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Research Article

A New Route to Obtain Perfluorodecalin Nanocapsules as An Oxygen Carrier in Cosmetic Formulations

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Abstract

The preparation of Perfluorodecalin (PFD) encapsulated silica nanocapsules via a two-step process method using a combination of emulsifiers (Pluronic 127, purified Soy Lecithin and Polysorbate 80) to obtain first stabilized water nanoemulsions of PFD as a template, followed by *"in situ"* precipitation of silica is demonstrated herein. Our method differs from previously published sol-gel processes starting from TEOS (Tetraethyl Orthosilicate) or TMOS (Tetramethyl Orthosilicate) because Sodium Silicate was used as departing material, hydrolyzed under strictly controlled conditions as we describe in what follows. To obtain uniform sized nanocapsules we found essential to slow down the hydrolytic process, controlling the temperature, pH and ionic strength. Successful incorporation of PDF (20% wt) into the silica nanocapsule core was evident from EDAX analysis. Our aim was to propose PFD charged nano-silica carriers as a new approach to topical treatment of aging skin due to intrinsic instability of perfluorocarbon emulsions.

Keywords

Aging Skin Treatment; Oxygen Carriers; Perfluorodecalin; Silica Nanocapsules

Introduction

Perfluorocarbon Compounds (PFC) are chemically and biologically inert compounds, widely known as oxygen carriers. Their high gas-dissolving capacity comes from fluorine's low polarizability. Their intermolecular forces are so weak that they behave as gas-like fluids, dissolving other substances of low cohesion, such as oxygen and nitrogen [1].

It has been described that they have oxygen solubilities similar to hemoglobin [2], which makes them ideal oxygen reservoirs for organ preservation before transplantation [3]. An interesting feature of PFC as oxygen transporters is that the dissolved oxygen degree is not affected by external factors such as temperature or pH, thus the oxygen uptake curve is linear [4,5]. PFC have been also used as artificial blood substitutes for transfusions, with few reported adverse side effects compared to the whole blood [5,6].

Most of PFC formulations have been developed and marketed for biomedical applications. Emulsions and micellar suspensions have been obtained using PEG-based surfactants, [R]Pluronic surfactants and/or phospholipids, together with high-pressure homogenization or sonication

to down-load droplet size. Fraker et al. found particle size to be a critical factor affecting oxygen mass transfer from PFC containing colloidal systems and optimized the manufacture of nano-scale emulsion [7]. PFC were also encapsulated into biodegradable nanoparticles composed of PEGylated PLGA, as enhancement agents for efficient re-oxygenation to cells and organisms [8].

Perfluorodecalin (PFD) is one of the most commonly used PFC. It was included in the first commercial PFC formulation (Fluosol DA 20%) developed for blood substitute [9]. PFD was also nano-encapsulated within a biodegradable polymeric shell, namely Poly (Lactide-co-Gylcolide) Acid (PLGA), to provide a more stable alternative to commercial ultrasound and NMR contrast agents [10]. A similar PFD-PLGA nano-system was proposed as an artificial oxygen carrier for blood substitute formulations [11]. Silica nanocapsules were also evaluated to stabilize PFD for ultrasound imaging applications [12]. A high encapsulation percentage was obtained, while it was stated that the size could be easily controlled by changing the [R]Pluronic concentrations.

However, in the field of cosmetics there are few scientific references, even if it has been shown that after introducing fully saturated PFD with O_2(g) into an emulsion for the treatment of skin, beneficial and measurable cosmetic effects have been observed: an increase in the Oxygen partial pressure, reduction of the wrinkles and an increase of the skin moisture content [13]. The limited knowledge and poor understanding of the chemistry of PFD and others perfluorocarbon molecules as oxygen carriers, as well as the intrinsic difficulties to formulate into cosmetics molecules that have both hydrophobic and lipophobic character, and do not tend to dissolve in either aqueous or lipid phases to produce stable emulsions, prevents its use [14].

Several interesting uses of nanotechnology in cosmetics have been proposed, and nanomaterial's are being employed or suggested as carriers of active substances [15]. In this line, Silica-based materials are some of the most commonly used. The synthetic varieties of Silica produced via sol-gel routes allow the design of nanoparticles which have highly ordered structures with controlled pore-size distribution, pore geometry, and pore network tortuosity [15].

Recently the preparation of PFD encapsulated Silica nanocapsules via a one-pot synthesis method starting from Tetramethyl Orthosilicate (TMOS) was proposed [12].

In this paper, we suggest a new route to obtain PFD encapsulated in Silica for the topical treatment of aging skin based on nano-silica carriers.

Experimental

Materials

Perfluorodecalin (PFD, 95.5% cis-trans mixture) was purchased from Inta-Trade (Germany). TEOS, Sodium Silicate, [R]Pluorinc F-68, [R]Pluoronic F-127, HCl and Urea PA were initially bought to Sigma-Aldrich. Sodium Phosphate buffer was prepared from P.A. drugs. Water was produced by Millipore Milli-Q water purification system.

Sigma's Sodium Silicate (cat. 33844-3) had the following composition expressed as oxides:
Na_2O 10.6%
SiO_2 26.5%
Density(25°C) = 1.39 gml^{-1}
Soy Lecithin was purified by solvent extraction (Acetone) from Lecsoy S provided by fabriQUIMICA (Argentina) and contained a small quantity of Polysorbate 80.

After the synthesis was optimized with pure drugs, for the scaling up we used commercial raw materials as follows:
PFD had the same origin as before.
[R]Pluronic 127 was provided by BASF (Germany).

Sodium Silicate was provided by Mejorsil (Argentina), with the following composition, expressed as oxides:
Na_2O 12.3%
SiO_2 32.4%
Density(25°C) = 1.51 gml^{-1}
Impurities analysis by Atomic Absortion (ppm) :Ni<0.03; Pb=0.23; Sb<0.6 ; Fe=17.8; Cd<0.02 ; Hg< 0.07. Demineralized water was bought from Torbidoni (Argentina) with a conductivity of 0.07 μS cm^{-1} and contents of Si<0.01 ppm.

Commercial Urea was purchased from Dalgar (Argentina) with a purity of 99.6%. The rest was essentially Biuret, coming as a by-product from its synthesis.

Synthesis of silica nanocapsules

First, we tried to follow the classical Stober work, preparing Silica nanoparticles by hydrolysis and condensation of TEOS on a template microemulsion of PFD + [R]Pluronic F127 or PFD + [R]Pluronicc 68 in water, buffered with Potassium Phosphate 0.1M. Gel formation was studied as a function of pH by adding HCl 4M solution at 4°C [16].

Due to poor results in the presence of PFD, we decided to follow an alternative route starting from Sodium Silicate.

The optimized procedure was as follows:
We dissolved [R]Pluronic F127 (2.5% w/w) in water (77% w/w) at 40-50°C to obtain a clear solution, we then added Soy Lecithin (0.5% w/w), followed by PFD (20% w/w). The last was added very slowly, while the temperature was elevated to 90°C stirring intensely at 3500 rpm. with an Ultra Turrax T 18 basic disperser (Astral GmbH D-7801, Germany). PFD was slowly incorporated into an emulsion.

The pH spontaneously obtained was in the range 6-7 and was then adjusted to pH 12 with NaOH 0.5 M. Always stirring at 80-90°C, we added 2.0 M solution of Urea to reach a final

concentration of Urea = 0.5 M, and at last Sodium Silicate solution 0.5 M to reach a final concentration of 0.02 M.

Silica nanoparticles containing PFD were slowly precipitated from the mass as a very fine white powder, changing the system from opalescent to milky-white.

The whole process lasted up to three days.

The obtained precipitate could not be filtered. It was separated by centrifugation at 3000 rpm. (Rolco, Argentina) and resuspended three times with water to wash out the impurities.

Then the precipitate was dried in an oven at 40°C.

Due to the fact that Urea is slowly hydrolyzed, acidifying the reaction media, Silica is precipitated on the Pluronic matrix incorporating emulsified PFD.

Characterisation

Dynamic light scattering: Dynamic Light Scattering (DLS) was employed at the beginning to determine the hydrodynamic diameter of the silica nanocapsules aggregates (NanoBrook 90 Plus Particle Size Analyzer-Brookhaven Instruments). We re-suspended them in water or in ethanol before the measurements.

Scanning electron microscopy: Scanning Electron Microscopy (SEM) was used to characterize the morphology and size of the silica nanocapsules. A droplet of solution containing the nanocapsules was deposited on a silicon wafer and dried. The samples were imaged with a FESEM microscope (Supra 40 Zeiss Field Emission Scanning Electron Microscope) operating at an acceleration voltage of 5 kV.

Transmission electron microscopy: Transmission Electron Microscopy (TEM) was also used to characterize the morphology and size of the silica nanocapsules. As the microscope used (Philips CM 200) was also provided with an EDAX Apollo Zaluzec probe for elements quantification, it also helped us to estimate the retaining PFD into the silica nanocapsules. Briefly, a droplet of a sample containing nanocapsules was deposited onto a carbon-coated copper grid and air dried. Then scanned with the microscope.

Attenuated Total Reflectance Infrared Spectroscopy (ATR): Was performed using a Nicolet 8700 FTIR spectrometer provided with ATR in the range 4000-525 cm^{-1} and OMNIC version 7.3 software (Thermo Electron Co.)

Surface area and pore size by N_2(g) adsorption: Was estimated on the samples using Micromeritcs Serial 1417 equipment at -196°C.

Results and Discussion

Tunning the synthesis of the empty silica nanoparticles: Effects of ᴿPluronic 127 (P127), urea, concentration, and pH on the size of the nanoparticles

As may be seen in figure 1 the size of the silica nanoparticles depends on the presence or not of P127 and Urea (experiments signaled as (+) mean that the reagents were present, while (-) means absence). The size of the particles, measured by DLS starting from a Sodium Silicate solutions without the former was always bigger than 100 nm. While if P 127 and Urea were present during the synthesis obtention of smaller sizes was possible.

The obtained polydispersity in these screening experiments was very low (0.046-0.053).

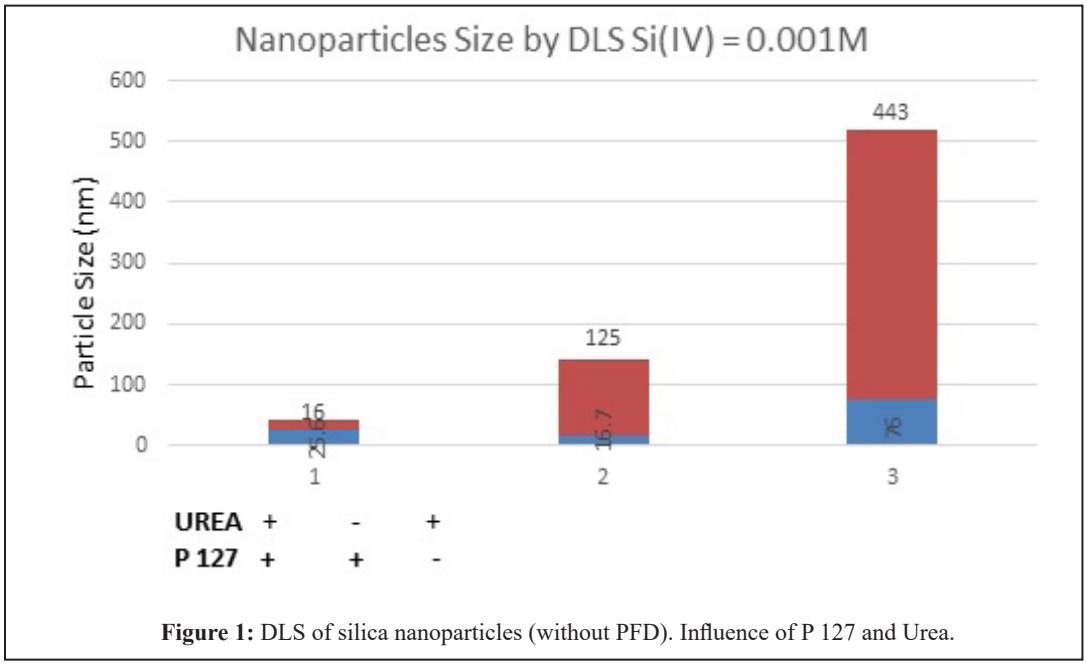


Figure 1: DLS of silica nanoparticles (without PFD). Influence of P 127 and Urea.

The size of nanoparticles, either with of without emulsion was also sensitive to the pH at which condensation occurs, as shown in figure 2 for microemulsion-free nanoparticles.

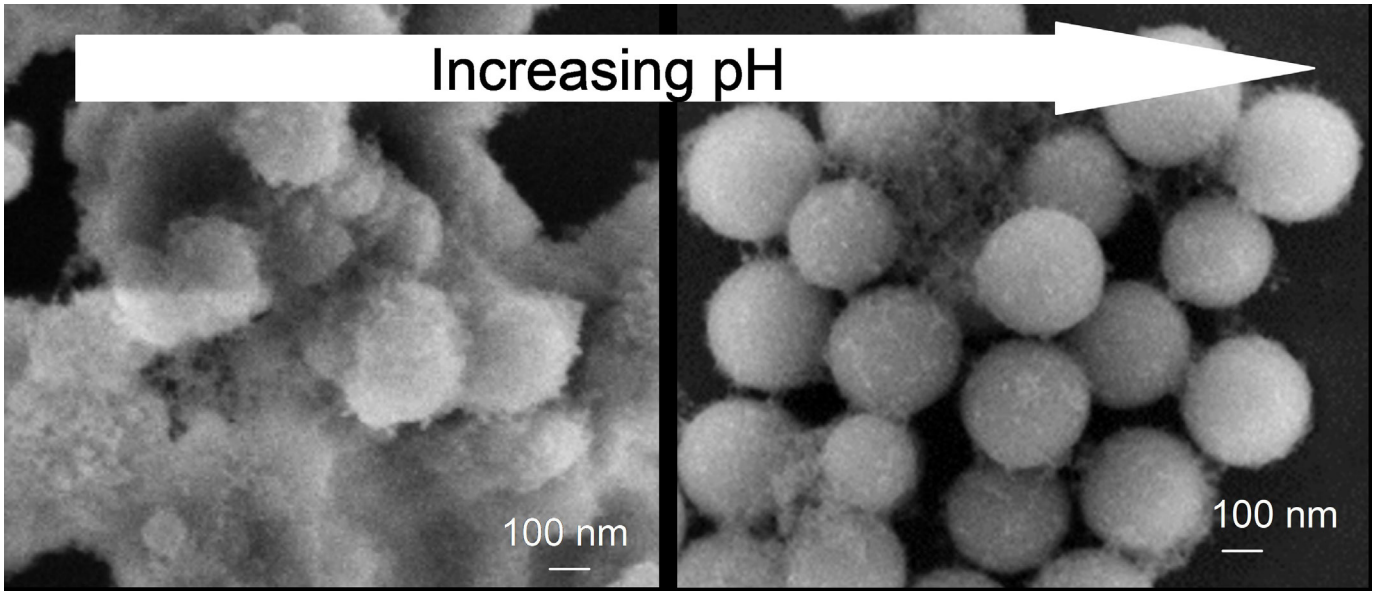


Figure 2: SEM images of empty silica nanoparticles synthesized at constant [Si] with variable pH without microemulsion.

At pH 12 translucent gels are obtained, which precipitate structured as long crystalline cylinders (Figure 3).

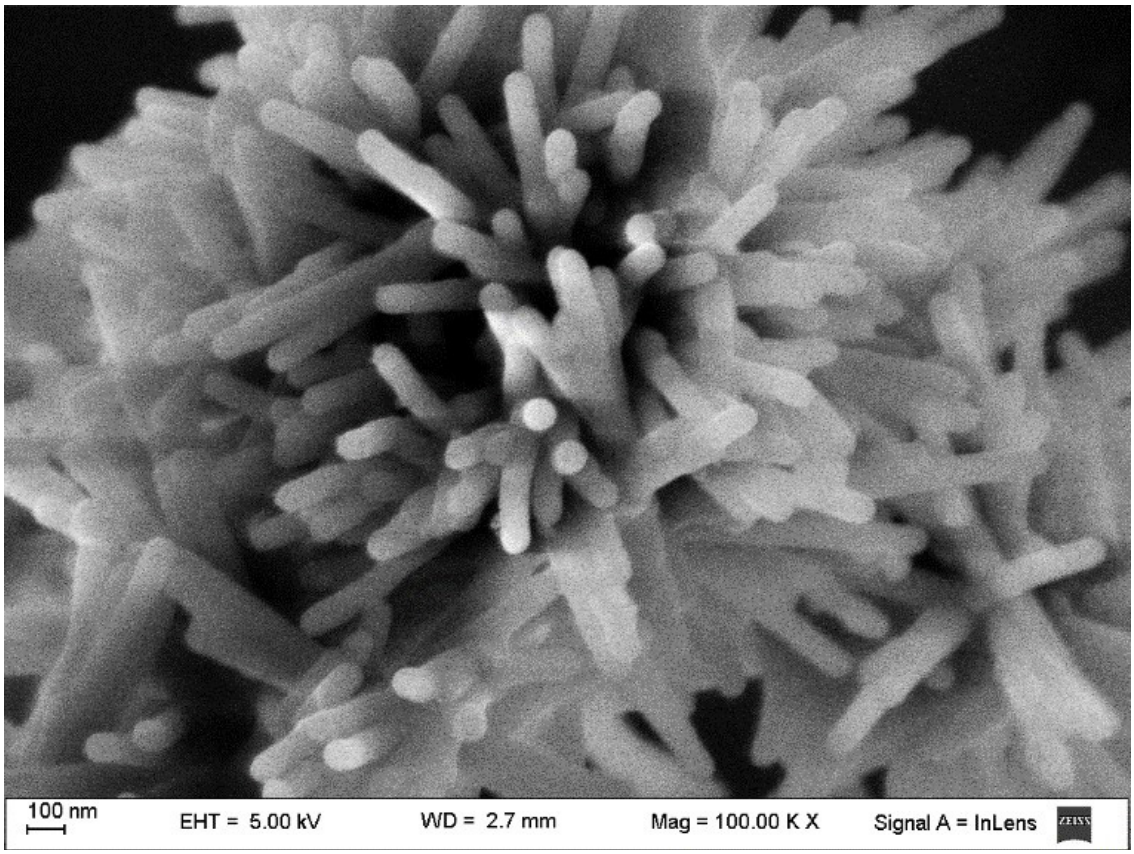


Figure 3: Silica precipitated from Na_2SiO_3 at pH 12. Final Si(IV) concentration = 0.0065M.

If the pH is buffered at a value around 8, big aggregates are obtained, mixed with spheres of circa 100 nm of diameter (Figure 4).

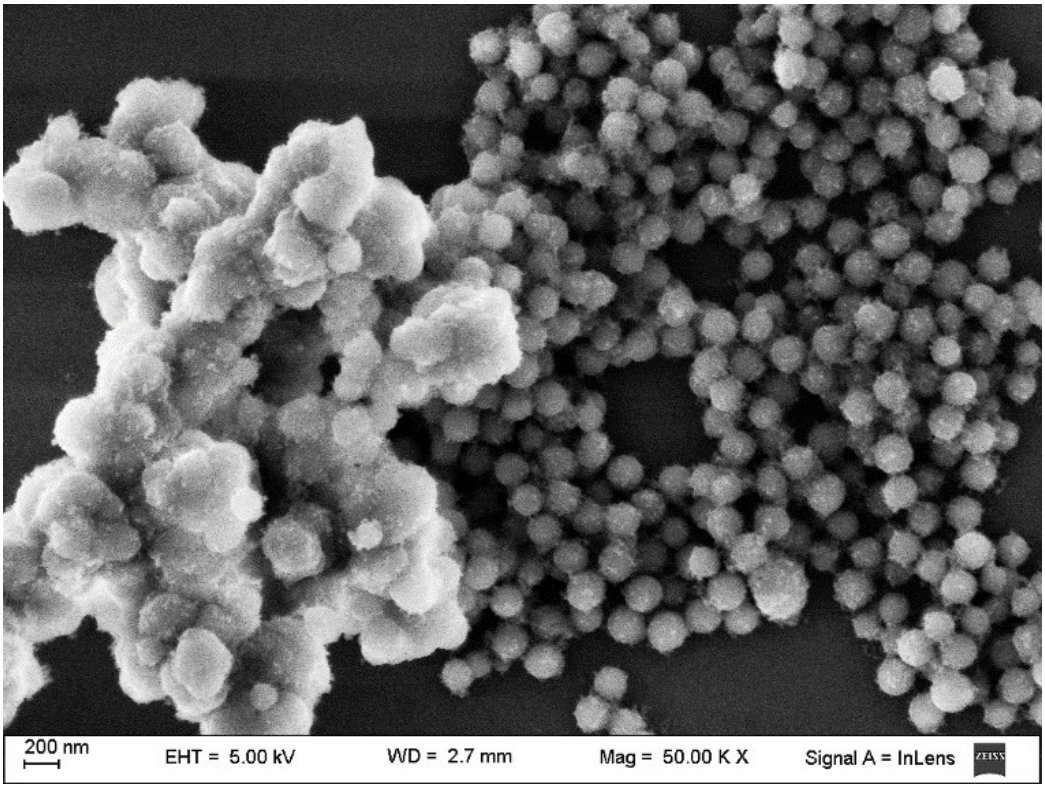


Figure 4: Silica precipitated from Na_2SiO_3 at pH 8. Final Si(IV) concentration = 0.0065M.

Finally, in the pH range from 8 to 9, we could obtain non-disperse spherical nanoparticles in the range of 50 to 100 nm (Figure 5).

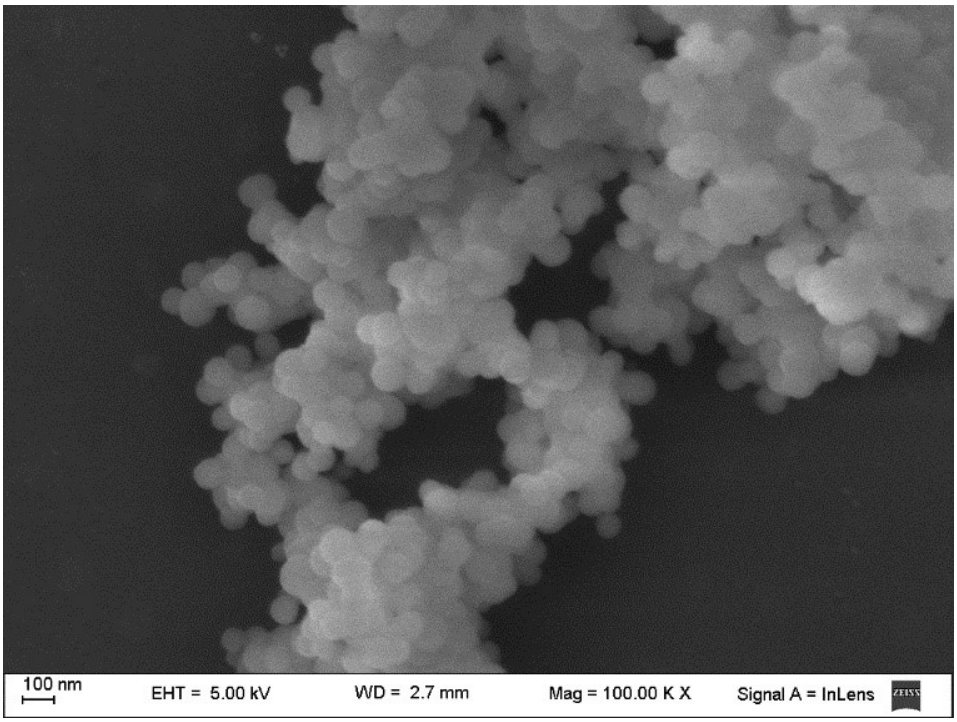


Figure 5: Silica precipitated from Na_2SiO_3 at pH 9. Final Si(IV) concentration = 0.005M.

Working at pH=9, if the final Si(IV) concentration increases, the size of the obtained nanoparticles becomes smaller and smaller (Figures 6 and 7).

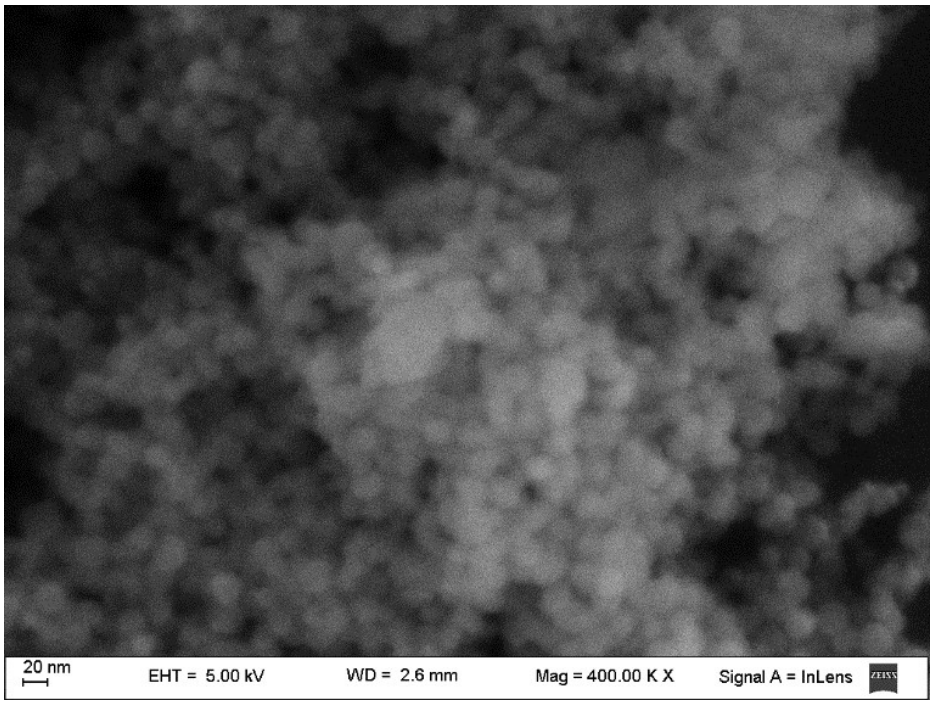


Figure 6: Silica precipitated from Na_2SiO_3 at pH 9. Final Si(IV) concentration = 0.075M.

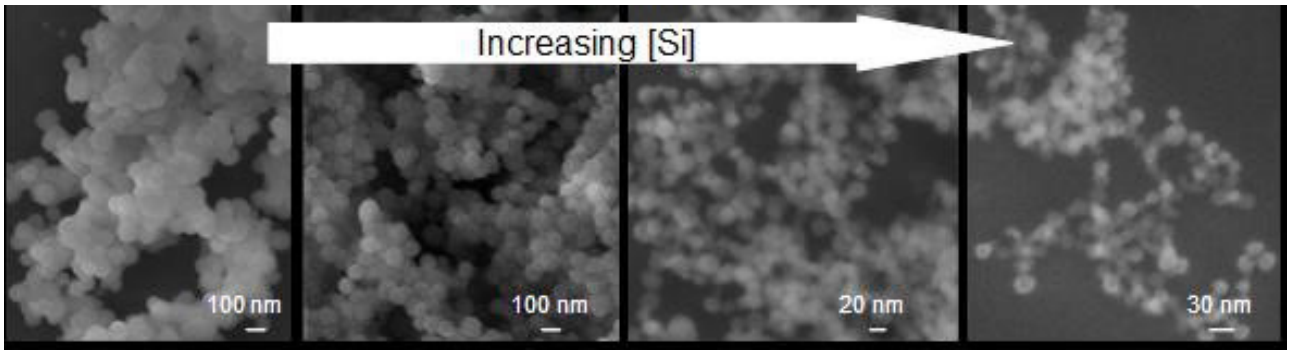
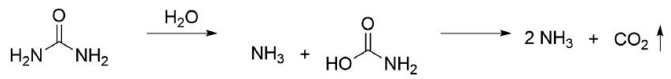


Figure 7: SEM images of silica nanoparticles synthesized at constant pH with variable [Si] with microemulsion.

The overall reaction follows the formation of silanol groups:
$Na_2SiO_3 + 3H_2O \rightarrow \equiv\equiv\equiv Si—OH$

Which undergoes further condensation reactions, giving rise to nanoparticle growth:
$\equiv\equiv\equiv Si—OH + \equiv\equiv\equiv Si—OH \rightarrow \equiv\equiv\equiv Si—O—Si \equiv\equiv\equiv$

To obtain small and uniform sized particles, we found essential to slow down the hydrolytic process, controlling the temperature, pH and ionic strength of the reaction media while maintaining continuous stirring throughout it.
The hydrolytic process and the pH is controlled by the decomposition of urea into the reaction media [17].



Synthesis and composition of PFD encapsulated silica nanocapsules

We employed the optimized operative conditions as described in section 4.2.
The size and external characteristics of the PFD charged nanocapsules was similar to the empty ones.
The presence of PFD was determined by EDAX analysis coupled to TEM microscope.

During the microscope scan, the different elements present were identified by their EDAX peaks. Carbon and copper peaks belonging to the sampling grid were subtracted from the total area. The FK peak at 0.6 KeV belonged to PFD, and allowed the system to calculate relative quantities of O, Si and F contained by the Nanocapsules (Table 1).

Thin Apx
Theoretical KAB, Elements, Model:
Zaluzec

Element	Weight %	Atomic %
OK	46.6	56.0
FK	22.7	23.0
SiK	30.7	21.0
Total	100.0	100.0

Element	Net Inte.	Backgrd	Inte. Error	P/B
OK	0.64	1.19	15.77	0.53
FK	0.23	1.01	37.81	0.23
SiK	0.83	0.75	10.67	1.10

PHILIPS CM200 with EDAX APOLLO

Table 1: EDX analysis of PFD containing silica nanoparticles.

It is interesting to note that even with the high vacuum employed in the transmission microscope, was found. ca. 1:1 F:Si atomic ratio. The same analysis performed with pyrogenic silica nanospheres (ca.30 nm diameter) containing 50% w/w PFD showed a 1:10 F:Si atomic ratio. TEM micrographs are shown in figure 8.

Once the optimal synthesis conditions were chosen, PFD encapsulation (20% w/w) gave rise to monodisperse spherical nanoparticles with (23±3) nm diameter with BET surface area of (38.9 ± 1.1) m^2/g, as determined from the $N_2(g)$ adsorption isotherms. The found surface area was coherently lower than the one declared for commercial pyrolytic-obtained silicas, as ^{R}Aerosil 200 (200 m^2/g) and ^{R}Cab O Sil 150 (150 m^2/g), which have a smaller mean size (12-14 nm).

The measured adsorption average pore width was of 11.6 nm. The isotherm adsorption and desorption plots showed very low hysteresis suggesting, as suspected, weak intermolecular forces between the gas and the PFD charged nanoparticles.

Our results with commercial sodium silicate, employing the optimized synthesis conditions, were nearly coincident with those obtained with the research reagents.

The relatively low level of impurities of the silicate, and biuret by-product coming from urea synthesis seemed not to interfere with our process. Biuret, once hydrolyzed renders $CO_2(g)$ and NH_3 as well, contributing to pH regulation too. Showing that commercially "pure" raw materials as those tested in our experiments (water, sodium silicate, urea) could also be used to obtain nano-silica carriers for cosmetic topical applications with cost advantage.

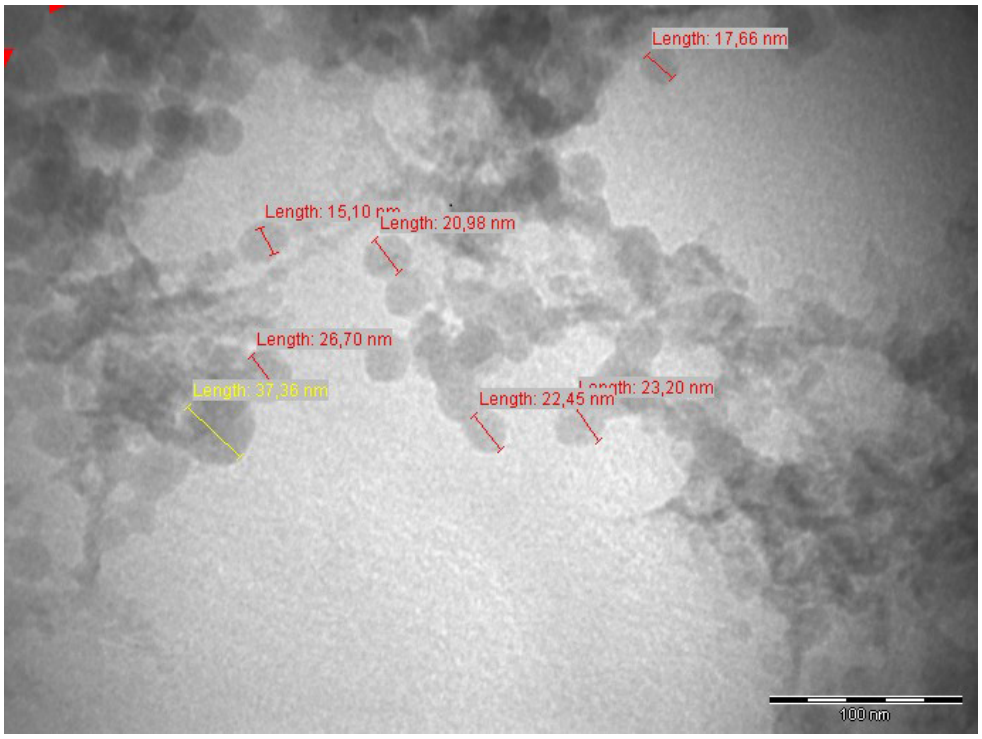


Figure 8: TEM micrographs of PFD containing silica nanoparticles. Note the homogeneous size and small diameter obtained.

Conclusion

A positive cosmetic effect on the skin by PFD as Oxygen carrier has been demonstrated, but it is barely used because of the intrinsic formulation instability. In recent years there has been a spate interest relative to the application of nanotechnology in cosmetics, with nanostructures used as active substances, carriers and formulation aids [15].

In this work, we obtained Silica nanoparticles that could be employed to deliver Perfluorodecalin. First, we have obtained relatively stable microemulsions using specific experimental conditions and combinations of emulsifiers [14]. Then we have molded on the former, Silica nanoparticles loaded with Perfluorodecalin via one-pot condensation.

The synthetic method used allowed us to obtain an essentially monodisperse Silica population, whose mean size is bigger than that of well-known commercial materials obtained by the pyrolytic pathway who are usually employed to formulate cosmetics. The latter claim a BET surface area in the range of 100 to 400 m^2/g, and particle diameters between 7 and 20 nm.

Our method can be easily scaled-up starting from commercially available materials with a cost advantage over other synthetic pathways [17].

Regarding potential toxicity for cosmetic use, commercial Silica nanoparticles as mentioned above - which are even smaller than those obtained by our condensation process- have been used in cosmetic formulations for long. CIR Expert Panel concluded that Silica and Hydrated Silica were safe as used in cosmetics and personal care products [18]. They are also considered safe by the FDA and accomplish the general provisions of the Cosmetic Directives of the European Union [19]. Due to their controlled size in the range of 20 to 50 nm, our nanoparticles should penetrate the skin enough to deliver PFD, but not enough to affect the human body in a systemic way. Recently *in vitro* assessment of analog silica nano-capsules cytotoxicity was investigated [12], without showing any cytotoxic effect at low concentrations towards HepG2 and 3T3 cell lines.

In summary, we have been able to stabilize Silica nanoparticles containing PFD, who could act as effective vectors to introduce the molecule into stable cosmetic formulations. The research should continue, to further verify the ability of our nano-device to help oxygenate the living human skin improving its barrier function.

Conflicts of Interest

There are no conflicts to declare.

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