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Hydrophilization and Functionalization of Fullerene C$_{60}$ with Maleic Acid Copolymers by Forming a Non-Covalent Complex

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Abstract: In this study, we report an easy approach for the production of aqueous dispersions of C$_{60}$ fullerene with good stability. Maleic acid copolymers, poly(styrene-*alt*-maleic acid) (SM), poly(N-vinyl-2-pyrrolidone-*alt*-maleic acid) (VM) and poly(ethylene-*alt*-maleic acid) (EM) were used to stabilize C$_{60}$ fullerene molecules in an aqueous environment by forming non-covalent complexes. Polymer conjugates were prepared by mixing a solution of fullerene in N-methylpyrrolidone (NMP) with an aqueous solution of the copolymer, followed by exhaustive dialysis against water. The molar ratios of maleic acid residues in the copolymer and C$_{60}$ were 5/1 for SM and VM and 10/1 for EM. The volume ratio of NMP and water used was 1:1.2–1.6. Water-soluble complexes (composites) dried lyophilically retained solubility in NMP and water but were practically insoluble in non-polar solvents. The optical and physical properties of the preparations were characterized by UV-Vis spectroscopy, FTIR, DLS, TGA and XPS. The average diameter of the composites in water was 120–200 nm, and the ξ-potential ranged from −16 to −20 mV. The bactericidal properties of the obtained nanostructures were studied. Toxic reagents and time-consuming procedures were not used in the preparation of water-soluble C$_{60}$ nanocomposites stabilized by the proposed copolymers.

Keywords: fullerene C$_{60}$ aqueous dispersions; maleic acid copolymers; C$_{60}$ nanocomposites

1. Introduction

Fullerene C$_{60}$, a spherical cage-shaped molecule, is one of the most studied in the family of carbon allotropes. Due to its exceptionally high symmetry, C$_{60}$ is highly stable, and its structure is the most thermodynamically efficient. The molecule in the shape of a truncated icosahedron has a diameter of 0.7 nm and contains 12 pentagonal faces and 20 hexagonal ones. The electronic structure of fullerene determines its unique properties: electron acceptor activity, high polarizability, radical scavenging activity and, vice versa, a generation of free radicals under light irradiation. In addition, C$_{60}$ has a large surface area with a large number of equivalent reaction sites [1]. Pure fullerene (pristine C$_{60}$) and a number of its derivatives are commercially available products [2]. Fullerenes have been used as building blocks for the creation of covalent or non-covalent 2D/3D carbon materials (third-generation solar cells); such materials have also found application in catalysis, spintronic, water treatment, etc. [3–6]. The presence of C$_{60}$ molecules leads to an increase in the photoconductivity of conducting polymers and organo-metallic compounds [7]. Due to their

spherical form and unique electron properties, fullerenes attract researchers in the fields of medicine and biology [8–11]. Nanomaterials based on C_{60} have good prospects in cancer therapy [12–14]. Under the influence of UV radiation, fullerene derivatives (malonates) can cause HeLa cells' death [15]. C_{60} fullerene compounds can provide radioprotection to radiosensitive mammalian cells [16]. A noticeable anti-inflammatory effect of an aqueous dispersion of C_{60} fullerene, which also acts as an antioxidant and an anti-aging antioxidant drug, has been shown [17–20]. The antibacterial properties and virucidal activity of fullerene and its derivatives have been described [21–25]. Fullerenes are able to reduce the apoptosis of neurons induced by reactive oxygen species (ROS). It is also assumed that by inhibiting the level of ROS, fullerenes can have an antiallergic effect [26]. However, none of the listed applications of aqueous and oil C_{60} dispersions are currently available to consumers, except skin creams [27]. A number of studies show that aqueous dispersions of pristine C_{60} do not possess acute or subacute toxicity [17,28–30].

For the use of C_{60} and its derivatives for biomedical purposes, it is preferable to disperse them in aqueous media due to the biocompatibility, non-toxicity and environmental friendliness of this solvent. The main approaches to the production of water-dispersible fullerene can be distinguished:

1. chemical modification of fullerene (derivatization),
2. direct dispersion in water and the solvent-exchange method,
3. non-covalent complexation of fullerene with a number of hydrophilic compounds,
4. encapsulation.

The most popular chemical modifications of fullerene are reactions of nucleophilic and radical additions, including cycloadditions, hydroxylation (fullerenol), carboxylation and amination (amino acid-containing adducts) [31–33]. The synthesis of covalent assemblies consisting of inherently chiral open inter–60 fullerenes has been demonstrated [34]. Fullerene molecules can also be incorporated into polymeric structures by covalent bonding. These include "pearl necklace" structures, "charm bracelet" structures, organometallic polymers, cross-linked polymers, end-capped polymers, star-shaped polymers and supramolecular polymers. Some of them have been shown to be soluble in water [35]. In cases of chemical modification, in order to give fullerene water-solubility, it is often necessary to introduce more than one functional group into its structure. Such fullerene derivatives are difficult to identify and separate because the system often contains a mixture of regioisomers. Such modification can change the chemical and physical properties of C_{60} molecules. In addition, methods of chemical C_{60} functionalization require harsh conditions, the use of catalysts or strong oxidizing agents, favoring cage opening.

The most striking individuality of fullerene molecules is manifested in an unmodified form. The dispersion of the fullerene in water was carried out in a series of experiments. At the same time, the prolonged mixing and sonication of fullerite and water were used [36–38]. Known methods for the formation of stable aqueous dispersions are based on the transfer of C_{60} from an organic solution (benzene, toluene, tetrahydrofuran) into an aqueous phase ("solvent exchange method") using ultrasonic treatment. The organic solvent is gradually displaced by intense ultrasound, which ensures the heating of the system and the evaporation of the solvent [39,40]. Such dispersions contain hydrated clusters of C_{60} molecules; their size depends on the characteristics of the method. However, an aromatic solvent is not suited for obtaining solutions for medical purposes, and it is also difficult to standardize ultrasonic technology, which depends on the sonication time, volume and geometry of the vessel with the dispersion. It is also difficult to completely remove traces of toluene due to its specific π-stacking interaction with fullerene molecules [41]. The above approach is quite labor- and energy-intensive, and the final concentration of fullerene is limited ($<10^{-5}$ M). It should be added that the dried C_{60} dispersions obtained by the abovementioned methods usually irreversibly lose their solubility in water.

An alternative approach for avoiding some of the above-stated problems is the conjugation of fullerene with a number of hydrophilic compounds without forming covalent bonds. The electron structure of fullerene is less affected during such bonding; hence, its

physical properties are retained to a significant extent. Some variants are the supramolecular encapsulation of fullerene with γ- or β-cyclodextrin and dendritic derivatives of cyclotriveratrilene and calixarenes [42–45]. However, the strength of such complexes does not always correspond to the required one. The solubilization of fullerene with proteins is another option [46,47]. The encapsulation of fullerene C_{60} in lipid micelles has been described by many researchers [48–51]. Although nonionic surfactants such as Triton and Twin provide the good solubilization of C_{60}, this method also uses toluene to initially dissolve the fullerene, which then must be removed [49].

The approach of including fullerene in polymers seems promising. Supramolecular chemistry approaches for the preparation of host–guest inclusion complexes with fullerenes have made significant progress over the past decade [3]. Polymer-fullerene nanocomposites are new materials with many applications in the biomedical field [8]. Fullerenes can be retained in the cavity of host polymer molecules due to van der Waals and hydrophobic interactions and π-π stacking, as well as electrostatic interactions [52]. It is known that polymers involving oxygen or (and) nitrogen (electron-donor elements) may form charge-transfer complexes with fullerene [53,54]. The inclusion of fullerene in the compositions of hydrophilic polymers can provide the ability for them to be dispersed in water; in addition, the manufacturability of such complexes increases, which facilitates their use in a variety of fields. The dispersibility of C_{60} in polar solvents may increase using solubilization with amphiphilic polymers, such as polyethylene glycol [55], poly(styrene-b-dimethylacrylamide) block copolymer [56] or poloxamer [10]. Water-soluble polymers of acrylamide and acrylic acid that contain fullerene have been prepared by the low-temperature radiation-induced living polymerization in organic solvents [57]. The most well-known are water-soluble fullerene complexes with polyvinylpyrrolidone (PVP) [58–62]. During the complexation of fullerene with PVP, the fullerene content in relation to the polymer usually does not exceed 1–1.5% [60].

Maleic acid copolymers have not yet been used for the hydrophilization of fullerene. Meanwhile, such copolymers have a number of positive properties: they are water-soluble and amphiphilic, and many of them are commercially available and nontoxic. The copolymers have a regular structure—polymer units clearly alternate. Previously, such copolymers were used to stabilize silver nanoparticles [63], colloidal nanohybrid structures of silver (gold) and zinc oxide [64,65]

In this work, we propose to use maleic acid copolymers for the hydrophilization of fullerene and the introduction of easily modified functional groups into the composition. The complexation of fullerene C_{60} to copolymers was carried out using the so-called "dialysis principle" [66].

2. Materials and Methods

2.1. Materials

Poly(N-vinyl-pyrrolidone-*alt*-maleic anhydride) M = 40,000 was prepared according to [67]. Poly(styrene-*alt*-maleic anhydride) M = 50,000 and poly(ethylene-*alt*-maleic anhydride) with an average molecular weight of M = 25,000 were purchased from Sterlitamak chemical plant (Sterlitamak, Russia) and Monsanto (Saint Louis, MO, USA), respectively. The copolymers of maleic acid (VM, EM and SM) were obtained by the hydrolysis of the corresponding copolymers of maleic anhydride by dissolution in deionized water followed by lyophilization fullerene C_{60} (the van der Waals diameter is about 1.1 nm, 99.9%, SES Research, catalog 600–9969, Houston, TX, USA), N-methylpyrrolidone (NMP) (99%, Panreac AppliChem GmbH, Ottoweg 4, D-64291 Darmstadt, Germany) and NaOH (analytical grade, Reakhim, Moscow, Russia), which were used without purification. The 1 kDa dialysis tubes were from Spectra/Por (Spectrum Labs, Pittsburgh, PA, USA).

The following tested microorganisms were used ("BD MicrotrolTM", Becton Dickinson, Franklin Lakes, NJ, USA): *Candida albicans* (*C. albicans*) NCPF3255/ATCC 10231, *Escherichia coli* (*E. coli*) NCTC 11954/ATCC 35218, *Pseudomonas aeruginosa* (*P. aeruginosa*) NCTC

12903/ATCC 27853, *Staphylococcus aureus* MRSA (*S. aureus MRSA*) NCTC 12973/ATCC 43300 and *Enterococcus faecalis* (*E. faecalis*) NCTC 12697/ATCC 29212.

2.2. Instrumentation

The pH values were determined using a Fisher Scientific 300 403.1 pH-meter (Waltham, MA, USA). FTIR spectra (KBr) were recorded using a Fourier-spectrometer Magna IR-720 (Nicolet, Parsons, WV, USA). The UV-visible absorption spectra were measured with a UVIKON-922 spectrophotometer (Zeiss Group, Baden-Württemberg, Germany). Registration was carried out without diluting the reaction solution, and a cuvette with a diameter of 0.2 cm was used for spectrophotometric measurements.

X-ray photoelectron spectroscopy studies were carried out on an Axis Ultra DLD spectrometer (Kratos Analytical, Manchester, UK) using monochromatic Al Kα (hν = 1486.6 eV) radiation. Survey spectra and high-resolution spectra were recorded at pass energies of 160 and 40 eV with steps of 1 eV and 0.1 eV, respectively. Survey and high-resolution spectra were recorded with steps of 1 eV and 0.1 eV, respectively. The sampling area was 300 × 700 μm^2. Samples were mounted on a titanium holder using a double-sided adhesive tape and studied at room temperature under a vacuum of <10^{-8} Torr. The energy scale of the spectrometer was calibrated according to the standard procedure based on the following binding energies: 932.62, 368.21 and 83.96 eV for Cu $2p_{3/2}$, Ag $3d_{5/2}$ and Au $4f_{7/2}$, respectively. To eliminate the effect of sample charging, the spectra were recorded using a neutralizer. Surface charging was corrected by referencing to the C-C/C-H peak identified in the C 1sspectra (285.0 eV). The background due to electron inelastic energy losses was subtracted by the Shirley method. Quantification was performed using atomic sensitivity factors included in the software of the spectrometer.

The elemental analysis was performed using an Elementar Vario MICRO cube C, H, N-analyzer (DKSH Group, Tokyo, Japan) equipped with a thermal desorption column and a UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

Measurements of ξ-potential and nanoparticle sizes were performed using dynamic light scattering (DLS) on a Zeta Sizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK). The thermal stability of the initial copolymers, the copolymer and C$_{60}$ mixture and the composites was studied by thermogravimetric analysis (TGA). TGA measurements were performed by a Derivatograth-C (MOM Szerviz, Budapest, Hungary) on samples of about 15 mg at a heating rate of 10 °C/min in argon.

2.3. Methods

2.3.1. Synthesis C$_{60}$/Copolymer Composites

Synthesis of C$_{60}$/VM: Initially, 6.25 mL of solution crystalline C$_{60}$ in NMP (0.8 mg/mL) was mixed with 4 mL of an aqueous solution of VM (2 mg/mL, pH 8) using a magnetic stirrer. The molar ratio of the maleic acid residues of copolymer to the mole C$_{60}$ was 5/1. After 0.5 h, the resulting solution was subjected to exhaustive dialysis (cutoff 1 kDa) against deionized water (1.5 L, four changes). The dried sample was obtained with subsequent lyophilic drying (−55 °C, 0.05 mbar). The samples C$_{60}$/SM and C$_{60}$/EM were prepared under similar reaction conditions and reagent concentrations; the molar ratios of monomeric units of maleic acid residues of the copolymer to C$_{60}$ were ~5/1 and 10/1, respectively.

2.3.2. Antimicrobial Activity Tests

The method of serial microdilution was used for the determination of the minimum inhibitory concentrations (MIC) of the preparations in relation to the strains of microorganisms in accordance with the standard procedure [68]. The initial concentrations of C$_{60}$/VM and C$_{60}$/SM were 0.46 and 0.37 mg/mL, respectively (C$_{60}$/EM—0.50 mg/mL). A detailed description of the experiment was given elsewhere [69].

3. Results

To obtain a stable colloidal solution of a fullerene–polymer complex, the choice of a polymer component (matrix) as a stabilizer is very important. To prevent the aggregation of fullerene nanoparticles by reducing their surface energy, the choice of a suitable polymer coating of nanoparticles is very important. The stabilization of fullerene nanoparticles can occur not only due to hydrophobic and/or π-π interactions; spatial and/or Coulomb stabilization are also significant. The selected amphiphilic maleic acid copolymers are suitable according to the above criteria. NMP was selected as an aprotic solvent in which fullerene C_{60} (fullerite) and polymers are sufficiently soluble due to its low toxicity (taking into account the medical aspect of this work) [70]. In a further description of the processes for obtaining stabilized aqueous dispersions of C_{60} and an analysis of their properties, for the purposes of the present article, the term "dissolution" will be used, with the resulting system being a colloidal solution (nanodispersion).

Polymer-stabilized fullerene complexes can be obtained in two main ways: the dissolution (co-dissolution) of the fullerene and copolymer in NMP or the addition of an aqueous polymer solution to a fullerene solution in NMP. The second variant is preferable because it takes less time and the final solution contains less NMP. Meanwhile, the co-dissolution of the fullerene and polymer in water at pH 6–8 did not result in a homogeneous solution. We used an approximately fivefold molar excess of the VM and SM copolymers relative to fullerene and a tenfold excess for the EM copolymer (calculated per dimer unit), which corresponded to a 1.6–2.0-fold excess by weight. The calculated content of fullerene in the samples was 33–38%. The values found according to the elemental analysis, gravimetric data and spectrophotometry of the corresponding solutions ($\lambda = 340$ nm, $\varepsilon = 68,000$ dm^3 mol^{-1} cm^{-1}) [71] were 30–33%. The increase in the fullerene content relative to the copolymer resulted in a stronger processes of aggregation in a solution and was accompanied by the appearance of large particles separated into the solid phase. The volume ratio of the NMP solution to water was equal to 1:1.2–1.6. The resulting polymer-fullerene solutions were subjected to exhaustive dialysis (cutoff 1 kDa) against deionized water to remove the organic solvent. Drying the yellow–brown dialysate yielded brown powders (Figure 1) that were soluble in water. According to XPS and elemental analysis, the product contained approximately 1–2% nitrogen, even in the case of composites with nitrogen-free copolymers. This indicated that the products contained solvated NMP, and this was not due to insufficient dialysis, since fullerene forms charge transfer complexes with NMP [38,72,73]. According to the elemental analysis data, the conjugates contained approximately 5–10% water. It is known that fullerene is also capable of complexing water [74].

After dialysis, the concentration of the complexes in an aqueous solution was 300–400 μg/mL (fullerene content—50–100 μg/mL). The stability of the aqueous solutions of the composites, according to our estimates, is at least 12 months when stored at 4–25 °C. The fullerene composite containing the most hydrophilic VM copolymer (C_{60}/VM) had the best solubility—approximately 500 μg/mL at pH 7.0. The solubilities of C_{60}/SM and C_{60}/EM were noticeably worse—about 100 and 50 μg/mL, respectively. The solubility of the complexes in NMP was approximately 300–400 μg/mL for C_{60}/SM and C_{60}/VM and 500 μg/mL for C_{60}/EM. Fullerene C_{60} is practically impossible to extract from dried composites with chloroform, benzene and toluene, and no extraction of C_{60} occurred from water solutions of composites with the use of such solvents. So, these complexes practically do not break down in these solvents. Figure 2 shows the UV-Vis spectra of the initial copolymers and obtained conjugates. Solutions of the copolymers VM, EM and SM are colorless and almost do not absorb at wavelengths greater than 300 nm (Figure 2(1–3)).
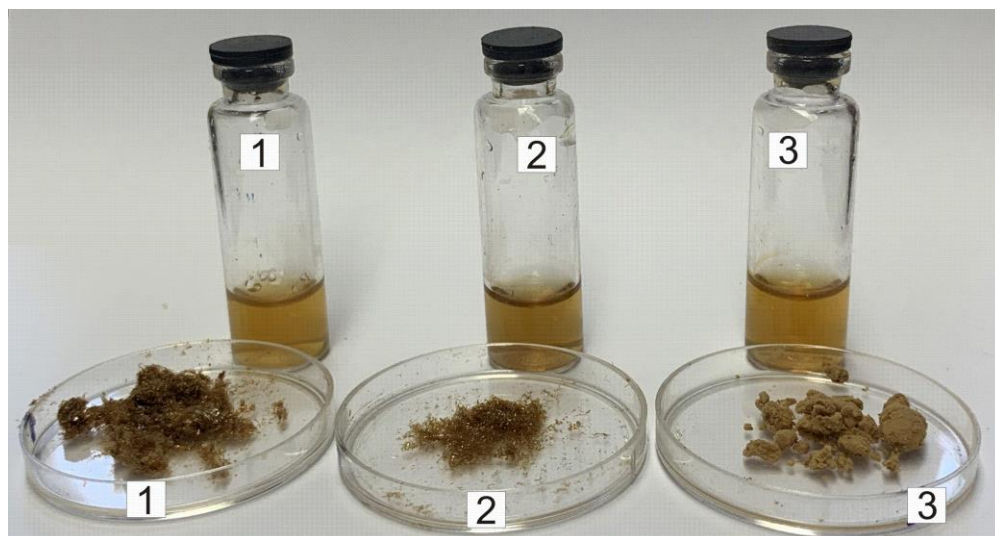
Figure 1. Aqueous solutions and dry forms of polymer–fullerene samples: 1—C_{60}/SM, 2—C_{60}/EM, 3—C_{60}/VM.
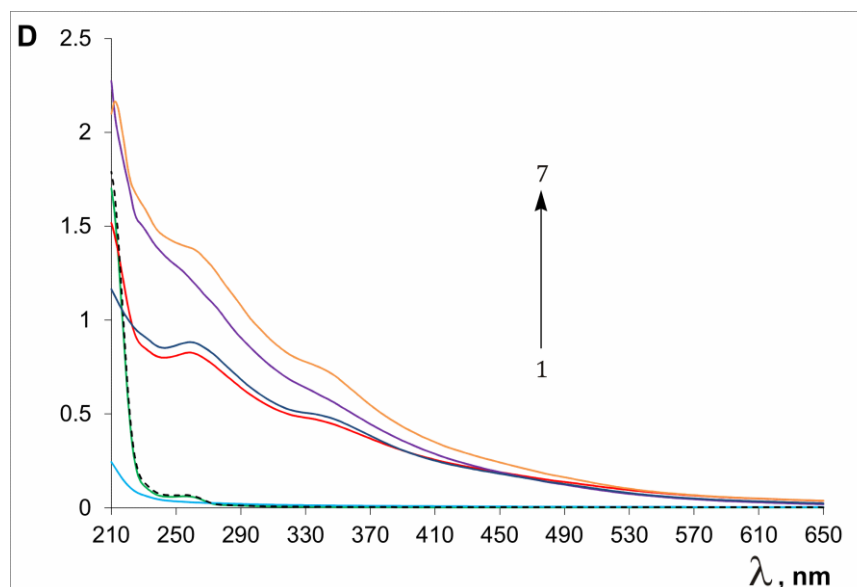

Figure 2. Absorption spectra of water solutions of copolymers and C_{60} conjugates: 1—EM, 2—SM, 3—VM; 4—C_{60}/SM, 5—C_{60}/VM, 6—C_{60}/EM; (C = 80–90 μg/mL, l = 0.2 cm); 7—C_{60}/VM after drying and dissolving (C = 100 μg/mL).

The absorption spectrum of fullerene C_{60} in nonpolar hexane (where its aggregation is minimal) is characterized by four maxima at 213, 257, 328 and 406 nm [75]. In our case, the spectra of the composites do not have such distinct maxima (Figure 2(4–7)); there are small hills in the region of 260 and 340 nm. This is probably due to the self-association of fullerene molecules, aggregation with copolymers, binding to NMP and water molecules and also some loss of the icosahedral symmetry of C_{60} molecules [76]. After several months of storage, the spectra of the obtained solutions did not change.

The hydrodynamic particle size of the diluted C_{60} samples measured by DLS ranged from 116 to 200 nm, with a polydispersity index (PDI) of 0.20–0.40 (Table 1). The distributions of scattered-light intensity over the particle size in the solutions of the hybrid macromolecular structures C_{60}/SM and C_{60}/VM were monomodal.

Table 1. The size and ζ–potential of hybrid macromolecular structures measured by the DLS method.

Sample [a]	Average Size (nm) [b]	PDI	ζ (mV)
C_{60}/SM	202.3	0.21	−19.5
C_{60}/VM	155.0	0.40	−17.6
C_{60}/EM	116.1; 1387	0.20; 0.40	−15.7

[a] The sample concentration was 80–90 μg/mL in deionized H_2O, T = 25 °C; [b] large particles were previously removed by filtration through a glass filter S2.

The size distributions of composite particles are different and are related to the structure of polymer chains. When using a copolymer EM as a stabilizer C_{60}, the distribution was bimodal, and the colloidal solution contained micron-sized particles. The presence of fullerene in composites apparently provides the greatest contribution to the aggregation of the resulting hybrid structures. The composite particle size may be related to the homo aggregation of fullerene $nC_{60} \leftrightarrow (C_{60})n$ [77–79]. At the same time, it was previously shown that maleic acid copolymers can aggregate in acidic and alkaline media, and the particle size distributions were bimodal, with the fast mode correlated with single chains (2–4 nm) and the slow mode correlated with polymer aggregates (60–120 nm). Meanwhile, EM copolymers aggregate to a noticeably greater extent [80,81]. Apparently, the polymer acts as a hydrophilic shell on the surface of fullerene aggregates. The used copolymers involve electron-donor elements, oxygen and nitrogen (in the case of VM), so they may form charge-transfer complexes with fullerene [53]. It can be assumed that the SM copolymer is able to bind to fullerene through π-π interactions. Since C_{60}, toluene and xylene contain sp^2-states, C_{60} acts as a π-electron acceptor when interacting with them [82]. Besides donor–acceptor interactions, Coulomb stabilization plays a role in the stabilization of the colloid solution of complexes. The copolymers of maleic acid contain two carboxyl groups with different pK values in the monomeric unit. We have shown previously [83] that in the pH range of 4–6, one carboxyl group of the maleic acid residue in copolymers was ionized by 60–70%, while the other was practically not ionized; however, at a neutral pH, the ionization of both carboxylic groups will be significant. The ξ-potential measured for all samples was from −16 to −20 mV. The obtained values of the zeta potentials indicate the good stability of the colloidal solution of the composites [84]. A negative zeta potential means that the composite surface has a negative charge, which may contribute to the high dispersion stability in the solution due to the inter-particle electrostatic repulsive forces. The FTIR spectra of the fullerene composites and initial copolymers are presented in Figure 3.

The characteristic absorption bands of fullerene C_{60} at 1429, 1181, 576 and 527 cm^{-1} are attributed to the C-C vibrational modes of the C_{60} molecules [85]. Against the background of the absorption bands of the copolymers in the IR spectra of hybrid structures, characteristic absorption bands of fullerene are observed at ν = 527, 576 (577) and 1419 (1421, 1426) cm^{-1} (Figure 3(a2–c2)). Due to the overlap of the polymer and fullerene bands, Figure 3 highlights the most intense bands of fullerene. This indicates the presence of native fullerene in the C_{60}–copolymer conjugates. The noticeable change in the FTIR spectra between the composites and copolymers is due to the position of the C=O bond of the carboxyl group. For example, in the VM spectrum (Figure 3(a1)), C=O stretching vibration at 1719 cm^{-1} corresponds to non-ionized carboxyl groups of maleic acid residues. In the FTIR spectrum of C_{60}/VM (Figure 3(a2)), the value C=O stretching vibration is shifted to the region at 1575–1576 cm^{-1}, which corresponds to the ionized form of the carboxyl group of the maleic acid residues of the copolymers [65]. For the composites C_{60}/EM and C_{60}/SM, this band is in the range of 1561–1569 cm^{-1}. The sample SM (Figure 3(c1)) also contained partially non-hydrolyzed anhydride groups (bonds 1779, 1860 cm^{-1}), which, in the composite, were also converted into ionized carboxyl groups.
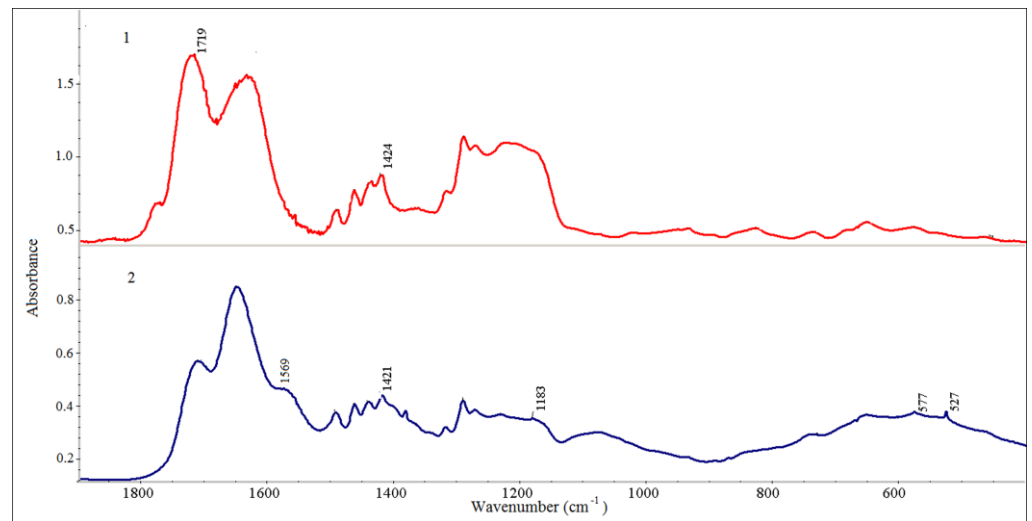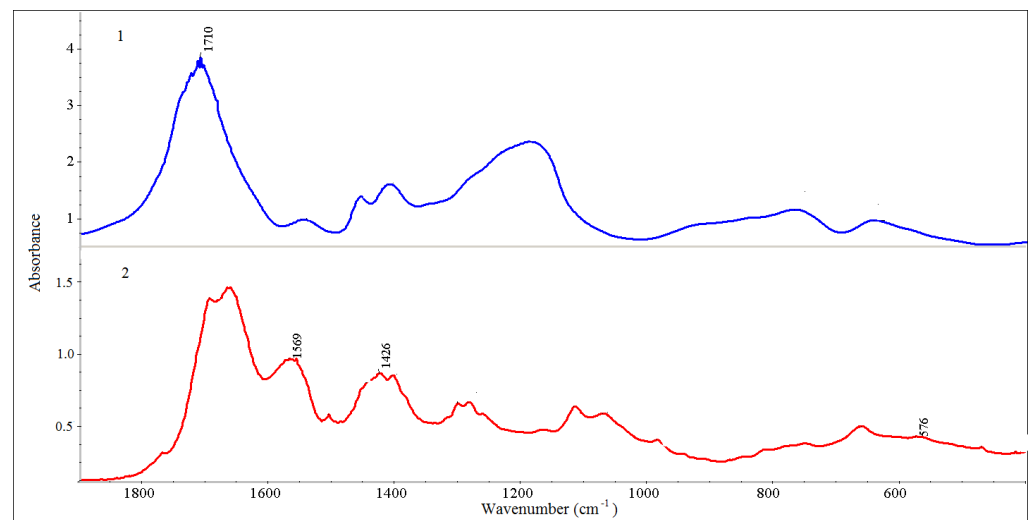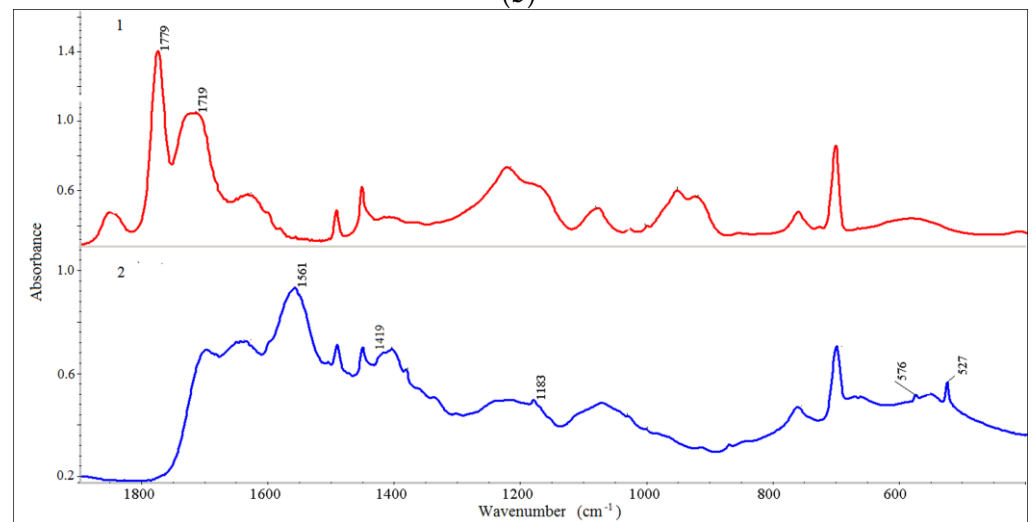
Figure 3. FTIR spectra of samples containing copolymers: (**a**) VM, (**b**) EM and (**c**) SM; initial copolymers (**a1–c1**) and fullerene conjugates (**a2–c2**).

A comparative analysis of the TGA curves of hybrid structures C_{60}/copolymer, C_{60}, copolymers and a mechanical mixture of copolymers with fullerene can serve as proof of the inclusion of fullerene in the structure of the polymer composite (Figure 4).
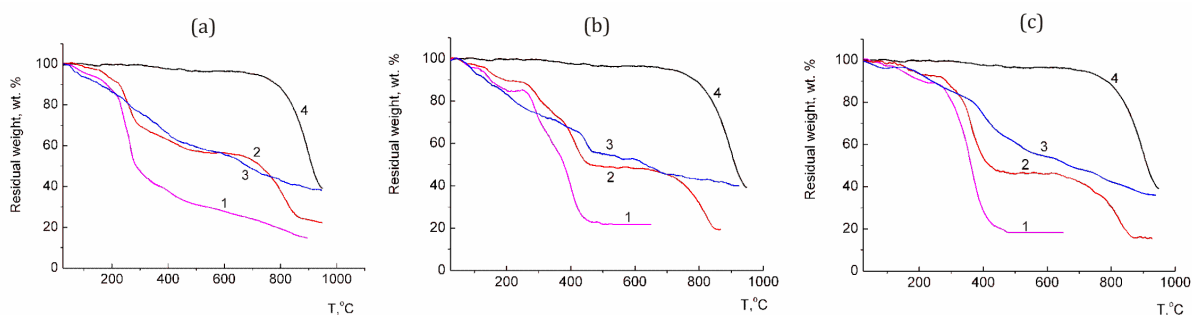

Figure 4. TGA curves of samples containing VM (**a**), EM (**b**) and SM (**c**) copolymers: 1—copolymers, 2—mechanical mixtures of copolymer with C_{60}, 3—hybrid structures and 4—C_{60}. Heating rate of 10 °C/min (argon atmosphere).

The native C_{60} was stable up to 800 °C, with almost no loss of mass (Figure 4a–c, curves 4). The initial copolymers decompose mainly at about 400 °C (Figure 4a–c, curves 1). The TGA curves of mechanical mixtures (~30 wt.% fullerene) practically repeat the curves of the individual components contained in the mixture. The decomposition degree of the fullerene-containing samples occurred at about 100 °C lower than that of individual C_{60}. The TGA curves of C_{60}–copolymer hybrid structures (Figure 4a–c, curves 3) differ from those of mixtures of system components in a smoother course. At low temperatures, the samples lost mass, probably as a result of the cleavage of solvent molecules. Table 2 shows comparative data on the decomposition of the studied samples.

Table 2. Characteristics of the samples and TGA analysis data.

Sample [a]	C_{60} (wt.%)		Residual Weight [b] (%)	
	Calculated	Found [a]	Found	Calculated
VM	-	-	27	-
C_{60}/VM complex	38	32	55	54
C_{60}/VM mixture	38	-	55	55
EM	-	-	22	-
C_{60}/EM complex	33	33	53	48
C_{60}/EM mixture	32	-	49	47
SM	-	-	14	-
C_{60}/SM complex	38	31	60	47
C_{60}/SM mixture	37	-	45	46

[a] The average values according to the methods of gravimetric and elemental analysis and spectrophotometry of the corresponding solutions (λ = 340 nm, ε = 68,000 dm^3·mol^{-1}·cm^{-1}) [71]; [b] at 600 °C.

We noted that, for composites, the last stage of destruction lasts longer and above 700 °C, in contrast to the mechanical mixtures and initial copolymers (Figure 4, Table 2). Previously, a similar effect was observed for C_{60}/epoxy polymer nanocomposites [86]. For composite samples, mainly, the found values of the residual weight at 600 °C exceeded those for the calculated values (Table 2). We conducted a control experiment in which fullerene was subjected to the same procedure that was used in the preparation of conjugates: the C_{60} solution in NMP was subjected to dialysis against water, followed by lyophilic drying. The resulting water-insoluble red–brown powder was also studied by the TGA method (Figure 5, curve 1).
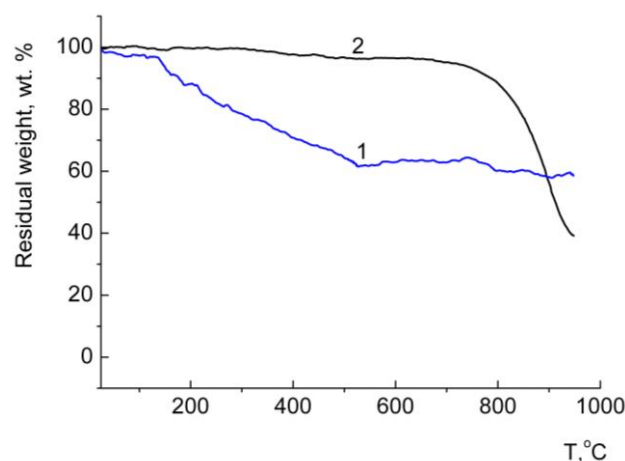
Figure 5. TGA curves: 1—C_{60} after dialysis and lyophilic drying, 2—pristine C_{60}.

The TGA curve for this compound differs from that of the original C_{60}, and its tendency toward decomposition is similar to that of C_{60} complexes with copolymers. In this case, the last stage of destruction of this sample also continues at temperatures above 700 °C; the decomposition stage of fullerene at this temperature is practically degenerate. In contrast to the C_{60} fullerene, at temperatures above 100 °C, a loss of mass is observed, probably due to the removal of bound water and NMP. Judging by the TGA curves, the composites do not include individual fullerene formations, and obviously, the fullerene component is associated with NMP molecules, which was also confirmed by DLS, elemental analysis and XPS data.

Figure 6 shows the high-resolution C 1s photoelectron spectra of the prepared composites. The spectra in Figure 6a are normalized by the intensity of the main peak. Figure 6b–d show the spectra of the samples C_{60}/SM, C_{60}/EM and C_{60}/VM fitted with several Gaussian profiles in accordance with their chemical formulas and reliable chemical shifts, respectively [87].
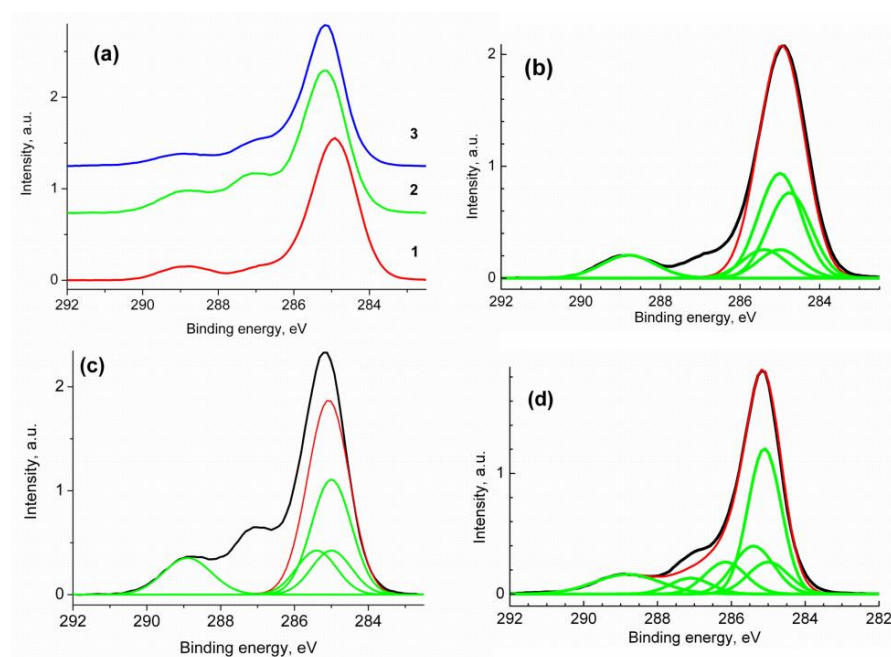

Figure 6. The high-resolution C 1s spectra normalized by the intensity of the main peak ((**a**) 1—C_{60}/SM, 2—C_{60}/EM, 3—C_{60}/VM), sample C_{60}/SM (**b**), sample C_{60}/EM (**c**) and sample C_{60}/VM (**d**). Black—experimental line, green—Gaussian profiles used for fitting, red—result of fitting.

The characteristics of the peaks are presented in Table 3. Based on the quantitative analysis data, the binding energies and Figure 6, it can be concluded that NMP impurity is present in all samples, mainly in the C_{60}/EM sample. The surface elemental compositions of the samples C_{60}/SM, C_{60}/EM and C_{60}/VM calculated from high-resolution XPS spectra are $C_{80.4}O_{18.2}Na_{0.5}N_{0.9}$, $C_{76.6}O_{21.4}Na_{0.7}N_{1.3}$ and $C_{82.4}O_{15.2}Na_{0.3}N_{2.1}$, respectively.

Table 3. Parameters of components in the C 1s photoelectron spectra of the studied samples: E_b—binding energy, W—Gaussian peak width and I_{rel}—relative intensity.

Sample	Group	-C=C-	CH$_2$	C$_{60}$	C*-C(O)	C-N	C(O)N	C(O)O
C_{60}/SM	E_b	284.76	285.0	285.0	285.4			288.0
	W	1.08	1.08	1.08	1.08			1.34
	I_{rel}	0.31	0.10	0.38	0.10			0.10
C_{60}/EM	E_b		285.0	285.0	285.4			288.9
	W		1.08	1.08	1.08			1.3
	I_{rel}		0.18	0.47	0.18			0.18
C_{60}/VM	E_b		285.0	285.0	285.4	288.16	287.1	288.8
	W		1.1	0.9	1.1	1.1	1.1	1.79
	I_{rel}		0.11	0.43	0.17	0.11	0.06	0.11

* Carbon atoms bonded to C(O) group.

The relative concentrations of C_{60} decrease in the sequence C_{60}/EM, C_{60}/VM and C_{60}/SM (Table 3). The -C=C- groups correspond to the benzene ring in the C_{60}/SM sample. CH_2 groups are present in all carbochain copolymers, as well as carboxyl groups of maleic acid residues -C(O)O (Table 3). Thus, an analysis of XPS spectra confirms the presence of fullerene in the resulting conjugates, as well as the composition of the samples.

The resulting composites were tested for antibacterial activity against conditionally pathogenic microorganisms by the serial microdilution method. We use *E. coli*—Gram-negative, facultative anaerobic bacteria; *S. aureus MRSA*—Gram-positive bacteria, resistant to oxacillin; *C. albicans*—yeast-like fungi; *P. aeruginosa*—aerobic non-fermenting Gram-negative bacteria and *E. faecalis*—Gram-positive, commensal bacterium as test microorganisms. At a sample concentration of 300–400 µg/mL, the bactericidal activity of the studied conjugates was not detected against all the strains of the microorganisms used. When using an increased concentration of C_{60}/VM up to 400 µg/mL, no activity against *C. albicans* was also observed. It may be noted that Japanese researchers showed carboxyfullerene (six carboxyl groups attached to fullerene C_{60}) to be effective in the treatment of both Gram-positive and Gram-negative infections in in vitro experiments in doses of 5–50 µg/mL [21]. At the same time, C_{60} fullerene in the form of a complex with polyvinylpyrrolidone does not affect the microflora as well as any of the tested microorganisms, including Gram-positive cocci, streptococci, enterococci and Gram-negative bacteria [22]. Although the resulting stabilized aqueous C_{60} dispersions did not exhibit antibacterial activity, their subsequent modification makes it possible to obtain a sample with a pronounced antimicrobial effect. When silver nanoparticles in complex with EM (EM/Ag^0) were introduced into the composite with fullerene, the MIC value in relation to *S. aureus* MSSA was 19.5 µg/mL or 6.7 µg/mL in terms of the silver content in the sample. For EM/Ag^0, the MIC value is 54.5 µg/mL or 23.4 µg/mL in terms of the silver content in this sample. Thus, the resulting aqueous C_{60} dispersions, on the one hand, are promising as potential non-toxic carriers of pharmacologically active substances, and on the other hand, they can be modified to impart antibacterial activity.

4. Conclusions

We have demonstrated the production of stable water-soluble composites of C_{60} containing significant amounts of fullerene. Available ionogenic amphiphilic carbon chain copolymers of maleic acid of a regular structure were used for stabilization and hydrophilization agents in such systems. The protocol for the preparation of C_{60} conjugates

eliminates the use of toxic organic solvents and time-consuming methods for their preparation, such as heating and prolonged stirring, ultrasonic treatment, etc. Such transformation is achieved under mild conditions when using the so-called "dialysis method" that is easily implemented. In a scalable version, a similar process, tangential ultrafiltration, can also be used. In the resulting nanostructures, guest molecules of C_{60} are confined by a polymer shell. The polymer shell provides the stabilization and solubility of encapsulated C_{60} in water through non-valent interactions—hydrophobic, steric and electrostatic. The resulting composites contained about 30% fullerene. The composite containing a more hydrophilic copolymer—poly(*N*-vinyl-pyrrolidone-*alt*-maleic acid) had better solubility in water. Although the composites had no activity as antibacterial agents, their fortification by introducing active bactericides, such as silver, can impart antimicrobial properties. The proposed approach may be used to obtain composites containing modified fullerene—for example, its amino acid adducts and other derivatives, which exhibit biological activity but poor solubility. There is the potential for using the obtained fullerene conjugates in other fields. The antioxidant properties of fullerene as part of a complex with non-toxic copolymers can be manifested in the creation of external products and can be used in cosmetology and dermatology [88].

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References

1. Prato, M. [60]Fullerene chemistry for materials science applications. *J. Mater. Chem.* **1997**, *7*, 1097–1109. [CrossRef]
2. Acquah, S.F.A.; Penkova, A.V.; Markelov, D.A.; Semisalova, A.S.; Leonhard, B.E.; Magia, J.M. The Beautiful Molecule: 30 Years of C60 and Its Derivatives. *ECS J. Solid State Sci. Technol.* **2017**, *6*, M3155–M3162. [CrossRef]
3. Chang, X.; Xu, Y.; Delius, M. Recent advances in supramolecular fullerene chemistry. *R. Soc. Chem. Chem. Soc. Rev.* **2024**, *53*, 47–83. [CrossRef]
4. Kramberger, C.; Begichev, I.; Burdanova, M.; Paukov, M.; Kharlamova, M.V. Functionalized Fullerenes and Their Applications in Electrochemistry, Solar Cells, and Nanoelectronics. *Materials* **2023**, *16*, 1276. [CrossRef]
5. Mumyatov, A.V.; Troshin, P.A. A Review on Fullerene Derivatives with Reduced Electron Affinity as Acceptor Materials for Organic Solar Cells. *Energies* **2023**, *16*, 1924. [CrossRef]
6. Kausar, A.; Ahmad, I.; Maaza, M.; Eisa, M.H. State-of-the-Art of Polymer/Fullerene C60 Nanocomposite Membranes for Water Treatment: Conceptions, Structural Diversity and Topographies. *Membranes* **2023**, *13*, 27. [CrossRef]
7. Lin, Y.; Li, Y.; Zhan, X. Small molecule semiconductors for high-efficiency organic photovoltaics. *Chem. Soc. Rev.* **2012**, *41*, 4245–4272. [CrossRef]
8. Buzea, C.; Pacheco, I. Chapter 11—State-of-the-art of polymer/fullerene nanocomposites in biomedical field. In *Micro and Nano Technologies, Polymer/Fullerene Nanocomposites*; Kausar, A., Ed.; Elsevier: Cambridge, MA, USA, 2023; pp. 211–250. [CrossRef]
9. Teradal, N.L.; Jelinek, R. Carbon Nanomaterials in Biological Studies and Biomedicine. *Adv. Healthc. Mater.* **2017**, *6*, 1700574. [CrossRef]
10. Shershakova, N.N.; Andreev, S.M.; Tomchuk, A.A.; Makarova, E.; Nikonova, A.; Turetskiy, E.A.; Petukhova, O.A.; Kamyshnikov, O.Y.; Ivankov, O.; Kyzyma, O.A.; et al. Wound healing activity of aqueous dispersion of fullerene C60 produced by "green technology". *Nanomed. Nanotechnol. Biol. Med.* **2023**, *47*, 102619. [CrossRef]
11. Cataldo, F.; Da Ros, T. (Eds.) *Medicinal Chemistry and Pharmacological Potential of Fullerenes and Carbon Nanotubes*; Springer: Dordrecht, The Netherlands, 2008; Volume 1, 408p.

12. Prylutska, S.V.; Burlaka, A.P.; Prylutskyy, Y.I.; Ritter, U.; Scharff, P. Pristine C(60) fullerenes inhibit the rate of tumor growth and metastasis. *Exp. Oncol.* **2011**, *33*, 162–164.

13. Injac, R.; Perse, M.; Cerne, M.; Potocnik, N.; Radic, N.; Govedarica, B.; Djordjevic, A.; Cerar, A.; Strukelj, B. Protective effects of fullerenol $C_{60}(OH)_{24}$ against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats with colorectal cancer. *Biomaterials* **2009**, *30*, 1184–1196. [CrossRef]

14. Andrievsky, G.V.; Burenin, I.S. On medicinal and preventive efficacy of small doses of hydrated C60 fullerenes at cancer pathologies. *Chem. Prepr. Arch.* **2002**, *6*, 53–68.

15. Yang, X.L.; Fan, C.H.; Zhu, H.S. Photo-induced cytotoxicity of malonic acid [C60] fullerene derivatives and its mechanism. *Toxicol. Vitr.* **2002**, *16*, 41–46. [CrossRef]

16. Theriot, C.A.; Casey, R.C.; Moore, V.C.; Mitchell, L.; Reynolds, J.O.; Burgoyne, M.; Partha, R.; Huff, J.L.; Conyers, J.L.; Jeevarajan, A.; et al. Dendro [C60] fullerene DF-1 provides radioprotection to radiosensitive mammalian cells. *Radiat. Environ. Biophys.* **2010**, *49*, 437–445. [CrossRef]

17. Shershakova, N.; Baraboshkina, E.; Andreev, S.; Purgina, D.; Struchkova, I.; Kamyshnikov, O.; Nikonova, A.; Khaitov, M. Antiinflammatory effect of fullerene C60 in a mice model of atopic dermatitis. *J. Nanobiotechnol.* **2016**, *14*, 1483–1493. [CrossRef]

18. Ma, H.; Liang, X.-J. Fullerenes as unique nanopharmaceuticals for disease treatment. *Sci. China Chem.* **2010**, *53*, 2233–2240. [CrossRef]

19. Andrievsky, G.V.; Bruskov, V.I.; Tykhomyrov, A.A.; Gudkov, S.V. Peculiarities of the antioxidant and radioprotective effects of hydrated C60 fullerene nanostuctures in vitro and in vivo. *Free Radical. Biol. Med.* **2009**, *47*, 786–793. [CrossRef]

20. Galvan, Y.P.; Alperovich, I.; Zolotukhin, P.; Prazdnova, E.; Mazanko, M.; Belanova, A.; Chistyakov, V. Fullerenes as Anti-aging Antioxidants. *Curr. Aging Sci.* **2017**, *10*, 56–67. [CrossRef]

21. Tsao, N.; Luh, T.-Y.; Chou, C.-K.; Chang, T.-Y.; Wu, J.-J.; Liu, C.-C.; Lei, H.-Y. In vitro action of carboxyfullerene. *J. Antimicrob. Chemother.* **2002**, *49*, 641–649. [CrossRef]

22. Aoshima, H.; Kokubo, K.; Shirakawa, S.; Ito, M.; Yamana, S.; Oshima, T. Antimicrobial activity of fullerenes and their hydroxylated derivatives. *Biocontrol. Sci.* **2009**, *14*, 69–72. [CrossRef]

23. Mashino, T.; Okuda, K.; Hirota, T.; Hirobe, M.; Nagano, T.; Mochizuki, M. Inhibition of E. coli growth by fullerene derivatives and inhibition mechanism. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2959–2962. [CrossRef]

24. Shoji, M.; Takahashi, E.; Hatakeyama, D.; Iwai, Y.; Morita, Y.; Shirayama, R.; Echigo, N.; Kido, H.; Nakamura, S.; Mashino, T.; et al. Anti-influenza Activity of C60 Fullerene Derivatives. *PLoS ONE* **2013**, *8*, e66337. [CrossRef]

25. Klimova, R.; Andreev, S.; Momotyuk, E.; Demidova, N.; Fedorova, N.; Chernoryzh, Y.; Yurlov, K.; Turetskiy, E.; Baraboshkina, E.; Shershakova, N.; et al. Aqueous fullerene C60 solution suppresses herpes simplex virus and cytomegalovirus infections. *Fuller. Nanotub. Carbon Nanostruct.* **2020**, *28*, 487–499. [CrossRef]

26. Ryan, J.; Bateman, H.R.; Stover, A.; Gomez, G.; Norton, S.K.; Zhao, W.; Schwartz, L.B.; Lenk, R.; Kepley, C.L. Fullerenes inhibit IgE-induced anaphylaxis in vivo. *J. Immunol.* **2007**, *179*, 665–672. [CrossRef]

27. Ngan, C.L.; Basri, M.; Tripathy, M.; Karjiban, R.A.; Abdul-Malek, E. Skin intervention of fullerene-integrated nanoemulsion in structural and collagen regeneration against skin aging. *Eur. J. Pharm. Sci.* **2015**, *70*, 22–28. [CrossRef]

28. Kyzyma, E.A.; Tomchuk, A.A.; Bulavin, L.A.; Petrenko, V.I.; Almasy, L.; Korobov, M.V.; Volkov, D.S.; Mikheev, I.V.; Koshlan, I.V.; Koshlan, N.A.; et al. Structure and Toxicity of Aqueous Fullerene C60 Solutions. *J. Surf. Investig. X-ray Synchrotron Neutron Tech.* **2015**, *9*, 1–5. [CrossRef]

29. Sayes, C.M.; Marchione, A.A.; Reed, K.L.; Warheit, D.B. Comparative pulmonary toxicity assessments of C60 water suspensions in rats: Few differences in fullerene toxicity in vivo in contrast to in vitro profiles. *Nano Lett.* **2007**, *7*, 2399–2406. [CrossRef]

30. Horie, M.; Nishio, K.; Kato, H.; Shinohara, N.; Nakamura, A.; Fujita, K.; Kinugasa, S.; Endoh, S.; Yamamoto, K.; Yamomoto, O.; et al. In vitro evaluation of cellular responses induced by stable fullerene C60 medium dispersion. *J. Biochem.* **2010**, *148*, 289–298. [CrossRef]

31. Bogdanović, V.; Stankov, K.; Icević, I.; Zikic, D.; Nikolić, A.; Solajić, S.; Djordjević, A.; Bogdanović, G. Fullerenol $C_{60}(OH)_{24}$ effects on antioxidative enzymes activity in irradiated human erythroleukemia cell line. *J. Radiat. Res.* **2008**, *49*, 321–327. [CrossRef]

32. Nakamura, E.; Isobe, H. Functionalized Fullerenes in Water. The First 10 Years of Their Chemistry, Biology, and Nanoscience. *Acc. Chem. Res.* **2003**, *36*, 807–815. [CrossRef]

33. Sitharaman, B.; Asokan, S.; Rusakova, I.; Wong, M.S.; Wilson, L.J. Nanoscale aggregation properties of neuroprotective carboxyfullerene (C_3) in aqueous solutions. *Nano Lett.* **2004**, *4*, 1759–1762. [CrossRef]

34. Hashikawa, Y.; Okamoto, S.; Murata, Y. Synthesis of inter-[60]fullerene conjugates with inherent chirality. *Nat. Commun.* **2024**, *15*, 514. [CrossRef]

35. Harris, P.J.F. Fullerene Polymers: A Brief Review. *J. Carbon Res.* **2020**, *6*, 71. [CrossRef]

36. Bouchard, D.; Ma, X.; Isaacson, C. Colloidal properties of aqueous fullerenes: Isoelectric points and aggregation kinetics of C60 and C70 derivatives. *Environ. Sci. Technol.* **2009**, *43*, 6597–6603. [CrossRef]

37. Labille, J.; Masion, A.; Ziarelli, F.; Rose, J.; Brant, J.; Villieras, F.; Pelletier, M.; Borschneck, D.; Wiesner, M.R.; Bottero, J.-Y. Hydration and Dispersion of C60 in Aqueous Systems: The Nature of Water-Fullerene Interactions. *Langmuir* **2009**, *25*, 11232–11235. [CrossRef]

38. Mchedlov-Petrossyan, N.O. Fullerenes in liquid media: An unsettling intrusion into the solution chemistry. *Chem. Rev.* **2013**, *113*, 5149–5193. [CrossRef]

39. Andrievsky, G.V.; Kosevich, M.V.; Vovk, O.M.; Shelkovsky, V.S.; Vashcenko, L.A. On the production of an aqueous colloidal solution of fullerenes. *J. Chem. Soc. Chem. Comm.* **1995**, *12*, 1281–1282. [CrossRef]

40. Maples, R.D.; Hilburn, M.E.; Murdianti, B.S.; HikkaduwaKoralege, R.S.; Williams, J.S.; Kuriyavar, S.I.; Ausman, K.D. Optimized solvent-exchange synthesis method for C60 colloidal dispersions. *J. Colloid. Interface Sci.* **2012**, *370*, 27–31. [CrossRef]

41. Konarev, D.V.; Litvinov, A.L.; Kovaltvsky, A.Y.; Drichko, N.V.; Coppens, R.N.; Lubovskaya, R.N. Molecular complexes of fullerene C60 with aromatic hydrocarbons: Crystal structures of (TPE)2C60 and DPA·C60. *Synth. Met.* **2003**, *133–134*, 675–677. [CrossRef]

42. Ikeda, A.; Iizuka, T.; Maekubo, N.; Aono, R.; Kikuchi, J.-I; Akiyama, M.; Konishi, T.; Ogawa, T.; Ishida-Kitagawa, N.; Tatebe, H.; et al. Cyclodextrin complexed [60]fullerene derivatives with high levels of photodynamic activity by long wavelength excitation. *ACS Med. Chem. Lett.* **2013**, *4*, 752–756. [CrossRef]

43. Murthya, C.N.; Geckeler, K.E. The water-soluble β-cyclodextrin–[60]fullerene complex. *Chem. Commun.* **2001**, *13*, 1194–1195. [CrossRef]

44. Nierengarten, J.F. Supramolecular encapsulation of [60]fullerene with dendritic cyclotriveratrylene derivatives. *Fuller. Nanotub. Carbon Nanostruct.* **2005**, *13*, 229–242. [CrossRef]

45. Hirsch, A. Amphiphilic architectures based on fullerene and calixarene platforms: From buckysomes to shape-persistent micelles. *Pure Appl. Chem.* **2008**, *80*, 571–587. [CrossRef]

46. Komatsu, T.; Nakagawa, A.; Qu, X. Structural and mutagenic approach to create human serum albumin-based oxygen carrier and photosensitizer. *Drug Metab. Pharmacokinet.* **2009**, *24*, 287–299. [CrossRef]

47. Liu, S.; Sui, Y.; Guo, K.; Yin, Z.; Gao, X. Spectroscopic study on the interaction of pristine C60 and serum albumins in solution. *Nanoscale Res. Lett.* **2012**, *7*, 433. [CrossRef]

48. Ikeda, A.; Doi, Y.; Nishiguchi, K.; Kitamura, K.; Hashizume, M.; Kikuchi, J.-I.; Yogo, K.; Ogawa, T.; Takeya, T. Induction of cell death by photodynamic therapy with water-soluble lipid-membrane-incorporated [60]fullerene. *Org. Biomol. Chem.* **2007**, *5*, 1158–1160. [CrossRef]

49. Torres, V.M.; Posa, M.; Srdjenovic, B.; Simplício, A.L. Solubilization of fullerene C60 in micellar solutions of different solubilizers. *Colloids Surf. B Biointerfaces* **2011**, *82*, 46–53. [CrossRef]

50. Ramakanth, I.; Patnaik, A. Characteristics of solubilization and encapsulation of fullerene C60 in non-ionic Triton X-100 micelles. *Carbon* **2008**, *46*, 692–698. [CrossRef]

51. Jeng, U.-S.; Hsu, C.-H.; Lin, T.-L.; Wu, C.-M.; Chen, H.-L.; Tai, L.-A.; Hwang, K.-C. Dispersion of fullerenes in phospholipid bilayers and the subsequent phase changes in the host bilayers. *Phys. B Condens. Matter.* **2005**, *357*, 193–198. [CrossRef]

52. Ikeda, A. Water-soluble fullerenes using solubilizing agents, and their applications. *J. Incl. Phenom. Macrocycl. Chem.* **2013**, *77*, 49–65. [CrossRef]

53. Wang, X.S.; Metanawin, T.; Zheng, X.Y.; Wang, P.Y.; Ali, M.; Vernon, D. Structure-defined c60/polymer colloids supramolecular nanocomposites in water. *Langmuir* **2008**, *24*, 9230–9232. [CrossRef]

54. Laiho, A.; Ras, R.H.A.; Valkama, S.; Ruokolainen, J.; Osterbaska, R.; Ikkala, O. Control of Self-Assembly by Charge-Transfer Complexation between C60 Fullerene and Electron Donating Units of Block Copolymers. *Macromolecules* **2006**, *39*, 7648–7653. [CrossRef]

55. Tabata, Y.; Murakami, Y.; Ikada, Y. Photodynamic effect of polyethylene glycol-modified fullereneon tumor. *Jpn. J. Cancer Res.* **1997**, *88*, 1108–1116. [CrossRef]

56. Metanawin, T.; Tang, T.; Chen, R.; Vernon, D.; Wang, X. Cytotoxicity and photocytotoxicity of structure-defined water-soluble C60/micelle supramolecular nanoparticles. *Nanotecnology* **2011**, *22*, 235604. [CrossRef]

57. Pakhomova, V.A.; Gordon, D.A.; Mikhailov, A.I. Synthesis of Water-Soluble Fullerene-Containing Vinyl Polymers by Low-Temperature Radiation-Induced Living Polymerization. *Polym. Sci. Ser. A* **2006**, *48*, 689–695. [CrossRef]

58. Behera, M.; Ram, S. Solubilization and stabilization of fullerene C60 in presence of poly(vinyl pyrrolidone) molecules in water. *J. Incl. Phenom. Macrocycl. Chem.* **2012**, *72*, 233–239. [CrossRef]

59. Ungurenasu, C.; Airinei, A. Highly stable C(60)/poly(vinylpyrrolidone) charge-transfer complexes afford new predictions for biological applications of underivatized fullerenes. *J. Med. Chem.* **2000**, *43*, 3186–3188. [CrossRef]

60. Krasnou, I.; Tarabukina, E.; Melenevskaya, E.; Filippov, A.; Aseyev, V.; Hietala, S.; Tenhu, H. Rheological Behavior of Poly(vinylpyrrolidone)/Fullerene C60 Complexes in Aqueous Medium. *J. Macromol. Sci. Ser. B* **2008**, *47*, 500–510. [CrossRef]

61. Vidanapathirana, A.K.; Thompson, L.C.; Mann, E.E.; Odom, J.T.; Holland, N.A.; Sumner, S.J.; Han, L.; Lewin, A.H.; Fennell, T.R.; Brown, J.M.; et al. PVP formulated fullerene (C60) increases Rho-kinase dependent vascular tissue contractility in pregnant Sprague Dawley rats. *Reprod. Toxicol.* **2014**, *49*, 86–100. [CrossRef]

62. Yamakoshi, Y.N.; Yagami, T.; Fukuhara, K.; Sueyoshia, S.; Miyata, N. Solubilization of Fullerenes into Water with Polyvinylpyrrolidone Applicable to Biological Tests. *J. Chem. Soc. Chem. Commun.* **1994**, *4*, 517–518. [CrossRef]

63. Samoilova, N.; Kurskaya, E.; Krayukhina, M.; Askadsky, A.; Yamskov, I. Copolymers of Maleic Acid and Their Amphiphilic Derivatives as Stabilizers of Silver Nanoparticles. *J. Phys. Chem. B* **2009**, *113*, 3395–3403. [CrossRef]

64. Samoilova, N.A.; Krayukhina, M.A.; Naumkin, A.V.; Korlyukov, A.A.; Anuchina, N.M.; Popov, D.A. Polymer-Stabilized Silver (Gold)-Zinc Oxide Nanoheterodimer Structures as Antimicrobials. *Appl. Sci.* **2023**, *13*, 11121. [CrossRef]

65. Samoilova, N.A.; Krayukhina, M.A.; Korlyukov, A.A.; Klemenkova, Z.S.; Naumkin, A.V.; Mezhuev, Y.O. One-Pot Synthesis of Colloidal Hybrid Au (Ag)/ZnO Nanostructures with the Participation of Maleic Acid Copolymers. *Polymers* **2023**, *15*, 1670. [CrossRef]

66. Andreev, S.; Purgina, D.; Bashkatova, E.; Garshev, A.; Maerle, A.; Andreev, I.; Osipova, N.; Shershakova, N.; Khaitov, M. Study of Fullerene Aqueous Dispersion Prepared by Novel Dialysis Method: Simple Way to Fullerene Aqueous Solution. *Fuller. Nanotub. Carbon Nanostruct.* **2015**, *23*, 792–800. [CrossRef]
67. Conix, A.; Smets, G. Ring opening in lactam polymers. *J. Polym. Sci.* **1955**, *15*, 221–229. [CrossRef]
68. Cockerill, F.R.; Wikler, M.A.; Alder, J.; Dudley, M.N.; Eliopoulos, G.M.; Ferraro, M.J.; Hardy, D.J.; Hecht, D.W.; Hindler, J.A.; Patel, J.B.; et al. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard—Ninth Edition*; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2012; Volume 32, M07-A9.
69. Samoilova, N.; Krayukhina, M.; Naumkin, A.; Anuchina, N.; Popov, D. Silver nanoparticles doped with silver cations and stabilized with maleic acid copolymers: Specific structure and antimicrobial properties. *New J. Chem.* **2021**, *45*, 14513–14521. [CrossRef]
70. Jouyban, A.; Fakhree, M.A.A.; Shayanfar, A. Review of pharmaceutical applications of N-Methyl-2-pyrrolidone N-methyl-2-pyrrolidone. *J. Pharm. Pharmaceut. Sci.* **2010**, *13*, 524–535. [CrossRef]
71. Andrievsky, G.V.; Klochkov, V.K.; Bordyuh, A.B.; Dovbeshko, G.I. Comparative analysis of two aqueous-colloidal solutions of C60 fullerene with help of FTIR reflectance and UV-Vis spectroscopy. *Chem. Phys. Lett.* **2002**, *364*, 8–17. [CrossRef]
72. Yevlampieva, N.P.; Biryulin, Y.F.; Melenevskaja, E.Y.; Zgonnik, V.N.; Rjumtsev, E.I. Aggregation of fullerene C60 in N-methylpyrrolidone. *Colloids Surf. A* **2002**, *209*, 167–171. [CrossRef]
73. Kyzyma, O.A.; Korobov, M.V.; Avdeev, M.V.; Garamus, V.M.; Petrenko, V.I.; Aksenov, V.L.; Bulavin, L.A. Solvatochromism and Fullerene Cluster Formation in C60/N-methyl-2-pyrrolidone. *Fuller. Nanotub. Carbon Nanostruct.* **2010**, *18*, 458–461. [CrossRef]
74. Rivelino, R.; Maniero, A.M.; Prudente, F.V.; Costa, L.S. Theoretical calculations of the structure and UV–vis absorption spectra of hydrated C60 fullerene. *Carbon* **2006**, *44*, 2925–2930. [CrossRef]
75. Bensasson, R.V.; Bienvenue, E.; Dellinger, M.; Leach, S.; Seta, P. C60 in model biological systems. A visible-UV absorption study of solvent-dependent parameters and solute aggregation. *J. Phys. Chem.* **1994**, *98*, 3492–3500. [CrossRef]
76. Vinogradova, L.V.; Melenevskaya, E.Y.; Khachaturov, A.S.; Zgonnik, V.N.; Kever, E.E.; Litvinova, L.S.; Novokreshchenova, A.V.; Sushko, M.L.; Klenin, S.I. Water-soluble complexes of C60 fullerene with poly(N-vinylpyrrolidone). *Polym. Sci. Ser. A* **1998**, *40*, 1152–1159.
77. Ghosh, H.N.; Sapre, A.V.; Mittal, J.P. Aggregation of C70 in solvent mixtures. *J. Phys. Chem.* **1996**, *100*, 9439–9443. [CrossRef]
78. Mrzel, A.; Mertelj, A.; Omerzu, A.; Čopič, M.; Mihailovic, D. Investigation of encapsulation and solvatochromism of fullerenes in binary solvent mixtures. *J. Phys. Chem. B* **1999**, *103*, 11256–11260. [CrossRef]
79. Prylutskyy, Y.I.; Durov, S.S.; Bulavin, L.A.; Adamenko, I.I.; Moroz, K.O.; Geru, I.I.; Dihor, I.N.; Scharff, P.; Eklund, P.C.; Grigorian, L. Structure and Thermophysical Properties of Fullerene C60 Aqueous Solutions. *Int. J. Thermophys.* **2001**, *22*, 943–956. [CrossRef]
80. Samoilova, N.A.; Krayukhina, M.A.; Klimova, T.P.; Babushkina, T.A.; Vyshivannaya, O.V.; Blagodatskikh, I.V.; Yamskov, I.A. Oxidation of glucose to gluconic acid using a colloidal catalyst containing gold nanoparticles and glucose oxidase. *Russ. Chem. Bull.* **2014**, *63*, 1009–1016. [CrossRef]
81. Samoilova, N.A.; Krayukhina, M.A.; Vyshivannaya, O.V.; Blagodatskikh, I.V. Investigation of the Binding of Lectins with Polymer Glycoconjugates and the Glycoconjugates Containing Silver Nanoparticles by Means of Optical Spectroscopy and Light Scattering. *Polym. Sci. Ser. A* **2022**, *64*, 267–279. [CrossRef]
82. Saraswati, T.E.; Setiawan, U.H.; Ihsan, M.R.; Isnaeni, I.; Herbani, Y. The Study of the Optical Properties of C60 Fullerene in Different Organic Solvents. *Open Chem.* **2019**, *17*, 1198–1212. [CrossRef]
83. Krayukhina, M.A.; Samoilova, N.A.; Yamskov, I.A. Complexation of chitosan with maleic acid copolymers. *J. Polym. Sci. Ser. A* **2010**, *52*, 243–250. [CrossRef]
84. Stubenrauch, C. Emulsions, Foams and Suspensions. Fundamentals and Applications. By Schramm, L.L. *Chem. Phys. Chem.* **2006**, *7*, 965. [CrossRef]
85. Fabian, J. Theoretical investigation of the C60 infrared spectrum. *Phys. Rev. B* **1996**, *53*, 13864–13870. [CrossRef]
86. Pikhurov, D.V.; Zuev, V.V. The Effect of Fullerene C60 on the Mechanical and Dielectrical Behavior of Epoxy Resins at Low Loading. *AIP Conf. Proc.* **2014**, *1599*, 453–456. [CrossRef]
87. Beamson, G.; Briggs, D. *High Resolution XPS of Organic Polymers: The Scienta ESCA300 Database*; John Wiley & Sons: Hoboken, NJ, USA, 1992.
88. Mousavi, S.Z.; Nafisi, S.; Maibach, H.I. Fullerene nanoparticle in dermatological and cosmetic applications. *Nanomed. Nanotechnol. Biol. Med.* **2017**, *13*, 1071–1087. [CrossRef]