Chapter 7

Congo Red Interactions with Single-Walled Carbon Nanotubes

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Abstract A new method of dispersion of single-wall carbon nanotubes (SWNT) in aqueous solution using supramolecular compounds is proposed in this chapter. The described system consists of SWNT overlaid by Congo red. SWNT are formed from a rolled layer of graphene, providing a large surface area for binding compounds with planar, aromatic structures (including drugs). Congo red is able to associate with proteins in the form of supramolecular, ribbon-like structures, and may bind various drugs by intercalation.

The study reveals strong interactions between Congo red and the surface of SWNT. The authors' aim was to explain the mechanism driving this interaction. Spectral analysis of the SWNT-CR complex, effects of sonication on CR binding, microscopic imaging and molecular modelling analyses are all discussed. Results indicate that binding of supramolecular Congo red to the surface of nanotubes is based on face-to-face stacking. Having attached itself to the surface of a nanotube, a dye molecule may attract other similarly oriented molecules, giving rise to a protruding supramolecular appendage. This explains the high affinity of CR for nanotubes and the resulting system's capability to bind drugs.

Analysis of complexes formed by SWNT-CR with the model drug (DOX) and with other planar compounds (EB, TY) indicates that it may be possible to construct complexes capable of binding multiple compounds simultaneously.

Keywords Single-walled carbon nanotubes • Supramolecular compounds • Bis-azo dye • Congo red • Chemical container • Drug delivery system • Shortening of carbon nanotubes • Pi-pi stacking • Face-to-face stacking

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7.1 Methods of Dispersing Carbon Nanotubes – The Search for Optimal Dispersion Methods

Carbon nanotubes (CNT) are used in electronics [1], composite materials research [2, 3], catalysis [4], textiles [5] and many other areas. A particularly interesting is their use in the field of biomedicine: in biosensors [6, 7], medical diagnostics [8, 9], transplantology [10, 11], tissue engineering [12] or in pharmacology – including the field of targeted therapies [13–16].

In studies on the use of CNT in biomedicine it is necessary to obtain their suspension in water media. CNT are most often found not in the form of single fibres, but in the form of bundles stabilized by van der Waals interactions and it is very difficult to evenly distribute them in the liquid phase. The purpose of research is thus to find the compounds that provide effective dispersion of carbon nanotubes and simultaneously bind other compounds, including some drugs. Numerous reports concern both covalent or non-covalent modifications of CNT [17].

The covalent modification based on the introduction of functional groups into the graphene surface of carbon nanotubes is an effective way to increase CNT dispersion. This may, however, lead to changes in the physicochemical properties of CNT and create defects in their structure [18–20].

Non-covalent modification is considered a less invasive method. Because of the fact, that there is no heating or acidic environment involved, virtually no damage is done to the structure of nanotubes [8, 21]. Among non-covalent CNT modifications one can distinguish: interactions with amphiphilic molecules – surfactants (e.g. SDS – sodium dodecyl sulphate, SDBS – sodium dodecylbenzenesulphonate, CTAB – cetyltrimethylammonium bromide, DDAB – dimethyldioctadecylammonium bromide, Triton, Pluronic [22]). The most effective interactions between CNTs and surfactants were observed for surfactants containing a benzene ring in the structure (e.g. SDBS), due to the presence of strong pi-pi stacking interactions between the phenyl ring and the graphene nanotube surface [23].

Other compounds used for the noncovalent functionalization and dispersing of CNT include: ionic liquids (ILs) – which form bucky gels, polymers – especially those with aromatic rings in their structure [24], other compounds with an aromatic ring [25] (for example ammonium salts containing pyrene rings [26]) and or deoxycholic acid sodium salts [27].

7.2 The Interaction of Congo Red with Carbon Nanotubes

CR, a bis-azo dye, similarly to the carbon nanotube dispersing compounds mentioned above, has aromatic rings in its structure. It is therefore possible to form a non-covalent pi-pi interaction between CR molecules and the graphene surface of carbon nanotubes. Reports can be found in literature on the interaction of CR with carbon nanotubes [28–31] and the surface of graphene oxide [32, 33].

Fig. 7.1 Binding of an increasing amount of CR by 1 mg of SWNT. (1,2,3,4 – samples)

Hu C. et al. [28] describe a complex of single-walled carbon nanotubes (SWNT) with CR (SWNT-CR), with water solubility of up to 3.5 mg/mL. The described complex was formed by prolonged grinding of SWNT and CR in the agate mortar, followed by drying. In this case CR probably interacts with the surface of nanotubes as single molecules bound to the nanotube surface via pi-pi interactions. The described method enables nanotubes dispersion, however the results may not be reproducible and the CR binding effectiveness is relatively low.

In another paper [31] authors point to the possibility of using CNT for adsorbing dyes from aqueous solutions – in post-production wastes containing textile dyes. CR was used as a model compound in these studies and the results show an effective binding of CR by carbon nanotubes (with a maximum adsorption of 500 mg/g).

The SWNT dispersion method that was used in our studies is based on the sonication of SWNT with CR solution and is much simpler and reproducible than the above described method [34]. It leads to the formation of complexes between graphene surfaces of carbon nanotubes and supramolecular ribbon-like structures created by CR molecules. An attempt was made to explain the mechanism of interaction between the ribbon-like supramolecular compounds like CR and the single-walled carbon nanotubes. The results indicate a very efficient binding of CR by SWNT (up to 35 mg CR per 1 mg of SWNT) (Fig. 7.1).

In contrast to the method proposed by Hu et al. the samples are not dried, which allows for efficient binding of CR – in the form of large supramolecular structures – to the SWNT surface. A model of SWNT-CR complex has been proposed based on a “face to face” interaction between CR and the carbon nanotube surface. In this model, the SWNT play a role of a scaffolding to the surface of which CR molecules are bound in the form of supramolecular, ribbon-like structures that protrude from the surface of the carbon nanotube. The nanotube-CR interaction involves the pi-pi stacking between the benzene rings (Fig. 7.2), as in the case of aforementioned surfactants.

The supramolecular character of CR bound to the carbon nanotube surface is indicated by the results obtained during the analysis of SWNT-CR complexes

Fig. 7.2 Model of interaction between single CR molecule (*left*) and supramolecular CR (*right*) with the SWNT surface (based on calculations presented in [30])

Fig. 7.3 Binding of CR to carbon nanotubes in solutions of different ionic strength. 0.05 M Tris/HCl buffer pH7.4 without NaCl or with 0.145 M NaCl. The total amount of CR added to the sample was taken as 100%

formed in solutions of various ionic strengths. The properties of CR as a supramolecular system depend on the ionic strength of the solution [35]. If the ionic strength is low, then the ability of CR to form a supramolecular structure is also low, as a result of the lack of shielding of the negatively charged sulphonic groups by Na^+ ions. In contrast, at high salt concentration the supramolecular structure is stabilized. In order to explain if single CR molecules are involved in the SWNT-CR interaction or whether the supramolecular structures are required, an analysis of the SWNT-CR interaction in solutions of various ionic strengths was performed. All samples were prepared in the 0.05 M Tris/HCl pH7.4 buffer. The significant differences in CR binding between samples to which 0.145 M NaCl was added and the NaCl-free samples were observed (Fig. 7.3).

Fig. 7.4 Birefringence of the CR bound on SWNT (polarized light microscopy)

Additional information concerning the mechanism of CR- nanotube interaction is provided by the birefringence of CR-nanotube complexes observed using polarized light microscopy. This phenomenon, which is typical for the amyloid containing preparations stained with CR, proves that the CR molecules bound to the fibres are ordered [36]. The presence of “apple-green” birefringence in histopathological samples is a diagnostic criterion for amyloidosis. In the case of the SWNT-CR complexes the birefringence also demonstrates the ordering of CR molecules bound to the carbon nanotube surface (Fig. 7.4) and supports the supramolecular, ribbon-like character of CR interacting with the carbon nanotube.

Samples containing carbon nanotubes sonicated with highly concentrated CR (above 5 mg/mL), presented gel-like properties. CR in these samples was bound to SWNT strongly enough not to be removed during filtration through the PTFE membrane (non-bonded CR can pass freely through this membrane) (Fig. 7.5A). SWNT-CR complexes in these samples slowly sedimented at the bottom of the test tube but could be easily dispersed even by gentle mixing (Fig. 7.5C). The formation of such big, sedimenting complexes can be explained by the presence of CR ribbons, which protrude from the surface of nanotubes and cross-link them to form stable gels, as in the case of *bucky gels* resulting from the interaction between CNT and ionic liquids (room-temperature ionic liquids, RILs) [24, 37]. In the case of solutions containing a lower concentration of CR the resulting solution is clearer, but also tends to separate over a longer period of time (Fig. 7.5B).

Spectral analyses have shown changes in the CR spectrum after binding to SWNT, demonstrating the interaction between CR and carbon nanotubes (Fig. 7.6). This effect is related to the change in the electron structure of the CR molecule after binding to carbon nanotubes and is explained by the transition of the CR molecule to the quinoid form [34, 38]. A similar phenomenon is described in the case of binding CR to the surface of graphene oxide [32]. According to the mechanism of the interaction between CR and SWNT described in our paper, not all molecules in the CR ribbon-like structure adhere directly to the surface of the nanotube, while the

Fig. 7.5 (A) SWNT-CR complexes – filtration on PTFE membrane; (B) SWNT-CR complex obtained from 1 mg of SWNT and 2 mg CR, after filtration, suspended in 1 mL of the buffer; (C) SWNT-CR complex obtained from 1 mg of SWNT and 5 mg CR, after filtration, suspended in 1 mL of the buffer; the *dashed line* indicates the sedimentation of the gel-like complex

Fig. 7.6 Absorption spectra of free CR and CR complexed with SWNT – maximum of the absorbance shifts from 490 to 530 nm

observed spectral effect – the shift of the absorbance maximum from 490 to 510 nm – suggests that all CR molecules (not only those that directly adhere to the nanotube surface) participate in the transfer of electrons (and switch to the quinoid form) [34].

The SWNT-CR complexes were studied using a variety of microscopic techniques. All analyses have shown that CR binding to the surface of the carbon nano-

Fig. 7.7 TEM micrographs of (A) a single SWNT, without CR; (B) a single SWNT functionalized with CR (obtained from 1 mg of SWNT and 10 mg CR, after filtration, suspended in 1 mL of the buffer) – CR layers evenly distributed at the surface of a carbon nanotube are visible (*dark lines* indicate the thickness of CR layer)

tubes significantly increased their diameter. Depending on the amount of CR added per portion of single-walled carbon nanotubes more or less uniformly loaded nanotubes were obtained [34]. The TEM, SEM and AFM images of the SWNT-CR complexes confirm that the interaction between CR and carbon nanotube surface is based on the “face to face” stacking. A comparison of transmission microscopy images of a free carbon nanotube (Fig. 7.7A) and a CR-loaded nanotube (Fig. 7.7B) shows a sixfold increase in nanotube diameter after CR binding. The image shows that addition of highly concentrated CR (10 mg/mL) leads to a uniform covering of the carbon nanotube with CR. This can be interpreted as a large number of short, “face to face” bound CR “ribbons”, attached side by side to the carbon nanotube.

The presence of supramolecular CR ribbons that protrude from the surface of carbon nanotubes was confirmed by the results obtained using the ternary complex, composed of CR, Titan yellow (TY) and silver ions (CR/TY/Ag) (see Chap. 6). Titan Yellow-silver ion complex (TY/Ag) intercalates between CR molecules within a supramolecular ribbon. This system was developed because CR molecules do not complex metal ions and thus cannot be directly used for contrasting in TEM images [39, 40]. Thanks to the use of silver contrast, the binding sites of the ternary complex have been made visible. EDS (energy dispersive spectroscopy) analysis of chemical composition, indicated the presence of silver and sulphur in these sites, which confirms the presence of both CR and TY/Ag. Electronically dense silver ions are visible primarily in places of higher CR/TY loading. Contrasting with silver ions also allowed to confirm the mechanism of supramolecular CR ribbons interaction with carbon nanotubes – silver is not directly attached to the carbon nanotube surface, but is bound to the dye that forms a sheath around the nanotube (Fig. 7.8).

Fig. 7.8 Complexes of SWNT-CR contrasted by intercalated Titan Yellow-silver ion complexes (obtained from 1 mg of SWNT and 5 mg CR/TY/Ag (1:1:0.8 molar ratio), after removal of dyes and Ag excess by filtration). The EDS analysis indicates the presence of CR-TY-Ag complexes in highly loaded places (indicated by arrows)

Fig. 7.9 SEM images of (A) SWNT and (B) SWNT-CR complex (obtained from 1 mg of SWNT and 5 mg CR, after filtration)

The images obtained by scanning electron microscopy (Fig. 7.9) and atomic force microscopy [34] indicate an increase in carbon nanotube diameter after CR binding to its surface. Unevenly distribution of dye on nanotubes is seen on Fig. 7.9B.

The SWNT-CR complexes were also analysed by molecular modelling, which has shown various possibilities of nanotube-CR interaction depending on nanotube diameter [30]. In the case of narrow nanotubes, CR partially retains the supramolecular ribbon structure, adhering to the outer nanotube surface, while some of the molecules are adsorbed individually on the surface (as shown in Fig. 7.2). In the case of wider nanotubes, individual CR molecules adsorb to the nanotube surface

and supramolecular ribbon-like structure of CR is lost. The geometry of the wide nanotube increases the contact surface between CR molecule and the nanotube which allows to ascribe the dominant role in the creation of the system's equilibrium structure to the pi-pi stacking. Nanotubes of smaller diameter have a smaller side wall area that is characterized by a greater curvature. For this reason, partial preservation of the supramolecular ribbon structure of CR is preferable. The molecular modelling results show a tendency for CR to group into ribbons, while simultaneously leaving a portion of the nanotube surface exposed, which was also observed in microscopic images. These results were also confirmed by analysis of the radial distribution functions (rdf) between CR and SWNT [30].

7.3 The Incorporation of Other Compounds by SWNT-CR Complexes – Examples of Possible Biomedical Use

A particularly interesting feature of carbon nanotube-CR complex is its ability to bind other molecules. CNT, as hollow structures, are excellent high volume lightweight containers, in which other molecules can be enclosed while their large surface allows for efficient adsorption of many compounds. CR (and similar compounds) that create ribbon-like supramolecular structures can bind numerous polyaromatic planar molecules that intercalate into supramolecular CR ribbon. Compounds that can be bound this way include e.g. antineoplastic drug doxorubicin, Titan yellow, rhodamine B. The combination SWNT and CR creates a “chemical container” characterized by significantly increased capacity and the ability to bind different molecules simultaneously. It could be used as a drug carrier, which limits the drug's toxicity and allows for the targeted delivery [41].

An interesting phenomenon of breaking carbon nanotubes resulting from the interaction with mixed supramolecular systems was observed. Mixed supramolecular systems (CR/EB and CR-DOX) interact with carbon nanotubes causing them to stiffen and break (Fig. 7.10). Dispersion of nanotubes by CR is not accompanied by their shortening. Similar dye – EB shows much lower nanotube dispersing capabil-

Fig. 7.10 Carbon nanotubes shortened after interaction with the mixed supramolecular complexes of CR/EB

ity and does not interact with their surface [42]. The effect of nanotube breaking can be explained by the mechanical weakening of the nanotube in the area where a large mixed supramolecular system is attached. This phenomenon can also be explained by changes in the electron structure of the nanotube and the supramolecular system bound to it.

The dispersion of carbon nanotubes based on their interaction with CR is simple, efficient and reproducible. Supramolecular systems (both pure CR and mixed) allow not only to disperse carbon nanotubes, but can also bind other compounds through intercalation. As carriers of different compounds (e.g. fluorescent dyes, drugs, metal ions), these complexes present an interesting alternative to the currently used systems. They can be used in diagnostics as well as targeted delivery systems for drugs or metal ions.

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