Optimization of the solvent-exchange process for high-yield synthesis of aqueous fullerene dispersions

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The ultrasound-assisted solvent-exchange technique for aqueous fullerene dispersions (AFD) of C_{60} (10^{-4} – 10^{-6} M) have been improved for high-yield synthesis, thereby achieving AFDs with total recovery over 90 %. Using ICP-AES, HPLC-UV, HGC-MS, the elemental and residual organic compounds have been estimated as not exceeding 3 ppm. The possible structure of fullerene clusters in AFD was assumed as $\{n[C_{60}]mC_6H_5COO^-(m-x)Na^+\}xNa^+$.

Keywords: solvent-exchange process, fullerene C_{60}, stability, atomic spectroscopy.

1. Introduction

Fullerene-based materials have already been used in new composite materials with superconducting properties, photoresist masks, lubricants, etc. [1–8]. Fullerenes find the application in medicine and cosmetology, as well as in such areas as theranostics, nanomedicine, drug delivery, etc. Among the most promising are aqueous fullerene dispersions (AFDs), with a wide range of applications in medicine and other fields that require fullerenes in aqueous media [9–13]. AFDs of unmodified fullerenes are preferable due to biocompatibility and safety: owing to their open surface without moieties, they are not involved in metabolic processes. This is especially the case of endohedral metallofullerene (EMF) AFDs, which are promising contrast agents.

Expanding the range of AFD applications requires improvement in their preparation. Derivatization and solubilization have been disregarded in this field. The most relevant techniques of AFD preparation are solvent exchange [11, 14, 15], dialysis [16], and mixing [17–19]. However, the total yield of fullerene transfer into water of 100 % has not yet been achieved, which leads to significant losses and increases for the cost of the final product, especially for EMFs. The ultrasound-assisted solvent-exchange procedure produces AFDs from a range of neat solvents, both polar and nonpolar, and that are stable for long periods. There was no previously described exact mechanism for the solubilization, although some research has dealt with the prediction of dispersion stability.

Apart from the yield, the main drawback for the solvent-exchange process is significant amounts of hazardous organic substances in AFDs [11, 14, 15]. However, not much attention is paid to the purity of the produced dispersions, which is unacceptable for biomedicine. Therefore, it is necessary to develop techniques for purification and determination of the residual amounts of organic substances. Here, the ultrasound-assisted solvent-exchange procedure has been optimized for preparing AFD with a yield close to 100 %. We selected widely available fullerene C_{60} and toluene due to its lower volatility compared to benzene and good solubility of C_{60}.

2. Materials and methods

To produce AFD and improve reagent dissolution during the preparation, a GRAD 180-35 (LLC Grad-Technology, Russia) ultrasonic bath was used. Weighing was performed on an Ohaus DV-114C analytical balance (Switzerland). Absorbance measurements in the UV/vis range were carried out using an Agilent Cary 4000 (Australia). Pure toluene Reakhim (Russia), ultra-pure water "type 1" from a Milli-Q Millipore Corporation water purification system, 18.2 m$\Omega\times$cm, pure potassium hydrogen phthalate "Merck" (Germany), fullerene C_{60} 99.5 % LLC NeoTechProdukt (Russia) were used. Elemental analysis was performed by an Agilent ICP-AES 720 (Malaysia) with ICP Expert software 2.0.5 according to ISO 11885:2007(en). All emission lines (Table 1) were measured simultaneously (a MultiCal mode). To estimate volatile organic compounds, Headspace Gas Chromatography – Mass Spectrometry (HGC-MS) Shimadzu, Germany was used. A CP Sil 5 CB column (25 m$\times$0.25 mm$\times$0.4 μM,

starting temperature 40 °C, isotherm for 5 min, temperature increase up to 130 °C, temperature pitch 9 °C/min, isotherm for 3 min). For benzoate detection in AFDs, Agilent 1200 HPLC-UV was used with an Eclipse XDB-C18 column (Agilent, USA) 150×4.6 mm, sorbent particle diameter, 5 μm; precolumn Security Guard C18 (4×3 mm; Phenomenex, USA); column temperature: 35 °C; sample volume: 100 μL; mobile phase: Eluent A, 0.2 % phosphoric acid (83 % v/v), Eluent B, acetonitrile (17 % v/v); mobile phase rate: 1 mL/min; detection conditions: wavelength 235 nm, spectral bandwidth 4 nm. LOD and LOQ were calculated according to IUPAC 1998 recommendations.

TABLE 1. ICP–AES measurements conditions

Instrumental conditions settings	
RF power (kW)	1.40
Plasma flow (L/min)	15
Axial flow (L/min)	1.50
Nebulizer flow (L/min)	0.70
Replicate read time (s)	5
Plasma stabilization delay (s)	15
Replicates	3
Sample introduction settings	
Sample uptake delay (s)	15
Pump rate (rpm)	15

3. Results and discussion

Aqueous fullerene C_{60} dispersions were obtained by the ultrasound-assisted solvent exchange process that is generally known in colloidal chemistry. This approach was previously applied for AFDs [11,12,20]. Nevertheless, here, we have applied and developed methods and approaches to improve the yield and physicochemical properties, as well as the sample purity. We have previously published [1–4] the data concerning fullerene state in pristine fullerene samples before ultrasound treatment and as well fullerene ultrasound-assisted solvent-exchange process. We have not observed any peaks with masses higher than the molecular ion peak $[M]^{+\cdot}$ (*e.g.* epoxy-, hydroxyl-, derivatives, and polymeric structures: dimeric, trimeric, etc.) for both pristine fullerene and their aqueous dispersions).

3.1. Quantitative determination of fullerenes

For the quantification of fullerenes both in the aqueous and in the organic phases, UV/vis spectroscopy was used. For toluene, the absorption spectra and the apparent molar absorptivity ($\varepsilon_{407} = 3.5 \pm 0.2$ M^{-1}cm^{-1}) coincide with the existing data [17,21,22]. For the quantification, a wavelength of 407 nm was used. LOD and LOQ for C_{60} fullerene in toluene are 100 and 400 nM. These values are acceptable for the optimization of solvent-exchange procedures for a wide range of concentrations up to micromolar. Quantification of fullerenes in the aqueous phase requires a strict control of carbon species. A drawback of solvent-exchange procedures is residual organic solvents, which result in overestimation of fullerenes by a TOC analyzer. By purging for 16 h, we reduced up to 44 % the total quantity of the volatile organic matter (Fig. 1 and Fig. 2). The initial content of toluene in AFD is 0.044 ± 0.005 ppm (Fig. 1) and about 25 hours are necessary to reduce its content to ppb level or less. To the best of our knowledge, it is the first description of purging for purification of AFDs C_{60} from nonpolar organic admixtures. This approach is cheaper and no less effective compared to solid-phase extraction (using C18-sorbents).

After checking the aqueous dispersions, a combination of TOC and UV/vis. measurements allowed us to calculate an apparent molar absorptivity (Table 2) [9,11]. These data have a good correlation with existing data that we used for fullerene quantification. LOD and LOQ were 100 and 300 nM, respectively (at 349 nm). Thus, due to the selected conditions with coincided performance parameters for fullerene C_{60} in toluene and in their AFD, we can proceed to the adjusting of optimal conditions for obtaining AFD in high-yield synthesis.
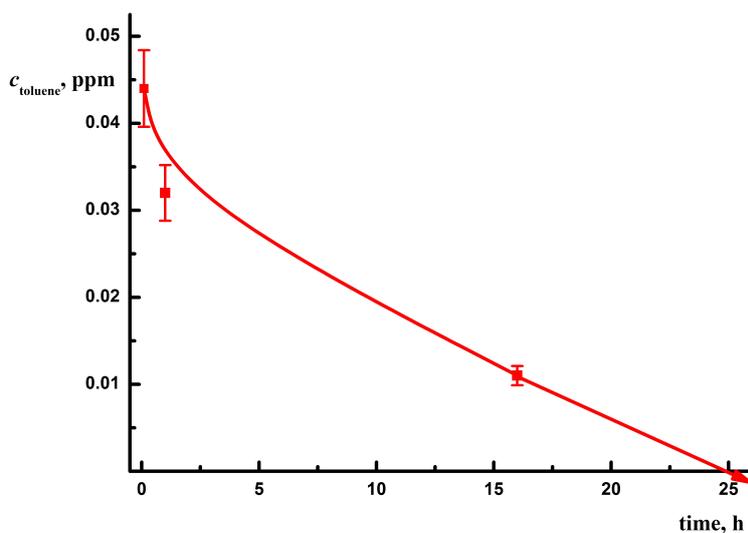
FIG. 1. Dependence of toluene concentration in AFD on the argon purging time (rate, 100 mL/min) by HGC-MS
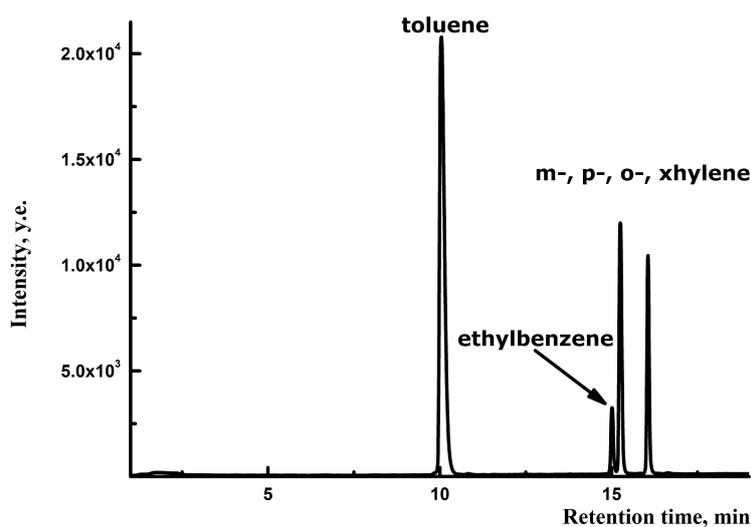
FIG. 2. Representative HS GC-MS chromatograms are registered on 91 Da m/z for non-purging AFD C_{60}

TABLE 2. Apparent molar absorptivities for C_{60} in water and its deviation from existing data

λ, nm	$\varepsilon \times 10^3$ M^{-1}cm^{-1}	$\varepsilon \times 10^3$ M^{-1}cm^{-1} [9,11]	$\delta\varepsilon$,%
349	49 ± 1	49 ± 2	0.7
268	71 ± 8	67 ± 6	5.6
220	69 ± 8	60 ± 6	13.0

3.2. Optimization of the solvent-exchange process for fullerene C_{60} AFD from toluene

Having calculated the LOD and LOQ for fullerene C_{60} in toluene and in AFD, we improved the conditions for obtaining AFD using ultrasound baths with higher electric power (up to 1 kW) and Erlenmeyer flasks with the lowest glass thickness (down to 3 mm). The main optimization point consisted of using different ratios of fullerene in toluene and water. It was found that the appropriate recovery values (more 50 %) was achieved for the fullerene

concentration in toluene in the range of $n \times 10^{-5}$ M. The concentration range from $n \times 10^{-6}$ M to $n \times 10^{-4}$ M was tested.

At different ratios of C_{60} in toluene to water, the total volume of the toluene-water system did not differ by more than 15 % by volume. Thus, we could use the same Erlenmeyer flasks for 250 mL to obtain more reproducible results. The average time of ultrasonic treatment was about 12 h at an electric power of 0.9 kW.

Hydrophobic fullerene nanoparticles in AFDs show a negative zeta-potential in water. In all cases, we found values < -20 mV. Polydispersity indexes for all solutions were $0.10 - 0.15$. These values are close to those for a material with a unimodal distribution of nanoparticles. The optimum conditions provide the transfer of fullerene up to 100 % into aqueous media for C_{60}. ICP-AES was used for determining elemental composition for major components of the glass vessel (sodium, silicon, calcium etc.) in the final solution; the total concentration of elements is 3 ppm (Table 3).

TABLE 3. The recovery of fullerenes in two parallel experiments, the average recovery value, ($n = 2$, $P = 0.95$) and the results of elemental analysis using the ICP-AES ($n = 3$, $P = 0.95$)

C_{60} toluene/water ratio	Recovery (I) C_{60} into aqueous phase, %	Recovery (II) C_{60} into aqueous phase, %	Average recovery (I) C_{60} into aqueous phase, %	Sodium, ppb[1]	Silicon, ppb[1]
$2 : 1$	29 ± 5	22 ± 3	26 ± 8	160 ± 24	102 ± 15
$1 : 2$	34 ± 5	26 ± 6	30 ± 11	580 ± 87	2081 ± 312
$1 : 1$	55 ± 2	49 ± 4	52 ± 6	199 ± 30	280 ± 42
$1 : 5$	91 ± 5	93 ± 5	92 ± 10	211 ± 32	550 ± 83
$1 : 10$	6 ± 2	3 ± 1	4 ± 3	266 ± 40	319 ± 48
$1 : 25$	1.5 ± 0.2	1.7 ± 0.3	1.6 ± 0.5	35 ± 5	36 ± 5

[1]Another elements concentration: Ag, Al, As, Ba, Be, Cd, Co, Cr, Fe, K, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Ti, Tl, V, W, Zr < 1 ppb, Cu < 3 ppb, K, Mg, Ca <80 ppb, B < 100 ppb

HPLC-UV showed that the total value of benzoate (a product of ultrasound oxidation of toluene) was no more 30 ppb. We assumed the possible clustered structure of the micelle which including $\{n[C_{60}]mC_6H_5COO^-(m-x)Na^+\}xNa^+$, which can provide, apparently, the most probable source of stabilization of fullerenes. It has no impact on increasing ionic strength and AFD stability. In certain conditions, there is a complete shift of the equilibrium in a one-component system towards AFD formation.

4. Conclusions

The conditions for high-yield preparation of all types of AFDs have been found. Our results highlighted optimization techniques for obtaining stable aqueous dispersions. Obviously, in the future, this technique should be employed for higher-order fullerenes and metallofullerenes. A special role in this, of course, will be played by metal-containing fullerenes. First, since the cost of the initial samples is now very high. Secondly, in view of the unique structure and their paramagnetic properties (*e.g.* Gd^{3+}) of aqueous dispersions, there are promising applications for studying and using them as contrast agents in MRI. Additionally, such work will provide a means to approach solutions for difficult problems, such as establish the nature of the fullerenes' stability in their AFDs.

Acknowledgements

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