Title: INCLUSION COMPLEX OF ARTEMISININ OR DERIVATIVES THEREOF WITH CYCLODEXTRINS

Abstract: The present invention relates to methods for preparing pharmaceutical compositions comprising artemisinin or derivatives thereof. The present invention also relates to pharmaceutical compositions comprising artemisinin or derivatives thereof and a cycloextrin, obtainable by the methods according to the invention. The invention further provides a kit comprising a pharmaceutical composition according to the present invention. The pharmaceutical compositions according to the present invention may be used in the preparation of a medicament for treating diseases including malaria, cancer, babesiosis, shistosomiasis, and fungal, viral and/or bacterial infections.
INCLUSION COMPLEX OF ARTEMISININ OR DERIVATIVES THEREOF WITH CYCLODEXTRINS

Field of the invention

The present invention relates to the field of pharmacology and clinical biology. In a first aspect the present invention relates to a method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof. In a second aspect the invention relates to the pharmaceutical composition comprising artemisinin or derivatives thereof and cyclodextrins obtainable by the method according to the invention. In a third aspect, the present invention relates to the kit comprising a pharmaceutical composition according to the present invention. The invention also relates to the use of pharmaceutical compositions according to the present invention in the preparation of a medicament for treating diseases including malaria, cancer, babesiosis, schistosomiasis, and fungal, viral and/or bacterial infections.

Background of the invention

Artemisinin (qinghaosu) is obtained from the leaves of the shrub plant Artemisia annua and is a naturally occurring sesquiterpene lactone containing an endoperoxide group (−O−O−C). Dihydroartemisinin (DHA) is the reduced lactol derivative of artemisinin and the derivatives artemether, arteether, artesunate and artelinate are ethers or esters of the lacton. In general, the endoperoxides present in all these derivatives, are a promising class of anti-malarial drugs which may meet the dual challenges posed by drug parasites and the rapid progression of severe malarial illness and complications which can prove fatal unless emergency treatment is instituted. The endoperoxides have several advantages over existing anti-malarial drugs. They show little or no cross-resistance to existing anti-malarials, are fast-acting and clear the peripheral blood of parasites more rapidly than any other available drug and finally resistance to the endoperoxides has not yet developed, despite widespread clinical trials. The attractive feature of the drugs is the lack of adverse reactions, including systemic neurotoxicity from which these drugs, based on data from animal studies, were suspected, at the clinically prescribed doses.

An important disadvantage of the natural substance artemisinin is its low solubility in water (0.46 mg/ml at 37°C) and oils. In order to overcome this difficulty, attempts have been made to convert it into a variety of derivatives in order to improve the bioavailability. Derivatives were semi-synthesized from the mother compound artemisinin, leading to the hydro soluble sodium salt artesunate and the liposoluble β-arteether, β-arteether, and dihydroartemisinin and to artemelic acid and artesunic acid. In the body, both artemisinin...
and artemunic acid are converted to dihydroartemisinin (DHA, artechol), which is the actual antimalarial active substance. Artemisinin and in particular artemunic acid but other artemisinin derivatives too, can therefore be regarded as prodrugs for dihydroartemisinin. However, the prepared derivatives still showed a low solubility in aqueous media. For instance, to dissolve the water insoluble derivative artesunate (artesunic acid) in an aqueous solution weak alkali is required. The most frequently used alkali therefor is sodium bicarbonate. However, the prepared solution decomposes rapidly in an aqueous solution after preparation and also at high temperature and therefore not suitable to prepare preparations for injection; they are only useful as ready-to-use preparations. For the other derivatives, no pure aqueous solutions exist.

In order to improve their bioavailability by enhancing their water solubility, the mentioned compounds can be dissolved in organic solvents like alcohol solutions, propylene glycol and polyethylene glycol. However, these solvents have limited therapeutic use in injectable preparations. Other therapeutic agents, such as oil/water-emulsions, can also be prepared, using oils of natural origin. However such agents require special treatments during production and sterilization due to particle size and their instable character (colour formation, rancidity) and are thus very expensive. Solubility enhancers such as Artasolve®, Cremophor®, sodium lauryl(ether)sulfate or Tween®, can enhance the solubility of artemisinin and derivative compounds in aqueous solutions but are known to be aggressive in vivo. Phlebitis at the injection place of the solution and other systemic allergic reactions are very common undesirable effects with this type of injections. Moreover the compounds precipitate after injection due to dilution.

There remains a great need in the art for improved pharmaceutical compositions comprising artemisinin or its derivatives, showing improved bioavailability of the active compounds and which are suitable for use in injectable preparations. There also remains a great need in the art for improved pharmaceutical compositions comprising artemisinin or its derivatives, for emergency treatment of malaria in children in whom the disease generates quickly to severe complications.

In general, it is therefore an object of the present invention to provide an improved method for preparing a composition comprising artemisinin or its derivatives. Another object of the present invention is to provide an improved pharmaceutical composition comprising artemisinin or its derivatives. It is in particular an aim of the present invention to provide a pharmaceutical composition, which provides improved availability of the active
components and which are suitable for parenteral, rectal, topical and oral use. The invention also aims to provide a pharmaceutical composition, which is very suitable for intravenous administration, in particular for use as an infuse.

5 **Summary of the invention**

One embodiment of the present invention is a method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of:

(A) dissolving a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, in a suitable amount of organic solvent or ammonia solution such that a solution comprising an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained, and

(B) spray-drying said solution such that a spray-dried inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

15 Another embodiment of the present invention is a method as described above, wherein said spray-dried inclusion complex is further dissolved in water and sterilised.

Another embodiment of the present invention is a method as described above, wherein the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

Another embodiment of the present invention is a method as described above, wherein said organic solvent is selected from the group comprising alcohols, 10% to 100% ethanol 96%, 10 to 80% v/v isopropanol, acetonitril, tetrahydrofuran and dimethyl sulphoxide.

Another embodiment of the present invention is a method as described above, wherein the pH of the said ammonia solution is between 9 and 12.

30 Another embodiment of the present invention is a method as described above, wherein a suitable amount of artemisinin or derivatives thereof and a suitable amount of cyclodextrin are dissolved in a suitable amount of solvent in the presence of a suitable amount of one or more pharmaceutical polymers.

Another embodiment of the present invention is a method as described above, wherein said polymer is selected from the group comprising hydroxypropylmethylcellulose, hydroxypropylcellulose and polyvinylpyrrolidone.
Another embodiment of the present invention is a method as described above, wherein the suitable amount of said polymer is that capable of providing a pharmaceutical composition that is comprised between 0.05 and 1% polymer w/w.

Another embodiment of the present invention is a method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and kneading said artemisinin or derivatives thereof and said cyclodextrin such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

Another embodiment of the present invention is a method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of bringing a suitable amount of artemisinin or derivatives thereof, bringing the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and grinding said artemisinin or derivatives thereof and said cyclodextrin, such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

Another embodiment of the present invention is a method as described above, wherein said inclusion complex is further dissolved in water and sterilised.

Another embodiment of the present invention is a method as described above, wherein the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

Another embodiment of the present invention is a method as described above wherein said artemisinin derivatives are selected from the group comprising artesunate, artemether, arteether, artelnic acid, artesunic acid, and dihydroartemisinin.

Another embodiment of the present invention is a method as described above, wherein the suitable amount of artemisinin or derivatives is that capable of providing a
pharmaceutical composition that is between 0.025 and 25 % w/w of artemisinin or derivatives.

Another embodiment of the present invention is a method as described above wherein said cyclodextrin is an alpha, beta or gamma-cyclodextrin.

Another embodiment of the present invention is a method as described above wherein said cyclodextrin is selected from the group comprising hydroxypropyl-b-cyclodextrin, hydroxypropyl-\(\gamma\)-cyclodextrin, sulfobutylether-b-cyclodextrin, maltosyl-b-cyclodextrin and b-cyclodextrin.

Another embodiment of the present invention is a method as described above, wherein the suitable amount of cyclodextrin is that capable of providing a solution of a pharmaceutical composition that is comprised between 0.1 and 40 % w/w cyclodextrin.

Another embodiment of the present invention is a method as described above, wherein the suitable amount of cyclodextrin is that capable of providing a tablet of a pharmaceutical composition that is comprised between 10 and 90 % w/w cyclodextrin.

Another embodiment of the present invention is a method as described above, wherein the molar ratio of artemisinin or derivatives thereof to cyclodextrin in said inclusion complex is comprised between 3:1 and 1:25.

Another embodiment of the present invention is a method as described above, wherein said lyophilised form of said inclusion complex is dissolved in a suitable amount of water or solvent for obtaining an injectable solution or infusion.

Another embodiment of the present invention is a pharmaceutical composition comprising a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, obtainable by the method as described above.

Another embodiment of the present invention is a pharmaceutical composition as described above comprising an artemisinin derivative selected from the group comprising artesunate, artemether, arteether, artelinic acid, artesunic acid, and dihydroartemisinin.
6
Another embodiment of the present invention is a pharmaceutical composition as
described above, comprising a cyclodextrin selected from the group comprising
hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin,
maltosyl-β-cyclodextrin and β-cyclodextrin.

5
Another embodiment of the present invention is a pharmaceutical composition as
described above, wherein the amount of artemisinin or derivatives thereof is between
0.025 and 25 % w/w artemisinin or derivatives thereof in said composition.

10 Another embodiment of the present invention is a pharmaceutical composition as
described above, suitable for use as a solution wherein the amount of cyclodextrin is
between 0.1 and 40 % w/w cyclodextrin in solution.

Another embodiment of the present invention is a pharmaceutical composition as
described above, suitable for use as a tablet wherein the amount of cyclodextrin is
between 10 and 90 % w/w cyclodextrin in the tablet.

15 Another embodiment of the present invention is a pharmaceutical composition as
described above wherein the molar ratio of artemisinin or derivatives thereof to
cyclodextrin is comprised between 3:1 and 1:25.

Another embodiment of the present invention is a pharmaceutical composition as
described above for parenteral, rectal, oral or topical use.

20 Another embodiment of the present invention is a pharmaceutical composition as
described above for intravenous, intramuscular, peritoneal, peridural, rectal or oral
application.

Another embodiment of the present invention is a pharmaceutical composition as
described above for use as an infuse.

25 Another embodiment of the present invention is a pharmaceutical composition as
described above for use as an injectable solution, a lyophylisate, a capsule, a tablet, an
ampoule, a hydrogel, a cream or suppository.
Another embodiment of the present invention is a use of a pharmaceutical composition as described above in the preparation of a medicament for treating malaria.

Another embodiment of the present invention is a use of a pharmaceutical composition as described above in the preparation of a medicament for treating cancer.

Another embodiment of the present invention is a use of a pharmaceutical composition as described above in the preparation of a medicament for treating babesiosis.

Another embodiment of the present invention is a use of a pharmaceutical composition as described above in the preparation of a medicament for treating schistosomiasis.

Another embodiment of the present invention is a use of a pharmaceutical composition as described above in the preparation of a medicament for treating fungal, viral and/or bacterial infections.

Another embodiment of the present invention is a kit comprising a pharmaceutical composition as described above.

Another embodiment of the present invention is a kit comprising a pharmaceutical composition in lyophilised form obtainable by a method as described above.

Another embodiment of the present invention is a kit as described above further comprising a pharmaceutically acceptable solvent.

Another embodiment of the present invention is a kit as described above wherein said composition and pharmaceutically acceptable solvent are present in separate containers.

Another embodiment of the present invention is a kit as described above further comprising at least one needle and syringe.

Another embodiment of the present invention is a kit as described above for parenteral use.

The present invention relates to methods for preparing a pharmaceutical active composition comprising artemisinin or derivatives thereof in a form acceptable for different
administration routes. The invention also relates to the pharmaceutically active composition obtainable by such methods.

A first method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof according to the present invention comprises the steps of dissolving a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, in a suitable amount of solvent such that an solution comprising an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained; and spray-drying said solution such that a spray-dried inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

A second method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof according to the present invention comprises the steps of bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and kneading said artemisinin or derivatives thereof and said cyclodextrin such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

A third method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof according to the present invention comprises the steps of bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and grinding said artemisinin or derivatives thereof and said cyclodextrin, such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

In a preferred embodiment, the inclusion complex obtained by any of the methods according to the present invention is further dissolved in water and sterilised. The obtained sterilised solution can be used as such, if stability of the included active compound is assured up to the moment of administration. In yet another preferred embodiment, the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained. In the freeze-dried form, the preparation can be stored as long as necessary before administration.
The methods of preparation according to the present invention allow industrial production of powder for injection as well as tablets and solutions for oral use.

In another aspect the invention relates to the pharmaceutical composition comprising artemisinin or derivatives thereof obtainable by a method according to the invention. This composition can be applied parenterally, rectally, orally or topically. Preferably, the composition may be used for intravenous, intramuscular, peritoneal, peridural, rectal or oral application. In a preferred embodiment, the pharmaceutical composition according to the invention is intended to be used as an infuse.

The pharmaceutical compositions according to the present invention are particularly suitable for use in the preparation of medicaments in the treatment of various diseases, including malaria and cancer. Those skilled in the art will immediate recognize the many advantages and the numerous possibilities for end uses of the present pharmaceutical compositions from the detailed description and non-limiting examples provided below.

**Detailed description of the figures**

Figures 1A-1C represent the amount of three cyclodextrin derivates required for forming an inclusion complex with the artemisinin derivates artemether, artesunate and dihydroartemisinin, respectively. Fig. 1A represents dissolved artemether (in mg/100ml) in function of the concentration of the HP-β-CD, SBE-7-β-CD and MS-β-CD cyclodextrins (in % w/w). Fig. 1B represents dissolved artensate (in mg/100ml) in function of the concentration of the HP-β-CD, SBE-7-β-CD and MS-β-CD cyclodextrins (in % w/w). Fig. 1C represents dissolved dihydroartemisinin (in mg/100ml) in function of the concentration of the HP-β-CD, SBE-7-β-CD and MS-β-CD cyclodextrins (in % w/w).

**Detailed description of the invention**

**Methods of preparation**

The present invention relates to methods for preparing a composition comprising artemisinin or derivatives thereof. The methods comprise the use of cyclodextrins for entrapping the artemisinin or derivatives thereof and improving their solubility. The cyclodextrins improve the solubility of the artemisinin or derivatives thereof by entrapping artemisinin or its derivatives in the cyclodextrin ring.
According to the present invention, the term "artemisinin derivatives" refers to active compounds or chemicals or combined products derived from artemisinin, which are pharmaceutically active. This term may include oxides, esters, salts, metabolites and epimers. The artemisinin derivatives comprise compounds, which have been synthesized starting from the natural compound artemisinin. As used herein artemisinin and its derivatives are also referred to as "active compounds." Examples of such derivatives according to the invention comprise ester-like compounds such as artesunate, ether-like compounds such as arteether and artemether, acid form such as artelonic acid and artesunic acid, and the reduced lactol derivative of artemisinin dihydroartemisinin (DHA, artecho). DHA is the active schizonticidal metabolite of artemisinin and the mentioned derivatives. As a consequence, artemisinin and its derivatives, with exception of DHA, can be regarded as pro-drugs for DHA. The term "artemisinin derivatives" also refers to alpha or beta epimers of the mentioned derivatives. The term "alpha and beta epimer" distinguishes two compounds, from which the spatial orientation of the different groups provided on only one carbon atom C10 differs.

The term "cyclodextrins" as used herein comprise all alpha, beta or gamma-cyclodextrins that are suitable for parenteral, topical, rectal or oral use. In a preferred embodiment, the cyclodextrins used in the method according to the invention are selected from the group comprising hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) and maltsyl-β-cyclodextrin (MS-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD). In an even more preferred embodiment, the cyclodextrin used in the method according to the invention is sulfobutylether-β-cyclodextrin. Sodium sulfobutylether-β-cyclodextrin contains an average of 7 sulfobutyl ether groups per cyclodextrin molecule. Because of the very low pKa of the sulfonic acid groups, the molecule carries multiple negative charges at physiologically compatible pH values. This phenomenon and the carbon butyl chains at the end group of the negative charges, prolonging the cyclodextrin cavity, often results in stronger binding to active compound candidates in comparison with other cyclodextrins. As a consequence this cyclodextrin provides a higher solubility and safety when used in compositions for parenteral use. Sodium sulfobutylether-β-cyclodextrin has been shown to be a very safe material for use in pharmaceutical formulations. Cyclodextrins are suitable for dissolving poorly water-soluble drugs. Cyclodextrins have been used extensively as effective in this domain. Examples of cyclodextrins involve α-, β, γ-cyclodextrins which consist of respectively 6, 7 and 8 glucose units, forming 3 dimensional polyglucose rings or "crowns". It is known that the solubilizing effect of
cyclodextrins is produced by the formation of complexes containing the active ingredients in the cyclodextrins, by entrapping the active ingredients in the cyclodextrin ring. Also various homologues of such cyclodextrins are known. Their water solubility and their capability to complex a certain drug depend on their structure.

Nevertheless, β-cyclodextrins are known to have limited water solubility. Therefore, many β-cyclodextrin derivates were developed improving water solubility but only a very few are suitable and available for pharmaceutical applications. Their availability is limited by the costly toxicological evaluations of the different cyclodextrins. For instance, safety profiles of most of the above-mentioned cyclodextrins have demonstrated that orally administrated α-, β-, and γ-cyclodextrins are practically non-toxic, due to the lack of absorption from the gastro-intestinal tract. Furthermore, other safety evaluation studies showed that only hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) and maltosyl-β-cyclodextrin (MS-β-CD) appear to be safe even when parenterally administered. However, the natural α- and β-cyclodextrins, and methylated β-cyclodextrins are not suitable for parenteral administration. In fact, there is a great need in the art for lowering the necessary amount of cyclodextrin in pharmaceutical preparations, not only for safety and toxicological reasons but also for reducing cost and for reasons of production capabilities.

The applicant has now provided methods for preparing compositions comprising artemisinin or derivatives thereof wherein a cyclodextrin is successfully applied, despite its above-mentioned property of limited water solubility. Also, the methods according to this invention result in pharmaceutical compositions, wherein the necessary amount of cyclodextrin to be used is reduced. In addition, the present invention provides methods that considerably reduce time and costs for preparation of the pharmaceutical compositions.

A first method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprises dissolving a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, in a suitable amount of solvent such that an solution comprising an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained; and drying said solution such that a dried inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained. The term
“drying” may comprise freeze-drying or spray drying. “Freeze-drying” refers to the process of drying a sample in a frozen state under vacuum, and the result of such process is a lyophilized product or powder. “Spray-drying” as used herein refers to the process of transformation of liquid solutions into powder or dried products by spraying into hot environments so that liquid phase of droplets vaporises to leave solid particles.

In a preferred embodiment, the first method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprises dissolving a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, in a suitable amount of solvent such that a solution comprising an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained; and spray-drying said solution such that a spray-dried inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

In this method, artemisinin or derivatives thereof and the cyclodextrin may be dissolved separately or dissolved together. An important criterion is the choice of the solvents. The inventors have found that the use of either organic solvents or an ammonia solution allows for direct and fast dissolution of both components, so that an industrial preparation is feasible in a suitable time and short time span. Moreover, the inclusion capacity (the maximum concentration or amount of active compound) using a given cyclodextrin concentration can be increased when either of these solvents is used. Also an improved efficacy and reduced toxicity can be obtained. The inclusion of the active compound shows a high solubility in water when either of these solvents are used. Therefore the concentration in tablets needed to be efficacious for antimalarial treatment, for example, can be diminished. Because a lower concentration of the active compound is required, a lower toxicity is obtained.

Another consideration is the choice of organic solvent with regards to toxicity. As small residual solvents are always present in a lyophilised product obtained according to the method of the invention, the choice of organic solvents was taken from class III (ICH, 1997). In a preferred embodiment, said solvents are selected from the group comprising: alcohols, ethanol 96°, ethanol 96°:water – wherein proportion by volume of ethanol is in the range 10 to 100 % v/v, 10 to 80 % v/v, 20 to 80 % v/v, 30 to 80 % v/v, 40 to 80 % v/v, 50 to 80 % v/v, 60 to 80 % v/v, 70 to 80 % v/v, 10 to 70 % v/v, 10 to 60 % v/v, 10 to 50 % v/v, 10 to 40 % v/v, 10 to 30 % v/v, 30 to 70 % v/v, 40 to 60 % v/v, 50 % v/v, and
isopropanol, water:isopropanol – wherein proportion by volume of isopropanol is in the range 10 to 80 % v/v, 20 to 80 % v/v, 30 to 80 % v/v, 40 to 80 % v/v, 50 to 80 % v/v, 60 to 80 % v/v, 70 to 80 % v/v, 10 to 70 % v/v, 10 to 60 % v/v, 10 to 50 % v/v, 10 to 40 % v/v, 10 to 30 % v/v, 30 to 70 % v/v, 40 to 60 % v/v, 50 % v/v, acetonitrile, tetrahydrofuran (THF) and dimethylsulfoxide (DMSO). In a more preferred embodiment, said solvent is DMSO. DMSO is particularly suitable, since this solvent is able to freeze-dry at relatively elevated temperature, around 0°C, and can rapidly evaporate, which considerably increases the preparation process of the composition.

Another consideration is the choice of the ammonia solvent with regards to the solubility of the artemisinin derivative and its degradation in such solution. In a preferred embodiment, the said ammonia solution is selected from a range of ammonia solutions from which the pH is comprised between 7 and 12, and preferable between pH 8 and 11 and more preferably between 9 and 10, and even more preferably 9.3 to 9.7.

The ammonia solution with artesunate is prepared as such that the required amount of artesunate can dissolve in a certain amount of water by dropwise addition of ammonia 25% (w/w) or a dilution of it.

In a preferred embodiment, the said spray-dried inclusion complex obtained according to the invention is further dissolved in water and sterilised. Sterilisation may comprise, but is not limited to, filtrating or autoclaving the dissolved residue such that a sterile solution is obtained. In another further embodiment of the invention the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

In another embodiment of the method according to the invention a suitable amount of artemisinin or derivatives thereof and a suitable amount of cyclodextrin are dissolved in a suitable amount of solvent in the presence of one or more pharmaceutical polymers. In a preferred embodiment, said polymer is selected from the group comprising hydroxypropylmethylcellulose, hydroxypropylcellulose and polyvinylpyrrolidone (PVP). Water-soluble pharmaceutical polymers are widely used as pharmaceutical excipients such as emulsifiers, suspending agents, flocculating agents, coating materials and binders.
Unexpectedly, it was found that the use of pharmaceutical polymers enhances the complexation efficacy of the cyclodextrins. Polymers provide a synergistic effect on the capacity of cyclodextrins to form complexes with the water-insoluble artemisinin or its derived compounds. Addition of polymers according to the invention enables to reduce the amount of cyclodextrins required for suitably entrapping said artemisinin or its derivatives.

In a preferred embodiment, the amount of pharmaceutical polymer, such as hydroxypropylmethylcellulose, hydroxypropylcellulose and polyvinylpyrrolidone (PVP) used in the method according to the invention is comprised between 0.05 and 1% w/w, and preferably between 0.1 and 0.5% w/w. In yet another preferred embodiment, the ratio of artemisinin or derivatives thereof to the polymer used in the method according to the invention is comprised between 6:1 and 3:10, and more preferably between 3:1 and 3:5. In another preferred embodiment, use of a polymer in the method according to the invention enhances the complexation of the active compound with 10 to 130%.

The use of polymers enhances the capacity of the cyclodextrins to include the therapeutically active compound. Thus, the use of polymers enables to reduce the amount of cyclodextrins necessary in the composition according to the invention. Lowering the necessary amount of cyclodextrin in pharmaceutical preparations is not only advantageous for safety and toxicological reasons but also for reducing costs and for reasons of production capabilities. In addition, the use of polymers can also induce a enhanced and more rapid release of the active compound out of the pharmaceutical composition.

A second method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprises the steps of bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and kneading said artemisinin or derivatives thereof and said cyclodextrin such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

A third method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprises the steps of bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and grinding said
15 artemisinin or derivatives thereof and said cyclodextrin, such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

Advantageously, in these methods, the problem of the limited solubility of artemisinin or its derivatives and of cyclodextrin is resolved by using a process, which does not require the dissolution of the components. Artemisinin or its derivatives and cyclodextrin are mixed together and kneaded or grinded to form an inclusion complex; only a small amount of solvent is used in this method. In a preferred embodiment, the inclusion complex obtained by the first or the second or the third method according to the present invention is further dissolved in water and sterilised, if required for the pharmaceutical formulation. Sterilisation may comprise, but is not limited to, filtrating or autoclaving the dissolved residue in order to obtain a sterile solution. In another further embodiment of the invention the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

The use of cyclodextrins for entrapping artemisinin or derivatives thereof according to the methods of the invention provides several beneficial effects. In first instance, it improves the solubility of artemisinin or derivatives thereof. Cyclodextrin also provide protection to the artemisinin or derivatives thereof from reacting with their environment since formation of the compounds/cyclodextrin complexes often has a stabilizing effect. The effects on the chemical stability can be suitable, especially for artesunate in this invention, because the water soluble salt of this active compound is known to be unstable in solution. Furthermore, the formation of inclusion complexes can alter the physical state, and consequently influence the bioavailability or absorption of the artemisinin or derivatives thereof and provide more convenient pharmaceutical dosage forms with improved chemical stability of the active compound. Cyclodextrins create and maintain stable homogeneous distributions of the encapsulated artemisinin or its derivatives and can reduce unpleasant side effects such as irritation caused by contact of more conventional excipients, used in injectable preparation with non-water soluble active compounds e.g. Cremophor®, polyethylene glycol with tissues e.g. extravasations at the injection site and gastro-intestinal tract irritations. Moreover, they lead to an enhanced bioavailability, and enable to lower the dosage of active compound, which needs to be administered for efficacy. Also, the formation of inclusion complexes with cyclodextrins has the advantages that precipitation after dilution of the active compounds can be avoided.
In another embodiment, the invention relates to a method wherein said artemisinin derivatives are selected from the group comprising artesunate, artemether, arteether, artelinic acid, artesunic acid, and dihydroartemisinin. In a preferred embodiment, the suitable amount of artemisinin or derivatives thereof is comprised between 0.025 and 25 % w/w, and preferably between 0.05 and 15 % w/w and even more preferred between 0.10 and 10 % w/w. Said percentages refer to the weight of artemisinin or derivative with respect to the weight of the final composition after reconstitution of the lyophilisate or in the solid (tablets) or dry forms, other forms (e.g. gel, spray) produced by the method.

In another embodiment, the invention relates to a method wherein said cyclodextrin is an alpha, beta or gamma-cyclodextrin. Preferably, said cyclodextrin is selected from the group comprising hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin and maltosyl-β-cyclodextrin. Even more preferably the β-cyclodextrins applied in this invention for parenteral purposes comprise hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) and maltosyl-β-cyclodextrin (MS-β-CD). Preferably, the cyclodextrins applied in this invention for oral use comprise natural α- and β-cyclodextrins, methylated β-cyclodextrins and derivates thereof, and the hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) and maltosyl-β-cyclodextrin (MS-β-CD).

The pharmaceutical composition according to the present invention may be used as a solution or in the form of a tablet. When used as solution, the suitable amount of cyclodextrin in said compositions is preferably comprised between 0.1 and 40 % w/w, and preferably between 2.0 and 35 % w/w, an even more preferred between 5.0 and 30 % w/w. When used as tablets, the suitable amount of cyclodextrin in said compositions is preferably comprised between 10 and 90 % w/w, and preferably between 12.5 and 85 % w/w, an even more preferred between 15 and 80 % w/w. The ranges for suitable amount of cyclodextrin are lower for applications in parenteral solutions than in tablets. For parenteral solutions it is required that all artemisinin or derivatives thereof is included in the inclusion complex in order to avoid precipitation of the composition. Also, the amount of CD is to be reduced in parenteral solutions in comparison to tablet, in order to limit hyper tonicity of the composition.
In yet another preferred embodiment, the molar ratio of artemisinin or derivatives thereof to cyclodextrin in said inclusion complex is comprised between 3:1 and 1:25, and preferably between 2:1 and 1:20, and even more preferred between 1:1 and 1:10.

According to a further embodiment, the lyophilised form of said inclusion complex is dissolved in a suitable amount of pharmaceutically acceptable solvent for obtaining an injectable solution or infusion or an oral or rectal formulation. In another preferred embodiment, the lyophilised form of said inclusion complex can be dissolved and further diluted up to 100 times, and preferably up to 1000 times and even to 10,000 times without occurrence of precipitation, which might take place in the human body (blood stream) at the moment of injection and which can occur when an infusion is prepared. In another preferred embodiment, the lyophilised form of said inclusion complex can be dissolved in less than 3 minutes, and preferably in less than 2 minutes, and even more preferred in less than 1 minute.

**Pharmaceutical compositions**

In another embodiment, the present invention relates to a pharmaceutical composition comprising a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin obtainable by the method according to the present invention. In a preferred embodiment, the pharmaceutical composition comprises an artemisinin derivative selected from the group comprising artesunate, artemether, arteether, artelnic acid, artesunic acid, and dihydroartemisinin. In another preferred embodiment, the pharmaceutical composition comprises a cyclodextrin which is an alpha, beta or gamma-cyclodextrin. In a more preferred embodiment, said cyclodextrin is selected from the group comprising hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin and maltosyl-β-cyclodextrin.

In another embodiment, the suitable amount of artemisinin or derivatives thereof in said pharmaceutical composition is comprised between 0.025 and 25% w/w and preferably between 0.05 and 15% w/w and even more preferred between 0.1 and 10% w/w of either a dry or solid form (e.g. a tablet, or powder), a solution, gel or other composition.

In a preferred embodiment, the suitable amount of cyclodextrin in said pharmaceutical composition when used as solution, is preferably comprised between 0.1 and 40 % w/w, and preferably between 2.0 and 35 % w/w, an even more preferred between 5.0 and 30 %
w/w. In another preferred embodiment, the suitable amount of cyclodextrin in said pharmaceutical composition when used as tablets, is preferably comprised between 10 and 90% w/w, and preferably between 12.5 and 85% w/w, even more preferred between 15 and 80% w/w.

In yet another embodiment, the molar ratio of artemisinin or derivatives thereof to cyclodextrin in the pharmaceutical composition according to the invention is comprised between 3:1 and 1:25, and preferably between 2:1 and 1:20 and even more preferred between 1:1 and 1:10.

The pharmaceutical compositions according to the invention can be provided in the form of a sterile solution, if stability is allowed by environmental conditions, or as a lyophilisate, i.e. in powder form. It is known that at least some artemisinin derivatives degrade in the presence of moisture. Providing artemisinin or its derivatives in lyophilised form has the advantage of avoiding their degradation. Also, in lyophilised powders cyclodextrins are good cryo-protectors. It is not necessary to add other excipients as cryo-protectors. The cyclodextrins contribute to the tonicity, required for the injectable solution, especially for high volume solutions and infusions. Starting from this lyophilised form injectable solutions of the composition can be obtained by dissolving the lyophilised form in water or aqueous solvent. Alternatively, also tablets and capsules can be made starting from the lyophilised form.

When the compositions according to the invention are used in the form of a solution, the solution as such can be injected directly (bolus injection). The advantage of preparing a solution starting from the lyophilised form is the fact that a real solution is obtained and not a suspension, which cannot be injected via intravenous mode, but only via intramuscular mode. The solution of the composition according to the invention may be further diluted in water according to the need of the dose required for the patient. In diluted form the active compounds will retain their activity and will not precipitate, as illustrated in example 3. In a preferred embodiment, the pharmaceutical composition according to the invention is used as an injectable solution. In a particularly preferred embodiment, the pharmaceutical composition according to the invention is used as an infuse.

Tablets or capsules can for instance be prepared starting from the lyophilised form, or from the products obtained by the first and second method, i.e. the spray-dried or the kneaded or grinded inclusion complex. Such formulations are, evidently, most suitable for oral administration. For such formulations the use of cyclodextrin not only enhances water
solubility of the active compounds but also improves their stability, increase their bioavailability and mask the taste and smell of the medication. The lyophilised products can optionally be mixed with the usual tablet excipients such as binders such as polyvinylpyrrolidone, microcrystalline cellulose, starch, clays, disintegrants such as crosscarmellose, crosspovidone, starch, starch derivatives; fillers such as lactose, dicalciumphosphate, or glidants like magnesium stearate. The products are generally compressed with direct compression or with the wet granulation method, dependent on the content of CD-complex in the tablet. Other acceptable formulations of the compositions according to the invention may include, but are not limited to ampoules, hydrogels, creams or suppositories.

In a preferred embodiment, the invention relates to pharmaceutical compositions that are suitable for parenteral, rectal, oral or topical use. The pharmaceutical compositions according to the invention formulated as solution are suitable for parenteral use. The term "parenteral" refers to the administration as a substance by means of an injection, e.g. by intra-dermal, subcutaneous, intra-muscular, intravenous injection, or non-enteral administration. In a preferred embodiment, the solutions are administered as intravenous (IV), intramuscular (IM), peritoneal, peridural injections or rectal administration such as enemas. It is even more preferred to administer the solution according to the invention via intravenous route. Such intravenous formulation is particularly advantageous since it acts immediately and provides a direct efficacy, as is explained in more detail below.

Topical use refers to the use of the composition by applying it onto the skin of a patient. The inclusion complex of artemisinin or its derivative and cyclodextrin according to the invention can be incorporated in a hydrogel or a cream for topical use. Hydrogels may include excipients for gel formation such as hydroxypropylcellulose. The hydrogel or cream can be directly applied on the skin and act systemically as a transdermal preparation.

The rectal administration may include the injection of a pharmaceutical composition in solution form, which results in fast absorption of the active compounds in vivo. Since, for parenteral application, IM injections are rather painful, administering the solution according to the invention via intravenous (IV) mode is a suitable alternative. Alternatively, for rectal administration acceptable formulations of the compositions according to the invention may also include suppositories.
For oral administration, ampoules containing the solution form of the pharmaceutical preparations can be used. In particular, non-lyophilised solutions of artemether and arteether are suitable here for, since these compounds do not degrade in non-lyophilised form. Preferably, a taste-masking product is added to mask the bitter taste originating from the artemisinin derivatives. Generally, for oral preparations sweeteners and flavouring agents can be added. Pharmaceutically acceptable sweeteners comprise bulk sweeteners and/or intense sweeteners. As bulk sweeteners glucose, sucrose, fructose, maltose, isomalt, sorbitol, mannitol, xylitol, hydrogenated glucose syrup, caramel of honey can be used, preferably sucrose is used. As intense sweeteners, saccharin and its salts sodium or calcium, sodium cyclamate, dihydrochalcone sweetener, stevioside or sucralose, and preferably acesulfame potassium can be used. The intense sweeteners are conveniently employed in low concentrations. In the case of sodium saccharin the concentrations may range from 0.1 to 0.4 % w/w. The flavouring agents, masking the bitter taste for oral preparations are preferably fruit flavors such as black current, raspberry, strawberry, tutti frutti, preferably tutti frutti. Each flavor may be present in a concentration ranging from 0.05 to 1 % (w/v)

Use of the compositions
The pharmaceutical compositions according to the invention can be used in the preparations of medicaments for treating various human as well as veterinary diseases. In an embodiment the pharmaceutical composition according to the present invention is usable in the preparation of a medicament for treating malaria. The compositions are intended to cure malaria in all their forms: i.e. non-complicated, complicated, non-severe, severe, cerebral and multi-resistant malaria.

Malaria is caused by protozoa of the genus *Plasmodium*. The four species of *Plasmodium* that infect humans are *Plasmodium vivax*, *P. malariae*, *P. ovale* and *P. falciparum*, the last one is responsible for producing severe complications and cerebral malaria, which can cause the patient to lapse into a coma and ultimately leads to death. In some parts of the world, some resistance of strains of *P. falciparum* was noticed for chloroquine, mefloquine, halofantrine, quinine and combination of sulfadoxin with pyrimethamine, the trusted drugs of choice for control of malaria. Likewise *P. vivax* infections resistant to chloroquine are emerging in different countries. More than 270 million people suffer from the disease, and 1.2-1.7 million deaths occur yearly. Mortality is more among children under 5 years of age who are particularly sensitive because of their lack of immunity to the disease.
Administration of the solution according to the invention, in particular by intravenous injection, is particularly beneficial in the treatment of malaria. For the treatment of malaria, and in particular for the treatment of fatal cerebral malaria, there has been a long felt need for a preparation of artemisinin derivatives that acts fast and attains high therapeutic levels. Also, the bioavailability is low. In the case of IM injections there are further limitations in respect of the therapeutic effect. In acute cases, especially in small children with severe cerebral malaria, the IM injection fail to deliver the therapeutic effect within a short period of time and the patient suffers sometimes immensely, resulting in loss of life. According to the invention a composition comprising artemisinin or derivatives thereof is provided which is particularly suitable for intravenous route of administration. Through the intravenous route the active compounds directly reach the site to kill parasites in the brain where the malaria parasite *Plasmodium falciparum*, has fatal effects.

In another embodiment the pharmaceutical composition according to the present invention is usable in the preparation of a medicament for treating cancer. Administration of the pharmaceutical composition as an injectable solution or infuse according to the invention by intravenous injection, is also particularly beneficial in the treatment of cancer. In particular, several cancer types including breast cancer, bone cancer and leukemia, may be treated by used the compositions according to the invention. It is known that artemisinin derivatives react with the high iron concentrations found in the malaria parasite, to develop free radicals, which attack cell membranes, by breaking them apart and so killing the single-cell parasite. Especially in human breast cancer, tumor cells also metabolize and retain high ferrous concentrations. Also in leukemia the cancer cell have high iron concentration. It is known from artemisinin that when in contact with iron, a chemical reaction is developed, generates free radicals which attack cell membranes. The level of iron in the leukemia cells is around 1000 times higher than normal cells.

In yet another embodiment the pharmaceutical composition according to the present invention is usable in the preparation of a medicament for treating babesiosis. Babesiosis refers to an illness caused by the parasite Babesia that is transmitted from animals to humans by ticks. The signs and symptoms include fever, chills, sweating, muscle aches, fatigue, enlargement of the liver and spleen, and haemolytic anaemia (anaemia due to break-up of red cells).

In yet another embodiment the pharmaceutical composition according to the present invention is usable in the preparation of a medicament for treating schistosomiasis.
Schistosomiasis, also called bilharzias, refers to diseases of liver, gastrointestinal tract and bladder caused by schistosomes, trematode worms that parasitize human. Infection is from infested water. There are three main species of these trematode worms (flukes), *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni*, that cause disease in humans.

The pharmaceutical composition according to the present invention is further usable in the preparation of a medicament for treating fungal infections, such as those caused by *Candida albicans*, and bacterial infections, e.g. avian coccidiosis in animals and viral infections such as aids, cytomegalus virus infection and influenza.

The pharmaceutical composition according to the present invention is further usable in the preparation of a medicament for lupus erythematosus.

In addition, the pharmaceutical composition according to the present invention is further usable in the preparation of a medicament for treating various other diseases including but not limited to the diseases discussed above and the complication thereof, skin diseases such as psoriasis, blistering skin disease, bullous skin disease, warts, *Molluscum contagiosum*, *Ecthyma contagiosum* and *Lupus erythematosus*, haemorrhoids, neosporosis, toxoplasmose, leishmaniasis.

**Kits**

In another embodiment, the present invention further provides a kit comprising a pharmaceutical composition according to any of the embodiments of the present invention.

In another embodiment, the invention provides a kit comprises a pharmaceutical composition in lyophilised form obtainable by a method according to the present invention.

A kit according to the present invention may further comprise a pharmaceutically acceptable solvent for dissolving said lyophilised form. The pharmaceutically acceptable solvent may be provided in a separate vial. Examples of pharmaceutically acceptable solvents include, but are not limited to water, saline, buffered water, glucose solution or any other solution that can be used to dissolve the lyophilisate to make an isotonic solution. Such kits enable the use of a lyophilised product of artemisinin or its derivative in combination with a cyclodextrin and to dissolve the lyophilisate in a suitable solvent at the moment of use. Both components of such kits are used in proportions such that a pH
value and tonicity are obtained which is suitable in accordance with the needs for injections. A kit may further comprise one or more syringes and needles.

A kit may provide or be used to prepare a composition suitable for administration by injection (parenteral use). A kit may provide or be used to prepare a composition suitable for administration by nasal spray. A kit may provide or be used to prepare a composition suitable for administration sublingually.

Another embodiment of the present invention is a kit for parenteral use. Another embodiment of the present invention is a use of a kit for parenteral administration.

**Examples**

The invention is further illustrated by the following non-limiting examples. Example 1 illustrates a method for determining the minimal amounts of cyclodextrins required for preparing the compositions according to the invention. Example 2 illustrates the preparation of several compositions according to the invention. Example 3 illustrates some properties of the compositions according to the invention.

20

*Example 1: Determination of minimal amounts of cyclodextrins required for preparing compositions according to the invention*

It is important to minimize the use of cyclodextrins, not only because of the high cost of cyclodextrins but even for requirements for injectable solutions, concerning their tonicity. This example illustrates the determination of the amounts of cyclodextrin to be used in preparation of compositions according to the invention.

In this example a composition comprising 300 mg of the compounds artemether, dihydroartemisinin or artesunate is to be made. In order to determine the amount of cyclodextrins required for suitable encapsulation of this concentration of the artemisinin derivatives, phase diagrams were drawn using the active compounds and different cyclodextrins. Such diagrams enable to determine the increase in water solubility of the artemisinin derivatives as a function of cyclodextrin concentration. The active compounds artemether (AM), dihydroartemisinin (DHA) or artesunate (ARS) were added in excess to aqueous solutions containing various amounts going from 0 to 40% w/w of the cyclodextrins hydroxypropyl-β-cyclodextrin (HP-β-CD), sulfobutylether-β-cyclodextrin (SBE-7-β-CD) or maltosyl-β-cyclodextrin (Maltosyl-β-CD). The suspensions were shaken
at 25 °C up to equilibrium for 14 days. Each sample was centrifuged for 10 min at 1512 g. From the clear supernatant, 2 ml was taken and appropriately diluted with acetonitrile to fall in the linear range of the calibration line for analysis. All dilutions were executed by weighing and using the densities of the solutions. The concentrations of the dissolved active compounds were analysed by HPLC. Figures 1A-1C represent the phase diagrams of artemether, artesunate and dihydroartemisinin with three cyclodextrin derivatives. For the amount of 300 mg active compound, the required minimum concentration of the cyclodextrin derivatives was calculated. This amount is representative for the amount of active compound, which is generally administered via infusion route. Results for the different cyclodextrins tested are summarized in Table 1.

<table>
<thead>
<tr>
<th>Active compound</th>
<th>CD derivative</th>
<th>% w/w CD to dissolve 300 mg active compound (0.3 % w/w) in 100 ml water</th>
<th>Ratio by weight Active compound : CD derivative</th>
<th>Molar ratio : Active compound : CD derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>HP-β-CD&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.71</td>
<td>1:25</td>
<td>1:5.73</td>
</tr>
<tr>
<td></td>
<td>SBE-7-β-CD&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.51</td>
<td>1:25</td>
<td>1:3.33</td>
</tr>
<tr>
<td></td>
<td>Maltosyl-β-CD&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.05</td>
<td>1:43</td>
<td>1:8.89</td>
</tr>
<tr>
<td>ARS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>HP-β-CD</td>
<td>7.42</td>
<td>1:25</td>
<td>1:7.11</td>
</tr>
<tr>
<td></td>
<td>SBE-7-β-CD</td>
<td>10.85</td>
<td>1:33</td>
<td>1:8.05</td>
</tr>
<tr>
<td></td>
<td>Maltosyl-β-CD</td>
<td>9.76</td>
<td>1:25</td>
<td>1:8.57</td>
</tr>
<tr>
<td>DHA&lt;sup&gt;c&lt;/sup&gt;</td>
<td>HP-β-CD</td>
<td>7.34</td>
<td>1:25</td>
<td>1:5.20</td>
</tr>
<tr>
<td></td>
<td>SBE-7-β-CD</td>
<td>8.01</td>
<td>1:26</td>
<td>1:3.39</td>
</tr>
<tr>
<td></td>
<td>Maltosyl-β-CD</td>
<td>25.96</td>
<td>1:83</td>
<td>1:16.86</td>
</tr>
</tbody>
</table>

<sup>a</sup> molecular weight of ARM is 298.4; <sup>b</sup> molecular weight of ARS is 384.4; <sup>c</sup> molecular weight of DHA is 284.3; <sup>d</sup> molecular weight of HP-β-CD is 1338; <sup>e</sup> molecular weight of SBE-7-β-CD is 2241; <sup>f</sup> molecular weight of Maltosyl-β-CD is 1459

With other cyclodextrins and other artemisinin derivatives similar data can be obtained. It is clear that, if lower concentrations of active compounds are required, e.g. in for types of administration, the necessary amounts of cyclodextrins can be calculated in the same manner.

In conclusion, in this example, the amount of cyclodextrin required for entrapment of artemether (AM), dihydroartemisinin (DHA) or artesunate (ARS) is preferably comprised in the range of 6 to 28 % w/w. These results were obtained by use of the shaking method according to the present invention in which an excess of active compound was shaken with an aqueous cyclodextrin-solution with a certain concentration and the presence of
complex is quantitatively determined. Lower concentrations can be obtained with the kneading method and grinding method according to the present invention.

Example 2  Preparation of a pharmaceutical compositions in lyophilised form
The present example illustrates different preparation methods for preparing the pharmaceutical compositions according to the invention comprising artemisinin or its derivatives in order to obtain a lyophilised form.

Example 2.1
In this example, the artemisinin derivates artemether, dihydroartemisinin or artesunate were dissolved with a cyclodextrin in a common solvent. The amount of artemisinin derivates applied comprised 0.3% w/w in the solution, prepared from the lyophylisate. The applied cyclodextrin comprised SBE-7-β-CD, in 9%, 12% and 15% w/w. The solvent comprised DMSO, which shows the advantage that it freezes at about 4°C. Use of DMSO as a solvent for dissolving SBE-7-β-CD together with either artemether, dihydroartemisinin or artesunate was highly efficient and enabled to obtain a very porous and dry composition. Dissolution was very quick. The mixtures were lyophilized and a lyophilized product was obtained.

Example 2.2
In this example the artemisinin derivates artemether, dihydroartemisinin or artesunate were dissolved with a cyclodextrin in a common solvent. The amount of artemisinin derivates applied comprised 0.3% w/w in the solution, prepared from the lyophylisate. The applied cyclodextrin comprised HP-β-CD, in 9%, 10% and 11% w/w. The solvent comprised ethanol (96 °). The solutions were dried under a stream of nitrogen, dissolved in water, freeze-dried at -45°C and lyophilized at -40°C. When water was added, the lyophilisates dissolved immediately.

Example 2.3
In this example, the artemisinin derivative, artesunate or any other derivative comprising an acidic moiety allowing the formation of ammonia salts, is dissolved in an ammonia solution (pH 9.5). The amount of artemisinin derivative applied comprised 0.3% w/w in the solution prepared from the lyophylisate. The applied cyclodextrin comprised SBE- β–CD
8% w/w and HP-β–CD 9% w/w, which were dissolved separately into a certain amount of water. The solvent for the artemisinin derivative comprised an ammonia solution pH 9.5 which shows the advantage that it freezes at about 0°C. Use of ammonia solutions with artesunate was highly efficient and enabled a very porous and dry composition with no organic residuals or only very low ammonia organic residual to be obtained.

The ammonia solution with artesunate and the solution of the cyclodextrin were mixed and lyophilized. When water was added, the lyophilsates dissolved immediately.

Example 2.4.
In this example, the artemisinin derivative, arteether, or any other derivative, and any other CD first dissolved in an alcoholic solution and then spray-dried. The amounts of arteether and CD are comprised as follows: HP-β–CD (ratio AM/ HP-β–CD : 1:4 or 1:9 w/w) or β–CD (ratio AM/ β–CD : 1:2.5 or 1:4 w/w) were respectively dissolved in alcohol 96° and isopropanol:water (1:1 v/v) and spray-dried at 50°C. An alternative for preparation of the inclusion complex is that the artemisinin derivative, arteether, or any other derivative, and β–CD were kneaded during 30 min while adding a small amount of water ratio. The amounts of the respective compounds are AM:CD:water at 7:15:11 w/w/w.

To this inclusion complex tablet excipients were added (lactose, Avicel PH102®, Crosscarmellose, Aerosil® 200, magnesium stearate) and mixed. A granulate was prepared with water as solvent and sieved. Secondly, the glidant magnesium stearate was added and mixed. From this mixture, tablets were prepared, having suitable tablet characteristics (hardness, friability, disintegration, dissolution). Additionally, based on the in vitro dissolution results an enhanced bioavailability can be suggested from the tablets with active compound present as inclusion complex compared to normal tablets.

Example 3   Properties of some prepared lyophilised pharmaceutical compositions

Properties of three lyophilised compositions obtained in example 2 were investigated and are represented in Table 2.

TABLE 2   Tested lyophilized compositions

<table>
<thead>
<tr>
<th>Composition</th>
<th>Solvent used in preparation</th>
</tr>
</thead>
</table>

No crystals were observed in the lyophilisates, which indicates the formation of solid inclusion complex. The lyophilisates were dissolved in water. The necessary water was added to prepare a solution with 0.3 % w/w active compound. The dissolution was very fast. All preparations dissolved in less than one minute. This is also an indication for the formation of a dry inclusion complex. The pH of the solutions was slightly acid and the preparations were hypotonic.

A suitable amount of phosphate buffer (NaH₂PO₄/ Na₂HPO₄), NaOH 0.1N and NaCl were added to adjust the pH and isotony of the composition after reconstitution. Results are given in Table 3.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Solvent</th>
<th>pH</th>
<th>MOsm/kg</th>
<th>Adjustment for 100 ml for isotony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemether - 9 % w/w SBE-7-β-CD</td>
<td>Alcohol 20 %</td>
<td>6.23</td>
<td>101</td>
<td>1.01 g NaH₂PO₄ * 0.21 g Na₂HPO₄ -</td>
</tr>
<tr>
<td>Artemether - 9 % w/w HP-β-CD</td>
<td>Alcohol 20 %</td>
<td>6.52</td>
<td>290</td>
<td>NaOH 0.01 N *</td>
</tr>
<tr>
<td>Artemether - 9 % w/w SBE-7-β-CD</td>
<td>Ammonia</td>
<td>4.34</td>
<td>84</td>
<td>0.648 % NaCl</td>
</tr>
<tr>
<td>Artemether - 8 % w/w SBE-7-β-CD</td>
<td>Ammonia</td>
<td>5.62</td>
<td>255</td>
<td>0.135 % NaCl</td>
</tr>
<tr>
<td>Artemether - 9 % w/w SBE-7-β-CD</td>
<td>Ammonia</td>
<td>5.76</td>
<td>300</td>
<td>----</td>
</tr>
</tbody>
</table>

* for pH adjustment to 7.4.

Analysis of residual solvents in cyclodextrin-complexes with GC (Table 4) revealed that an extra drying step for inclusion complexes, prepared with alcohol is required to lower the residual amount of alcohol within acceptable limits (ICH: maximal daily dose of ethanol is 50 mg). Most of the residual ammonia solution disappeared during the lyophilisation step, which is proven by the low residual values.
Table 4. The amount of residual solvents, ethanol and ammonia in CD-samples of AM and AS

<table>
<thead>
<tr>
<th>CD preparation</th>
<th>Time 0</th>
<th>After drying*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residual ethanol</td>
<td></td>
</tr>
<tr>
<td>AM/SBE-7-β-CD 9%/alcohol 20%</td>
<td>7.15</td>
<td>2.24</td>
</tr>
<tr>
<td>AM/HP-β-CD 9%/alcohol 20%</td>
<td>6.38</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Residual ammonia</td>
<td></td>
</tr>
<tr>
<td>AS/HP-β-CD 9%/ammonia</td>
<td>5.68</td>
<td>5.09</td>
</tr>
<tr>
<td>AS/ SBE-7-β-CD 8%/ammonia</td>
<td>5.88</td>
<td>5.54</td>
</tr>
<tr>
<td>AS/ SBE-7-β-CD 9%/ammonia</td>
<td>3.92</td>
<td>4.36</td>
</tr>
</tbody>
</table>

* drying step includes 2 days of drying in an silicagel exsiccator at 25°C

No differences were noted for solubility of the active compounds and the cyclodextrin derivatives with or without buffer. Cyclodextrins can be autoclaved. The compositions comprising artemether were investigated for stability at 121 °C. Results indicate no degradation of the active compound artemether at this condition. Also, sterile filtration of the solutions with a 0.22 µm filter is possible. Furthermore, it was investigated whether the solutions can be diluted without precipitation of the compounds. Due to the existing equilibrium between cyclodextrins and active compounds, the compounds could precipitate after dilution. This dilution takes place in the blood stream in human when the reconstituted solution is injected or when an infusion to administer from the lyophilisate is prepared. The solutions obtained from the lyophilised compositions (Table 2) were diluted in a solution of 0.9 % w/w NaCl, 5 % w/w sucrose and phosphate buffers at pH 7.4, and verified for precipitation. None of the reconstituted solutions showed precipitation. The lyophilisates could be diluted up to 10000 times without occurrence of precipitation. Such diluted forms of the solutions are particularly suitable for use as infuses.

In conclusion, the applicants have surprisingly found that the long felt need of an effective injectable, anti-malarial formulation, especially intravenous, and which can be diluted for infuse administration, may be obtained by providing artemisinin, artesunate, arteether, artelinic acid, artesunic acid, and dihydroartemisinin in combination with cyclodextrins, providing clear aqueous solutions, which are economically beneficial and safe to inject, attaining the concentration range required for antimalarial action and for many other diseases, when dosage regimens for efficacy are determined, such as schistosomiasis, many viral, fungal and viral infections, and cancer.
29

CLAIMS

1. Method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of:

(A) dissolving a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, in a suitable amount of organic solvent or ammonia solution such that a solution comprising an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained, and

(B) spray-drying said solution such that a spray-dried inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

2. Method according to claim 1, wherein said spray-dried inclusion complex is further dissolved in water and sterilised.

3. Method according to claim 2, wherein the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

4. Method according to any of claims 1 to 3, wherein said organic solvent is selected from the group comprising alcohols, 10% to 100% ethanol 96°, 10 to 80% v/v isopropanol, acetonitril, tetrahydrofuran and dimethyl sulfoxide.

5. A method according to any of claims 1 to 4, wherein the pH of the said ammonia solution is between 9 and 12.

6. A method according to any of claims 1 to 5, wherein a suitable amount of artemisinin or derivatives thereof and a suitable amount of cyclodextrin are dissolved in a suitable amount of solvent in the presence of a suitable amount of one or more pharmaceutical polymers.

7. A method according to claim 6, wherein said polymer is selected from the group comprising hydroxypropylmethylcellulose, hydroxypropylcellulose and polyvinylpyrrolidone.

8. A method according to claims 6 or 7, wherein the suitable amount of said polymer is that capable of providing a pharmaceutical composition that is comprised between 0.05 and 1% polymer w/w.
9. Method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of
   bringing a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and
   kneading said artemisinin or derivatives thereof and said cyclodextrin such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

10. Method for preparing a pharmaceutical composition comprising artemisinin or derivatives thereof comprising the steps of
   bringing a suitable amount of artemisinin or derivatives thereof, bringing the therapeutically active compound in said pharmaceutical composition, into contact with a suitable amount of cyclodextrin, and
   grinding said artemisinin or derivatives thereof and said cyclodextrin, such that an inclusion complex of said artemisinin or derivatives thereof and said cyclodextrin is obtained.

11. Method according to claim 10, wherein said inclusion complex is further dissolved in water and sterilised.

12. Method according to claim 11, wherein the sterilised solution is freeze-dried such that a lyophilised form of said inclusion complex is obtained.

13. A method according to any of claims 1 to 12 wherein said artemisinin derivatives are selected from the group comprising artesunate, artemether, arteether, artelinic acid, artesunic acid, and dihydroartemisinin.

14. A method according to any of claims 1 to 13, wherein the suitable amount of artemisinin or derivatives is that capable of providing a pharmaceutical composition that is between 0.025 and 25 % w/w of artemisinin or derivatives.

15. A method according to any of the previous claims 1 to 14 wherein said cyclodextrin is an alpha, beta or gamma-cyclodextrin.
16. A method according to claim 15 wherein said cyclodextrin is selected from the group comprising hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin, maltosyl-β-cyclodextrin and β-cyclodextrin.

17. A method according to any of claims 1 to 16, wherein the suitable amount of cyclodextrin is that capable of providing a solution of a pharmaceutical composition that is comprised between 0.1 and 40 % w/w cyclodextrin.

18. A method according to any of claims 1 to 16, wherein the suitable amount of cyclodextrin is that capable of providing a tablet of a pharmaceutical composition that is comprised between 10 and 90 % w/w cyclodextrin.

19. A method according to any of claims 1 to 18, wherein the molar ratio of artemisinin or derivatives thereof to cyclodextrin in said inclusion complex is comprised between 3:1 and 1:25.

20. A method according to any of the previous claims 1 to 19, wherein said lyophilised form of said inclusion complex is dissolved in a suitable amount of water or solvent for obtaining an injectable solution or infusion.

21. Pharmaceutical composition comprising a suitable amount of artemisinin or derivatives thereof, being the therapeutically active compound in said pharmaceutical composition, and a suitable amount of cyclodextrin, obtainable by the method according to any of claims 1 to 20.

22. A pharmaceutical composition according to claim 21 comprising an artemisinin derivative selected from the group comprising artesunate, arteether, artemether, artelinic acid, artesunic acid, and dihydroartemisinin.

23. A pharmaceutical composition according to claim 22, comprising a cyclodextrin selected from the group comprising hydroxypropyl-β-cyclodextrin, hydroxypropyl-γ-cyclodextrin, sulfobutylether-β-cyclodextrin, maltosyl-β-cyclodextrin and β-cyclodextrin.

24. A pharmaceutical composition according to any of claims 21 to 23, wherein the amount of artemisinin or derivatives thereof is between 0.025 and 25 % w/w artemisinin or derivatives thereof in said composition.
25. A pharmaceutical composition according to any of claims 21 to 24, suitable for use as a solution wherein the amount of cyclodextrin is between 0.1 and 40 % w/w cyclodextrin in solution.

26. A pharmaceutical composition according to any of claims 21 to 24, suitable for use as a tablet wherein the amount of cyclodextrin is between 10 and 90 % w/w cyclodextrin in the tablet.

27. A pharmaceutical composition according to any of claims 21 to 26 wherein the molar ratio of artemisinin or derivatives thereof to cyclodextrin is comprised between 3:1 and 1:25.

28. Pharmaceutical composition according to any of claims 21 to 27 for parenteral, rectal, oral or topical use.

29. Pharmaceutical composition according to any of claims 21 to 28 for intravenous, intramuscular, peritoneal, peridural, rectal or oral application.

30. Pharmaceutical composition according to any of claims 21 to 29 for use as an infuse.

31. Pharmaceutical composition according to any of claims 21 to 29 for use as an injectable solution, a lyophylisate, a capsule, a tablet, an ampoule, a hydrogel, a cream or suppository.

32. Use of the pharmaceutical composition according to any of claims 21 to 31 in the preparation of a medicament for treating malaria.

33. Use of the pharmaceutical composition according to any of claims 21 to 31 in the preparation of a medicament for treating cancer.

34. Use of the pharmaceutical composition according to any of claims 21 to 31 in the preparation of a medicament for treating babesiosis.

35. Use of the pharmaceutical composition according to any of claims 21 to 31 in the preparation of a medicament for treating schistosomiasis.
36. Use of the pharmaceutical composition according to any of claims 21 to 31 in the preparation of a medicament for treating fungal, viral and/or bacterial infections.

37. A kit comprising a pharmaceutical composition according to any of the claims 21 to 31.

38. A kit comprising a pharmaceutical composition in lyophilised form obtainable by a method according to any of claims 1 to 20.39. A kit according to claim 38 further comprising a pharmaceutically acceptable solvent.

40. A kit according to claim 39 wherein said composition and pharmaceutically acceptable solvent are present in separate containers.

41. A kit according to claim 40 further comprising at least one needle and syringe.

42. A kit according to any of claims 37 to 41 for parenteral use.
Fig. 1A

Fig. 1B
Fig. 1C
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48 A61K31/365 A61P33/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base unde, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, CHEMABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>CH 685 391 A (ACHILLE BENAKIS) 30 June 1995 (1995-06-30) the whole document</td>
<td>1-42</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C.

Date of the actual completion of the international search
18 June 2004

Date of mailing of the international search report
05/07/2004

European Patent Office, P.B. 5318 Patentliaan 2 NL - 2330 HV Rijswijk
Tel. (+31-70) 940-0040, Tlx. 31 651 epo nl, Fac. (+31-70) 940-3016

Name and mailing address of the ISA
Pacreu Largo, M
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WONG J W ET AL: &quot;Improved oral bioavailability of artemisinin through inclusion complexation with beta- and gamma-cyclodextrins.&quot; INTERNATIONAL JOURNAL OF PHARMACEUTICS (KIDLINGTON), vol. 227, no. 1-2, 2001, pages 177-185, XP002248960 ISSN: 0378-5173 page 178, right-hand column</td>
<td>1-20,42</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>1-42</td>
</tr>
</tbody>
</table>

Form PCT/IBA210 (continuation of second sheet) (January 2004)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
| Y        | DUCHENE D ET AL: "PHARMACEUTICAL USES OF CYCLODEXTRINS AND DERIVATIVES"   
DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY,   
NEW YORK, NY, US, 
vol. 16, no. 17, 1990, pages 2487-2499,   
XP001084629 
ISSN: 0363-9045 
page 2490, paragraph 2 | 1-42 |
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH 685391 A</td>
<td>30-06-1995</td>
<td>CH 685391 A5</td>
<td>30-06-1995</td>
</tr>
<tr>
<td>US 2002147177 Al</td>
<td>10-10-2002</td>
<td>NONE</td>
<td></td>
</tr>
</tbody>
</table>