymer is from the group of polymers containing oxaloacetete, citrate, isocitrate, oxalosuccinate, ketoglutarate, succinate or malate or a derivative of these.

7.
A formulation according to claims 1-6, **characterizedby**, that the matrix preparation is in the form of a capsule, cylinder, microsphere, complex or a solution.

8.
The use of the formulation according to claims 1-7 for biologically active substances.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE88/00183

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 47/00, 9/22

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	A 61 K 47/00, 9/16, /20, /22, /32, /58
US Cl	424:14, 31, 32

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,Y,	EP, A, 102 265 (THE STOLLE RESEARCH AND DEVELOPMENT CORPORATION) 7 March 1984 see claims, page 4, lines 11-27 & JP, 59161316 US, 4530840 US, 4542025 CA, 1218306	1,4,5,7,8
X	SE, B, 328 670 (SMITHKLINE & FRENCH LABORATORIES) 21 September 1970 see example	1,2,7,8
X	US, A, 4 479 911 (SANDOZ) 30 October 1984 see claims, col. 2, lines 30-40 and 54-62, col. 5, lines 1-7	1,2,4,5,7,8
X	Patent Abstract of Japan Vol. 9, No. 180 (C-293), abstract of JP 60-48923, published 16 March 1985	1,4,5,7,8

.../...

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1988-06-09	1988 -06- 23
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Agneta Tannerfeldt

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	SE, B, 413 578 (AS ALFRED BENZON) 9 June 1980 see example 1, page 1, lines 4-8 & NL, 7404134 FR, 2223047 DE, 2414868 GB, 1468172 CH, 585556 CA, 1029658 JP, 50029729	1,7,8
Y	WO, A, 84/00294 (SCHRÖDER ULF) 2 February 1984 see claims & EP, 0113749 AU, 567434 US, 4713249	3
A	EP, A, 26 599 (ELI LILLY AND COMPANY) 8 April 1981	
A	EP, A, 147 335 (LABORATOIRES D'HYGIENE ET DE DIETETIQUE) 3 July 1985	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [ ] Claim numbers ....., because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claim numbers 1, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claim, which defines the polymer with its solubility property relating to the activ component is too vague and unspecified. The search has therefore been incomplete.

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.