Polymer 51 (2010) 2465–2471

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

UV-assisted grafting of polymers: A method towards biocompatible carbon nanotubes

Petar Petrov [a,*], Georgi Georgiev [a], Denica Momekova [b], Georgi Momekov [b], Christo B. Tsvetanov [a]

[a] Institute of Polymers, Bulgarian Academy of Sciences, Akad. G. Bonchev Street 103A, 1113 Sofia, Bulgaria
[b] Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav Street, 1000 Sofia, Bulgaria

ARTICLE INFO

Article history:
Received 1 February 2010
Received in revised form
15 April 2010
Accepted 15 April 2010
Available online 22 April 2010

Keywords:
Carbon nanotubes
Biocompatible polymers
UV irradiation

ABSTRACT

A strategy for covalent grafting of biocompatible polymers onto sidewalls of multi-walled carbon nanotubes (MWNTs) via UV-initiated free-radical polymerization is presented. The effects of the irradiation doze(time) and monomer/MWNTs ratio on the stability of the corresponding aqueous dispersions were investigated. It was found that stable dispersions of MWNTs modified with polyacrylamide, poly(*N*-isopropylacrylamide), poly[poly(ethylene glycol) methacrylate] and poly(sodium methacrylate) can be obtained by irradiation with UV light for at least 5 min at an irradiation dose rate of 5.7 J/cm^2 min at a minimum monomer/CNTs ratio of 200:1. Biocompatibility of polymer-modified MWNTs was assessed using the standard MTT-dye reduction assay and compared to pristine MWNTs. As a rule, all polymer-functionalized nanotubes examined in this study were non-cytotoxic up to concentration 150 μg/mL and, remarkably, MWNTs-g-PNIPAAm did not exhibit cytotoxicity even at the highest concentration studied (300 μg/mL). MWNTs modified with stimuli-sensitive polymers underwent a reversible transition from well-dispersed nanotubes in water to precipitate triggered by changes in temperature or pH.

1. Introduction

Carbon nanotubes (CNTs) [1] have attracted much attention in various disciplines due to the unique properties and potential for application in electronic devices, field emission display, hydrogen storage, polymeric composites, etc. [2]. The pristine CNTs, however, are entangled bundles that interact mutually via van der Waals forces which hampers their dispersibility in liquids and processing. Functionalization or surface modification of carbon nanotubes seems to be the best approach for improving their dispersibility in a wide range of solvents and the performance of the nanocomposite materials [3]. In particular, the modification of carbon nanotubes enables preparation of stable aqueous dispersions and, on the other hand, can overcome the apparent cytotoxicity of non-modified CNTs [4,5] that makes these materials of special interest for biochemical and biomedical applications [6,7,8,9]. For instance, CNTs have been solubilized in water either by supramolecular complexation with starch [10], Gum Arabic [11] and η-cyclodextrin [12] or by covalent attachments of glucosamine [13], nucleic acids [14], amino acids [15] and proteins [16]. The functionalization of CNTs with synthetic hydrophilic macromolecules is of particular interest, since this is a facile route to obtain stable aqueous dispersions of CNTs even at a low degree of functionalization. Water-soluble polymers like poly(ethylene glycol) [17], poly(vinyl alcohol) [18], etc., have been successfully attached onto CNTs by the "grafting to" method. Atom transfer radical polymerization (ATRP) technique has been employed to obtain poly(acrylic acid) and polystyrene-*block*-poly(acrylic acid) grafted onto the surface of CNTs by the "grafting from" approach [19,20]. Based on $\pi-\pi$ stacking between CNTs and pyrene moieties, water-soluble multi-walled carbon nanotubes (MWNTs) have been prepared by side-wall functionalization of full-length MWNTs with pyrene-carrying poly(2-dimethylaminoethyl methacrylate) [21]. Beside the successful modification, most of the abovementioned studies involve multistep procedures, sometimes the preparation protocols are rigorous and tedious. Meanwhile, techniques that allow grafting of hydrophilic polymers onto CNTs in a simpler and more economical manner are still limited. Recently, gamma-ray irradiation has been exploited for covalent grafting of poly(acrylic acid) onto MWNTs [22]. Despite the fact that the length of grafted chains cannot be controlled precisely, this method enables an effective grafting of polymer onto CNTs in a simple way and can be easily developed for large-scale production of water-soluble CNTs.

This work aims at presenting a facile strategy for preparation of biocompatible water-soluble MWNTs based on the UV irradiation

* Corresponding author. Tel.: +35929792281; fax: +35928700309.
E-mail address: ppetrov@polymer.bas.bg (P. Petrov).

0032-3861/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2010.04.033

technique. Various hydrophilic and temperature-responsive polymers were covalently attached onto full-length multi-walled carbon nanotubes via UV-initiated free-radical polymerization. The cytotoxicity of polymer-grafted MWNTs was assessed by means of MTT-dye reduction assay test.

2. Experimental

2.1. Materials

Multi-walled carbon nanotube produced by the CVD method (carbon content >95%; O.D.×I.D.×L: 20–30 nm × 5–10 nm × 0.5–200 μm), acrylamide, *N*-isopropylacrylamide, 2-hydroxyethyl methacrylate, poly(ethylene glycol) methacrylate (avg. M_N *ca.* 526), sodium methacrylate, benzophenone, and *N,N*-dimethylformamide (DMF, 99.8%) were purchased from Aldrich and used without further purification. The human tumor cell lines OPM-2 and HT-29 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany).

2.2. Grafting of polymers onto MWNTs

Pristine MWNTs were added to a large glass beaker containing DMF (0.07 mg/mL) and irradiated with 20 kHz ultrasound for 2 min to form a meta-stable dispersion (thickness *ca.* 10 mm). Given amounts of monomer and benzophenone (10 wt.% with respect to the monomer) were added and the mixture was purged with argon for 40 min. Then, the dispersion was sonicated for additional 30 s and irradiated with full spectrum UV–vis light with a "Dymax 5000-EC" UV curing equipment with 400 W metal halide flood lamp for given time (irradiation dose rate = 5.7 J/cm^2 min; input power = 93 mW/cm^2) under stirring. The reaction mixture was first vacuum-filtered through a 0.1 μm Nylon membrane, and the collected black solids were thoroughly washed several times with DMF and water in order to remove any residual monomer and non-grafted polymer. Finally, MWNTs-g-polymer were re-dispersed in water by ultrasonication.

2.3. Turbidity measurements

Turbidity measurements were performed on a Perkin–Elmer UV–vis spectrophotometer at a wavelength of 500 nm 7 days after preparation of the aqueous MWNTs-g-polymer dispersions. The concentration of MWNTs was kept nearly constant at 0.1 mg/mL. Water was used as the reference for all the measurements.

2.4. Transmission electron microscopy

A drop of dispersion was deposited on a TEM copper grid (3.05 mm 200 mesh) coated with a Carbon film, and the solvent was allowed to evaporate. A JEOL JEM-1011 Transmission Electron Microscope was used, at an accelerating voltage of 100 kV.

2.5. Fourier transformation infrared analyses

FTIR spectra were measured with an attenuated total reflection (ATR) spectrometer (IRAffinity-1, Shimadzu, Japan).

2.6. Thermal gravimetric analysis

TGA was carried out with a Perkin–Elmer TGA-7 thermal analyzer from 30 to 700 °C at a heating rate of 10 °C min^{-1} under air flow.

2.7. Cell lines and culture conditions

The human tumor cell lines OPM-2 and HT-29 were cultured under standard conditions – RPMI-1640 liquid medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine, in cell culture flasks, housed at 37 °C in an incubator 'BB 16-Function Line' Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO_2. The cells cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly.

2.8. Cytotoxicity assessment (MTT-dye reduction assay)

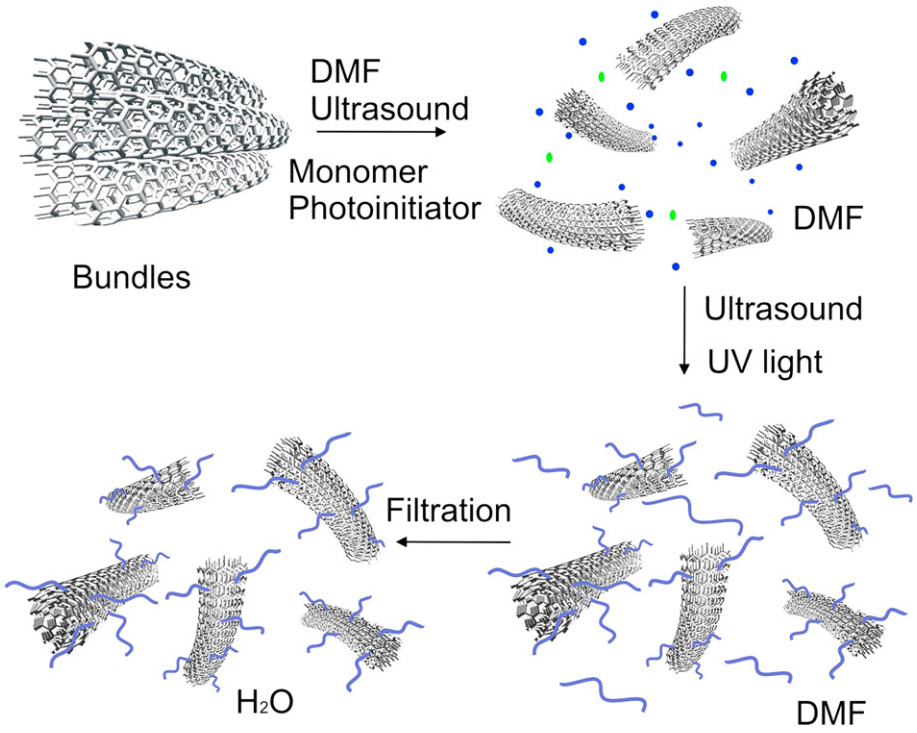
Stock dispersions of the nanotubes were freshly prepared and diluted with RPMI-1640 medium to yield the desired final concentrations. The cellular viability and proliferation after exposure to the tested nanotubes were assessed using the standard MTT-dye reduction assay. Exponentially growing cells were seeded in 96-well flat-bottomed microplates and after 24 h incubation at 37 °C they were exposed to various concentrations of the tested nanotubes for 48 h. For each concentration at least 8 wells were used. After the incubation with the test compounds 10 μl MTT solution (10 mg/ml in PBS) aliquots were added to each well. The microplates were further incubated for 4 h at 37 °C and the MTT-formazan crystals formed were dissolved by adding 100 μl/well 5% HCHO-acidified 2-propanol. The MTT–formazan absorption was determined using a microprocessor controlled microplate reader (Labexim LMR-1) at 580 nm. Cell survival fractions were calculated as percentage of the untreated control. The cell survival data were normalized as percentage of the untreated control (set as 100% viability). The statistical processing of cytotoxicity data included the Student's *t*-test with $p \leq 0.05$ set as significance level.

3. Results and discussion

The UV irradiation technique has been extensively explored by our group in the preparation of biocompatible polymer materials like stabilized polymeric and hybrid micelles, hydrogels and cryogels [23,24,25,26,27]. The main advantages of the UV irradiation are the very low capital outlay and the extremely short reaction time. In this work, the UV-irradiation technique was employed for the preparation of biocompatible water-soluble MWNTs via covalent grafting of polymers. Most of the experiments in our study were focused on polyacrylamide, poly(*N*-isopropylacrylamide) and poly(2-hydroxyethyl methacrylate), however, the method is not restricted to these polymers as described below. The polymers were covalently grafted onto multi-walled carbon nanotubes during the UV-initiated free-radical polymerization of given monomer carried out in a dispersion of MWNTs. The grafting mechanism involves generation, propagation and simultaneous addition of macrochains onto CNTs surface due to the strong tendency of macroradicals to react with the unsaturated double bonds of MWNTs [28] (Scheme 1).

The modification was carried out in DMF which is a common solvent for both the monomers and polymers and, on the other hand, allowed preparation of relatively stable dispersion of pristine MWNTs (disentangled bundles) within several minutes prior to modification. The reaction mixture was irradiated with UV light for given time, then the modified MWNTs were collected by repeated washing and filtration and re-dispersed in water by ultrasonication. Noteworthy, it was impossible to perform the modification directly in water, since our efforts to disperse the highly hydrophobic pristine MWNTs failed.

The visual inspection of MWNTs dispersions provides roughly an evidence whether sufficient polymer chains are grafted (Fig. 1 A). The aqueous dispersion of pristine MWNTs is totally unstable with a solid



Scheme 1. Preparation of stable aqueous dispersions of MWNTs via UV assisted *in situ* grafting of polymers.

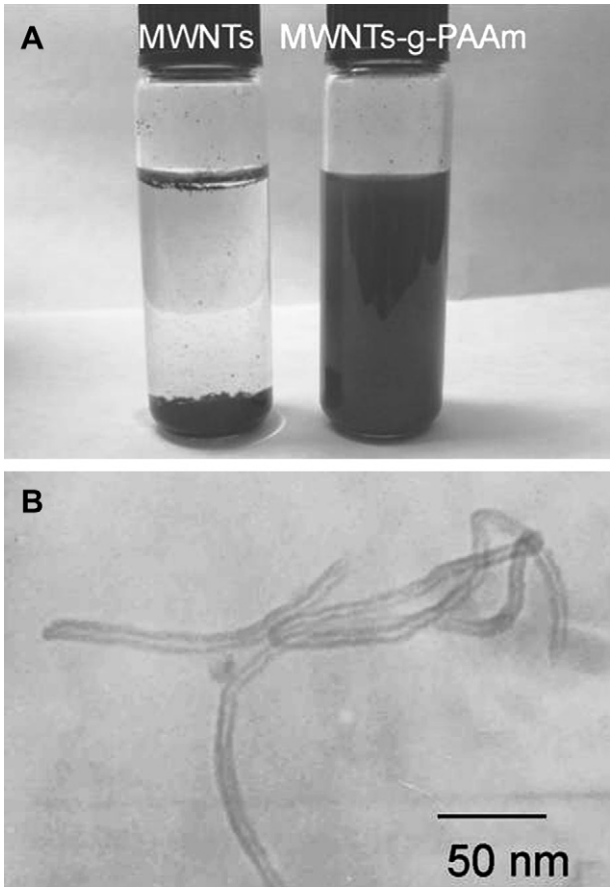


Fig. 1. Photographs of (A) aqueous dispersions of pristine MWNTs (left) and PAAm-grafted MWNTs (right), and (B) TEM microphotograph of individually dispersed MWNTs-g-PAAm.

precipitate and a nearly transparent liquid supernatant. In sharp contrast, the polymer-modified MWNTs form a stable dispersion, which makes the solvent deeply black. In general, the polymer chains attached to the surface of CNTs form an effective steric barrier against reagglomeration and the MWNTs are individually dispersed in water, as visualized by TEM analysis (Fig. 1B).

Initially, the experiments were focused on establishing the minimum irradiation dose(time) and the proper monomer/MWNTs ratio required to obtain stable aqueous dispersions of MWNTs for each polymer. The influence of both the irradiation dose(time) and the monomer/CNTs ratio on the stability of aqueous dispersions of modified MWNTs was studied by turbidity measurements. All measurements were performed 7 days after the samples preparation. The irradiation dose plays an important role for achieving a fast and effective grafting of polymers onto CNTs. As mentioned above, the pristine MWNTs bundles can be disentangled in the organic solvent by ultrasound and after that the dispersion is stable for several minutes. Therefore, it is important to ensure an irradiation dose capable to initiate the polymerization process and grafting of polymer chains, respectively, in the first few minutes of UV irradiation. It was found that 5 min irradiation with UV light at a dose rate of 5.7 J/cm^2 min is adequate for preparation of stable aqueous dispersions of PAAm- and PNIPAAm-grafted MWNTs (Fig. 2). Specifically, the aqueous dispersions of MWNTs-g-PHEMA exhibit more complicated behavior that are discussed in detail below.

The turbidity of the dispersions of MWNTs modified with PAAm, PNIPAAm and PHEMA as a function of monomer/MWNTs mass ratio is plotted on Fig. 3. At a lower monomer/MWNTs mass ratio the turbidity increases gradually with the increase of ratio up to 200 and, then, the MWNTs-g-PAAm and MWNTs-g-PNIPAAm dispersions exhibit nearly constant turbidity and stability, respectively. Most probably, above that ratio the critical grafting density and/or length of grafted chains are achieved and no re-agglomeration occurs within several weeks. In contrast, the MWNTs-g-PHEMA dispersions obtained at monomer/MWNTs mass ratios >200 exhibit a remarkable decrease in their turbidity value and a noticeable

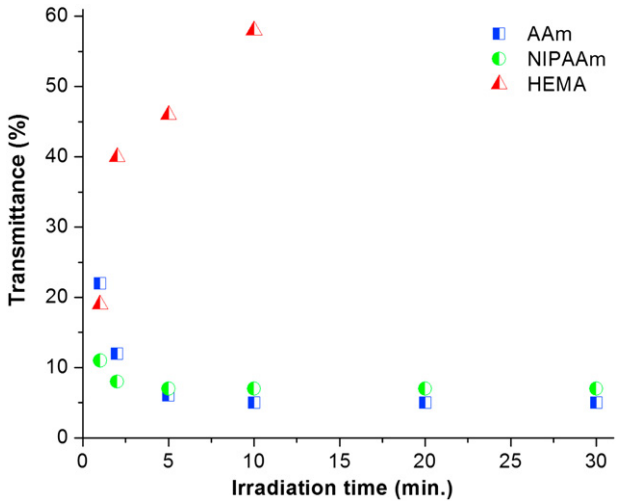


Fig. 2. Effect of the irradiation time(dose) on the turbidity of aqueous dispersions of MWNTs-g-polymer (monomer:MWNTs mass ratio 400:1).

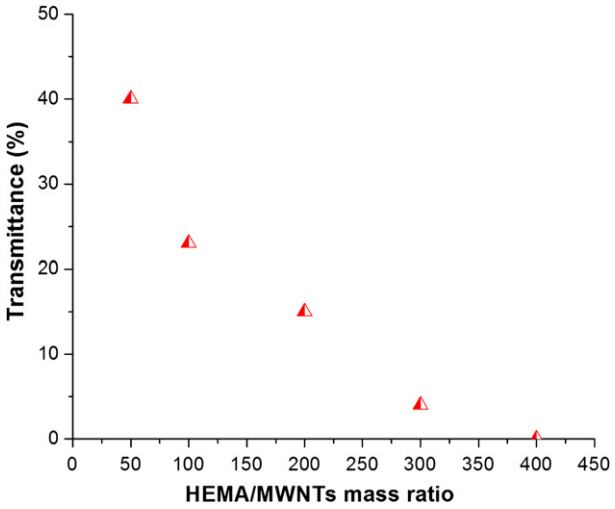


Fig. 4. Effect of HEMA:MWNTs mass ratio on the turbidity of MWNTs-g-PHEMA dispersions in DMF (10 min irradiation with UV light).

precipitation is observed. This behavior can be attributed to either insufficient grafting of polymer or poor solubility of PHEMA chains in water. To clarify this phenomena additional measurements of MWNTs-g-PHEMA dispersions in DMF were performed (Fig. 4).

Definitely, the most turbid system is obtained at the highest amount of HEMA, which is a direct proof that PHEMA is effectively grafted onto MWNTs surface at monomer/MWNTs mass ratio >200. Thus, the reason for the poor stability of MWNTs-g-PHEMA dispersions in water can be addressed to the solubility of the grafted PHEMA macromolecules. Principally, PHEMA is considered to be more water-swellable rather than water-soluble polymer. Furthermore, a systematic study relevant to the solubility of PHEMA homopolymers of different degree of polymerization (DPn) pointed out that up to DPn = 40 the linear PHEMA is water-soluble at 20 °C and above that value some precipitation occurs [29]. Therefore, one may conclude that stable aqueous MWNTs-g-PHEMA dispersions (Figs. 2 and 3) can be obtained only via grafting of relatively short PHEMA chains (low monomer conversion), whereas, above certain length PHEMA does not improve the macroscopic stability of the system.

The chemical structures of the polymer-grafted MWNTs were further characterized by FTIR spectroscopy (Fig. 5).

Although the amount of grafted polymers was not sufficient to record spectra of very good quality, the most characteristic absorption peaks of PAAm and PNIPAAm (C═O stretching vibration of the amide group at 1660 cm^{-1}; the bending vibration of the amide group at 1630 cm^{-1}) were detected. Since the modified MWNTs were thoroughly washed to remove the non-grafted polymer, the FTIR results indicate that the presence of polymers is due to the successfully grafted chains onto the surface of MWNTs via UV-induced free-radical polymerization. Further evidence for the relative amount of polymers grafted on to MWNTs was provided by TGA analysis. Fig. 6 shows the TGA weight loss curves of MWNTs-g-PNIPAAm obtained at different irradiation time. One should mention that the pristine MWNTs are stable without evident weight loss below 500 °C and, therefore, the weight loss in the temperature interval from 250 to 500 °C corresponds to the decomposition of polymer. Generally, the grafting ratios (GR), defined as the mass ratio of grafted polymer to nanotubes, for different polymers

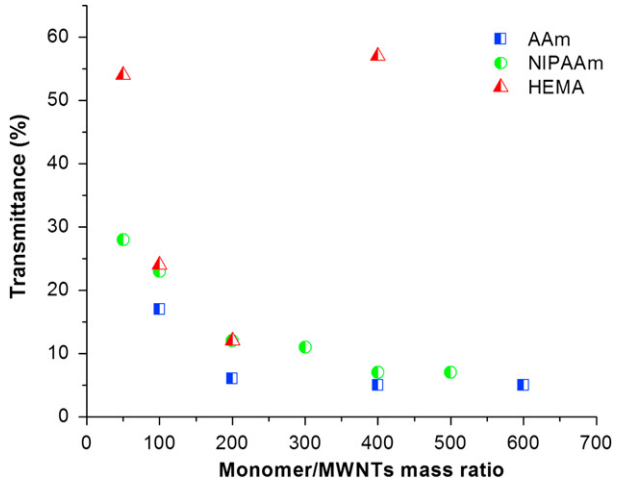


Fig. 3. Effect of monomer:MWNTs mass ratio on the turbidity of aqueous dispersions of MWNTs-g-polymer (10 min irradiation with UV light).

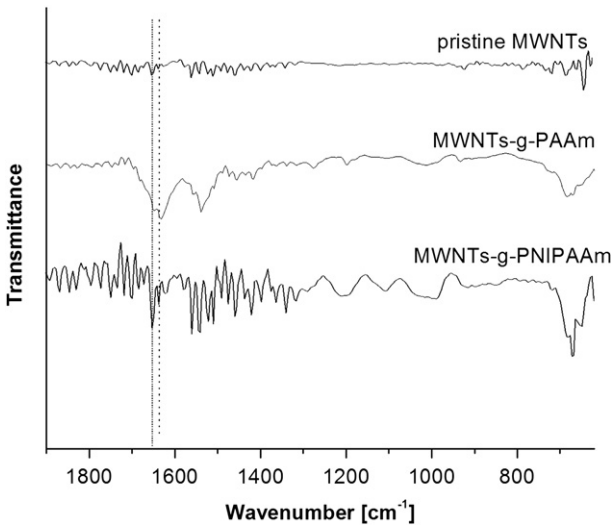


Fig. 5. FTIR spectra of the pristine MWNTs, MWNTs-g-PAAm and MWNTs-g-PNIPAAm.

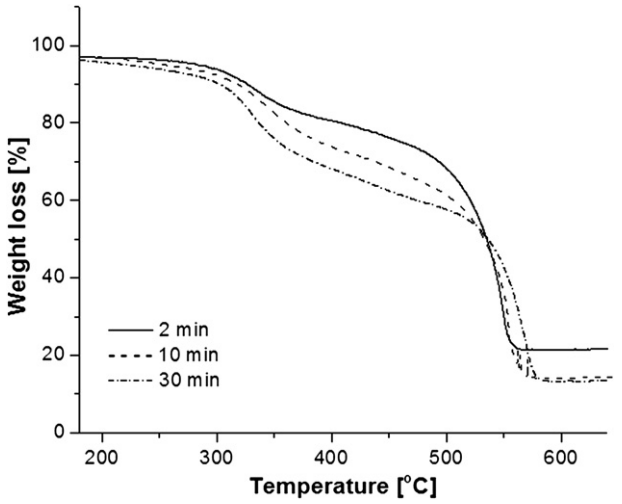


Fig. 6. TGA curves of MWNTs-g-PNIPAAm obtained at different irradiation time.

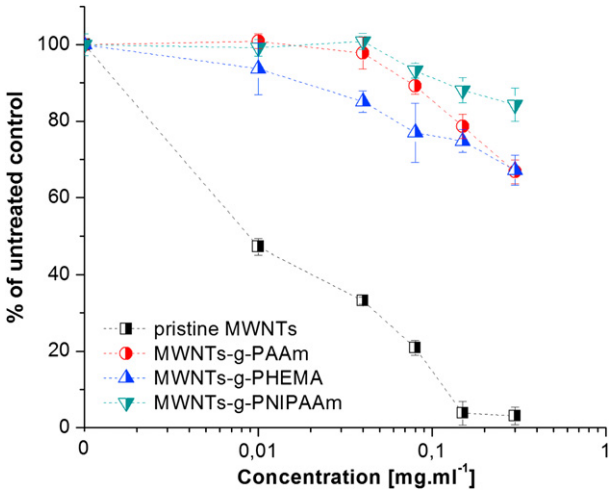


Fig. 8. Cytotoxic effects of: pristine MWNTs, MWNTs-g-PAAm, MWNTs-g-PHEMA and MWNTs-g-PNIPAAm against the human multiple myeloma-derived cell line OPM-2 after 72 continuous exposure (MTT-dye reduction assay). Each column represents the arithmetic mean ± sd from 8 independent experiments.

estimated by TGA (Fig. 7) increases with the increase of the irradiation dose. This observation is an indication for continuous grafting of polymer chains during polymerization within the time interval studied. On the other hand, these results are in agreement with the turbidity measurements and show that a considerable part of polymer is grafted in the first few minutes of UV irradiation. Clearly, stable aqueous dispersions of MWNTs-g-PAAm and MWNTs-g-PNIPAAm are obtained at GR above 0.3, while MWNTs-g-PHEMA system is stable only at GR in the 0.23–0.25 range at the experimental conditions reported.

The toxicity of polymer-grafted MWNTs was tested on human tumor cells. The cellular viability and proliferation after exposure to the tested nanotubes were assessed using the standard MTT-dye reduction assay as described by Mosmann [30] with some modifications [31]. The method is based on the reduction of the yellow tetrazolium salt MTT to violet crystals product by the mitochondrial succinate dehydrogenase in viable cells. Two human tumor cell lines, namely OPM-2 (multiple myeloma) and HT-29 (colon carcinoma) were exposed to pristine MWNTs and functionalized nanotubes for 48 h. The results obtained, expressed as percentage of the untreated control (set as 100% viable) and the corresponding concentration-response curves are depicted on Figs. 8 and 9. In both cell lines the pristine MWNTs evoke strong, concentration-dependent cytotoxicity, with almost total eradication of viable cells at concentrations higher than 100 μg/ml. In a dissimilar fashion the polymer-functionalized nanotubes are non-cytotoxic, i.e., do not induce statistically significant effects upon cellular viability and proliferation at concentrations lower than 150 μg/ml. At the higher concentrations, PNIPAAm-grafted MWNTs have only marginal cytotoxicity even at the highest value studied (300 μg/ml) in both cell lines, while more pronounced cytotoxicity is observed for MWNTs-g-PAAm and MWNTs-g-PHEMA. These findings unambiguously indicate that the grafting of polymers on to carbon nanotubes via UV-assistance is consistent with significant loss of CNTs cytotoxicity and, thus, this is a valuable strategy for preparation of biocompatible CNTs-based materials.

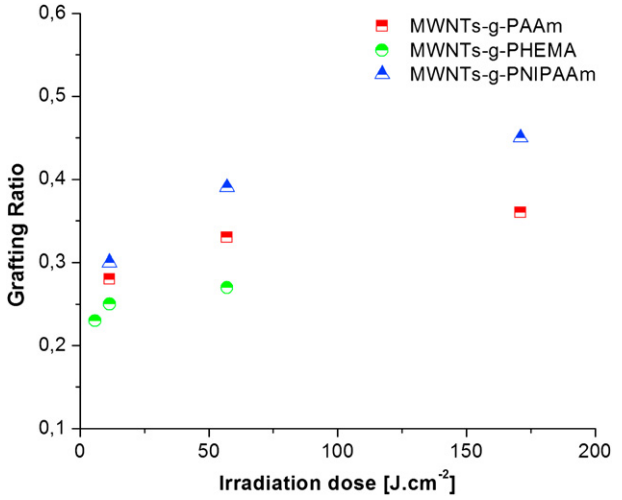


Fig. 7. Effect of the irradiation dose on the grafting ratio of different polymer-grafted MWNTs.

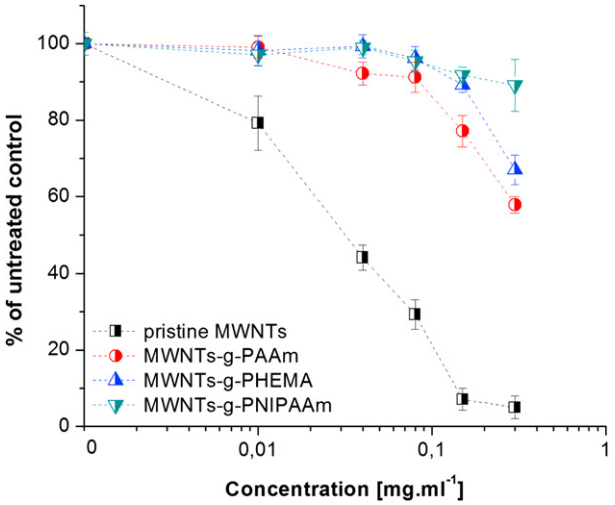


Fig. 9. Cytotoxic effects of: pristine MWNTs, MWNTs-g-PAAm, MWNTs-g-PHEMA and MWNTs-g-PNIPAAm against the human colon carcinoma-derived cell line HT-29 after 72 continuous exposure (MTT-dye reduction assay). Each column represents the arithmetic mean ± sd from 8 independent experiments.

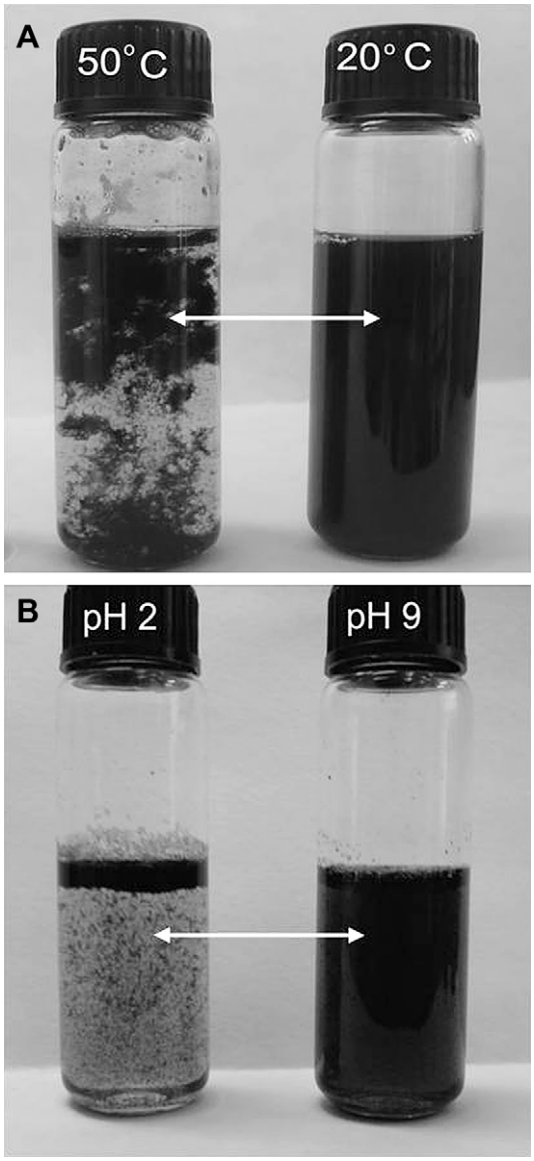


Fig. 10. Photographs of aqueous dispersions of (A) MWNTs-g-PNIPAAm at different temperature and (B) MWNTs-g-poly(sodium methacrylate) at different pH.

The versatility of UV irradiation technique was further examined by grafting of another two water-soluble polymers – poly[poly (ethylene glycol) methacrylate] and poly(sodium methacrylate). In both cases, stable aqueous dispersions were obtained for at least 5 min irradiation and minimum monomer/CNTs ratio of 200:1, which seems to be the common feature of the polymer-grafted MWNTs in our study at the specified experimental conditions. It should be mentioned that the grafting of stimuli-sensitive polymers onto MWNTs provides the herein prepared dispersions with additional functionality and potential. Fig. 10 demonstrates a reversible temperature- (a) and pH (b) triggered transition from well-dispersed MWNTs to precipitate. Indeed, at temperatures above the LCST (>32 °C) [32] the hydrophilicity of PNIPAAm outer layer is substantially decreased and the efficiency of the steric barrier against agglomeration is remarkably reduced. However, the stable aqueous dispersion of MWNTs-g-PNIPAAm can be regained by a gentle treatment with ultrasound at 20 °C for 20 s. Similar, pH-induced, behavior is observed for MWNTs-g-poly(sodium methacrylate). At pH<5 poly(sodium methacrylate) loses its anionic character [33], i.e., the repulsive interaction between polymer chains is decreased resulting in macroscopic precipitation of CNTs, while in alkaline solution polymer is ionized and CNTs can be re-dispersed again.

4. Conclusions

The UV irradiation is a facile method for effective covalent grafting of different hydrophilic polymers onto MWNTs which prevents the agglomeration of individual CNTs in water and improves the long-term stability of the system. Indeed, stable aqueous dispersions of MWNTs are obtained from samples irradiated with UV light for at least 5 min at an irradiation dose rate of 5.7 J/cm^2 min at minimum monomer/CNTs ratio of 200:1. The modified MWNTs exhibit remarkably improved biocompatibility, especially PNIPAAm-grafted MWNTs which are non-cytotoxic even at concentration of 300 μg/mL. Grafting of stimuli-sensitive polymers onto MWNTs allows a reversible precipitation upon external stimuli.

Acknowledgements

This work was supported by the Bulgarian Ministry of Education and Science, Structural Funds and Educational Programs Directorate, Project "Support for the development and realization of PhD-students, post-docs and young researchers in the field of polymer chemistry, physics and engineering", Grant 51, financed by the Operational Program "Human resources development" at the scheme BG051PO001/07/3.3-02. P.P. thanks Prof. AHE Müller and Dr J. Yuan (MCII, University of Bayreuth) for TGA measurements.

References

[1] Iijima S. Nature 1991;354:56–8.
[2] Baughman RH, Zakhidov AA, de Heer WA. Science 2002;297:787–92.
[3] (a) Tasis D, Tagmatarchis N, Bianco A, Prato M. Chem Rev 2006;106:1105–36; (b) Kong H, Gao C, Yan D. J Am Chem Soc 2004;126:412–3; (c) Baskaran D, Mays JW, Bratcher MS. Angew Chem Int Ed 2004;43:2138–42.
[4] Magrez A, Kasas S, Salicio V, Pasquier N, Seo JW, Celio M, et al. Nano Lett 2006; 6:1121–5.
[5] Dumortier H, Lacotte S, Pastorin G, Marega R, Wu W, Bonifazi D, et al. Nano Lett 2006;6:1522–8.
[6] Lacerda L, Bianco A, Prato M, Kostarelos K. Adv Drug Deliv Rev 2006; 58:1460–70.
[7] Saito N, Usui Y, Aoki K, Narita N, Shimizu M, Hara K, et al. Chem Soc Rev 2009; 38:1897–903.
[8] Bianco A, Kostarelos K, Partidos CD, Prato M. Chem Commun; 2005:571–7.
[9] Pastorin G. Pharm Res 2009;26:746–69.
[10] Star A, Steuerman DW, Heath JR, Stoddart JF. Angew Chem Int Ed 2002;41:2508–12.
[11] Bandyopadhyaya R, Nativ-Roth E, Regev O, Yerushalmi-Rozen R. Nano Lett 2002;2:25–8.
[12] Dodziuk H, Ejchart A, Anczewski W, Ueda H, Krinichnaya E, Dolgonos G, et al. Chem Commun 2003;9:986–7.
[13] Pompeo F, Resasco DE. Nano Lett 2002;2:369–73.
[14] Nguyen CV, Delzeit L, Cassell AM, Li J, Han J, Meyyappan M. Nano Lett 2002; 2:1079–81.
[15] Georgakilas V, Tagmatarchis N, Pantarotto D, Bianco A, Briand J-P, Prato M. Chem Commun 2002;24:3050–1.
[16] Huang W, Taylor S, Fu K, Lin Y, Zhang D, Hanks TW, et al. Nano Lett 2002; 2:311–4.
[17] Sano M, Kamino A, Okamura J, Shinkai S. Langmuir 2001;17:5125–8.
[18] Riggs JE, Guo Z, Carroll DL, Sun Y-P. J Am Chem Soc 2000;122:5879–80.
[19] Kong H, Luo P, Gao C, Yan D. Polymer 2005;46:2472–85.
[20] Kong H, Gao C, Yan D. J Mater Chem 2004;14:1401–5.
[21] Petrov P, Stassin F, Pagnoulle C, Jerome R. Chem Commun; 2003:2904–5.
[22] Chen S, Wu G, Liu Y, Long D. Macromolecules 2006;39:330–4.
[23] Petrov P, Bozukov M, Tsvetanov ChB. J Mater Chem 2005;15:1481–6.
[24] Petrov P, Yuan J, Yoncheva K, Müller AHE, Tsvetanov ChB. J Phys Chem B 2008;112:8879–83.
[25] Doycheva M, Petrova E, Stamenova R, Tsvetanov ChB, Riess G. Macromol Mater Eng 2004;289:676–80.
[26] Petrov P, Petrova E, Tchorbanov B, Tsvetanov ChB. Polymer 2007;48:4943–9.
[27] Petrov P, Petrova E, Tsvetanov ChB. Polymer 2009;50:1118–23.
[28] Qin S, Qin D, Ford WT, Herrera JE, Resasco DE. Macromolecules 2004; 37:9963–7.

[29] Weaver JVM, Bannister I, Robinson KL, Bories-Azeau X, Armes SP, Smallridge M, et al. Macromolecules 2004;37:2395–403.
[30] Mosmann T. J Immunol Methods 1983;65:55–63.
[31] Konstantinov SM, Eibl H, Berger MR. Br J Haematol 1999;107:365–80.
[32] Dimitrov I, Trzebicka B, Müller AHE, Dworak A, Tsvetanov CB. Prog Polym Sci 2007;32:1275–343.
[33] Holappa S, Andersson T, Kantonen L, Plattner P, Tenhu H. Polymer 2003; 44:7907–16.