The present invention provides a stable water-in-oil microemulsion pharmaceutical formulation comprising a continuous oil phase and a dispersed aqueous phase containing a biological. The microemulsion formulation of the present invention is stable under refrigerated conditions, have acceptable organo-leptic properties and improve the absorption of biologicals through mucous membrane.
RELATED APPLICATION

This application is related and takes priority from Indian provisional application 1600/MUM/2009 filed 6 July 2009 titled 'Microemulsion Composition for Biologicals' and is herein incorporated in its entirety.

FIELD OF THE INVENTION

The present invention relates to pharmaceutically acceptable, optically clear and transparent water-in-oil (w/o) microemulsion formulation comprising solubilized biological and their use thereof. The invention further describes w/o microemulsion system where the aqueous phase droplets are dispersed in the continuous oil phase containing different ratios of surfactants. The microemulsion of the present invention is stable under refrigerated conditions and has acceptable organo-leptic properties. The formulation described herein improves the absorption of biologicals through mucous membrane.

BACKGROUND OF THE INVENTION

Biologicals e.g. proteins, peptide, nucleotides, vaccines, hormones, small interfering ribonucleic acid (siRNA), Deoxyribonucleic acid (DNA), enzymes, etc. are important as they are used to cure a number of diseases including diabetes (e.g. Insulin), cancers (e.g. Interferon, monoclonal antibodies), heart attacks, strokes, cystic fibrosis (e.g. Enzymes, Blood factors), inflammation diseases (e.g. Tumor Necrosis Factors), anemia (e.g. Erythropoietin), hemophilia (e.g. Blood clotting factors) etc. Formulating these biologicals is usually a difficult task as these macromolecules are large and unstable and are easily modified or denatured even under mild stress conditions.

Biologicals are generally not suitable for administration through oral route as they are poorly absorbed due to their large size and polarity and charge distribution, and may undergo denaturation/ enzymatic degradation in the gastrointestinal tract. As a result, most biologicals are available as aqueous injectable compositions which require repeated
injections and frequent visits to the medical service providers. This results in patient non-compliance and increased healthcare costs. Thus, there is a need to develop a delivery system for these macromolecules which can be administered through non-parenteral route. The development of such a self-administrable delivery system would enable patients to avoid invasive route of administration (intravenous/intramuscular/subcutaneous) thereby increasing the patient comfort and improving patient compliance.

An emulsion is a mixture of two or more immiscible liquids. One liquid (the dispersed phase) is dispersed in the other (the continuous phase). Emulsions tend to have a cloudy appearance, because the many phase interfaces (the boundary between the phases is called the interface) scatter light that passes through the emulsion. Emulsions are unstable and thus do not form spontaneously. Energy input through shaking, stirring, homogenizing, or spray processes are needed to form an emulsion. Over time, emulsions tend to revert to the stable state of the phases comprising the emulsion.

Compared to emulsions, microemulsions are thermodynamically stable, isotropically clear dispersions of two immiscible liquids stabilized by interfacial films of surface-active molecules usually called surfactants. The formation of microemulsions usually involves a combination of three to five components, viz., oil, water, a surfactant, a co-surfactant and an electrolyte. The tendency towards water-in-oil (w/o) or an oil-in-water (o/w) microemulsion is dependent not only on the volume of dispersed and dispersant (or continuous) phase but also on the properties of the surfactants. Surfactants are conveniently classified on an empirical scale known as the hydrophilic-lipophilic balance (HLB) which runs from 1 to 20. In general, w/o microemulsions are formed using surfactants which have an HLB value in the range of about 3 to 8.

Microemulsions are characteristically transparent or opalescent when viewed by optical microscopic means. In the undisturbed state, they are optically isotropic when examined under polarized light. The dispersed phase typically comprises particles or droplets which are normally between 5 and 200 nm in size which gives them optical transparency. These particles are usually spherical, although other structures are feasible.
The role of co-surfactant is to increase the interfacial fluidity by penetrating the surfactant film and consequently creating a disordered film due to the formation of void space among surfactant molecules. The use of a co-surfactant or a co-solvent in microemulsions is optional.

There are many advantages to the use of microemulsions over conventional emulsions as drug formulations. Microemulsions form spontaneously, without the need for a high energy input and are therefore easy to prepare and scale up for commercial applications; they have thermodynamic stability due to their small particle size and therefore have a long shelf life; they have an isotropically clear appearance and therefore the stability can be monitored by spectroscopic means; additionally the relative low viscosity of microemulsions facilitate easy to mixing, filling, transporting and administering.

EP0597007B describes a pharmaceutically acceptable, stable, self-emulsifying (w/o) microemulsions comprising (i) a lipophilic phase comprising a medium-chain fatty acid triglyceride and a low HLB surfactant, (ii) an aqueous-based hydrophilic phase containing a water-soluble therapeutic agent, and (iii) a high HLB surfactant having improved drug-delivery characteristics. This patent describes about a self-emulsifying (w/o) microemulsion which on exposure to water or gastrointestinal fluids forms an emulsion for oral delivery of proteins and peptides. Microemulsion described in this patent comprises an aqueous phase of less than 10%. However, for the microemulsion system to be successful it should solubilize therapeutically effective amount of biologicals. Unlike small drug molecules, mostly biologicals are complex and large having high molecular weight. To solubilize, therapeutic effective quantity, usually higher aqueous phase solubilization (as much as 30-40% v/v) is required.

US6916785 discloses a pharmaceutical composition suitable for oral administration in the form of a homogeneous solution which on exposure to water or gastrointestinal fluids forms an emulsion having a particle size of less than 5 microns, the solution containing: (a) a pharmaceutically effective amount of a cyclosporin, in particular Cyclosporin A, (b)
a carrier medium which is a triglycerol monoester of a fatty acid having from 6 to 30
carbon atoms or mixtures thereof, (c) polyethylene glycol, (d) a non-ionic surfactant
having a hydrophilic lipophilic balance (HLB) greater than 10, and (e) optionally, a
viscosity reducing agent, the composition being substantially free from ethanol. The
preferred carrier medium is triglycerol monooleate. Examples of the viscosity reducing
agent are glycerol monocaprylate and glycerol monooleate. This patent discloses a self
emulsifying formulation containing triglycerides which do not contain any aqueous or
hydrophilic phase.

US6761903 discloses an invention relating to pharmaceutical compositions and methods
for improved solubilization of triglycerides and improved delivery of biologicals. The
compositions presented include a carrier formed from a combination of a triglyceride and
at least two surfactants, at least one of which is hydrophilic. Upon dilution with an
aqueous medium, the carrier forms a clear, aqueous dispersion of the triglyceride and
surfactants. This patent mainly focuses on o/w microemulsion and polysaccharide and
low molecular drugs which can be dissolved in the oil phase. This invention is more
towards triglycerides containing formulations and their drawbacks.

US5824638 discloses stable water in oil (w/o) oral insulin microemulsion, comprising
continuous phase of hydrophobic material selected from the group consisting of a long
chain carboxylic acid or ester or alcohol dispersed in aqueous phase or having
hydrophilic discontinuous phase of a long chain carboxylic acid or alcohol. This patent
discloses the oral delivery of insulin wherein the aqueous hydrophilic phase is present in
an amount of about 5.1 to about 9.9 weight percent of the emulsion which may not be
adequate for loading of therapeutically effective amount of proteins/peptides. Further,
this invention discloses use of long chain carboxylic acid or ester or alcohol as
hydrophobic phase which is not used in the current invention.

Indian patent application 681/MUM/2004 relates to the microemulsion compositions for
transmucosal administration of proteins and peptides and process thereof. In particular
this invention relates to oil in water (o/w) microemulsion compositions of peptides such
as insulin and the process of making the same. This invention describes a microemulsion system where protein is present in the continuous aqueous phase and not solubilized in the dispersed phase. The composition also contains permeation enhancer and there is hardly any role of microemulsion in enhancing the absorption of biological/s through mucosal membranes.

As is seen from the available prior art, there is scarcity with regard to formulations having the required characteristics of ideal microemulsion for delivery of biologicals through non-parenteral route e.g transmucosal membranes. The objective of the present invention is to develop a microemulsion formulation having high aqueous phase solubilization capacities, stable under refrigerated, room and at elevated temperatures, with high loading of biologicals, acceptable organo-leptic properties, and uniform size distribution of the droplets in the aqueous phase. Furthermore, the present invention also aims to increase the absorption of the biologicals solubilized inside the microemulsion composition through mucosal tissue.

SUMMARY OF THE INVENTION

The present invention provides pharmaceutically acceptable, optically clear and transparent water-in-oil (w/o) microemulsion formulation comprising solubilized biological and their use thereof.

In one aspect, the invention provides a stable, water-in-oil (w/o) microemulsion pharmaceutical formulation comprising:

a. a continuous oil phase having a fatty acid derivative of glycerol and a low HLB surfactant;
b. one or more high HLB surfactant;
c. a dispersed aqueous phase containing a water soluble biological alone or in combination dissolved in stabilizing buffer and pharmaceutically acceptable excipients;
d. a co-solvent;
e. optionally a flavoring agent or a taste-masking agent; and
f. optionally a mucoadhesive or a permeation enhancing agent.
The fatty acid derivative of glycerol is glycerol caprylate caprate, propylene glycol dicaprate, propylene glycol dicaprylate/dicaprate, propylene glycol dilaurate, glyceryl tricaprylate, glyceryl caprate, glyceryl tricaprylate, glyceryl caprate, glyceryl stearate or polyoxylglycerides.

The low HLB surfactant is glyceryl mono/dicaprate, glyceryl caprylate/caprate, glyceryl oleate, glyceryl monooleate, glyceryl monostearate, propylene glycol monocaprylate, propylene glycol monolaurate, sorbitan esters, glycerol monocaprylocaprate, sodium lauroyl dilactate, sodium stearoyl dilactate or α-D-tocopherol.

In another aspect, the oil phase of the microemulsion makes up to an amount of about 10-50% v/v and preferably at about 20-40% v/v and the ratio of the the medium-chain fatty acid derivative to the low HLB surfactant is in the range of 1:0.01 to 1:5.

In yet another aspect the value of the low HLB surfactant is in the range of 3-8 while the value of high HLB surfactant is at least 9. It is also provided that the high HLB surfactant is present in an amount of about 5-50% v/v, preferably at about 10-25% v/v.

The microemulsion of the invention has a pH ranging from 3 to 9, preferably from 3 to 6 in its aqueous phase.

Further, the microemulsion formulation of the invention encompasses biologicals at molecular weight ranging from about 1 kDa to 100 kDa. Examples of the biologicals include protein, peptide, nucleotide, vaccine, hormone, siRNA and enzyme. The invention further provides co-solvents selected from the group consisting of ethanol, isopropanol, methanol, n-butanol, isobutanol propylene glycol, polyethylene glycol preferably ethanol and isopropanol.

The microemulsion further exhibits a droplet size of less than 500nm.

The microemulsion of the present invention exhibits acceptable organo-leptic properties.
and thus consists of flavoring and/or taste-masking agents as well as sweetening agents.

In another aspect, the microemulsion is administered transmucosally by means of solution, drops, sprays, gels, bioadhesive gels and bioadhesive sprays.

The present invention also provides a process of preparing water-in-oil (w/o) microemulsion as claimed in claim 1, comprising a continuous oil phase with dispersed aqueous droplets for delivery of biologicals through mucosal route wherein the biologicals are solubilized in aqueous droplets, comprising the steps:

a) Dissolving the biologicals in an aqueous phase of pH 3-9 to obtain a preferred concentration of 0.1-5mg/ml for solubilization in w/o microemulsion;

b) In first glass vial, taking 8-15% v/v of high HLB surfactants and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;

c) In second glass vial, taking 19-22% v/v of oil and low HLB surfactant and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;

d) Addition of contents of second vial dropwise into first vial and mixing all the ingredients on a magnetic stirrer at 500 rpm for 5 minutes at room temperature to obtain a clear mixture of oil and surfactants; and

e) Dropwise addition of 5-40% v/v of biologicals containing aqueous phase into oil and surfactant mixture from step (d) with continuous stirring on the magnetic stirrer to get optically transparent w/o microemulsion.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 shows the Pseudo ternary phase diagram of representative microemulsion

Figure 2 shows the Globule size distribution of representative microemulsion

Figure-3: in vitro release profile of PTH from w/o microemulsion. The data is presented as % release as a function of time (minutes).
DESCRIPTION OF THE INVENTION

The present invention relates to pharmaceutically acceptable water-in-oil (w/o) microemulsions comprising a biological, process for their preparation and their use for mucosal delivery of the same.

In one embodiment, the water-in-oil microemulsions of the present invention, comprises a stable, water-in-oil (w/o) microemulsion pharmaceutical formulation, comprising:

a. a continuous oil phase having a fatty acid derivative of glycerol and a low HLB surfactant;

b. one or more high HLB surfactant;

c. a dispersed aqueous phase containing a water soluble biological alone or in combination dissolved in stabilizing buffer and pharmaceutically acceptable excipients;

d. a co-solvent;

e. optionally a flavoring agent or a taste-masking agent; and

f. optionally a mucoadhesive or a permeation enhancing agent.

The microemulsion of the present invention comprises a continuous oil phase consisting fatty acid derivatives of glycerol. In one embodiment the fatty acid derivative of glycerol is a medium-chain triglyceride and a low HLB surfactant with HLB values ranging between 3 to 8 in which the ratio of the medium chain triglyceride to the low HLB surfactant ranges from about 1:0.01 to about 1:5. Suitable medium-chain triglycerides for use in the present invention are of natural, semi-synthetic or synthetic origin and include but not restricted to blends of different medium-chain fatty acid triglycerides. In another embodiment, the oil phase in the microemulsion is present in an amount of about 10-50% volume/volume (v/v), more preferably at 20-40% v/v.

The term "medium-chain fatty acid" as used in the present disclosure refers to a fatty acid having 6 to 12 carbon atoms, preferably 8 to 10 carbon atoms which may be branched or unbranched and which may be optionally substituted such as including but not restricted to glycerol caprylate caprate, propylene glycol dicaprate, propylene glycol
dicaprylate/dicaprate, propylene glycol dilaurate, glycercyl tricaprylate, glycercyl caprate, glycercyl triacetate, glyceryl caprate, glyceryl caprylate, glyceryl caprylate, glyceryl caprate, glyceryl stearate and polyoxylglycerides. The triglyceride preferably comprises of 50 to 100% (w/w) of Caprylic (C8) and in the range of 0 to 50% (w/w) of capric (C10) acid triglycerides.

Suitable examples include but not limited to Captex 350, Captex 355, Captex 300, Captex 850, Captex 8000, Miglyol 810, Miglyol 812, Miglyol 818, Mazol 1400, Labrafac™, Lipophile W1 1349, Labrasol, and so on. Preferably, the triglyceride is Captex 355.

Suitable low HLB surfactants for use in the present invention include glycercyl mono/dicaprate, glycercyl caprylate/caprate, glycercyl oleate, glycercyl mono oleate, glycercyl monostearate, propylene glycol monocaprylate, propylene glycol monolaurate, sorbitan esters, glycerol monocaprylocaprate, sodium lauroyl .dilactate, sodium stearoyl dilactate and α-D-tocopherol. Suitable examples include but not limited to Capmul MCM, Span 20, sorbitan monooleate, sorbitan trioleate, sorbitan sesquioleate, etc. The preferred low HLB surfactant is Capmul MCM.

Suitable high HLB surfactants for use in the present invention include non-ionic surfactants such as (a) polyoxyethylene fatty acid esters (b) polyoxyethylene sorbitan fatty acid esters (c) polyoxyethylene glycol long-chain alkyl ethers (d) polyoxyethylene glycol long chain alkyl esters (e) polyoxyl hydrogenated vegetable oil. For use in the present microemulsion formulation, the high HLB surfactants preferably have HLB values 9 and above. In the present invention the value of high HLB is at least 9 with an upper range of up to 20. Preferably the value of high HLB surfactant is between 9 to 20. Stable w/o microemulsions are formed when HLB value of the microemulsion is between 9 to 12. To achieve this, high HLB surfactants in combination with low HLB surfactants are used in the present invention. Suitable examples of high HLB surfactants include but not limited to Polysorbate 20, Polysorbate 60, Polysorbate 80, Cremophor RH 40, Cremophor EL, Polaxamer 127, Polaxamer 188, Capryol™ 90, Capryol™ PGMC, etc. In the present formulation of microemulsion one or more high HLB surfactants are used. The preferred high HLB surfactants are polysorbate 20, polysorbate 80 or Cremophor RH40.
Suitably, the blend of low and high HLB surfactants will have a HLB value in the range of 6 to 15.

Protein stabilizing buffers of the invention include but not restricted to acetate, citrate, histidine, glycine, methionine, tartarate, lactate, succinate either alone or in combination thereof.

Suitable co-solvents for use in the present invention include alcohols selected from the group consisting of ethanol, isopropanol, methanol, n-butanol and isobutanol, propylene glycol, polyethylene glycol and isopropyl myristate.

Flavouring agents or taste masking agents are essential to make the formulation organo-leptically acceptable. Flavoring agents (flavors) well known to those skilled in the art are used. These flavoring agents may be chosen from synthetic flavor oils and aromatics, and/or oils, oleo resins and extracts derived from plants, leaves, flowers, fruits and so forth, and combinations thereof. Representative oil soluble flavoring agents include: spearmint oil, cinnamon oil, oil of wintergreen (methylsalicylate), peppermint oils, clove oil, bay oil, anise oil, eucalyptus oil, thyme oil, cedar leaf oil, oil of nutmeg, oil of sage, oil of bitter almonds, peanut butter flavor, chocolate flavor, rum flavor, cassia oil, cinnamon mint flavor, corn mint oil, cardamom flavor, ginger flavor, cola flavor, cherry cola flavor, etc.

Preferable mucoadhesive agents would increase the viscosity and allow for the more contact time of the formulations with the mucosal tissue. Another reason is the possible ionic interactions between the mucoadhesives and mucosal membranes. As a result of these interactions, the formulation would stay for longer duration at the site of application. These agents are selected from natural polymers (e.g. chitosan, gelatin, cellulose, collagen and their derivatives etc) or synthetic polymers (e.g. vinyl based polymers, acrylate based polymers and their derivatives etc).
The mucosal surfaces consist of the nasal, buccal, ocular, vaginal, and rectal mucosae etc. and they usually act as a barrier to the absorption of macromolecules. Permeation enhancers are usually employed to increase the flux/absorption of drugs through the mucosal membrane. These permeation enhancers enhance the paracellular permeability of mucosal epithelia by transiently opening the tight junctions, thereby increasing the paracellular absorption of hydrophilic and macromolecular drugs. A clinically accepted permeation enhancer must increase membrane permeability without causing toxicity and permanent membrane damage. These include cyclodextrins, bile salts, surfactants, fusidic acid derivatives, etc. either alone or in combination.

As described herein, the term "pharmaceutically acceptable", as used herein, means the formulations of the invention that are prepared from USFDA approved / GRAS listed excipients and are safe when administered in vivo and can effectively deliver the biologicals having desired biological activity across the mucosal tissues.

As described herein "stable" refers to optical clarity / transparency i.e. no change in physical appearance of the (w/o) microemulsion by visual inspection and droplet size measured by using Malvern Zetasizer with time stored under refrigeration (5±3 °C), room (25±2 °C) and at elevated temperature (40 ± 3 °C). No change in physical appearance by visual inspection implies to optical clarity / transparency i.e. non-existence of haziness, cloudiness, opaqueness, sedimentation, precipitation, crystallization and phase separation, etc.

As used herein, "globule size" is used to refer to the size of droplets in the composition in diameter, as measured by conventional particle size analyzers well known to those skilled in the art, such as, photon correlation spectroscopy, laser light scattering or dynamic light scattering technology and by using transmission electron microscope (TEM) or scanning electron microscope (SEM). By "uniform droplet size distribution" it is meant that all the droplets in the formulation have an average diameter of globule is less than 500nm, preferably in the range of 8 - 150 nm when measured by above mentioned techniques. As
used herein, the term "nm" refers to nanometer, size less than 1 micron, wherein micron is a unit of measure of one one-thousandth of a millimeter.

The term "biological or biologicals" refer to proteins, peptide, nucleotides, vaccines, hormones, siRNA, enzymes, etc. which are derived either from natural or recombinant sources having biological activity, is soluble in the aqueous phase and has a HLB value of at least equal to that of the high HLB surfactant used in the formulation, to ensure that the drug is preferentially dissolved in the hydrophilic rather than the lipophilic phase. Biologicals that are formulated as microemulsion of the present invention have a molecular weight of about 1 kDa to about 100 kDa. Biologicals of the invention include but not restricted to interleukin-2, PEG-IFN-α2b, gonadotropins, human growth hormone (hGH), IFN-β, Rituximab, Etanercept, erythropoietin (EPO), bevacizumab, teriparatide, exenatide and monoclonal antibodies etc.

The microemulsion of the invention further consists of sweetening agent. Sweetening agents may be selected from the group consisting of saccharin, aspartame, sucralose, neotame, acesulfame potassium and stevia, preferably aspartame.

The microemulsion formulation of the invention is administered transmucosally using the means of solution, drops, sprays, gels, bioadhesive gels or bioadhesive sprays.

In yet another embodiment, the invention provides a process of preparing a water-in-oil microemulsion as disclosed herein comprising a continuous oil phase with dispersed aqueous droplets for delivery of biologicals through mucosal route wherein the biologicals are solubilized in aqueous droplets, comprising the steps:

a) Dissolving the biologicals in an aqueous phase of pH 3-9 to obtain a preferred concentration of 0.1-5mg/ml for solubilization in w/o microemulsion;

b) In first glass vial, taking 8-15% v/v of high HLB surfactants and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;
In second glass vial, taking 19-22% v/v of oil and low HLB surfactant and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;

d) Addition of contents of second vial dropwise into first vial and mixing all the ingredients on a magnetic stirrer at 500 rpm for 5 minutes at room temperature to obtain a clear mixture of oil and surfactants; and

e) Dropwise addition of 5-40% v/v of biologicals containing aqueous phase into oil and surfactant mixture from step (d) with continuous stirring on the magnetic stirrer to get optically transparent w/o microemulsion.

The invention will now be further described with reference to the following Examples, it being understood that these are intended to illustrate the invention, and in no way to limit its scope.

Examples

Example 1: Preparation of the representative water-in-oil microemulsions

Water-in-oil (w/o) microemulsions for transmucosal delivery of proteins and peptides were prepared having the following composition (Table 1):

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Category</th>
<th>Type of Excipients</th>
<th>Example</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oil phase</td>
<td>Medium chain triglycerides</td>
<td>Captex 355</td>
<td>10 - 50 % v/v</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low HLB surfactant</td>
<td>Capmul MCM</td>
<td>10 - 50 % v/v</td>
</tr>
<tr>
<td>2.</td>
<td>Surfactant phase</td>
<td>High HLB surfactant 1</td>
<td>Super refined polysorbate 80</td>
<td>05 - 40 % v/v</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High HLB surfactant 2</td>
<td>Cresmer RH40</td>
<td>05 - 40 % w/v</td>
</tr>
<tr>
<td>3.</td>
<td>Aqueous phase</td>
<td>Water</td>
<td>Buffer system</td>
<td>0 - 50 % v/v</td>
</tr>
</tbody>
</table>

Microemulsion of the present invention was prepared by accurately weighing the required amount of Cresmer RH 40 in a 5 ml sterile glass vial (USP Type - 1). To this vial, required quantities of Captex 355, Capmul MCM and polysorbate 80 were added.
Then the contents in the vial were blended at room temperature on a magnetic stirrer for 3 - 5 minutes until all the ingredients were properly dissolved to form a clear viscous solution. Aqueous buffer was then added drop-wise to above mixture with continuous stirring on magnetic stirrer until a clear and transparent w/o microemulsion was obtained. The buffer got solubilized in the aqueous droplets of the reverse micelles formed by the blend of surfactants in the oils. It was observed that up to 34.8% of aqueous buffer could be easily solubilized to form a stable microemulsion. However, although higher volumes of aqueous buffer may be solubilized inside the microemulsion such a formulation would have lesser stability and thus higher percentage than the above was not opted for solubilization.

Example 2: Entrapment of FITC Dextran (MoI. Wt. 4.0 kDa) in w/o microemulsion

Table 2 Compositions of the different FITC-Dextran entrapped microemulsions prepared

<table>
<thead>
<tr>
<th>Excipient / formulation code</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Captex 355 (% v/v)</td>
</tr>
<tr>
<td>ME-1</td>
<td>21.7</td>
</tr>
<tr>
<td>ME-2</td>
<td>25.0</td>
</tr>
<tr>
<td>ME-3</td>
<td>21.7</td>
</tr>
<tr>
<td>ME-4</td>
<td>20.0</td>
</tr>
<tr>
<td>ME-5</td>
<td>36.5</td>
</tr>
<tr>
<td>ME-6</td>
<td>26.5</td>
</tr>
<tr>
<td>ME-7</td>
<td>16.6</td>
</tr>
<tr>
<td>ME-8</td>
<td>21.7</td>
</tr>
<tr>
<td>ME-9</td>
<td>21.7</td>
</tr>
<tr>
<td>ME-10</td>
<td>21.7</td>
</tr>
</tbody>
</table>

ME: Microemulsion

FITC Dextran, (MoI. Wt. 4kDa) (FITC-Dex) was selected as a surrogate molecule to entrap inside the aqueous droplet of reverse micelles. Different amount of FITC Dextran was entrapped inside the w/o microemulsion. The process of preparation of microemulsions remained same as explained in Example 1. In brief, required quantities of oils and
surfactants (as given in Table 2) were mixed using magnetic stirrer as described above. To this mixture, an aqueous buffer containing FITC-Dex, instead of plain buffer, was solubilized. FITC-Dex being a water soluble molecule got entrapped easily inside the w/o microemulsion. However, the loading amount of FITC-Dex depends on the concentration of the FITC solution used. In this way, the desired loading amount can be achieved for therapeutic applications by varying the FITC-Dex concentration.

Example 3: Preparation of Phase diagram

The pseudo-ternary phase diagram was constructed to identify the formation of w/o microemulsions. The regions of the phase diagram in which microemulsions according to the present invention exist were determined by titrating a mixture of the oil, low HLB surfactant and two high HLB surfactants against the aqueous phase, noting points of phase separation, turbidity and transparency. Due to the relatively low viscosity of the particular oil and low HLB surfactant, these components were readily formulated at room temperature. Figure 1 represents typical pseudo-ternary phase diagram for the microemulsions of the present invention. A combination of Capmul MCM and Captex 355, as an oil phase, is shown in the phase diagram. As shown in this figure, a clear and transparent microemulsion existed in the w/o shaded area, whereas outside this region the microemulsion formed were turbid. The optimal w/o microemulsion was selected from this area based on the maximum solubilization capacity of the microemulsions and stability of the microemulsions thus formed.

Example 4: Physico-chemical characterization of the microemulsions

(A) Measurement of size and size distribution: For measuring of the particle size, the refractive index and viscosity of oil phase as was available from the supplier certificate of analysis (CoA) were introduced into the Nano-S workstation program (Nano-S, Malvera Instruments Inc.) to calculate the mean diameter and polydispersity of the droplets. Figure 2 shows the typical size distribution of the ME-8 droplets. It showed that the ME size was less than 20 μm and were highly mono-dispersed (Figure 2).
(B) Conductivity and pH measurements: The electrical conductivity (σ) of the prepared microemulsion (me-8) was determined at ambient temperature with a digital conductivity meter (Seven multi, Mettler Toledo). Similarly pH was measured using digital pH meter (Seven multi, Mettler Toledo). Table 3 summarizes these details.

**Table 3: Physical properties of representative microemulsion**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>Conductivity</td>
<td>196 μS/cm</td>
</tr>
<tr>
<td>3</td>
<td>Globule size</td>
<td>15.2 ± 2.0nm</td>
</tr>
<tr>
<td>4</td>
<td>Polydispersity</td>
<td>Monodispersed (PDI: &lt;0.4)</td>
</tr>
</tbody>
</table>

Example 5: Taste Masking of the representative microemulsion

Taste is one of the most important parameter governing patient compliance. Oral administration of bitter drugs with an acceptable degree of palatability is the key issue for health care providers especially for pediatric patients. Many oral pharmaceutical products have unpleasant and bitter taste. So, a pharmaceutical formulation with pleasing taste is important for therapeutic value for the patient. The microemulsions developed above were further improved organo-leptically as the ingredients used may give some bitterness.

Within the scope of the present invention taste masking of the formulations was done by using sweetening / flavoring agent alone or in combination. The microemulsion was stored at 2-8°C and evaluated by visual appearance and globule size at regular intervals of time. The compositions are given below in Table 4.

**Table 4: Composition details of the taste masked Microemulsions**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
<th>ME-11</th>
<th>ME-12</th>
<th>ME-13</th>
</tr>
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<tbody>
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<td>19.23</td>
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<td>22</td>
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<tr>
<td>Lauroglycol 90 (% v/v)</td>
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<td>-</td>
<td></td>
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<tr>
<td>Cresmer RH 40 (% w/v)</td>
<td>11</td>
<td>11</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 80 (% v/v)</td>
<td>11</td>
<td>11</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>Peppermint oil (% v/v)</td>
<td>2</td>
<td>2</td>
<td>0.96</td>
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Example 6: Physical stability of Microemulsions
The Microemulsions prepared in Example 2 were evaluated for stability at three different temperatures (i.e. 2-8°C, 25°C and 40°C) by visual appearance and globule size distribution over a period of 6 months. All the MEs were optically transparent and were having uniform size distribution at the time of preparation and remained so till the end of the stability studies. From these formulations, ME-8 and ME-11 which were having high solubilization capacity and were stable (w. r. t. optical transparency and globule size) even after 6 months were selected for protein loading and further development.

Example 7: Parathyroid Hormone (PTH) loading in w/o microemulsions
The microemulsions when loaded with higher concentration of drug act as efficient drug delivery system. PTH entrapped microemulsion was prepared by the method as explained in Example 2. For this purpose, ME-8 and ME-11 were used for protein loading. As was seen in previous Examples, different concentrations may be entrapped inside the MEs without compromising on the stability of the MEs. In place of FITC-Dex, aqueous solution of PTH was used for entrapment. The PTH entrapped microemulsion was stored at 2-8°C and evaluated for stability by visual appearance and globule size at regular intervals of time. The formulations were found to be optically transparent and having uniform size distribution at all the loading concentrations from 0.1 —1.0 mg/ml. Table 5 gives the detailed composition of the formulations.

Table 5: Composition of PTH entrapped MEs

<table>
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<tr>
<th>Ingredients</th>
<th>Quantity ME-14</th>
<th>Quantity ME-15</th>
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<tbody>
<tr>
<td>Captex 355 (% v/v)</td>
<td>21.7</td>
<td>24</td>
</tr>
<tr>
<td>Capmul MCM (% v/v)</td>
<td>21.7</td>
<td>22</td>
</tr>
<tr>
<td>Cresmer RH 40 (%)</td>
<td>10.9</td>
<td>11</td>
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Example 8: Extraction of PTH from w/o microemulsion

The protein from the microemulsion needed to be extracted to ensure its native confirmation and stability. The ability of w/o microemulsion solution to isolate and selectively extract proteins is well known. However, the recovery of entrapped proteins from the w/o microemulsion solution can be difficult. Ion-exchange chromatography technique was developed to extract protein from the microemulsion. The emulsion of the present invention was diluted (1:10 dilution) with Acetate buffer (pH 5) and loaded in the ion exchange column and eluted with Tris buffer (pH 9.0). The fractions were collected and evaluated by RP-HPLC. Microemulsions were prepared with different loadings and extracted by ion-exchange chromatography to determine % recovery of protein from the microemulsion by using the above mentioned method. More than 80% recovery of PTH was achieved using this method with less than 2% decrease in purity of PTH as a result of extraction process. It was observed that recovery of PTH increases with increase in loading amount.

Example 9: Stability of Protein inside w/o microemulsion at 2-8°C

The purpose of stability studies is to ascertain how the quality of product varies as function of time and under the influence of a variety of environmental factors (i.e. during storage, distribution, dispensing and use). PTH was loaded inside w/o microemulsions as given in Example 6. The microemulsion was stored at 2-8°C and evaluated by visual appearance globule size and purity of protein at regular interval of time. PTH was extracted at different time points and analyzed through RP-HPLC as explained in Example 5. Percentage recovery was calculated with respect to initial purity values on zero days. The results of stability are given in Table 6.

Table 6: % recovery of PTH (1-34) inside w/o microemulsion with time at 2-8°C

<table>
<thead>
<tr>
<th></th>
<th>PTH Concentration (µg/ml of emulsion)</th>
<th>Polysorbate 80 (% v/v)</th>
<th>Peppermint oil (% v/v)</th>
<th>Acqueous buffer (% v/v)</th>
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<tr>
<td></td>
<td></td>
<td>10.9</td>
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<table>
<thead>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Time Points (Days)</td>
<td>Percentage Recovery ME-14</td>
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<tr>
<td>-------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>0</td>
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</tr>
<tr>
<td>7</td>
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<td>15</td>
<td>95.44</td>
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<td>30</td>
<td>93.64</td>
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Claims

We claim

1. A stable, water-in-oil (w/o) microemulsion pharmaceutical formulation comprising:
   a. a continuous oil phase having a fatty acid derivative of glycerol and a low HLB surfactant;
   b. one or more high HLB surfactant;
   c. a dispersed aqueous phase containing a water soluble biological alone or in combination dissolved in stabilizing buffer and pharmaceutically acceptable excipients;
   d. a co-solvent;
   e. optionally a flavoring agent or a taste-masking agent; and
   f. optionally a mucoadhesive or a permeation enhancing agent.

2. The microemulsion of claim 1, wherein the fatty acid derivative of glycerol is a medium-chain triglyceride selected from the group consisting of glycerol caprylate caprate, propylene glycol dicaprate, propylene glycol dicaprylate/dicaprate, propylene glycol dilaurate, glyceryl tricaprylate, glyceryl caprate, glyceryl tricaprylate, glyceryl caprate, glyceryl stearate and polyoxylglycerides.

3. The microemulsion of claim 1, wherein the low HLB surfactant is selected from the group consisting of glyceryl mono/dicaprate, glyceryl caprylate/caprate, glyceryl oleate, glyceryl mono oleate, glyceryl monostearate, propylene glycol monocaprylate, propylene glycol monolaurate, sorbitan esters, glycerol monocaprylocaprate, sodium lauroyl dilactate, sodium stearoyl dilactate and α-D-tocopherol.

4. The microemulsion of claim 1, wherein the oil phase comprises an amount of about 10-50% v/v and preferably at about 20-40% v/v.

5. The microemulsion of claim 1, wherein the value of low HLB surfactant is in the range of 3-8.
6. The microemulsion of claim 1, wherein the ratio of the medium-chain fatty acid derivative to the low HLB surfactant is in the range of 1:0.01 to 1:5.

7. The microemulsion of claim 1, wherein the value of high HLB surfactant is at least 9.

8. The microemulsion of claim 1, wherein the high HLB surfactant is selected from the group consisting of polyoxyethylene fatty acid esters, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene glycol long-chain alkyl ethers, polyoxyethylene glycol long chain alkyl ester and polyoxyl hydrogenated vegetable oil either alone or in combination.

9. The microemulsion of claim 1, wherein the high HLB surfactant is present in an amount of about 5-50% v/v, preferably at about 10-25% v/v.

10. The microemulsion of claim 1, wherein there is one or more HLB surfactant, the ratio of first high HLB surfactant to second high HLB surfactant is in the range of 1:0.1 to 1:10, preferably 1:0.5 to 1:2.

11. The microemulsion of claim 1, wherein the aqueous phase consisting of water soluble biological is dissolved in protein stabilizing buffer selected from the group consisting of acetate, citrate, histidine, glycine, methionine, tartarate, lactate and succinate or a combination thereof.

12. The microemulsion of claim 10, wherein the aqueous phase consists of pH ranging from 3 to 9, preferably from 3 to 6.

13. The microemulsion of claim 1, wherein the biological is selected from the group consisting of protein, peptide, nucleotide, vaccine, hormone, siRNA and enzyme.

14. The microemulsion of claim 1, wherein the biological consists of molecular weight ranging from about 1 kDa to 100 kDa.
15. The microemulsion of claim 1, wherein the biological is teriparatide or exenatide.

16. The microemulsion of claim 1, wherein the co-solvent is selected from the group consisting of ethanol, isopropanol, methanol, n-butanol, isobutanol propylene glycol, polyethylene glycol preferably ethanol and isopropanol.

17. The microemulsion of claim 1, wherein the flavoring agent or taste-masking agent is selected from the group consisting of spearmint oil, cinnamon oil, oil of wintergreen (methylsalicylate), peppermint oils, clove oil, bay oil, anise oil, eucalyptus oil, thyme oil, cedar leaf oil, oil of nutmeg, oil of sage, oil of bitter almonds, peanut butter flavor, chocolate flavor, rum flavor, cassia oil, cinnamon mint flavor, corn mint oil, cardamom flavor, ginger flavor, cola flavor, cherry cola flavor and peppermint oil.

18. The microemulsion of claim 1 further comprising a sweetening agent selected from the group consisting of saccharin, aspartame, sucralose, neotame, acesulfame potassium and stevia, preferably aspartame.

19. The microemulsion of claim 1, wherein the said microemulsion has a droplet size less than 500 nm.

20. The microemulsion formulation of claim 1, wherein the formulation is administered transmucosally by the means selected from the group consisting of solution, drops, sprays, gels, bioadhesive gels and bioadhesive sprays.

21. A process of preparing water-in-oil (w/o) microemulsion as claimed in claim 1, comprising a continuous oil phase with dispersed aqueous droplets for delivery of biologicals through mucosal route wherein the biologicals are solubilized in aqueous droplets, comprising the steps:

f) Dissolving the biologicals in an aqueous phase of pH 3-9 to obtain a preferred concentration of 0.1-5mg/ml for solubilization in w/o microemulsion;
g) In first glass vial, taking 8-15% v/v of high HLB surfactants and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;

h) In second glass vial, taking 19-22% v/v of oil and low HLB surfactant and mixing the contents of the vial using magnetic stirrer at 500 rpm for 5 minutes at room temperature;

i) Addition of contents of second vial dropwise into first vial and mixing all the ingredients on a magnetic stirrer at 500 rpm for 5 minutes at room temperature to obtain a clear mixture of oil and surfactants; and

j) Dropwise addition of 5-40% v/v of biologicals containing aqueous phase into oil and surfactant mixture from step (d) with continuous stirring on the magnetic stirrer to get optically transparent w/o microemulsion.
Figure 3.

*In-vitro release of PTH from ME*

- **Cumulative % Release**
- **Time (min)**

The graph shows the cumulative release of PTH over time, with a steady increase from 0% to 100% release by 140 minutes.
INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN 2010/000447

A. CLASSIFICATION OF SUBJECT MATTER
IPC: A61K 9/107 (2006.01); A61K 47/44 (2006.01); A61K 38/26 (2006.01); A61K 9/29 (2006.01)
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: A61K

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 and, where practicable, search terms used)
EPDOC, WPI, TXTE, TXTG, TXTF

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<td></td>
<td>Claims 1-4, 6; Description Page 6 Line 32 - Page 7 Line 24, Page 10 Lines 17-26</td>
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Li Further documents are listed in the continuation of Box C.

* Special categories of cited documents:
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  "T" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered and is not 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

Date of the actual completion of the international search

Date of mailing of the international search report
20 December 2010 (20.12.2010)

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HUNGER U.
Telephone No. +43 / 1 / 534 24 / 363
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