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Approaches to the determination of C_{60} and C_{70} fullerene and their mixtures in aqueous and organic solutions

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The solvent-exchange process from toluene was used for preparing aqueous dispersions of C_{60} and C_{70} without preconcentration with final concentrations of 180 ± 2 and 62 ± 1 μM, respectively, which exceeds the previously reported maximum concentrations for C_{60} more than 6-fold; for C_{70} such an aqueous dispersion is prepared for the first time. The residual quantity of the organic solvent and low-molecular compounds determined by headspace GC-MS was not more than 1 ppb. The procedure for the determination of fullerenes in aqueous dispersions is developed using a total organic carbon analyzer and absorption spectra; LOD, 50 nM, LOQ, 200 nM by TOC. Spectrophotometric determination of fullerenes in their aqueous dispersions was optimized: for C_{60} at 268 nm: LOD, 0.1 μM, LOQ, 0.3 μM, for C_{70} at 218 nm: LOD, 0.1 μM, LOQ, 0.3 μM. RSD mixture quantification by Vierordt's method in the range of 2 – 20 μM does not exceed 0.14 for C_{60} and 0.09 C_{70}. RSD for toluene fullerene mixtures by Vierordt's method in the range of 2 – 20 μM does not exceed 0.10 for C_{60} and 0.06 for C_{70}.

Keywords: fullerene C_{60}, fullerene C_{70}, fullerene mixture, UV/vis. fpectroscopy, GC/MS.

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1. Introduction

Aqueous fullerene dispersions (AFDs) are powerful antioxidants that adsorb free radicals and possibly prevent various pathologies [1–3] and might have utility at the cellular and organism levels for the immobilization and transport of medicines [1,4,5]. However, the relevant question is to understand the composition of the initial and target fullerene materials [6]. Currently, the most common approaches are UV/vis-spectrometry and liquid chromatography; for mixture analysis in organic solvents, UV/vis and Raman spectroscopies are also used [7,8]. However, these approaches were not used for AFDs. In addition, the use of organic solvents in AFD production calls for controlling the residual solvent and low-molecular compounds as these components contribute to the chemical behavior and safety of the materials [9–12]. Thus, the aim of this study is to (*i*) develop procedures for the determination of fullerenes and their mixtures in AFDs and (*ii*) to carry out the chromatographic characterization of the dispersions and residual solvents.

2. Experimental

2.1. Materials and methods

Fullerene C_{60}, 99.5 % and fullerene C_{70}, 99 %, (LLC Neo Tech Product, Russia), and an unseparated extract (technical mixture, lot PD-1) of fullerenes C_{60} (90 ± 5 %, estimated value) and C_{70} (10 ± 5 %, estimated value) from Fullerene Technology Company (Russia) were used.

Chemically pure toluene and benzene (Reakhim, Russia) were used throughout. Reagents were weighed on an Ohaus Discovery analytical balance (Switzerland). Solution preparation and sampling were done with Biohit Proline Plus mechanical automatic dispensers (Biohit Group, Finland). All solutions were prepared in Class A glass volumetric flasks with volumes of 25 and 50 mL (Labtekh, Russia). For sampling, 15 mL polypropylene test tubes were used (Axygen, Mexico). A GRAD 180-35 ultrasonic bath (Grad-Technology, Russia) was used to improve the dissolution of fullerenes and to prepare aqueous fullerene dispersions. Dynamic light scattering was measured using a Malvern Zetasizer nano ZS. Spectrophotometric measurements were made using an Agilent Cary 60 spectrophotometer (Australia). A liquiTOC II from Elementar Analysensysteme GmbH (Germany) was used for total organic carbon analysis and calibrated with standard solutions of potassium hydrogen phthalate in the range of 1 ppm to 200 ppm. The calibration plot of total organic carbon is $c(\text{ppm}) = (10.3 \pm 0.1)S$ ($P = 0.95$, $n = 7$, $r = 0.9986$), where S is the area of the peak of carbon, a.u. The limits of detection (3σ) were calculated using a standard protocol. The measurement results are presented in accordance with the requirements of ISO 5725:1994 (2012) [13].

2.2. Experimental Techniques

Procedure 1. Solvent-exchange aqueous fullerene dispersions [6]. A weighed portion of fullerene (0.5 – 0.6 g) was placed into a 200 mL volumetric flask, dissolved in 100 mL of toluene, and sonicated at 0.30 kW for 2 h at 353 K; next, the solution was diluted to mark with toluene. After sonication, the toluene solution was placed into a conical flask, 1 L of Milli-Q water was added, and the solution was sonicated at 0.90 kW for 3 days (12 h per day) at 353 K until complete elimination of toluene. Next, the solution was heated for 30 min, boiled for 15 min, and filtered through a Schott filter followed by a 0.45-μm microporous membrane filter. The concentration of fullerene was measured by the total organic carbon technique. Additionally, the residual toluene was measured by headspace gas chromatography technique.

Procedure 2. Analysis of residual organic solvent in aqueous fullerene dispersion by headspace gas chromatography. The test specimen (2 ml) was placed into a vial which was then sealed by a crimper. The instrumental parameters were as follows: temperature control time for sample – 20 min; thermostat temperature – 80 °C; sample injection volume – 1 ml. *Conditions of gas chromatographic separation:* column ZB-5 MS (30 m × 0.25 mm × 0.25 μm). Injector temperature – 250 °C; volumetric flow rate – 1 ml/min; septum purge flow – 6 ml/min. The initial column temperature was 40 °C (isothermal for 3 minutes), then the temperature was raised at a gradient of 2 °C/min to 60 °C (1 min isothermal). Sample input was done without dividing the carrier gas flow. *Conditions of mass spectrometric detection:* chromatograms registration mode by selected ion m/z 78 (benzene), 91 (toluene); the registration data 0.2 s. Source temperature 200 °C; interface temperature 250 °C.

Procedure 3. Stock fullerene C_{60} and C_{70} solutions preparation in (a) aqueous media (b) toluene media for the analysis by Vierordt's method.

(a) Aqueous dispersions were analyzed using TOC and GC-MS to determine fullerene concentration. Next, the aqueous samples were diluted to 10 μM. The aqueous fullerene mixture solutions were prepared with the following ratios of C_{60} : C_{70} fullerenes: 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1 in deionized water (Table 1). Next, the *Vierordt's method* for analysis at wavelengths 420 and 320 nm was used. The content of fullerenes in the mixture was checked by UV/vis spectroscopy.

(b) A weighed portion of fullerene C_{60} (21.6 mg) and C_{70} (25.2 mg) was placed into a 100 mL volumetric flask, dissolved in 50 mL of toluene, and sonicated at 0.30 kW for 0.5 h

at 353 K. Next, the sample was cooled to room temperature and diluted up to volume with toluene. For analysis, solutions were diluted 10-fold. The concentration of fullerene was 30 μM. The toluene fullerene mixture solutions were prepared with the following ratios of C_{60} : C_{70} fullerene: 1 : 1, 1 : 2, 2 : 1, 1 : 3, 3 : 1 in toluene (Table 1). Next, Vierordt's method was used to determine fullerene content by UV/vis spectroscopy at 337 and 406 nm.

TABLE 1. Composition of working solutions of fullerenes in toluene and aqueous mixtures for the analysis by UV/vis for Vierordt's method

Ratio		$c(C_{60}) \cdot 10^5$, M	$c(C_{70}) \cdot 10^5$, M	$c(C_{60}) \cdot 10^5$, M	$c(C_{70}) \cdot 10^5$, M
C_{60}	C_{70}	Toluene		Aqueous	
1	1	1.50	1.50	0.500	0.500
1	2	1.00	2.00	0.333	0.667
2	1	2.00	1.00	0.667	0.333
1	3	0.75	2.25	0.250	0.750
3	1	2.25	0.75	0.750	0.250

Procedure 4. Preparation of the toluene solution of technological mixture of the C_{60} and C_{70} for the analysis by Vierordt's method. A weighed portion of C_{60} and C_{70} mixture (30.2 mg) was added to a 100 mL volumetric flask, dissolved in 50 mL of toluene, and sonicated at 0.30 kW for 0.5 h at 353 K. The sample was then cooled to room temperature and diluted to volume with toluene. For the analysis, solutions were diluted 10-fold.

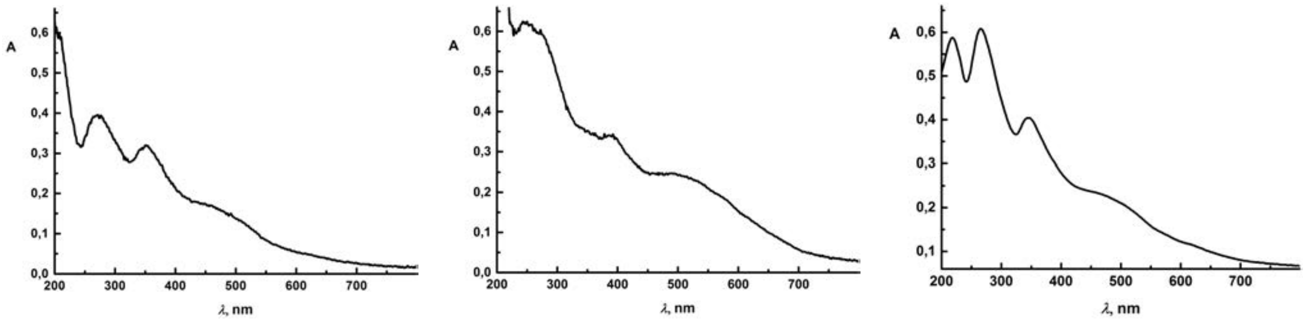
3. Results and discussion

3.1. Characterization of aqueous fullerene dispersions

The aqueous fullerene dispersions were made by the standard protocol [6] from toluene by a solvent-exchange process. The fullerene concentrations were 180 ± 2 and 62 ± 1 μM, respectively, which exceeded the previously-achieved maximum concentrations for C_{60} by more than 6-fold, and for the C_{70} fullerene, this was the first time that such an aqueous was prepared. This was confirmed by the TOC-analyzer. We postulate that the high fullerene concentrations were achieved by optimization of the sonication conditions and ratio of the organic to the aqueous phase. The total yields were calculated for C_{60} and C_{70} 54 ± 2 % and 45 ± 1 %, respectively. The absorption spectra are in a good agreement with existing data for C_{60} (see Fig. 1A), C_{70} (see Fig. 1B). The absorption spectra for the aqueous fullerene dispersion of the technical mixture were obtained for the first time (see Fig. 1C). The AFD were characterized in terms of the physico-chemistry parameters: size clusters distribution, ζ-potential, PDI-indices (see Table 2). These data are in good agreement with existing data for the solvent-exchange process [9, 14–16]. The slight differences may be a result of different preparation conditions or changes in the preparation technique.

3.2. Residual organic solvent analysis in aqueous fullerene dispersions

The residual organic solvent content has an effect not only on human health, but also on the total fullerene concentration in aqueous dispersions (TOC-analysis). We estimated the residual quantity of the solvents by GC-MS analysis. The organic compounds in AFD hardly ever detect and quantify. However, in [17] an effort was made to quantify the residual solvents



(A) (B) (C)

FIG. 1. Absorbance spectra $A(\lambda)$ of the aqueous fullerene dispersions: (A) 35 ± 2 μM; (B) 23 ± 2 μM; (C) C_{60} 59 ± 2 μM, C_{70} 4.4 ± 0.3 μM

TABLE 2. The main colloidal parameters of the aqueous fullerene dispersions

Characteristics	AFD-60	AFD-70	AFD (C_{60}/C_{70})
c, μM	180 ± 2	62 ± 1	60 ± 1 ($C_{60} + C_{70}$)
ζ, mV	-32.9 ± 1.4	-34.4 ± 0.7	-41.8 ± 1.2
%RSD(ζ)	1.24	1.58	0.65
d, nm	145 ± 3	175 ± 5	89 ± 5
%RSD (d)	1.98	1.32	0.56
PDI	0.10 ± 0.01	0.11 ± 0.02	0.26 ± 0.01

TABLE 3. The GC-MS analysis of the aqueous fullerene dispersions ($n = 3$, $P = 0.95$)

c, ppt	Retention time, min	AFD-60	AFD-70
Toluene	2.38	97 ± 8	69 ± 6
Benzene	4.15	55 ± 5	46 ± 6

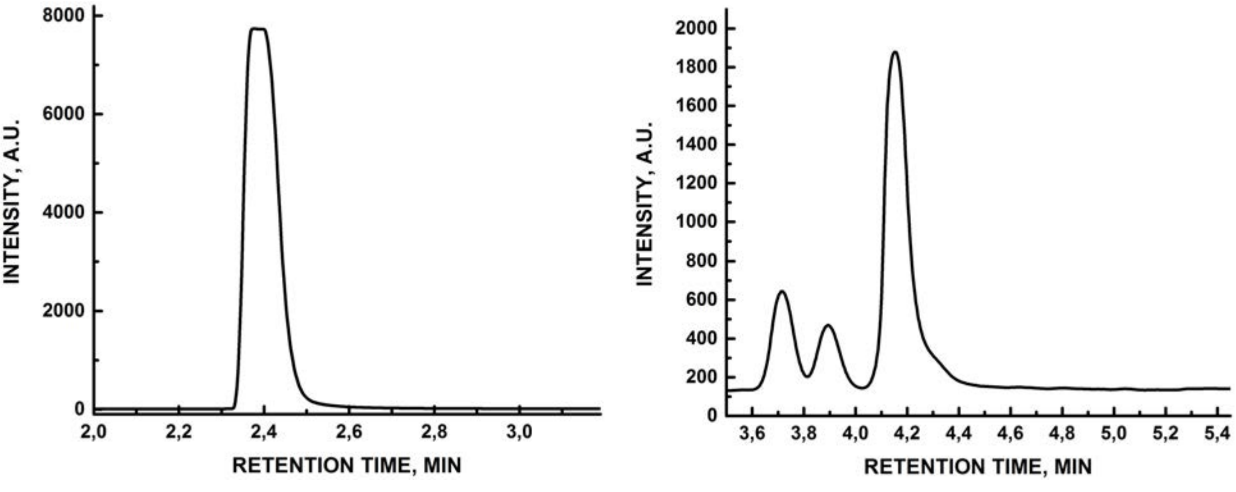
using solid-phase extraction with GC-analysis. The LOD of the THF was 1 ppb. Analysis of the samples showed that the AFDs samples contained solvents (benzene and its derivative toluene) below 100 ppt (see Table 3 and amount in AFDs by TOC (*vide infra*) Fig.2). Thus, the solvent analysis allowed us to determine the total fullerene levels much more precisely.

3.3. UV/vis Spectroscopy of individual aqueous fullerene dispersions

The concentrations of fullerenes in the resulting aqueous dispersions were determined by TOC-analyzer with preliminary residual solvent characterization by GS-MS (*vide supra*). The parameters of the fullerene determination are LOD, 50 nM, LOQ, 200 nM by TOC. Comparison of the calculated LOD and LOQ (see Table 4) at 268 and 349 nm shows a decrease in the LOD 3 times at 268 nm, and at 349 nm, the LOD was the same, as was discussed in [18,19] for C_{60}.

3.4. Application of Vierordt's method for C_{60}, C_{70}, and their technological mixtures

The dependences obtained for $\varepsilon_1^\lambda/\varepsilon_2^\lambda = f(\lambda)$ rely on the absorbance spectra of the fullerene in neat solvent over the range of 300 – 850 nm according to procedure 3b. It is characterized by the following extreme points, maximum at 355, 406 nm, a minimum at 320 and 337 nm, at these wavelengths, the ratios of the molar absorption coefficients have maximum great



(a) (b)

FIG. 2. GC-MS analysis of the aqueous fullerene dispersions sample. Characteristic chromatogram obtained by m/z analysis (a) m/z 78 benzene; (b) m/z 92 toluene

TABLE 4. Metrological parameters for the aqueous fullerene dispersion determination ($P = 0.95$, $n = 10$)

λ, nm	Calibration curves	$LOD \cdot 10^7$,	$LOQ \cdot 10^7$,
	AFD 60		
220	$A = (6.0 \pm 0.6) \cdot 10^4 c$, $r = 0.9977$	2	8
268	$A = (6.7 \pm 0.6) \cdot 10^4 c$, $r = 0.9975$	1	3
349	$A = (4.9 \pm 0.2) \cdot 10^4 c$, $r = 0.9969$	4	10
	AFD 70		
218	$A = (7.5 \pm 0.2) \cdot 10^4 c$, $r = 0.9970$	1	3
248	$A = (6.4 \pm 0.6) \cdot 10^4 c$, $r = 0.9916$	3	12
387	$A = (3.5 \pm 0.3) \cdot 10^4 c$, $r = 0.9940$	5	16
486	$A = (2.5 \pm 0.2) \cdot 10^4 c$, $r = 0.9934$	8	25

difference between $\varepsilon_1^{\lambda_1}/\varepsilon_2^{\lambda_1} - \varepsilon_1^{\lambda_2}/\varepsilon_2^{\lambda_2}$ [20]. Analysis of the mixture by Vierordt's method at 355, 406 and 320, 337 nm showed that the deviation of the calculated concentration in the mixture was more than 15 %. For 337 and 406 nm, the relative error (δc %) concentration ranged from 5 – 10 % (see Table 5). Therefore, for analysis of selected absorption maxima, the fullerene mixture in toluene was evaluated at 337 and 406 nm. Currently, the combination of UV/vis and cumbersome calculation permitted an accuracy of a hundredth of a percent [8]. However, for the technological purposes, such a value for the relative error is sufficient for monitoring fullerene mixture composition after synthesis. We confirmed this by analyzing a real sample of an unseparated fullerene mixture with a known mass ratio. We have calculated the ratio as C_{60} 92 ± 3 % and C_{70} 8 ± 2 % , and this data was in a good agreement with the real sample's certificate. Thus, analysis of the organic solutions permitted expansion to the analysis of the aqueous dispersions. The usage of a TOC-analyzer allowed to us to determine the fullerene content in the dispersions. The combination of the TOC and UV/vis-spectroscopy allowed calculation of the apparent molar absorptivity and then analysis of the aqueous dispersion mixture

TABLE 5. C_{60} and C_{70} determination in aqueous dispersion by Vierordt's method at 320/420 nm

Concentration		Calculation		δC, %	
$c(C_{60}) \cdot 10^6$, M	$c(C_{70}) \cdot 10^6$, M	$c(C_{60}) \cdot 10^6$, M	$c(C_{70}) \cdot 10^6$, M	C_{60}	C_{70}
5.14	5.30	5.75	5.14	11.9	3.0
5.14	2.65	5.70	2.38	10.9	10.2
2.57	5.30	2.91	5.10	13.3	3.9
6.17	2.12	6.82	1.92	10.5	9.3
2.06	6.36	2.32	6.25	12.9	1.7

by Vierordt's method. The dependences $\varepsilon_1^{\lambda}/\varepsilon_2^{\lambda} = f(\lambda)$ were obtained based on the absorbance spectra of the fullerene in neat solvent from 300 – 850 nm according to procedure 3(a). This was characterized by maxima at 241, 320, 420 nm, and at these wavelengths, the ratios of molar absorptivities had the maximum difference between $\varepsilon_1^{\lambda_1}/\varepsilon_2^{\lambda_1}$ – $\varepsilon_1^{\lambda_2}/\varepsilon_2^{\lambda_2}$ [20]. Analysis of the mixture by Vierordt's method at 241 and 320 nm showed that the deviation of the calculated concentration in the mixture was more than 25 %. Therefore, analysis was done at 320 and 420 nm for the selected absorption maxima of the fullerene mixture in toluene. For the maxima at 320 and 420 nm, the value of relative error (δc %) was below 15 % over the of 2 – 7 μM range (see Table 5).

The determination of fullerenes in toluene by Vierordt's method enabled us to proceed with our mixture analysis of the AFDs. Data obtained for the fullerenes in AFD showed that the relative error in the determination of fullerenes was higher than for toluene solutions of (1.5 times), due to the high light scattering by clustered structures in the AFDs. However, this relative error is acceptable to aim at biology and medicine for establishing the reinforcing action of fullerene in the mixture [21,22]. This was the first time Vierordt's method for analysis was applied to a two-component system of AFDs (C_{60} and C_{70}).

4. Conclusions

The technique of organic solvent determination is applicable for biological purposes and total AFDs characterization, which can be successfully used for a number of biomedical applications. The results obtained here indicate that we may apply the developed technique for mixture analysis with satisfactory accuracy. Continuing research into this field appears fully justified because no one method totally characterized such object akin to AFDs; rather, this was accomplished by applying a few physico-chemical analytical methods. In our opinion, for biological purposes, we should apply a number of physico-chemical methods to completely describe these aqueous fullerene systems.

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