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Statins Solubilized in Fish Oil: Versatile and Simple Combinations by Innovative Formulations

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Abstract

Dyslipidemia or hyperlipidemia comprised mainly hypertriglyceridemia and/or hypercholesterolemia; it is now well established as their control is considered to have an impact on public health. Therefore, in an overcrowded field to find appropriate answers to lower blood levels of cholesterol and triglycerides, here we report findings of formulation studies, aimed to combine statins and n-3 PUFA, overcoming drawbacks related to solubility and stability. A new formulation has been made creating a solvent system that allows for a complete solubilization of statin drugs such as atorvastatin, rosuvastatin, and pitavastatin. With simvastatin, a preliminary dehydration with molecular sieves was also needed for stability purpose. These findings are of great importance and could be applied widely in all cases where a co-administration of the two active therapeutic ingredients are routinely required.

Keywords: Fish oil; n3-PUFA; Statins; Stable fluid formulation; Statin solubilization; Ionic emulsifier

Abbreviations: PUFA: Omega-3 Polyunsaturated Fatty Acid; CVD: Cardiovascular Disease; WHO: World Health Organization; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; LC/PDA/MS: Liquid Chromatography/ Photo-Diode Array / Mass Spectrometry; HPLC: High-Performance Liquid Chromatography; DAD: Diode Array Detector; PTFE filter: Phenex® Teflon® filter; RH: Relative Humidity; INN: International Nonproprietary Names; LDL: Low Density Lipoprotein; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid

Introduction

The sedentary lifestyle of Western populations is closely associated to the use of hyper caloric and hyperlipidemic diets, is one of the important risk factors for Cardiovascular Disease (CVD), the major cause of mortality in the world.

Western lifestyle and its spread to a growing number of people worldwide, is increasingly worrying the World Health Organization (WHO) that recently revealed that ischemic heart disease and cerebrovascular disease would be in first and second position, respectively, in rank order of deaths in the frame time from now through the year 2030. Fortunately, CVD, as well as type 2 diabetes mellitus incidence, cancers and other terrible diseases, could be prevented with appropriate interventions to reduce the effects of risk factors. Dyslipidemia represents an important risk factor of CVD, manifested by elevation or attenuation of plasma concentration of lipoproteins [1,2]. Dyslipidemia is, therefore, an extremely important public health problem, involving great cost to both individuals and their families, as well as representing a huge burden to the health care systems. Control of triglycerides and cholesterol levels in blood is one of the most followed practices for preventing Dyslipidemia [3,4]. Statins, the most therapeutically and commercially successful class of drugs of the last century, are used to lower blood levels of cholesterol, and PUFAs (omega-3 polyunsaturated fatty acids) which are mainly fish oil, are widely used to reduce triglycerides.

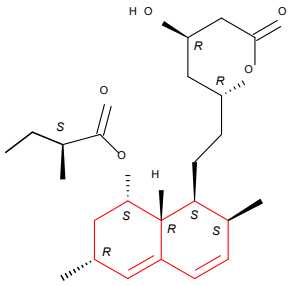
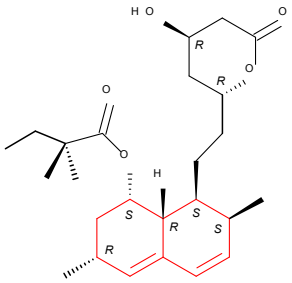
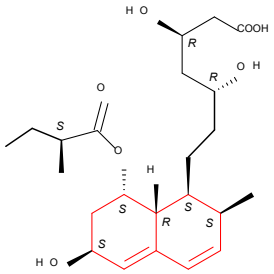
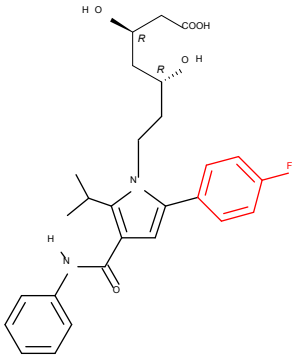
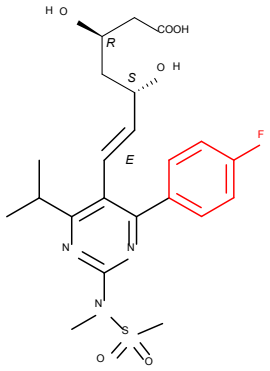
Normally, in patients with high cholesterol and triglycerides levels, doctors prescribe statins and PUFAs at different dosage, evaluated on a case by case basis, and this is why there are no fixed-dose combinations of these drugs [5,6]. A second reason is due to technological limitations: statins are usually poorly soluble and also unstable in fish oil. To obviate all this, different techniques have been proposed, mainly based on microencapsulation of statins prior to be mixed in fish oil [7].

Here we report the results of a research project aimed at developing a technology for the preparation of stable fluid solutions of statins in fish oil.

Statins

The discovery of HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, an important enzyme in the metabolic pathway of cholesterol synthesis, and the statins as its inhibitors, represent a breakthrough in the prevention of hypercholesterolemia and related diseases. So far this has been amply demonstrated by clinical evidence [8]. The statins on the market are fully or partially fermentation-derived (lovastatin, simvastatin and pravastatin) or entirely synthetic (atorvastatin, rovustatin, pitavastatin and fluvastatin). All the statins are relatively unstable, and their degradation is catalyzed by several factors like oxygen, humidity, acidity and temperature.

The purpose of this paper is not to analyze the different statins according to their absorption and bioavailability, pharmacokinetic and metabolism, but to describe statins according to their chemical structure being responsible of different limitations in the fish oil formulation. Statins can be grouped into two types: type A (decalin-ring derivatives) and type B (fluorophenyl derivatives). Another important parameter regards the equilibrium between the lactone forms (closed, prodrug) or the anionic carboxylate forms (open; active) (Table 1). With this information in mind few statins, belonging to both types, were investigated for their solubility and stability in fish oil solutions.

Origin	Type	Administered as	Chemical Structure	INN	Trade Name
Natural	*Type 1*	Prodrug *(lactone form)* *20-40 mg*		*Lovastatin*	*Mevocar®*
Natural	*Type 1*	Prodrug *(lactone form)* *20-40 mg*		*Simvastatin*	*Zocar®*
Natural	*Type 1*	Drug *(Free acid form)* *20-40 mg*		*Provastatin*	*Pravachol®*
Synthetic	*Type 2*	Drug *(Free acid form)* *10-20 mg*		*Atorvastatin*	*Lipitor®*
Synthetic	*Type 2*	Drug *(Free acid form)* *5-10 mg*		*Rosuvastatin*	*Crestor®*

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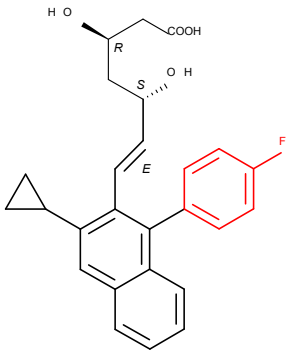
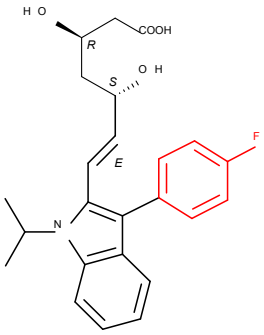
Synthetic	*Type 2*	Drug *(Free acid form)* *1-4 mg*		*Pitavastatin*	*Livalo®*
Synthetic	*Type 2*	Drug *(Free acid form)* *40-80 mg*		*Fluvastatin*	*Lescol®*

Table 1: Currently available statins on the market, classified according to their chemical structure. INN (International Nonproprietary Names) and trade name. Typical doses for reduction of LDL (low-density lipoprotein or the "bad cholesterol") in the range of 25-45%, are also reported.

Fish oil

Marine and freshwater fish oil are the primary sources of omega-3 polyunsaturated fatty acids (n-3 PUFA), composed mainly of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), which have been found to regulate lipid metabolism and lower blood triglyceride levels in a dose-dependent manner, demonstrating beneficial effects in the prevention of cardiovascular events. According to both primary and secondary prevention studies, consumption of omega-3 fatty acids, fish, and fish oil reduces all causes of mortality and various CVD outcomes such as sudden death, cardiac death, and myocardial infarction, with the evidence being largely in favor of fish and fish oil supplements [9]. In the literature it is possible to find many examples where n-3 PUFA from food or dietary-supplement showed a positive effect in the prevention and treatment of several diseases, but this is not the topic of this paper. Interested readers are encouraged to consult recent reviews to get a broader understanding of this area [10-12].

Statins in fish oil

Here we report the results of a study on fluid formulations (solutions) containing n-3 PUFA and statins; although the synergistic effects of statins associated to PUFA (fish oil) have been documented in several papers [13-18], it is also known that certain statins are sensitive to acidic environment causing their degradation to their lactone forms and various isomers. For example, pravastatin, atorvastatin, and fluvastatin are converted to their lactone forms in an acidic environment; meanwhile statins in the lactone form, e.g. lovastatin and simvastatin, are sensitive to alkaline environment causing this conversion into their acid form. On the other hand, fish oil also tends to oxidize easily, forcing the use of antioxidants to prevent rancidity. Taken together, it emerges that combining statin and fish oil is a particularly difficult task. So, two critical points must be overcome in order to combine these two active principles, solubility and stability. Three kinds of formulation have been reported: solutions, suspensions and solid formulations. However, to protect the components from a possible degradation, most of them employ techniques of microencapsulation of at least of one component. Statins are normally poorly soluble in fish oil and this is associated to their poor bioavailability; with formulations where they are microencapsulated, further reduction of their bioavailability was observed. Therefore, to overcome these limitations it might be useful to have homogeneous solutions. To prepare solutions of statins in fish oil it is necessary to overcome the limits of their poor solubility, in particular for the latest generations of statins. In fact, while simvastatin in fish oil has a solubility of 11mg/ml, atorvastatin (calcium salt) is <0.1 mg/ml soluble. In both cases, the solubility is far from being suitable to get an optimal therapeutic dosage. A second drawback to be overcome is the stability: a solution of simvastatin in fish oil is unstable.

The objective of this study was therefore to identify excipients and/or a technique to improve the solubility of the statins in fish oil, as well as their stability. Through a systematic study aimed at testing a set of emulsifying agents and co-solvents, the best results were obtained with a purified soybean lecithin, which mainly consists of phosphatidylcholine. It is very likely that the process of solubilization and stabilization is due to the formation of reverse micelles. Indeed, the hydrophobic medium in lecithin is organized to form reverse micelles where the hydrophobic chains are external and polar residues are internal. These systems can improve the solubility of the statins. With the simvastatin, more soluble than others, the main problem to overcome was the stability. The solution to the problem came with the anhydrification of the solution, on molecular sieves.

Materials and Methods

The omega-3 polyunsaturated fatty acids (n-3 PUFA) are a mixture of ethyl esters of polyunsaturated fatty acids with content in EPA and DHA greater than 85%, in a ratio EPA/DHA comprised between 0.9 and 1.5;

product provided by Pronova Norway. The statins used in this study were all commercially available: atorvastatin as calcium amorphous salt and simvastatin, by Biocon (India); rosuvastatin as calcium amorphous salt, by Biocon (India); pitavastatin by MSN Laboratories Pvt. LTD (India). The polyoxyethylene (20) sorbitan monooleate (Tween® 80) and the sodium docusate, were purchased from Sigma-Aldrich; the polyoxyethyleneglyceroltrihydroxy- stearate (Cremophor® RH 40) and the polyethylene glycol (15) hydroxystearate (Solutol® HS 15), by BASF Italia Srl. The mixture of glyceryl and polyethylene glycol esters (Labrasol®), the 2-(2-ethoxyethoxy)ethanol (Transcutol® P) and the propylene glycol monolaurate (Lauroglycol® 90), were purchased from Gattefossè Italia, S.r.l (Milan, Italy). The hydrogenated phosphatidyl choline (Epikuron® 200 SH) and the deoiled lecithin-enriched phosphatidylcholine (Epikuron® 200) used were both furnished by Cargill, and the solid natural lecithin (Lipoid S PC-3) by Lipoid GmbH.

LC/PDA/MS

A high-performance liquid chromatography (HPLC) instrument equipped with diode array detection (DAD) combined with mass spectrometry, was used for the quantification of statins and fish oil components. The liquid chromatographic apparatus comprised the following modular components: Alliance system 2695, Photodiode array Waters 2996 and mass system Waters *Quattro Micro (Waters USA)*.

LC conditions for simvastatin, atorvastatin, rosuvastatin

The HPLC column was an Intersil® ODS-3 (4.6 × 250 mm; 5 µm) column, and the mobile phase used was acetonitrile, water and trifluoroacetic acid (70:30:0.1, v/v/v). For pitavastatin, acetonitrile, water and trifluoroacetic acid (30:70:0.1, v/v/v). The flow used was 1ml/min (Table 2).

HPLC method for omega-3 polyunsaturated fatty acids (n-3 PUFA)

The HPLC column was a Waters Symmetry C-18 4.6 × 150 mm 5 µm (Waters USA). The mobile phase consisted of acetonitrile, methanol, water and trifluoroacetic acid (45:45:10: 0.1, v/v/v/v). The flow used was 1 ml/min. The UV detection and quantitation of EPA and DHA were performed at λ=215 nm (Figure 1).

Formulations with non-ionic surfactants (Comparative Examples)

Non-ionic surfactants have no charge on their hydrophilic end, which helps make them superior oily soil emulsifiers. In this study Lauroglycol® 90, Tween® 80, Cremophor® RH40, Labrasol®, and Solutol® HS15 were used as non-ionic surfactants. The emulsifier was dissolved in n-3 PUFA and left under mechanical stirring. Then, the statin (i.e., atorvastatin) was added and the appearance of the solution was evaluated. The emulsifiers used were all excipients already used in approved pharmaceutical products, and commercially available. In some cases, a co-surfactant (i.e., Transcutol® P) was added in a second step to further enhance the solubility of atorvastatin (Table 3).

Formulations with ionic surfactant-emulsifier (sodium docusate - Epikuron® 200)

These surfactants (cationic or anionic) emulsifier possess a charge on their polar end. The emulsifier was dissolved directly into the n-3 PUFA and left under mechanical stirring. Then, the statin (i.e., atorvastatin) was added and the appearance of the solution was evaluated (Table 3).

With poorly soluble statins (i.e., atorvastatin), a co-surfactant like Transcutol® P was added in a second step, to further enhance the solubility, however, without reaching this goal.

Solubility test procedure

For the four statins, atorvastatin, rosuvastatin, pitavastatin and simvastatin, the maximum concentration reached in fish oil was also calculated following another procedure: an increasing amount of statin was added to n-3 PUFA (1 ml) up to a point when a precipitate formed. Such mixture was left under agitation for 18 hours and then was filtered on 0.22 µm PTFE filter and analyzed by HPLC, according to the method reported above (Figure 2). Results are summarized in (Table 4).

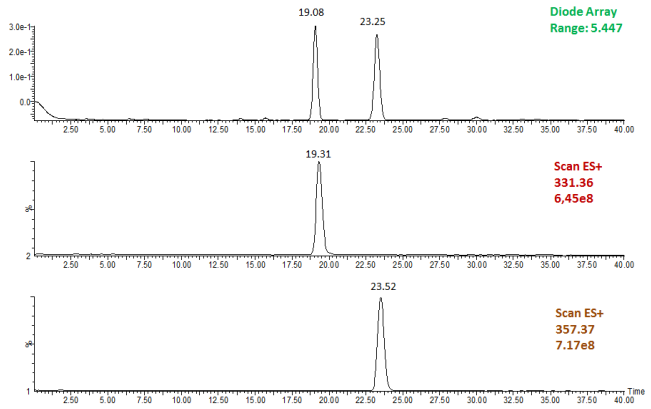


Figure 1: Chromatographic profile of n-3 PUFA analyzed at l=215nm. a) two main peaks are highlighted: at 19.08 e 23.25 min. b) The first isolated peaks, corresponding to EPA EE (EPA ethyl ester), with ES-MS: ES+ m/z 331.36; and c) the second to DHA EE (DHA ethyl ester), with ES-MS: ES+ m/z 357.37.

Form. No.	Statin	Detector UV/VIS	Retention Time (min)	Max. concentration of the "statin" in solution (mg/ml)°
Form 1	Atorvastatin	λ =244nm	5.8^	0.10
Form 2	Rosuvastatin	λ =241nm	3.9^	0.02
Form 3	Pitavastatin	λ =245nm	5.2*	2.30
Form 4	Simvastatin	λ =238nm	16.9^	10.10

Table 2: HPLC data, with the maximum amount of statins dissolved in n-3 PUFA analyzed. Intersil® ODS-3 (4.6 × 250 mm; 5 µm) column and a solution of CH_3CN/H_2O (70/30 + 0.1% of CF_3COOH) or CH_3CN/H_2O (30/70 + 0.1% of CF_3COOH) as eluent, with flow rate at 1ml/min.
^: HPLC methods for simvastatin, atorvastatin, rosuvastatin
*: HPLC method for Pitavastatin
°: Results are the means of a minimum of two independent experiments. ± SD less than 10%.

Formul. No.	Emulsifier		Statin		Solubility
	Component	Amount (g)	Component	Amount (g)	
Form 5	Transcutol® P	0.1	Atorvastatin	0.02	Not soluble
Form 6	Lauroglycol® 90	0.1	Atorvastatin	0.02	Not soluble
Form 7	Tween® 80	0.1	Atorvastatin	0.02	Not soluble
Form 8	Cremophor® RH 40	0.1	Atorvastatin	0.02	Not soluble
Form 9	Solutol®HS 15	0.1	Atorvastatin	0.02	Not soluble
Form 10	Labrasol®	0.1	Atorvastatin	0.02	Not soluble
Form 11	Lipoid S PC-3	0.1	Atorvastatin	0.02	Not soluble
Form 12	Epikuron® 200	0.1	Atorvastatin	0.02	**Soluble**
Form 13	Sodium docusate	0.1	Atorvastatin	0.03	**Soluble**
Form 14	Epikuron® 200 Sodium docusate	0.09 0.01	Atorvastatin	0.02	**Soluble**
Form 15	Transcutol® P	0.1	Rosuvastatin	0.02	Not soluble
Form 16	Lauroglycol® 90	0.1	Rosuvastatin	0.02	Not soluble
Form 17	Tween 80®	0.1	Rosuvastatin	0.02	Not soluble
Form 18	Cremophor® EL	0.1	Rosuvastatin	0.02	Not soluble
Form 19	Labrasol®	0.1	Rosuvastatin	0.02	Not soluble
Form 20	Epikuron® 200	0.1	Rosuvastatin	0.02	**Soluble**
Form 21	Sodium docusate	0.1	Rosuvastatin	0.02	**Soluble**
Form 22	Transcutol® P	0.1	Pitavastatin	0.02	Not soluble
Form 23	Lauroglycol® 90	0.1	Pitavastatin	0.02	Not soluble
Form 24	Tween 80®	0.1	Pitavastatin	0.02	Not soluble
Form 25	Cremophor® EL	0.1	Pitavastatin	0.02	Not soluble
Form 26	Epikuron® 200	0.1	Pitavastatin	0.02	**Soluble**
Form 27	Sodium docusate	0.1	Pitavastatin	0.02	**Soluble**
Form 28	Epikuron® 200 Sodium docusate	0.09 0.01	Pitavastatin	0.02	**Soluble**
Form 29	Transcutol® P	0.1	Simvastatin	0.02	Soluble
Form 30	Lauroglycol® 90	0.1	Simvastatin	0.02	Soluble

Table 3: Solubility testing of statins (atorvastatin, rosuvastatin, pitavastatin and simvastatin) and fish oil (n-3 PUFA; 0.9 g), in the presence of 10% co-solvent.

Stability tests

For the preliminary stability tests, the samples were placed in a climatic chamber at 5, 25 and 40°C and at 60% Relative Humidity (RH). The stability of the solutions (clear solution from visual inspection) was monitored for a time up to six months (1st, 3rd and 6th month). A suitable amount of the fish oil formulated was diluted with methanol and analyzed by HPLC (Table 5).

To increase the stability

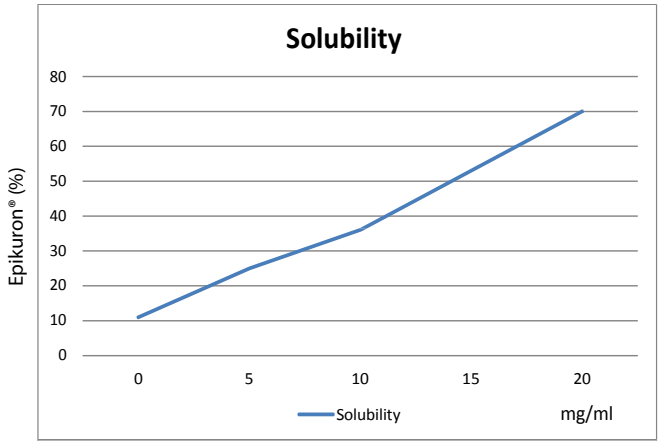
In order to increase the stability, a small amount (10%) of adipic acid and palmitic acid or citric acid was added to the simvastatin solution. The presence of said acids did not however enhance the stability (Table 6).

Drying of fish oil solution with molecular sieves. The molecular sieves (0.4 nm, beads about 2 mm), were previously activated under vacuum at 150°C for 18 hours, then added into the solution and the system was left overnight without mechanical stirring. The oily solution was finally obtained by decantation. Stability tests gave surprising results, which are reported in Table 7.

Results and Discussion

The objective of this study was to prepare homogeneous solutions of statins in fish oil to overcome the challenge associated with their sparing solubility and instability.

Different emulsifiers have been tested, demonstrating that with statins scarcely soluble in fish oil, like atorvastatin, rosuvastatin or pitavastatin,



% Epikuron®	Solubility (mg/ml)
0	11
5	25
10	36
15	53
20	70

Figure 2: Solubility test of Simvastatin

the addition of ionic emulsifiers, like Epikuron® 200 or sodium docusate, significantly increases their solubility, reaching concentrations greater than 20 mg/ml (Form 12 and 13; 20 and 21; 26 and 27). A mixture of them was equally efficient (Form 28). Such concentration are at least ten times greater than the analogue solutions formulated with non-ionic surfactants-emulsifier (Tween 80, Cremophor® RH40, Solutol HS 15, Labrasol, etc.), which in turn do not improve even after addition of a surfactant (i.e. Transcutol® P, 20%; data not shown) (Tables 3 and 4).

With more soluble statins, like simvastatin, the effect of ionic and non-ionic surfactant was also verified. The results showed that the maximum solubility reached with different solvents was in the same range (Form 29-31, 33, 34) (Table 7). With the formulation comprising Epikuron® 200 solubility proportionally increased with the amount of ionic emulsifier added (see examples in S.I.): with 20% of Epikuron® the solubility reached 70 mg/ml (Form 32); for the non-ionic solvents, Transcutol® P and Lauroglycol® 90, the solubility was not increased despite using a higher amount of solvent (Tables 3 and 4).

From this study, it is clearly demonstrated that to solubilize statins, above all those poorly soluble in fish oil, ionic emulsifiers, like Epikuron® 200 and sodium docusate, are necessary. With simvastatin, the addition of 20% Epikuron® greatly increases its solubility up to 70 mg/ml. Even greater was the effect observed with the latest generation of statins, being less soluble, where the formulation with ionic emulsifiers increase by up to 2 Log units their solubility: see Form 1 vs. Forms 12 and 13; Form 2 vs. Forms 20 and 21; Form 3 vs. Forms 26 and 27 (Table 4).

In conclusion, the maximum solubility in fish oil of the new statins (e.g. atorvastatin) is extremely low (<0.1 mg/ml) while with somatostatin higher solubility has been obtained (~11 mg/ml), however not therapeutically useful. Here we have clearly demonstrated the feasibility to increase the solubility of all statins to reach therapeutic dosages (≥ 20 mg/ml), with stable solutions.

As regards to the chapter of stability, for these new proposed formulations, two main aspects were monitored: degradation of n-3 PUFA and statins stability. Fish oil is mainly represented (>85%) by two n-3 PUFA, namely EPA and DHA, as shown in the chromatographic profile in Figure 1. In none of the formulations proposed here were the findings of fish oil degradation significant (Table 5). In the same table, the stability data of statins, with Epikuron® 200 (Form 12, 20, 26 and 31) or with sodium docusate (Form 13), monitored at 5°C, 25°C and 40°C, up to six months are showed. At room temperature, for statins in their free acid form, atorvastatin, rosuvastatin and pitavastatin, the only degradation product was the closed lactone derivative (10%; ± 3). Otherwise, statins in their lactone forms, i.e., simvastatin, the only degradation product was the opened lactone derivative (5%; ± 2). (S.I Figure 1). All statins followed- up for a month at 5°C showed complete stability. The free acid form statins in fish oil solution led to only a small percentage of lactone (<5%) (Form 12, 20, 26) (Table 5).

An independent study was done with the simvastatin. In this case the challenge that had to be overcome was the stability. Simvastatin is administered as an inactive lactone prodrug: hypothesized that acidic conditions could stabilize the lactone, citric acid, adipic acid and palmitic acid, all being excipients commonly used in pharmaceutical formulations, even in the solid formulations containing simvastatin already available on the market, which were added to the formulation. However, contrary to what was expected, in fish oil solutions the presence of a carboxylic or hydroxy acid did not enhance the stability (Table 6). The only degradation product observed was the carboxylic acid derivative resulting from the opening of the lactone ring. Chemically, degradation of lactone can be explained by the presence of water, so removal of traces of water from the solution could potentially stabilize the statin. To this end, we proceeded with anhydrification of the fish oil solution by the addition of suitably dried molecular sieves. The stability tests gave surprising results (Table 7).

The stability study was then performed with the two co-solvents individually for a month at various temperatures. The results confirm that the removal of traces of water from the solution is sufficient to stabilize simvastatin, regardless of the type of co-solvent used (Table 7). It is noteworthy that the only anhydrification of the fish oil solution with activated molecular sieves, it is not enough to stabilize simvastatin and that only the combination of the two co-solvents (Epikuron® 200 + Sodium docusate) ensures excellent stability for up to six months. Using different surfactants such as Transcutol® or Lauroglycol® (formulations described in the patent literature; WO2006/096806), the resulted solutions, left under the analogues conditions, were less stable than the ones described here (Table 7).

The in vivo stability of statins solubilized in fish oil was not the purpose of this study because it was expected to be similar as when fish oil and statins are co-administered. However, a preliminary study in simulated biological fluids (SGF, gastric and SIF, intestinal) with simvastatin has been done. The results, reported in S.I. (test in simulated biological fluids) showed that the statin solubilized in fish oil has a stability profile, in both vehicles, analogous to that reported in the literature.

In conclusion, the approaches here reported were equivalent to that used for solubilizing drugs in water solutions: indeed, co-solvents like alcohol, ethylene glycol, Transcutol®, etc. or surfactants, compounds that lower the surface tension between a liquid and a solid, are among the numerous approaches available and reported in the literature to enhance the solubility of poorly water-soluble drugs. In our case, attempting to enhance the solubility of statins in fish oil co-solvents were investigated with poor results, as well as emulsifying agents where a few of them, Epikuron® 200, sodium docusate, Tween 80, Solutol HS 15, were able to enhance the

Formul. No.	Emulsifier		Statin	maximum amount of solubilized (mg/ml)°
	Component	Amount (g)		
Form 1	-	-	Atorvastatin	0.10
Form 7*	Tween® 80	0.1	Atorvastatin	2.85
Form 8*	Cremophor® EL	0.1	Atorvastatin	3.15
Form 9*	Labrasol®	0.1	Atorvastatin	3.72
Form 12*	Epikuron® 200	0.1	Atorvastatin	> 20
Form 13*	Sodium docusate	0.1	Atorvastatin	> 40
Form 2	-	-	Rosuvastatin	0.02
Form 17*	Tween® 80	0.1	Rosuvastatin	1.98
Form 18*	Cremophor® EL	0.1	Rosuvastatin	2.47
Form 19*	Labrasol®	0.1	Rosuvastatin	2.63
Form 20*	Epikuron® 200	0.1	Rosuvastatin	20.05
Form 21*	Sodium docusate	0.1	Rosuvastatin	40.64
Form 3	-	-	Pitavastatin	2.30
Form 26*	Epikuron® 200	0.1	Pitavastatin	20.02
Form 27*	Sodium docusate	0.1	Pitavastatin	> 60
Form 28*	Epikuron® 200 Sodium docusate	0.09 0.01	Pitavastatin	> 20
Form 4	-	-	Simvastatin	10.10
Form 29*	Transcutol® P	0.1	Simvastatin	34.03
Form 30*	Lauroglycol® 90	0.1	Simvastatin	33.24
Form 31	Epikuron® 200	0.1	Simvastatin	36.02
Form 32	Epikuron® 200	0.2	Simvastatin	70.63
Form 33	Sodium docusate	0.1	Simvastatin	22.02
Form 34	Epikuron® 200 Sodium docusate	0.09 0.01	Simvastatin	35.41

Table 4: Solubility testing: maximum amount of statins (atorvastatin, rosuvastatin, pitavastatin and simvastatin) solubilized (mg/ml) in fish oil (n-3 PUFA; 0.9 g).
(*) The same formulation indicated in Table 3, increasing the amount of statin up to reach its maximum concentration in solution.
(°): Results are the means of a minimum of two independent experiments. ± SD less than 10%.

solubility up to 20% p/p. Other emulsifying agents like Cremophor® RH40 and Labrasol were less effective.

The physic-chemical mechanisms responsible for enhancing solubility of the statins in fish oil could be due to the formation of reverse micelles, as mentioned previously. From an analysis of the various emulsifying agents here investigated, it was clear that the chemical characteristics of the surfactant, in particular the polar head, were very important for solubility of the statins, mainly for those of the new generation (atorvastatin, pitavastatin, rosuvastatin, fluvastatin). However, such behaviour was also observed with more soluble statins, such as simvastatin. In conclusion, to enhancing the solubility of the statins in fish-oil, emulsifying agents play an important role, participating with their hydrophobic component in the interaction with the oil molecules and with their hydrophilic component (head) in the interaction with the statin molecules, forming

Formulation No.	Temp. (°C)	n-3 PUFA (% recovery after 6 months)		Atorvastatin (% lactone formation)°		
		%EPA	%DHA	after 1 month	after 3 months	after 6 months
Form 12 (*Epikuron® 200*)	5	100	100	0	0.64	1.65
Form 13 (*Sodium docusate*)	5	>98	>98	0	1.20	3.35
				Rosuvastatin (% lactone formation)		
Form 20 (*Epikuron® 200*)	5	100	100	0	1.3	4.43
Form 20 (*Epikuron® 200*)	25	>98	>98	3.24	12.1	21.6
Form 20 (*Epikuron® 200*)	40	>95	>95	8.38	18.8	28.7
				Pitavastatin (% lactone formation)		
Form 26 (*Epikuron® 200*)	5	100	100	0	0.75	2.23
Form 26 (*Epikuron® 200*)	25	>98	>98	1.18	3.20	7.14
				Simvastatin (% lactone opening)		
Form 31 (*Epikuron® 200*)	5	100	100	0	ND	ND
Form 31 (*Epikuron® 200*)	25	>98	> 98	1.90	ND	ND
Form 31 (*Epikuron® 200*)	40	>95	> 95	10.0	ND	ND

Table 5: Stability testing of statins (atorvastatin, rosuvastatin, pitavastatin and simvastatin), solubilized in n-3 PUFA, up to 6 month storage at 5°C, and at temperatures up to 40°C (accelerated storage condition).

Formulation No.	Solvent	Temperature (°C)	Recovery of simvastatin (%)º
Form 4	(no solvent)	25	65.4
		40	50.0
Form 31	Epikuron® 200	25	98.1
		40	90.0
Form 35	Epikuron® 200 + Citric acid	25	79.3
		40	76.1
Form 36	Epikuron® 200 + Adipic acid	25	75.2
		40	68.0
Form 37	Epikuron®200 + Palmitic acid	25	65.2
		40	60.0

Table 6: Stability testing of simvastatin, solubilized in fish oil, alone or with Epikuron® or Epikuron® and organic acids, after one month at two temperatures, 25 and 40º C.

º: Results are the means of a minimum of two independent experiments. ± SD less than 10%.

Form. No.	Solvent	Dehydrated system	Time (month)	Temperature (ºC)	Recovery of simvastatin (%)º
Form 29	Transcutol®	Yes	1	40	75.0
Form 30	Lauroglycol®	Yes	1	40	82.1
Form 31	Epikuron® 200	No	1	25	98.1
				40	90.0
Form 31	Epikuron® 200	Yes	1	25	**99.8**
				40	**98.3**
Form 33	sodium docusate	No	1	25	98.5
				40	94.4
Form 33	sodium docusate	Yes	1	25	**99.4**
				40	**99.3**
Form 34	Epikuron® 200+ Sodium docusate	No	1	25	98.9
				40	93.1
			3	25	86.3
				40	81.6
			6	25	73.7
				40	70.1
Form 34	Epikuron® 200+ Sodium docusate	Yes	1	25	99.7
				40	99.4
			3	25	98.5
				40	97.4
			6	25	98.0
				40	97.4

Table 7: Long-term stability testing of simvastatin with Epikuron® or/and sodium docusate, solubilized in fish oil in not dehydrated versus dehydrated solutions. Transcutol® and Lauroglycol® was also evaluated.

(º): Results are the means of a minimum of two independent experiments. ± SD less than 10%.

reverse micelles. It was also observed that solutions of statins prepared as their prodrug (lactone form, see simvastatin) once dried, through removed traces of water, were particularly stable without the need to mix in additives.

Taken together, the experimental data confirms that it is possible to devise an association between statins and fish oil, through robust formulations, homogenous and orally administrable, enabling pharmaceutically exploitable concentrations, in line with therapeutic dosages (Figure 3). Besides, these solutions are obtainable in short times and with low costs, rendering the process particularly suitable for industrial applicability. These formulations may represent a new tool for physicians and patients in the treatment of pathologies related to hyperlipidemia and hypertriglyceridemia, hypercholesterolemia, pathologies for which statins and n-3 PUFA are already prescribed individually in standard treatment protocols. For example, in patients on a long-term CVD prevention, taking a single pill could improve the compliance towards therapy compared to taking the two drugs separately. These results have been patented [19,20].

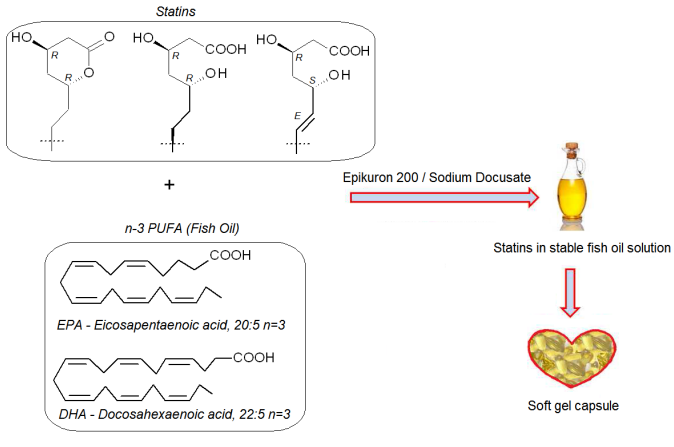


Figure 3: Table of Contents (TOC) Art

And last but not least, formulation with sodium docusate, useful medication against symptomatic treatment of constipation, could be useful in patients treated with statins, where constipation is a common adverse effect [21].

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Author Disclosure Statement

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Associated Content

* Supporting Information

Chromatographic profile of simvastatin in fish oil solution and their corresponding degradation product observed; examples of sample preparation of pharmaceutical formulations; test in simulated biological fluids (SGF and SIF).

This material is available free of charge via the Internet at ….

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