Title: NOVEL HIGHLY BIOAVAILABLE, WATER SOLUBLE AND SUSTAINED RELEASE NANOFORMULATIONS OF HYDROPHOBIC PLANT DERIVED COMPOUNDS AND EXTRACTS

Abstract: The present invention discloses novel highly bioavailable, water soluble, sustained release nanoformulation(s) comprising unique proportion of the hydrophobic plant derived compound(s) in an emulsifier phase and aqueous phase to achieve the sustained release over a 24hr time period and more. The present invention further discloses process for preparation of said novel water soluble, highly bioavailable and sustained release nanoformulation thereof.
Declarations under Rule 4.17:

— as to applicant’s entitlement to apply for and be granted a patent (Rule 4.17(H))

— as to the applicant’s entitlement to claim the priority of the earlier application (Rule 4.17(iii))
"NOVEL HIGHLY BIOAVAILABLE, WATER SOLUBLE AND SUSTAINED RELEASE NANOFORMULATIONS OF HYDROPHOBIC PLANT DERIVED COMPOUNDS AND EXTRACTS"

TECHNICAL FIELD OF THE INVENTION:

The present invention relates to novel highly bioavailable, water soluble, sustained release nanoformulation(s) comprising unique proportion of the hydrophobic plant derived compound(s) in an emulsifier phase and aqueous phase to achieve the sustained release over a 24hr time period and more.

The present invention is further directed towards formulating novel sustained release nanoformulation(s) containing unique proportion of the hydrophobic plant derived compound(s) in an emulsifier phase and aqueous phase to achieve ideal hydrophilic - lipophilic balance (HLB) for providing sustained release over a 24hr time period and more.

The invention also relates to highly soluble, highly bioavailable and highly sustained release nanoformulations of plant derived hydrophobic compounds capable of delivering significantly higher amount of active molecules to the human and/or animal system more specifically to plasma and maintaining their concentration in the plasma for longer duration, thus rendering enhanced therapeutic efficacy with lower doses.

BACKGROUND OF THE INVENTION:

The biodiversity observed on the planet earth provides vast varieties of plants/trees which are explored over the centuries to understand their use in daily life both as source of food and medicine. The use of plants as a source of medicine came to limelight with Ayurveda which was developed between 2500 and 500 BC in India. India, a land known for tradition and herbal resource, recorded around 20,000 medicinal plants out of which only 35 - 40% are explored by the traditional communities.
Medicinal plants like Aswagandha, Amla, Brahmi, Guggul, Long pepper, Tulsi, Henna, Haridra, Neem and many more have been traditionally in use for treating various ailments via Ayurveda, Siddha, Unani and other traditional methods. The traditional medicinal system used these rich herbal sources either alone or in combination, together with other required ingredients to treat various conditions. Despite these uses of medicinal plants over the years there has been a lag to deliver a therapeutically efficacious drug/nutraceutical from a plant source. The traditional system of medicine had little scientific effort to validate these anecdotal uses that are traditionally known.

Despite the long historical use, little has been achieved in treating the diseases. The major problem associated with hydrophobic plant compounds and extracts is their poor bioavailability, leading to poor or decreased efficacy. The major hydrophobic compounds with poor bioavailability in plant extracts belong to phenolic compounds, flavonoids, stilbenes and lignans. Flavonoids may themselves be divided into 6 subclasses based on the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). Most of the hydrophobic compounds like Curcuminoids, Boswellic acids, Resveratrol, Hypericin, Bacosides, Xanthorrhizol, Ginseng extract, Gingko biloba extract and many others are proven to have several therapeutic benefits like anti-inflammatory, anti-oxidant, anti-obese, memory enhancing, anti-allergic, anti-microbial, anti-cancerous and many other medicinal activities. But, little has been achieved with respect to these molecules for the prevention and treatment of diseases due to their poor bioavailability and lack of sustained release in the body.

Many of the plant molecules as discussed above are hydrophobic in nature and hence are not water soluble. The poor bioavailability of these molecules reflects the lack of efficient natural drugs in the market, in spite of their traditionally known benefits. On the other hand, biopharmaceuticals have been suffering from instability and biological degradation before reaching the target site.

One of the best and thoroughly studied molecule that can be exemplified here is Curcumin. Curcumin and derivatives like Bisdemethoxycurcumin, Demethoxycurcumin,
Bis-o-demethylcurcumin have been widely acknowledged as a botanical supplement with great potential to prevent and treat wide spectrum of therapeutic conditions. In addition, they have been proved to be remarkably safe in animal studies and in many clinical evaluations even at high doses (up to 12 g/day). However, the major problem limiting the commercial exploitation of their therapeutic effects is their low bioavailability and their fast elimination from the body, very often as quickly as in 30mins.

The reasons for the poor bioavailability of curcuminoids may be attributed to poor absorption, high rate of metabolism and/or rapid elimination and clearance from the body. This is the same case with any of the hydrophobic plant molecules/extracts. Several studies failed to detect these compounds in the blood plasma-serum even after administration of high doses in animals and humans. Most of the phytochemicals such as curcumin and resveratrol show bioavailability of less than 1%. Curcumin when taken orally get metabolized to form curcumin glucoronide, curcumin sulphate, which are not biologically active and thus are eliminated from the system, which inturns leads to poor efficacy.

Many of the existing curcumin products in market are unformulated turmeric extract or formulated with suitable excipients to enhance the bioavailability. One of well known and patent pending (US 12/281994) curcumin product uses Phospholipids to enhance the bioavailability of curcumin. Similarly, US 5861415 disclose curcumin formulation using piperine to enhance the bioavailability. Patent (US 7883728) disclose use of curcumin and purified essential oil of turmeric.

The main issue to be addressed with the hydrophobic compounds is not only the bioavailability but also their availability in the systemic circulation for longer period (24 hours or more) in an biologically active form for sustained efficacy. An ideal formulation would be the one which can be retained in the body over a period of 12 - 24 hours and more, with significant amount of the active compound in the blood stream to provide the required therapeutic benefits. This could minimize the expenditure due to reduced dose and patient compliance of convenient dosing.
The time to reach maximum concentration ($T_{\text{max}}$), Maximum concentration ($C_{\text{max}}$), Area under the curve (AUC) and Half-life ($T_{1/2}$) are some of the important parameters to establish the systemic bioavailability of a particular drug/formulation. For a drug intended to provide sustained release and enhanced bioavailability, AUC should be higher with higher value for $T_{1/2}$. Higher $T_{1/2}$ indicates the longer stay of drug in the body and hence long lasting efficacy.

The product of above features will reduce the dose levels and also achieves enhanced bioavailability leading to enhanced therapeutic efficacy compared to existing products in market. The currently available phyto pharmaceutical compositions are way behind in providing a highly bioavailable and sustained release formulation, which is stable and water soluble.

Moreover the conventional methods and regular solubilization techniques are not efficient enough to solubilize high concentration of the plant molecules/extracts and also not successful in providing sustained release. Due to their lipophilic and hydrophobic nature, the choice of the right excipients, right combination of excipients and process of formulating such product is key to achieve the desired product.

Emulsifiers, which are classified under surfactants and also lipids are widely used for solubilizing hydrophobic compounds and in formulating nanoemulsions. Some of the well known prior arts include,

- US 10/444935 - Pharmaceutical compositions and dosage forms for administration of hydrophobic drugs.

Currently many curcuminoid products in market claim high bioavailability which are formulated using phospholipids or plant derived oils and so on. Some of the well known prior art includes;
However, none of the prior arts discloses a nanoformulation containing unique proportion of the plant derived hydrophobic active compound(s) in an emulsifier phase and aqueous phase to achieve sustained release over a 24hr time period and more.

Further, none of the prior art discloses the use of aqueous phase in combination with emulsifier phase to achieve a nanoemulsion with smaller particle size for sustained release and enhanced efficacy.

Hence, inventiveness of the present invention lies in arriving at such unique proportion of the plant derived hydrophobic active compound(s) in an emulsifier phase and aqueous phase to provide enhanced efficacy and sustained release over a 24hr time period and more.

Accordingly the present invention aims to provide a potential successor in the field of drugs, biopharmaceuticals, nutritional/dietary supplements for human and/or animal application with a novel nanoemulsified composition with enhanced bioavailability and sustained release.

**SUMMARY OF THE INVENTION:**

In an important aspect the invention provides novel sustained release nanoformulation(s)/delivery system having a unique proportion of the plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase to achieve sustained release over a 24hr time period or more.
In yet another aspect, the invention provides a water soluble nanoemulsified formulation(s) comprising one and/or combinations of plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase to achieve enhanced and long lasting efficacy at low dose and low cost.

In yet another aspect, the invention provides a water soluble nanoemulsified formulation(s) comprising one and/or combinations of plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase for protection from biological degradation.

In yet another aspect, the invention provides a water soluble nanoemulsified formulation(s) comprising one and/or combinations of plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase for various therapeutic, preventative and general health supplement applications in animals and human beings.

In yet another aspect, the invention provides a water soluble nanoemulsified formulation(s) comprising one and/or combinations of plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase for use as dietary supplements or nutraceuticals/health supplements/general supplements/OTC products.

In yet another aspect, the invention provides a water soluble nanoemulsified formulation(s) comprising one and/or combinations of plant derived hydrophobic active compound(s)/extract(s) in an emulsifier phase and aqueous phase either in liquid, semisolid or solid dosage form.

**BRIEF DESCRIPTION OF THE DRAWINGS:**

**Figure 1:** Malvern Particle Size analysis of CurQlife®.

**Figure 2:** Morphological Characterization of CurQlife® by Scanning Electron Microscopy (SEM).

**Figure 3:** Confocal Microscopy of CurQlife®

**Figure 4:** Preliminary dissolution study (pH-1.2) results of CurQlife® in comparison with
other curcuminoid products in the market.

**Figure 5**: Preliminary dissolution study (pH-6.8) results of CurQlife® in comparison with other curcuminoid products in the market.

**Figure 6**: CurQlife® Pharmacokinetics Graph in SD Rats

**Figure 7**: Curcumin Concentration Time Graph for CurQlife® and curcuminoids in Human Pharmacokinetic study

**Figure 8**: Free Curcumin (C-I) $C_{max}$ for CurQlife® in Human PK Study

**Figure 9**: Free Curcumin (C-I) $AUC_{0-24h}$ for CurQlife® in Human PK Study

**DETAILED DESCRIPTION OF THE INVENTION:**

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be fully understood and appreciated.

In a preferred embodiment, the invention describes nanoformulation(s)/delivery composition having a unique proportion of the plant derived hydrophobic active compounds(s), emulsifier phase and aqueous phase to achieve sustained release over a 24hr time period and more.

The plant derived hydrophobic compound(s) as disclosed can be either purified molecule(s) or extract(s).

In yet another embodiment, the invention describes the use of the said compound(s) either alone or in combination thereof.

The present invention is directed to nanoformulation(s) and method of producing such nanoformulation to improve the bioavailability with sustained release for use in humans and/or animals as drug and/or dietary/nutritional supplement/OTC products/health supplements/Ayurvedic (botanical) medicine.
The novel nanoformulations and the process of producing the same enables to attain unique nano size which allows to cross the required biological barriers to provide higher amount of active ingredient for higher beneficial effect. The essential biological barriers might include, gut mucosa, buccal lining, nasal mucosa, cell membranes, blood brain barrier, skin, respiratory and urinary and anal lining.

The invention further relates to nanoformulations of plant derived hydrophobic compounds with poor bioavailability.

The invention relates to hydrophobic plant molecules/extracts which are selected from but not restricted to phenolic acids, flavonoids, stilbenes, and lignans. Flavonoids may be isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins). The plant molecules/extracts are selected from but not restricted to Curcuma longa extract, Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, Bis-demethylcurcumin, and derivative of curcumin, Boswellia Serrata extract, Boswellic acids, Beta boswellic acid, keto beta boswellic acid, acetyl keto beta boswellic acid, Resveratrol, Hypericin, Bacopa monnieri extract, Bacoside A, Bacoside A3, Bacoside B, Xanthorrhizol, Ginseng extract, Genistein, Gingko biloba, Coenzyme Q10, Pycnogenol, Luteolin, Kaempferol, Capsaicin, Rubia cordifolia extract, Lycopene, Pyrogallol, Lutein, Lawsennia iermis extract, Aloe vera extract, Beta carotene, Piperine and any other hydrophobic plant molecules/extracts.

The plant derived hydrophobic compound(s) described above can be obtained either naturally and/or by synthetic and/or semi synthetic process.

In yet another embodiment, the invention is directed to nanoformulations of hydrophobic compound(s) having anti-inflammatory, anti-allergic, anti-oxidant, memory enhancing, anti-obese, neuro protective, anti-diabetic, anti-cancerous, cardio protective, eye protective and anti-microbial activity.

In yet another aspect of the invention, the said hydrophobic plant molecules/extracts, can be used either alone or in combination to formulate into
nanoemulsion(s)/nanof ormulation(s) giving rise to the oral, nasal, anal, topical, vaginal, ocular, buccal dosage forms.

The invention further relates to nanoformulations of the hydrophobic compounds using unique process to achieve water soluble, bioavailable and sustained release formulation(s).

In another embodiment, the invention describes the method of obtaining such nanoformulations.

As used herein the term emulsifier(s) enhance the solubility of hydrophobic/lipophilic compounds/extracts.

The use of emulsifier(s) for solubilizing the hydrophobic compounds is well known in the prior art. However, the inventive step of the present invention lies in arriving at a unique proportion of the hydrophobic active compound(s), emulsifier phase and aqueous phase, which is the main principal in solubijizing higher concentrations of such hydrophobic compounds with higher bioavailability and sustained release.

The concentration of the emulsifier phase in the nanoformulations ranges from 60 to 95 % and more preferably 80 %.

Further, the invention relates to sustained release nanoformulation(s) with unique hydrophilic lipophilic balance (HLB), to achieve nanoparticle of smaller size for enhanced efficacy.

In yet another aspect, the invention describes the use of emulsifiers(s) which are anionic, cationic or non-ionic selected from but not limited to Polysorbates preferably Polysorbate 80 and Polysorbate 20, Polyethylene glycols preferably Polyethylene glycol 200 and Polyethylene glycol 400, Polyethylene glycol esters and Glycerol esters.
For the purpose of the present invention, the emulsifier(s) can be used alone or in combination to maintain the total HLB of the nanoformulation between 13 - 18.

The inventiveness of the present invention further lies in preparing the nanoemulsion(s) with unique particle size, which enhance the bioavailability of the said hydrophobic compounds and also to provide sustained release of the same over a 24 hour time period. The prior art teaching does not provide a method or a product to develop such nanoformulations, with enhanced bioavailability and sustained release.

The higher the HLB value the more water soluble or hydrophilic the emulsifiers are. Hence, it is important that the nanoformulations developed ideally comprise emulsifier(s) with a HLB value ranging from 13 - 18 to achieve enhanced bioavailability and sustained release. The inventiveness of the current invention relies on the Hydrophilic - Lipophilic balance of the emulsifier. The inventors of the present invention have found that nanoformulation with emulsifier phase HLB below 13 or above 18 could not achieve sustained release bioavailable formulations.

The concentration of the aqueous phase in the nanoformulations ranges from 5 to 20 % and more preferably 5 - 10 %.

In yet another embodiment, the invention provides a process to solubilize hydrophobic compounds with the help of energy in the form of sonication, heating, vortexing, shaking or any other forms of energy.

According to the inventive process, the emulsifier phase is preheated to a temperature below melting point of the hydrophobic active followed by addition of desired concentration of hydrophobic compound(s) to the emulsifier phase. The mixture is subjected to sonication/heating/vortexing/shaking to solubilize the hydrophobic compound(s). Upon solubilization, distilled water of desired concentration is added to the mixture to form nanoformulation(s). For the purpose of solubilization, heating is more preferable as it is quick and effective process. Heating the mixture of hydrophobic
compounds and surfactants for a period of 2 - 3 hours and preferably between 30 - 60 mins at a temperature ranging between 50 - 200 °C and preferably between 100 - 140 °C and subsequent adding distilled water upon cooling to room temperature.

The hydrophobic plant compound(s)/extract(s) can be solubilized between 0.0001- 50% using the method of the present invention and more preferably between 12 - 20 %.

The nanoformulation(s) of the present invention entraps the active compound within the spherical droplets which are in nanometer range. This entrapment offers bioprotection for the active compound(s) from hydrolytic and/or enzymatic degradation.

Solubilizing the hydrophobic compounds using surfactants with the aid of heat energy disperses the hydrophobic compounds from the larger crystal lattice into individual molecules there by reducing the particle size leading to complete solubilization upon heating. Hence, the nanoformulations are completely soluble in water, which no other existing products could achieve.

In yet another embodiment, the present invention discloses nanoformulations in a free flowing solid powder form, which is obtained by subjecting the liquid nanoformulations to techniques not limited to encapsulation, nanospray drying, thin layer drying, freeze drying, using carriers like Microcrystalline cellulose, Precipitated Silica, Fujicalin, Nuclcin, Mannitol, Hydroxypropyl Methylcellulose, Arbocel, Silica derivatives.

In yet another embodiment, the present invention discloses nanoformulations in a semi solid gel, lotion or cream form, which is obtained by formulating the liquid formulation with suitable polymers not limited to Hydroxypropyl Methylcellulose, Isopropyl myristate, Collagen, Glycerol, Cetyl alcohol, Sterates of magnesium, Zinc, Calcium and Carbopol.

In yet another embodiment, the invention is directed to nanoformulations of hydrophobic compounds/extracts with enhanced bioavailability and sustained release over a period of 24 hours compared to any existing products.
The nanoformulations of the present invention further are effective in delivering high concentrations of the active compound which was not disclosed in any of the known prior art. For the said reasons, the nanoformulations of the present invention are far superior to any of the existing products in terms of bioavailability, sustained release profile, low dose and low cost.

In yet another embodiment, the invention is directed to nanoformulations of hydrophobic compound(s) as drugs, dietary/nutritional supplement for use in humans and animals.

In yet another embodiment, the invention is directed to nanoformulations of hydrophobic compound(s) for the treatment and/or prevention of inflammation, osteoarthritis, allergy, obesity, neuro degenerative disorders, diabetes, cancer, cardio vascular disorders and microbial disorders.

In yet another embodiment, the invention is directed to nanoformulations of hydrophobic compound(s) which can be administered as pharmaceuticals/nutraceuticals/ayurvedic/dietical compositions to the subject in need thereof.

Various formulations were developed using one or combination of hydrophobic compound(s) along with single or combination of surfactants which are exemplified herein. Having described the invention with reference to certain preferred embodiments, other embodiments will become apparent to one skilled in the art from consideration of the specification.

The invention is further defined by reference to the following examples describing in detail the methods of preparation and use of the invention. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the invention.
Example 1: Process of Preparing 15% Curcuminoids Formulation

a) 15gms of accurately weighed Curcuminoids was used for obtaining nano formulation.

b) Surfactants (12g) Tween 80, (6g) Tween 20 and (62g) PEG 400 were accurately weighed and heated to 120 °C.

c) To the preheated surfactants, curcuminoids (15g) was added and stirred continuously till the curcuminoids are completely dissolved.

d) Once the curcuminoids are completely solubilized, it's cooled to room temperature and distilled water (5g) is added and stirred well to obtain a nanoemulsified formulation.

Example 2: Process of Preparing 10% Curcuminoids Formulation (CurQlife®)

a) 10gms of accurately weighed Curcuminoids was used for obtaining the nanof ormulation.

b) Surfactants (PEG 200 (13g), Tween 20 (67g)) were accurately weighed and heated to 120 °C.

c) To the preheated surfactants, curcuminoids (10g) was added and stirred continuously till the curcuminoids are completely dissolved.

d) Once the curcuminoids are completely solubilized, it's cooled to room temperature and distilled water (10g) is added and stirred well to obtain a nanoemulsified formulation.

Example 3: Process of Preparing 15% Bis-o-demethyl curcumin (BDMC) formulation

a) 15gms of accurately weighed BDMC was used for obtaining nano formulation.

b) Surfactants (PEG 200 (13g), Tween 20 (67g)) were accurately weighed and heated to 120 °C.

c) To the preheated surfactants, BDMC (15g) was added and stirred continuously till the BDMC is completely dissolved.

d) Once the BDMC is completely solubilized, it's cooled to room temperature and distilled water (5g) is added and stirred well to obtain a nanoemulsified formulation.
Example 4: Composition of Nanoformulations of Hydrophobic compounds

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Hydrophobic Compound</th>
<th>Emulsifier Phase</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Hydrophobic Active</th>
<th>Aqueous Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>Resveratrol</td>
<td>PEG20 0</td>
<td>1.6</td>
<td>11.8</td>
<td>0.6</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6-10</td>
<td>Curcumin</td>
<td>PEG40 0</td>
<td>3.4</td>
<td>12.4</td>
<td>1.2</td>
<td>0</td>
<td>2</td>
<td>2</td>
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<tr>
<td>11-15</td>
<td>Hypericin</td>
<td>Twee n 20</td>
<td>1.4</td>
<td>11.6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>16-20</td>
<td>Bisdemethylicurcumin</td>
<td>Twee n 80</td>
<td>2</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>21-25</td>
<td>Boswellic Acid</td>
<td>Acconon</td>
<td>2</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>26-30</td>
<td>Curcuminoid extract</td>
<td></td>
<td>2.6</td>
<td>12.4</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
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<tr>
<td>31-35</td>
<td>Curcumin Bacoside A</td>
<td></td>
<td>0</td>
<td>11.4</td>
<td>3.6</td>
<td>0</td>
<td>2</td>
<td>3</td>
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<tr>
<td>36-40</td>
<td>Curcuminoids</td>
<td></td>
<td>0.91</td>
<td>3.77</td>
<td>9.32</td>
<td>0</td>
<td>3</td>
<td>1</td>
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<tr>
<td>41-45</td>
<td>Xanthorrhizol</td>
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<td>0</td>
<td>10</td>
<td>0</td>
<td>6</td>
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<td>2</td>
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<td>46-50</td>
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<td>Pyrogallol</td>
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<td>56-60</td>
<td>Kaempferol</td>
<td></td>
<td>0.6</td>
<td>3.6</td>
<td>9.6</td>
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<td>61-65</td>
<td>Emolin</td>
<td></td>
<td>0.8</td>
<td>2.6</td>
<td>3</td>
<td>9.6</td>
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<td>2</td>
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<td>66-70</td>
<td>Genistein</td>
<td></td>
<td>0</td>
<td>2</td>
<td>14</td>
<td>0</td>
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<td>Ellagic Acid</td>
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<td>Psoralen</td>
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<td>0</td>
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<td>0</td>
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<td>2</td>
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<tr>
<td>81-85</td>
<td>Catechin</td>
<td></td>
<td>0</td>
<td>11.4</td>
<td>0</td>
<td>3.6</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Example 5: Particle Size Analysis of CurQlife

Dynamic Light Scattering (sometimes referred to as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is a technique for measuring the size of particles typically in the sub-micron region. DLS measures Brownian motion and relates this to the size of the particles. The scattered light is then detected by the detector and translated to an auto-correlator. Freshly prepared sample was transferred to Cuvette and the sample was analysed in 90 PLUS Particle Size Analyzer (Brookhaven Instrument Corporation) under the below mentioned conditions.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Angle of diffraction</td>
<td>90°</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>No of cycles</td>
<td>5</td>
</tr>
<tr>
<td>Time duration</td>
<td>1 min for each cycle</td>
</tr>
</tbody>
</table>

The mean particle size of CurQlife (10%) was found to be 102.6 nm, upon diluting the CurQlife sample with water in the ratio of 1:1000. The particle size in CurQlife in this dilution ranges from -81-181 nm as depicted in Figure 1.


SEM allows visualization and characterization of particles by providing better information on its structure, shape and distribution. SEM is an important technique for measuring the size of particles typically in the sub-micron region. In SEM, primary beam of electron scans the surface of the sample and then the secondary electrons generated from the sample are detected by the detectors to analyze the surface morphology of sample.

Freshly prepared and diluted sample was air dried on the glass slide and the sample was analyzed using FEI Quanta 400 FEG, as it has got better magnification and extended low-vacuum capabilities for the really challenging samples and less surface charging of the sample as compared to Quanta 200 FEI. The image was taken on the 500 nm scale bar and at the magnification of 60,000X.
SEM results show clearly spherical shaped particles. SEM image shows particles of more or less evenly sized particles distributed evenly throughout the test sample tested (Fig 2). These particles are found to be in nano-meter range. Majority of the particles are less than 100nm size and few are found to be around 100nm range.

Example 7: Morphological Characterization of CurQlife (10%) Using Confocal Microscopy

Confocal microscopy allows visualization and characterization of structures on the surface and also inside the particles, provided that the material is sufficiently transparent and fluorescent or fluorescent labeled. Curcuminoids loaded particles can be visualized by using confocal microscope, since Curcumin is naturally fluorescent in the visible green spectrum, no further labeling of Curcumin will be needed.

0.1ng/ml concentration of CurQlife product in water was used for the study. Diluted sample was filtered using 0.45µ Sartorius filter. 2-3 drops of sample was placed on the slide and was observed under the Confocal microscope.

Freshly prepared sample was transferred to the slide and was analyzed by using LSM (Laser Scanning Microscope) 710, under dark condition.

<table>
<thead>
<tr>
<th>Model name</th>
<th>LSM 710 Laser Scanning Microscope</th>
</tr>
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<tbody>
<tr>
<td>Company</td>
<td>Carl Ziess, Germany</td>
</tr>
<tr>
<td>Objective</td>
<td>Plan-ApoChromat 40X 0.95 Korr M27</td>
</tr>
<tr>
<td>Laser</td>
<td>Argon 488</td>
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<td>Laser Power</td>
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</tbody>
</table>
Spherical green fluorescence was observed under the confocal microscope including the presence of curcuminoids. Working test sample with 0.1ng/ml concentration of CurQlife product in water, showed clear individual particles with green fluorescence. These particles were found to be in proper spherical shape of different sizes. Almost all the particles were found to have regular spherical shaped structure (Fig. 3a-d).

**Example 8: Solubility Studies**

Solubility of curcuminoids in water is an important quality which all the bioavailable curcuminoids formulation is expected to possess. Hence, CurQlife® was compared with different bioavailable curcuminoid products available in the market to test their solubility in water.

Solubility studies were carried out in comparison with marketed products by dissolving respective products in the concentration of 1mg/ml of water. Vortexed for 5 minutes filtered using 0.2µ filter paper and the filtrate was tested for curcuminoid content using HPLC. Results of HPLC analysis for Curcuminoids are shown in Table.1 Below is the list of marketed curcuminoid products tested;

- Meriva (CP-1) - Batch No FG-9212
- C3 Complex (CP-2) - Batch No 1103071
- C3 Complex + Bioperine (CP-3) - Batch No 2011001-A

<table>
<thead>
<tr>
<th>Table 1. Results of consolidated solubility study of CurQlife® in comparison with other bioavailable curcuminoids product in market</th>
</tr>
</thead>
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<td><strong>Sl. No.</strong></td>
</tr>
<tr>
<td>1.</td>
</tr>
<tr>
<td>2.</td>
</tr>
</tbody>
</table>
Result (Table 1) showed that among all the bioavailable products tested, CurQlife® showed high solubility and the percentage solubility or recovery of curcuminoids in water. CurQlife® was found to be superior in terms of solubility in water compared to all other products. Interestingly none of the other bioavailable curcuminoid products were soluble in water in the concentration (mg/ml) tested.

Example 9: Analysis of Nature of Curcuminoid in Water

It is important to retain the structure, chemical and biological properties of curcuminoids after dissolution in water so as to retain its biological activity. To evaluate the change in the structure, chemical properties and biological properties, curcuminoids products were dissolved in water, filtered and filtrates were tested for the change in $\lambda_{max}$ using spectrophotometer.

Interestingly except for CurQlife®, there is significant change in $\lambda_{max}$ value for other marketed bioavailable curcuminoid products (Table 2). This may be due to complexation of curcuminoids with other excipients during formulation or might be due to degradation of curcuminoids in water. This clearly infers that CurQlife® doesn't change the nature of Curcuminoids and hence indicates the higher therapeutic activity. Below is the list of marketed curcuminoid products tested:

- Meriva (CP-1) - Batch No FG-9212
- C3 Complex (CP-2) - Batch No 1103071
- C3 Complex + Bioperine (CP-3) - Batch No 2011001-A

Table 2. Xmax of curcuminoids from different bioavailable curcuminoid formulations in water.

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Product</th>
<th>Xmax (nm)</th>
<th>Absorbance (OD) at $\lambda_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CurQlife</td>
<td>422</td>
<td>0.566</td>
</tr>
</tbody>
</table>
Example 10: Dissolution Study of CurQlife®

In order to assess the bioequivalence and to predict the bioavailability of CurQlife® in comparison to other bioavailable curcuminoid products in market, dissolution study was carried out. Dissolution study is a standard method for measuring the rate of drug release from dosage form. The preliminary dissolution study results, described in Fig 4 and Fig 5 at different pH of 1.2 and 6.8, reflecting stomach and intestine environment respectively, confirms that CurQlife® possess far superior dissolution properties compared to all other marketed products. Hence based on the dissolution study results, CurQlife® is expected to have much better bioavailability and pharmacokinetic properties than other products in the market.

Two of the dissolution bowls were filled with 500 mL of dissolution medium each. One bowl with 500 mL dissolution medium serves as blank. The dissolution apparatus was programmed so that the temperature was 37°C and paddle rotation of 100 rpm. After the temperature in the bowls reached 37°C, 500 mg of test substance was added to one dissolution bowl. Samples of 5 mL were withdrawn at time intervals of 0, 10, 20, 30, 40, 50, 60, 120, 300, 600, 900, 1800, 2700, 3600 sec. The samples were centrifuged at 2000 rpm to facilitate easy filtration through 0.22 micron syringe filters. Centrifuged samples were filtered using 0.22 micron filters to remove finely suspended particles. Filtered samples were analyzed by HPLC to determine % release with time. A graph with time versus % release was plotted (Fig 4 and Fig 5)

Below is the list of marketed curcuminoid products tested in the dissolution study:

- Meriva (CP-1) - Batch No FG-92 12
- C3 Complex (CP-2) - Batch No 1103071
- C3 Complex + Bioperine (CP-3) - Batch No 201 1001-A
- Vivomeric (CP-4)
Example 11: Oral Bioavailability Study of CurQlife® in SD Rats

Pharmacokinetics (PK) is a fundamental scientific discipline that underpins applied therapeutics. Drugs with poor PK are reported to be poorly absorbed into the biological system and hence are therapeutically inefficient. Current study was conducted to evaluate the bioavailability of orally administered CurQlife® and marketed curcuminoid products in Sprague Dawley rats. Total of 36 animals were divided into 6 groups having six numbers of animals in each group. The test substances were administered orally to the animals once on Day -1 at 500mg/Kg body weight dose equivalent to Active ingredient. Control or 0th time blood samples were collected from all the animals before dosing the test substance, followed by blood sampling at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24hrs after dosing the test substance by sinus orbital plexus under anaesthesia. Serum was separated from blood by centrifugation. Serum was acidified with Concentrated HCl and was extracted with methanol and centrifuged to collect the supernatant. The supernatant was subjected to LC-MS/MS analysis for estimation of curcumin concentration in serum samples.

Below is the list of marketed curcuminoid products tested in the study:

- Meriva (CP-1) - Batch No FG-6558
- C3 Complex + Bioperine (CP-3) - Batch No 201 1001-A
- BCM-95 (CP-5) - Batch No 66250
- Theracurmin (CP-7) - Batch No 0120-957-145

The results obtained confirmed superior bioavailability of CurQlife® compared to unformulated curcuminoids and other marketed products. CurQlife® was found to be 20 fold more bioavailable compared to unformulated curcuminoids and 3.8 fold more compared to closest marketed product (CP-1).

The summary of PK results is tabulated in Table 3. CurQlife® provided sustained release compared to other products and also delivers higher amount of curcuminoids over a period...
of 24hrs (Fig 6). The study confirmed the superior bioavailability of CurQlife®, which can be correlated to its enhanced efficacy.

**Table 3:** Pharmacokinetic parameters of CurQlife® and other marketed products

<table>
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<tr>
<th>Products</th>
<th>AUC&lt;sub&gt;0-&lt;i&gt;t&lt;/i&gt;</th>
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<tbody>
<tr>
<td>Curcuminoids</td>
<td>173 ± 38</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>CP-1</td>
<td>1111 ± 239</td>
<td>257 ± 16</td>
</tr>
<tr>
<td>CP-3</td>
<td>238 ± 15</td>
<td>40 ± 9</td>
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<tr>
<td>CP-5</td>
<td>113 ± 7</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>CP-7</td>
<td>2319 ± 376</td>
<td>330 ± 40</td>
</tr>
<tr>
<td>CurQlife®</td>
<td>3570 ± 499</td>
<td>828 ± 108</td>
</tr>
</tbody>
</table>

CP - Curcuminoid Product in market

**Example 12: Human Pharmacokinetic Study of CurQlife®**

A clinical study was conducted to determine the bioavailability of different doses of CurQlife in healthy, adult, human, subjects under fasting conditions. Different formulations used in this study are CurQlife 250mg, CurQlife 500mg, CurQlife 1g, CurQlife 2g, Curcuminoids 1g and Curcuminoids 2g. The bioavailability of Curcumin in blood was estimated at periodical intervals over a period of 24 hours, 0.00 (predose), 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.00, 4.00, 8.00, 12.00 and 24.00 hours (post-dose). The Pharmacokinetic parameters assessed were Cmax, Tmax, t½, AUC<sub>0-<i>t</i></sub>, AUC<sub>0-<i>inf</i></sub>. A total of 24 subjects, after fulfilling inclusion and exclusion criteria (4 subjects in each group x 6 groups) were screened and enrolled in the study as per the randomization number. Curcumin plasma concentrations were estimated in the samples using LCMS-MS. All the formulations were well tolerated in the subjects. The Bioavailability results confirmed that the pharmacokinetic parameters like Cmax, Tmax, t½, AUC<sub>0-<i>t</i></sub>, AUC<sub>0-<i>inf</i></sub> of Curcumin, were superior in CurQlife dosed subjects as compared to unformulated curcuminoids. (Fig No. 7). AUC and Cmax graphs of CurQlife are depicted in Figure 8 & 9. Curcumin bioavailability in CurQlife treated group is 48-fold higher (on a per mg basis) than unformulated curcumin.
We Claim,

1. A sustained release nanoformulation of hydrophobic plant molecule(s) and/or extract(s) with enhanced bioavailability comprising:
   a) an hydrophobic active in the range of 0.0001 to 50%;
   b) an emulsifier phase having HLB value of 13 to 18 with a concentration range of 60-95%; and
   c) an aqueous phase with a concentration range of 5 to 20%,
   wherein, the recovery of the hydrophobic plant molecules/extracts from said nanoformulation is in the range of 75 to 90%, when added into water.

2. The sustained release nanoformulation according to claim 1, wherein the said nanoformulation allows sustained release over a 24hr time period and more.

3. A process for formulating sustained release nanoformulation of hydrophobic plant molecule(s) and/or extract(s) comprises;
   a) preheating the emulsifier phase to a temperature below the melting point of the hydrophobic active;
   b) adding required quantity of hydrophobic active to preheated emulsifier phase and completely solubilizing the mixture in the emulsifier phase;
   c) cooling down the said mixture to room temperature; and
   d) adding desired quantity of aqueous phase followed by mixing to obtain a nano emulsified formulation of hydrophobic active.

4. The process according to claim 3, wherein the mixture of step (b) is heated at a temperature in the range of 50-200°C to obtain complete solubilization of hydrophobic active in an emulsifier phase.

5. The process according to claim 3, wherein the mixture of step (b) is subjected to sonication to obtain complete solubilization of hydrophobic active in an emulsifier phase.
6. The process according to claim 3, wherein the mixture of step (b) is subjected to vortexing to obtain complete solubilization of hydrophobic active in an emulsifier phase.

7. The process according to claim 3, wherein the mixture of step (b) is subjected to ultra-high pressure homogenization to obtain complete solubilization of hydrophobic active in an emulsifier phase.

8. The sustained release nanoformulation according to claim 1, wherein, the hydrophobic active(s) are natural and/or synthetically derived.

9. The sustained release nanoformulation according to claim 1, wherein, the hydrophobic active(s) is selected from group comprising, curcuminoids, curcumin, demethoxycurcumin, bisdemethoxycurcumin, bis-o-demethy1 curcumin, Boswellic acid(s), Resveratrol, Hypericin, Bacoside(s), Xanthorhizol, Luteolin, Pyrogallol, Genistein, Wogonin, Morin, Kaempferol either alone or in combination.

10. The sustained release nanoformulation according to claim 1, wherein the hydrophobic active(s) is derived from the plant(s) comprising Curcuma longa, Ginseng, Ginkgo biloba, Garcinia mangostana, Ocimum basilicum, Zingiber officinale, Tribulus terrestris, Sphaeranthus indicus, Annona Squamosa, Moringa oleifera, Murraya koenigii either alone or in combination.

11. The sustained release nanoformulation according to claim 1 and 3, wherein, the emulsifier(s) is selected from the group comprising anionic, cationic and/or non-ionic surfactants.

12. The sustained release nanoformulation according to claim 11, wherein, the emulsifier(s) is selected from the group consisting of Polysorbates such as Polysorbate 80 and Polysorbate 20, Polyethylene glycols such as Polyethylene glycol 200 and Polyethylene glycol 400, Polyethylene glycol esters, Glycerol esters either alone or in combination thereof.
13. The sustained release nanoformulation according to claim 1 and 3, wherein said nanoformulation can be formulated in liquid, semisolid or solid dosage form together with pharmaceutically acceptable excipients.

14. The sustained release nanoformulation(s) according to claim 1 and 3, wherein the said nanoformulation can further be converted to solid powder form using carriers comprising Microcrystalline cellulose, Precipitated Silica, Fujicalin, Nucelin, Mannitol, Hydroxypropyl Methylcellulose, Arbocel, Silica derivatives.

15. The sustained release nanoformulation(s) according to claim 1 and 3, wherein the said nanoformulation can further be converted to semi-solid gel and/or cream using suitable polymers comprising, Hydroxypropyl Methylcellulose, Isopropyl myristate, Collagen, Glycerol, Cetyl alcohol, Sterates of magnesium/zinc/calcium and Carbopol.

16. A method of treating and/or preventing inflammation, osteoarthritis, allergy, obesity, neuro degenerative disorder(s), diabetes, cancer, cardio vascular disorder(s) and microbial disorder(s) comprising administering sustained release formulation consisting of hydrophobic plant molecule(s) and/or extract(s) of claim 1, to a subject in need thereof.

17. Use of sustained release nanoformulation comprising of hydrophobic plant molecule(s) and/or extract(s) according to claim 1, useful as drug, dietary supplement, nutraceutical, health supplement, ayurvedic medicine and over the counter product(s).

18. Use of sustained release nanoformulation comprising of hydrophobic plant molecule(s) and/or extract(s) according to claim 1, useful for the treatment and/or prevention of inflammation, osteoarthritis, allergy, obesity, neuro degenerative disorder(s), diabetes, cancer, cardio vascular disorder(s) and microbial disorder(s).
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Operator ID | dinesh
Elapsed Time | 00:05:00
Mean Diam. | 102.6 nm
Rel. Var. | 0.035
Skew | 11.276

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**Figure 1:** Malvern Particle Size analysis of CurQlife®.

**Figure 2:** Morphological Characterization of CurQlife by Scanning Electron Microscopy (SEM)
Figure 3: Confocal Microscopy of CurQlife

Figure 4: Preliminary dissolution study (pH-1.2) results of CurQlife® in comparison with other curcuminoid products in the market.
Figure 5: Preliminary dissolution study (pH-6.8) results of CurQlife® in comparison with other curcuminoid products in the market.
IV/V

**Figure 6:** CurQlife® Pharmacokinetics Graph in SD Rats

**Figure 7:** Curcumin Concentration Time Graph for CurQlife and curcuminoids in Human Pharmacokinetic study
Fig 8. Free Curcumin (C-I) Cmax for CurQlife in Human PK Study

Fig 9. Free Curcumin (C-I) AUC_{0-24h} for CurQlife in Human PK Study
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/107 (2013.01)
USPC - 424/455

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(8) - A61K 9/10, 9/107 (2013.01)
USPC - 424/455, 457, 725; 514/938; 516/53; 977/801, 906

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 9/107, 9/1075 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>Y</td>
<td>US 2008/01 99523 A1 (FINNIE et al) 21 August 2008 (21.08.2008) entire document</td>
<td>1, 2, 8-10, 16-18</td>
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</table>

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

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

**A** document member of the same patent family

Date of the actual completion of the international search
14 October 2013

Date of mailing of the international search report
18 OCT 2013

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Blaine R. Copenheaver
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: 11-15
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

□ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
□ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
□ No protest accompanied the payment of additional search fees.