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Ubidecarenone nanoemulsified composite systems

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Abstract

The nanoemulsified composite (NEC™) delivery system is a patented technology based on the incorporation of a double microemulsion into microporous carrier. This approach was applied on the very water insoluble ubidecarenone drug. The resulting composite powder showed good technological properties such as flowability; also good stability was evidentiated, with size of the nano-droplets released from the systems maintained equal to the starting size also after a long storage. Furthermore very good biopharmaceutical properties were originated, with water solubility concentrations up to 50-fold higher than pure ubidecarenone and oral absorption in rats up to three-fold greater than standard commercial products in terms of plasma levels and AUC.

Keywords: Solid double microemulsion; Coenzyme Q10; Oral absorption; Sub-micron droplets; Stability

1. Introduction

Approximately 40% of new drug candidates have poor water solubility and the oral delivery of such drugs is frequently associated with low bioavailability, high intra and inter subject variability (Robinson, 1996).

To overcome these problems various formulation strategies are reported in the literature including the use of surfactant systems as micellar solutions, multilayer vesicles (liposomes, niosomes), micronised powder, inclusion complexes (cyclodextrins and derivatives), co-precipitates with water soluble polymers (Aungst, 1993; Robinson, 1996).

In recent years much attention has been focused on lipid based formulations such as emulsions, microemulsions, and self-emulsifying compositions (Humberstone and Charman, 1997).

Mostly attractive are microemulsions and self emulsifying drug delivery systems (SEDDS) which guarantee a high surface area when in contact with the physiological fluids.

SEDDS are isotropic mixtures of oil, surfactant, co-surfactant and drug, that form fine oil/water microemulsion when in contact with physiological fluids (Shah et al., 1996).

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Small oil droplets provide a large interfacial area for pancreatic lipase to hydrolyse triglycerides and thereby promote a faster release of the drug and/or formation of mixed micelles of the bile salts containing the drug (Tarr and Yalkowsky, 1989). In most cases the surfactant used for this kind of formulation increase the possibility to improve the bioavailability, stimulating different mechanisms, such as improved drug dissolution (Costantinides, 1985), increased intestinal epithelial permeability (Swenson and Curatolo, 1992), increased tight junction permeability (Lindmark et al., 1995).

Microemulsions are thermodynamically stable, isotropically clear dispersions of two immiscible liquids as oil and water, stabilized by an interfacial film of surfactant molecules (Eccleston, 1992).

Double microemulsions are multiple microemulsion whose dispersing phase can contain droplets of another phase, leading to water/oil/water, or oil/water/oil systems (Florence and Attwood, 1998).

Coenzyme Q10, ubidecarenone is a very good example of water insoluble drug with consequent poor absorption from the gastro-intestinal tract (Greenberg and Fishman, 1990).

Coenzyme Q10, a naturally occurring molecule, is the cofactor in the electron transport chain, the biochemical pathway in cellular ATP respiration from which most of the body's energy is derived and is considered essential for the health of all the body cells, tissues and organs (Folkers and Yamamura, 1980).

Many different approaches for formulating CoQ10 have been reported, some of these based on oil-based or powder filled capsule formulations or self nanoemulsified drug delivery systems (Nazzal et al., 2002), or dry emulsions (Takeuchi et al., 1992).

Remedia has developed a proprietary technology (NEC™, Nanoemulsified Composites) based on the incorporation of a double microemulsion into a powder solid microporous carrier (Carli and Chiellini, 2003). The advantages offered by this approach are: good processing and storage properties, easy redispersibility in water and maintenance of the sub-micron size of the released droplets. This paper reports data on the application of the Remedia technology on the development of a highly bioavailable formulation of coenzyme Q10 (Cardium™).

2. Materials and methods

2.1. Materials

CoQ10 was purchased from Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan), polyglycolized glycerides (Labrasol, Labrafil 2125M CS, Labrafil 1944 M) were obtained from Gattefosse (Milano, Italy). Miglyol 812 (Caprylic/Capric triglyceride) was obtained from Sasol Germany, Akoline (mono, di-triglyceride mixtures) was obtained from Karlshamns AB-Sweden.

As surfactant was used Polysorbate 80 (Tween 80), obtained from Sigma–Aldrich (Milan, Italy), as cosurfactant was used soya lecithin (Lipoid S 40) purchased from Lipoid GMBH (Ludwigshafen, Germany). Polyvinil pirrolidone crosslinked (Kollidon Cl M) was purchased from BASF Italia Spa. All materials used in the formulation of the double microemulsion (o/w/o) and the carrier are GRAS.

2.2. Preparation of coenzyme Q10 composite powder

CoQ10 is incorporated in the oily phase, microemulsified with water and surfactant, or a combination of surfactant/cosurfactant, under paddle stirring at the rate of 300 rpm for 3 h; the resulting o/w microemulsion is subsequently redispersed in an oily phase by stirring at 300 rpm for 2 h. The resulting double o/w/o microemulsion was loaded onto the PVP-Cl swellable polymer powder using a high shear mixer (Roto P10, Romaco), by adding slowly 900 ml of the double microemulsion to 1000 g of the polymer powder kept under stirring at the speed rate of 40 rpm.

2.3. Drug content

For quantitative determination of ubidecarenone in the powder a HPLC analytical method was used. The mobile phase was acetonitrile:tetrahydrofuran (80:20, v/v). The analysis was performed at a flow rate of 1.5 ml/min with UV detector at 275 nm, using a Reverse Phase C-18 column Novapack® (3.9 mm × 110 mm, 5 μm) from Waters Spa (Italy). The quantitative determination of the drug is determined by suspending the powder in the mobile phase using an ultrasound bath for 30 min. The suspension is then filtered with a Sartorius Regenerated Cellulose membrane 0.45 μm. The

concentration of the CoQ10 in the filtrate is determined by HPLC analysis.

2.4. Powder density characterization

The apparent and tapped densities of composite powders were analyzed by measuring the volume of the powder in a 50 ml cylinder before and after tapping (1250 taps) with adequate tapping instrument (Giuliani mod.IG/4).

2.5. Solubilization kinetics

The solubilization kinetics of the composite formulations is determined by adding an excess quantity of the samples to pH = 7.5 buffer solution at the temperature of 37 °C in Erlenmeyer flask under mild agitation in a water bath shaker.

The samples (5 ml) are collected at different time levels, and filtrated with Sartorius regenerated cellulose membrane 0.45 μm.

The concentration of ubidecarenone in each sample is determined using a HPLC technique.

2.6. Droplets size measurement

The size of the droplets released from the composite systems during the solubilization kinetics experiments is determined by centrifuging at the speed rate of 2000 rpm, for 30 min, the samples collected at different times and subsequently filtrating the supernatant with cellulose regenerated syringe filter (Sartorius 1.2 μm). The size of the sub-micron dispersed liquid phase is determined by using a laser light scattering technique (Coulter mod.N4 Plus).

2.7. Absorption studies

The absorption of experimental formulations (the emulsified nanocomposite formulation 1, the standard product marketed by Swiss Natural Sources) was carried out in Sprague–Dawley rats weighing 250–280 g fasted overnight with free access to water.

The aqueous suspension of the composite powder is administered with a gavage, in a single dose of 100 mg/kg for each animal.

Blood samples are collected from animal abdominal aorta at different time intervals in heparinized tubes. The experiment is run on groups of five animals for each time level.

The plasma fraction were separated from the blood sample by centrifugation at the speed rate 2500 rpm, for 15 min, and the drug quantity absorbed is determined using HPLC analytical method (Scalori et al., 1998).

3. Results and discussion

3.1. Micrometrics of nanoemulsified composite powders

The composition of the nanoemulsified composite powders prepared is reported in Table 1.

The composite powders resulting from loading the double o/w/o microemulsion onto the polymer were analyzed by optical microscopy (Karl Zeiss): essentially spherical particles were observed, with the largest percentage of particles within the size range of 1–10 μm.

The flowability properties of the composite dry powders prepared are reported in Table 2: the nanoemulsified composite systems showed good flowability characteristics, even if no glidant such as silica gel is added.

Table 1
Composition of the tested nanoemulsified composite powders

Component (mg)	Formulation		
	I	II	III
Ubidecarenone	10.6	10.4	20.4
PVP-Cl M	52.6	51.6	54.9
Colloidal silica gel	–	1.96	–
Polyglycolized glycerides	29.4	28.8	–
Mono, di, tri-glycerides mixtures	–	–	23.1
Soya lecithin	5.9	5.8	–
Polysorbate 80	0.46	0.45	0.48
Water	1.07	1.05	1.12

Table 2
Flow properties of nanoemulsified ubidecarenone composite systems

Formulation	Bulk density before tapping (g/ml)	Bulk density after tapping (g/ml)	Carr index (%) (Carr, 1965)
I	0.369	0.443	17.0
II	0.381	0.452	15.9

3.2. Drug content

The ubidecarenone content in the composite powders, determined by HPLC analytical method (USP XXIV), was found to be 10.19, 10.0 and 20.13 g of drug /100 g of powder for formulation I, II, and III, respectively, confirming the very good yield of the process (99%). Furthermore no degradation products were detected in the chromatograms, showing that the stability of the drug is maintained during the preparation process.

The three reported formulations differ in terms of solubilization level of ubidecarenone and flowability characteristics.

Formulation I was chosen for the "in vivo" absorption studies being the system with the best properties.

3.3. Size of double microemulsion droplets released

The size of the droplets released from the carrier "in vitro" (pH = 7.5, $T = 37\,^{\circ}C$) were found to be in the range of 200–400 nm (Fig. 1). Different sizes can be originated depending on the emulsifying properties of the oily external phase of the double microemulsion.

The oily phases selected in the present study showed good microemulsifying properties when in contact with buffer solution, not overcoming dimensions of 200–400 nm.

It is particularly interesting to observe that the size of the dispersed system released from the carrier remains constant during storage at room temperature for 12 months, showing the good stabilization effect originated by the microporous structure of the carrier where the nanodroplets are stored (Fig. 2).

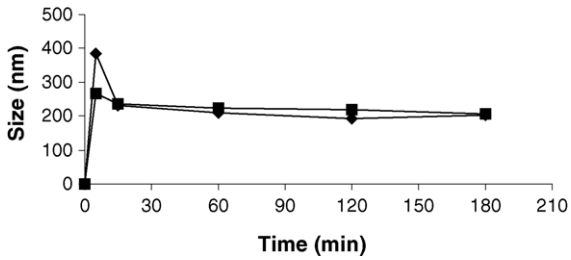

Fig. 1. Size of the droplets released from the composite powders ($T = 37\,^{\circ}C$, pH = 7.5); key: (■) nanoemulsified composite formulation I, (♦) nanoemulsified composite formulation III. Each reported value is the mean of six replicates.

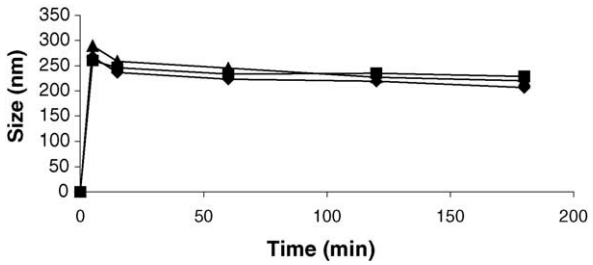

Fig. 2. Size of the droplets released from the composite powders ($T = 37\,^{\circ}C$, pH = 7.5); key: (♦) composite formulation I at $t = 0$ of storage ($T = 25\,^{\circ}C$, R.H. = 60%), (■) composite formulation I $t = 3$ months, (▲) composite formulation I at $t = 12$ months of storage. Each reported value is the mean of six replicates.

It was also observed that the size of the released double microemulsion droplets is practically equal to the size of the double microemulsion before the loading onto the carrier (Fig. 3).

3.4. Solubilization kinetics

The solubilization kinetics profile of the nanoemulsified composites is markedly higher than the kinetics of the pure drug as shown in Fig. 4; at 24 h the composites show a solubility of 50 μg/ml compared to less than 5 μg/ml for the pure ubidecarenone. It is interesting also to observe that the drug is gradually released up to 4 h, reaching almost the final equilibrium concentration. Another important feature reported in Fig. 4 is that no significant change in the kinetics profile is observed also after 1 year of storage.

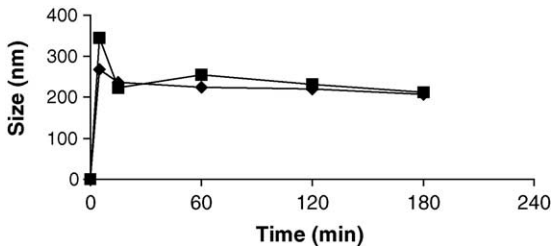

Fig. 3. Size of the droplets released from the composite powder ($T = 37\,^{\circ}C$, pH = 7.5); key: (♦) composite formulation I, (■) microemulsion of formulation I before incorporation in the microporous carrier. Each reported value is the mean of six replicates.

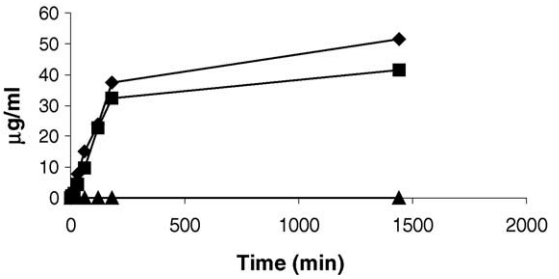

Fig. 4. Mean ubidecarenone concentrations after solubilization at pH = 7.5, $T = 37\,^{\circ}C$; key: (♦) nanoemulsified composite formulation I $t = 0$ (■) nanoemulsified composite formulation I ($T = 25\,^{\circ}C$, R.H. = 60%, $t = 12$ months) (▲) pure drug (no detectable concentrations). Each reported value is the mean of six replicates.

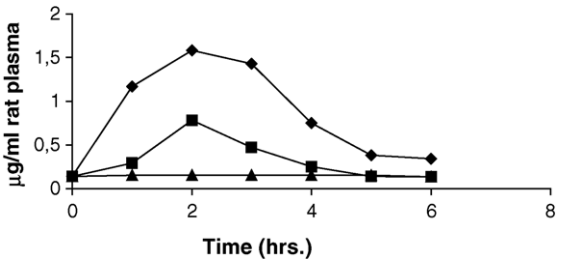

Fig. 5. Mean ubidecarenone plasma concentrations after oral administration to fasted rats; key: (♦) nanoemulsified composite I, (■) marketed ubidecarenone reference product (Swiss Natural Sources), (▲) endogenous level. Each reported value is the mean of five replicates (blood levels from five rats at each time).

3.5. Absorption studies

The nanoemulsified composite formulation I and the standard marketed coenzyme Q10 product were administered orally to Whistar rats and the plasma levels determined up to 6 h. As reported in Fig. 5 and Table 3 the composite system originated plasma levels up to three times higher than the marketed product with an $AUC_{0-6\,h}$ 2.8-fold greater than the commercial capsules.

Table 3
Ubidecarenone pharmacokinetics parameters after oral administration of formulations to fasted rats

Parameter	Nanoemulsified composite formulation I	Reference marketed product
AUC (µg h/ml)	0.913 ± 0.475	0.33 ± 0.209
C_{max} (µg/ml)	1.580 ± 0.320	0.78 ± 0.240
T_{max} (h)	2	2

4. Conclusion

The results of this study show that the application of the nanoemulsified composite system (NEC^{TM}) approach (double o/w/o microemulsion loaded into polymeric carrier) on ubidecarenone originates a powder with good technological properties, e.g. flowability and dispersibility, but above all with very good biopharmaceutical characteristics, i.e. high solubilization kinetics in aqueous buffers and high bioavailability after oral administration in comparison to the pure drug and standard products. These very good biopharmaceutical properties are probably related to the very fine sub-micron size of the droplets released from the carrier, which is maintained also after a long storage period indicating the important stabilization effect exerted by the tight cross-linked polymeric nature of the carrier.

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