Beni-Suef University Journal of Basic and Applied Sciences

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Self-assembled surfactant-based nanoparticles as a platform for solubilization and enhancement of the photothermal activity of sepia melanin

Nayera Mohamed El Ghoubary, Maha Fadel and Doaa Ahmed Abdel Fadeel*

Abstract

Background Sepia melanin (SM) is a natural photothermal biopolymer. Its biomedical applications are limited due to its poor solubility and bioavailability. This study aims to prepare a soluble formulation of sepia melanin to enhance its solubility, in turn, its bioavailability, and its use in photothermal therapy of cancer. SM was extracted from a sepia ink sac and prepared as insoluble powdered (SM) which is identified by FTIR, ^{1}H-NMR, thermogravimetric analysis (TGA), and scanning electron microscope. SM was self-assembled using tween 80 into dispersed nanoparticles (SM-NP-Tw). The prepared SM-NP-Tw were fully characterized. The photothermal performance of SM-NP-Tw was assessed. Dark and photocytotoxicity of SM-NP-Tw was studied on HepG2 cells using two wavelengths (660 nm and 820 nm).

Results The insoluble powdered (SM) exhibited a spherical nanoparticle-like shape as revealed by scanning electron microscope and was soluble only in an alkaline aqueous solution. TGA of SM showed high resistance to thermal degradation indicating good thermal stability. The prepared SM-NP-Tw exhibited a spherical shape with mean sizes of 308 ± 86 nm and a zeta potential of -25 mv. The cell viability decreased significantly upon increasing the concentration and upon radiation at 820 nm. The results of UV–Vis spectroscopy and the photothermal performance revealed that melanin can absorb light in a wide range of wavelengths including near the IR region; thus, it can emit sufficient heat to kill cells through the photoheat conversion effects.

Conclusion Sepia melanin nanoparticles self-assembled into tween-based nanostructures could be a promising natural platform for photothermal cancer therapy.

Keywords Sepia melanin, Melanin nanoparticles, Self-assembled nanoparticles, Photothermal therapy, Photocytotoxicity

1 Background

Melanin is a dark brown- to black-colored natural biopolymer [1]. It is found in nearly all living organisms. It can be classified, according to the origin and the location, as eumelanin, pheomelanin, neuromelanin, allomelanin, and pyomelanin [2].

One of the most studied melanin is sepia melanin (SM) which is extracted from the ink sac of cuttlefish (Sepia Officinalis L.) [3]. It is considered the main source of natural eumelanin, which is a promising natural biopolymer

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due to its natural abundance, bio renewability, biocompatibility, biodegradability, biostability, and low cost [3, 4]. It has antimicrobial, antioxidant, and antitumor activities [5, 6]. Moreover, it acts as an ultraviolet absorber, amorphous semiconductor, cation exchanger, and contrast agent for imaging [7].

Photothermal therapy (PTT) is a noninvasive cancer treatment. It depends on the presence of a molecule (photosensitizer) that can generate heat upon excitation by light in the near-infrared (NIR) region [8]. PTT has shown superior characteristics over conventional surgery and chemotherapy due to its localized tumor thermal ablation, minimal heating damage to the adjacent normal tissues, short recovery period, and low recurrence rate [9, 10]. Moreover, PTT can induce an immune response by activation of macrophages and T cells, thus aggravating the direct thermal effect [11]. Various types of nanoparticles are being studied to act as efficient PTT agents, such as metal nanoparticles and carbon nanotubes. However, many uncertainties concerning their safety and their accumulation in the body's organs limit their applications [12, 13]. Many efforts were devoted to searching for safe photothermal agents from natural origin to avoid the limitations of the aforementioned nanoparticles.

SM attracted more attention as a natural photothermal agent with high photothermal conversion efficiency as it can absorb a wide range of wavelengths, especially in the NIR region, and can convert the absorbed NIR energy into heat [9, 11]

However, SM is only soluble in strongly alkaline aqueous solutions. It is insoluble in water, in most organic solvents, and physiological fluids [9]. This poor solubility is a major drawback that limits the use of SM as a photothermal agent in biomedical applications [12].

The nanoparticles prepared from synthetic melanin are widely used as they are water-soluble, and their properties can be easily tailored by controlling the synthesis procedure. Nevertheless, the natural melanin nanoparticles extracted from living organisms remain superior to the synthetic ones due to the high biosafety and biocompatibility [9, 11]. Several previous studies tried to solve the problem of natural melanin insolubility by encapsulating it in different carriers such as nanovesicles [12], PEG nanoparticles [14], and liposomes [10, 15].

In this study, we tried to overcome the major problem of SM-NP insolubility with a very simple non-sophisticated technique. Firstly, SM was extracted from the sac ink of cuttlefish by applying a double precipitation technique to obtain pure powdered SM, which exhibited a spherical nanoparticle-like shape under the scanning electron microscope and was soluble only in an alkaline aqueous solution (pH 12). Secondly, the obtained powder was self-assembled, in a neutral aqueous solution, into stable, dispersible nanostructures using Tween 80 as a nonionic surfactant (SM-NP-Tw). They have a hydrophobic core and a hydrophilic shell; thus, they can solubilize the insoluble compounds in their core [16]. Polysorbates, such as Tween 80, are widely used for micelles preparation with low toxicity and high drug loading efficiency [17, 18]

Briefly, in this study, we introduced a novel approach for preparing water-dispersible natural melanin nanoparticles by preparation of self-assembled tween-based nanostructures. Unlike other studies that used multistep formulation processes, this novel approach is very simple, economic, and does not include the use of any organic solvents. The prepared SM-NP-Tw have further tested in vitro for their photothermal activity.

2 Materials and methods

2.1 Materials

Fresh cuttlefish was purchased from the local market in Egypt. Methanol was purchased from Sdfcl SD Fine Chem (India). Ethanol, absolute 99.8%, was purchased from Fisher Chemical (UK). Sodium hydroxide (NaOH) was purchased from Alamia company for chemicals (Egypt). HEPES buffer [N-(2-Hydroxyethyl) piperazine-N′-(2-ethane sulfonic acid)], chloroform, dimethyl sulfoxide (DMSO), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (USA). Tween 80 was purchased from Riedel–de Haen (Atlas Chemie) (Italy).

2.1.1 Cell line and culture conditions

The hepatocellular carcinoma cell line (HepG2) was obtained from Nawah Scientific Inc. (Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin, and 10% of heat-inactivated fetal bovine serum in humidified, 5% CO_2 atmosphere at 37 °C.

2.2 Extraction of sepia melanin

Sepia melanin (SM) was extracted from cuttlefish (*Sepia Officinalis L.*) ink sac as previously reported [1, 3, 7] with few modifications. Briefly, the ink sac was obtained by dissection from the fresh cuttlefish. The ink was centrifuged for 15 min at 6000 rpm to remove any debris and aggregates. The supernatant containing melanin was solubilized by stirring for 4 h in 50 ml, 10 M NaOH (pH 13).

Pure SM was precipitated upon reducing the pH to 2, by adding 10 ml 5 M HCL. Then the precipitate was separated by centrifugation at 10,000 rpm for 30 min at room temperature. To deproteinize the extract, the precipitate was redissolved in 10 M NaOH and chloroform. Melanin was precipitated by lowering the pH to 2 and collected by centrifugation. The obtained black precipitate was washed with methanol, ethanol, and distilled water.

Excess water was removed by drying using an oven at 40 °C for 24 h to obtain dried SM powder (Fig. 1a).

The dispersity of SM powder was studied in different solvents. Weighed amounts of SM (0.01 g) were added to 30 ml of each solvent included in the study under stirring at 400 rpm for 1 h. The solvents included in this study were ethanol, methanol, acetone, and dimethyl sulfoxide (DMSO) as organic solvents and HEPES buffer of pH 2, 7, 9, and 12 as an aqueous solution. The pH was adjusted using 0.1N HCL and 0.1N NaOH and measured by digital pH meter type HANNA HI9811-5, RI, USA.

2.3 Self-assembly of the extracted melanin into a stable dispersible nanosuspension (SM-NP-Tw)

The insoluble SM was self-assembled in water using Tween 80 (Fig. 1b) as a surfactant, as previously described by Ravichandran et al. [17]. Stock solutions from Tween 80 in HEPES buffer (pH 7.4) of different concentrations (from 1 to 10% w/v) were prepared and heated at 35 °C for 30 min under stirring. Then 0.1 gm of SM powder was added to 10 ml of the prepared Tween 80 solutions with continuous stirring. The complete dispersion of SM was attained in a 10% Tween solution. The obtained suspension was continued stirring for a further 30 min, then filtered through a 0.45 µm filter, and stored in the refrigerator for further use.

2.4 Characterization of the extracted SM

Fourier transform infrared spectroscopy was used to identify the functional groups and interpret the structure of the melanin in powdered form. IR measurements were taken using an FTIR spectrometer (JASCO FT/IR-4100 type A, Japan) in the wavenumber range of 400–4000 cm^{-1} [15].

For further confirmation of the molecular structure of the extracted melanin, proton nuclear magnetic resonance analysis (^{1}H-NMR) measurements were taken using Mercury-300 (BioSurplus, Inc., USA) [19, 20]. The sample was suspended in dimethyl sulfoxide (DMSO) before measurement.

Thermogravimetric analysis (TGA) was carried out using a thermogravimetric analyzer (DTG-60H, Shimadzu, Japan) to investigate the thermal stability of the extracted melanin. 5 mg of SM powder was heated from room temperature (25 °C) to 800 °C at a heating rate of 10 °C/min under nitrogen flow [3, 7].

The particle size and morphology of SM powder was examined using a scanning electron microscope (SEM) (Quanta FEG 250, USA) and operated at an acceleratory voltage of 20 kV. The powder sample was coated with a platinum layer using a vacuum sputter coater to increase the conductivity of the samples [7, 21].

2.5 Characterization of the prepared SM-NP-Tw

The morphology of the SM-NP-Tw suspension was observed under transmission electron microscopy (TEM, Jeol, Jem-1400, Tokyo), after negative staining, at an acceleratory voltage of 80 kV [18].

Particle size and zeta potential of SM-NP-Tw were measured by dynamic light scattering employing Malvern Zeta-sizer, at room temperature and flow rate 0.5. The polydispersity index (PI) was also determined as a measurement of particle size homogeneity of them [22, 23].

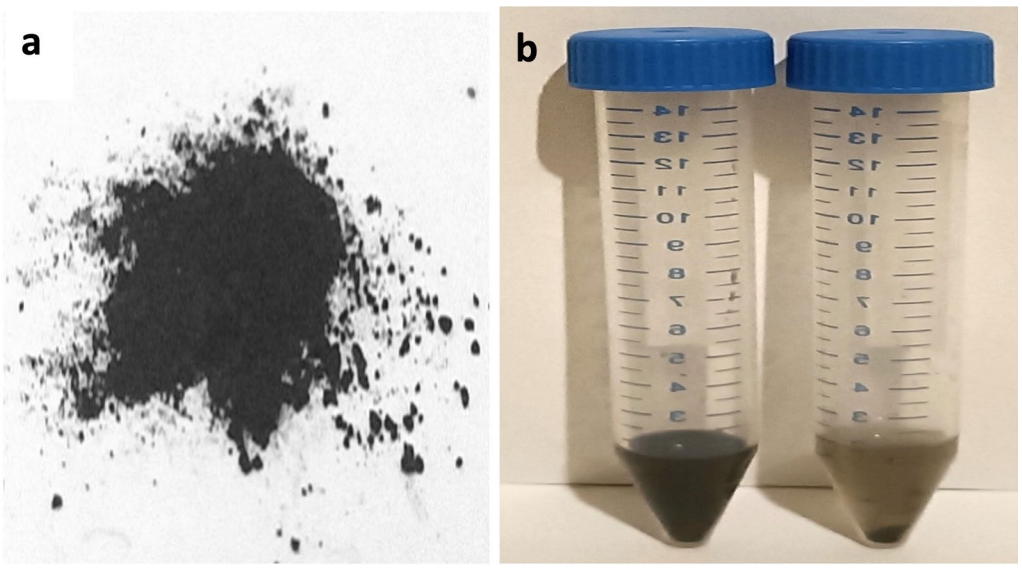

Fig. 1 **a** A photograph of the extracted sepia melanin powder, **b** a photograph of SM suspended in Hepes buffer at pH 7 (right) and SM-NP-Tw (left)

The absorption spectra of dilute aqueous suspensions of SM (pH 12) and SM-NP-Tw (pH 7.4) were recorded using a double-beam spectrophotometer (Rayleigh 2601, China), within the appropriate scan range (200–1000 nm). The spectra were taken against aqueous NaOH as a solvent reference for SM suspension and HEPES buffer as a solvent reference for SM-NP-Tw. Measurements were taken at room temperature [7, 24].

The photothermal conversion efficiency of the prepared SM-NP-Tw was evaluated by irradiation using white light. The SM-NP-Tw suspension was placed in a glass cuvette and irradiated with white light from a halogen lamp source (1.6 W/cm^2) for 12 min at room temperature. The distance between the light source and the sample was fixed at 10 cm. The temperature changes throughout the experiment were measured and recorded [25]. Temperature changes were compared with that of distilled water [1, 12]. The experiment was repeated three times, and data were represented as mean ± SD.

To examine the storage stability, the prepared SM-NP-Tw were placed at 4 °C for 10 months. Particle size, zeta potential, and polydispersity index were measured at different time points, using dynamic light scattering [13].

2.6 In vitro *release of SM from SM-Np-Tw*

The in vitro release of the melanin from the prepared SM-NP-Tw was studied under sink conditions using dialysis membrane tubing (molecular weight cutoff 12,000–14,000) presoaked in HEPES buffer for 24 h at room temperature. One ml of the SM-NP-Tw was placed in 5 cm^2 membrane tubes closed with clamps and suspended in a 10 ml acceptor medium of HEPES buffer (pH 7) containing 1% Tween 80 [25]. The solution was continuously stirred at 400 rpm, and the temperature was adjusted to 37 °C to mimic the in vivo conditions. Afterward, aliquots were collected from the acceptor solution and replaced with 1 ml of HEPES buffer at different time points [12]. The aliquots were measured spectrophotometrically at 340 nm to determine the amount of melanin released. The concentration of melanin released from the formulation was calculated from a standard calibration curve. The experiment was repeated three times, and the mean cumulative release of melanin was calculated and plotted as a function of time. For studying the release kinetics of melanin from SM-NP-Tw, the data were fitted to zero, first and Higuchi's diffusion control models using coefficient of variation for data analysis StatistiXL for MS Excel software. By applying the highest linear correlation coefficient (R^2) and the least coefficient of variation, the release kinetics was evaluated, and the release rate constant (K) was calculated.

2.7 In Vitro *dark and photocytotoxicity assay*

Aliquots of 100 µL HepG2 cell suspension (5×10^3 cells) were seeded in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 µL media containing SM-NP-Tw at various melanin concentrations (10,100 and 500 µg/ml) and incubated for 48 h.

After the incubation period, the cytotoxicity of SM-NP-Tw was assessed by sulforhodamine B (SRB) assay after fixation with 10% TCA (trichloroacetic acid), as previously described [26].

To assess the photothermal effect of SM-NP-Tw, another two groups of cells were irradiated, after 48 h incubation with the same sample concentrations under the same conditions, with a diode laser (SIM-MED, United Kingdom) for 10 min at 350 mW/cm^2. One group was irradiated at 660 nm, while the other group was irradiated at 820 nm [1, 12, 14]. Three replicates were conducted for each group. 24 h post-irradiation, an SRB assay was conducted.

2.8 Statistical analysis

Data were represented as mean ± standard deviation (SD), all experiments were performed in triplicate, and data were statistically analyzed by ANOVA followed by Tukey–Kramer test using SPSS software version 16. The level of significance was defined at $p < 0.05$.

3 Results

3.1 Characterization of the extracted SM

The solubility of the extracted SM powder was tested in several organic solvents and in an aqueous buffer at different pH values. SM powder was insoluble in all the tested organic solvents. In addition, it was insoluble aqueous media (HEPES buffer) at pH 7 and was precipitated in acidic PH. It started to disperse upon a gradual increase in PH. Complete dispersion took place at pH 12.

The FTIR spectrum of the extracted SM (Fig. 2) shows typical melanin signatures as reported by Xin et al. [27], Caldas et al. [6], and Roy and Rhim who prepared melanin nanoparticles from sepia ink [21]. These signatures may be recognized as a broadband corresponding to N–H and O–H groups at 3415 cm^{-1}, aliphatic C–H groups stretching vibrations at 2922 cm^{-1} which may be due to the residues of lipid and amino acid during extraction [28], C=C and C=O at 1625 cm^{-1}, phenolic COH, indolic and phenolic NH at 14,600–1390 cm^{-1} and C=C at 700–600 cm^{-1}.

The ^{1}H-NMR spectrum of SM (Fig. 3) shows several chemical shifts that support the identification of melanin molecular structure. In the aromatic regions, the signals between 7 and 8 ppm were assigned to the aromatic

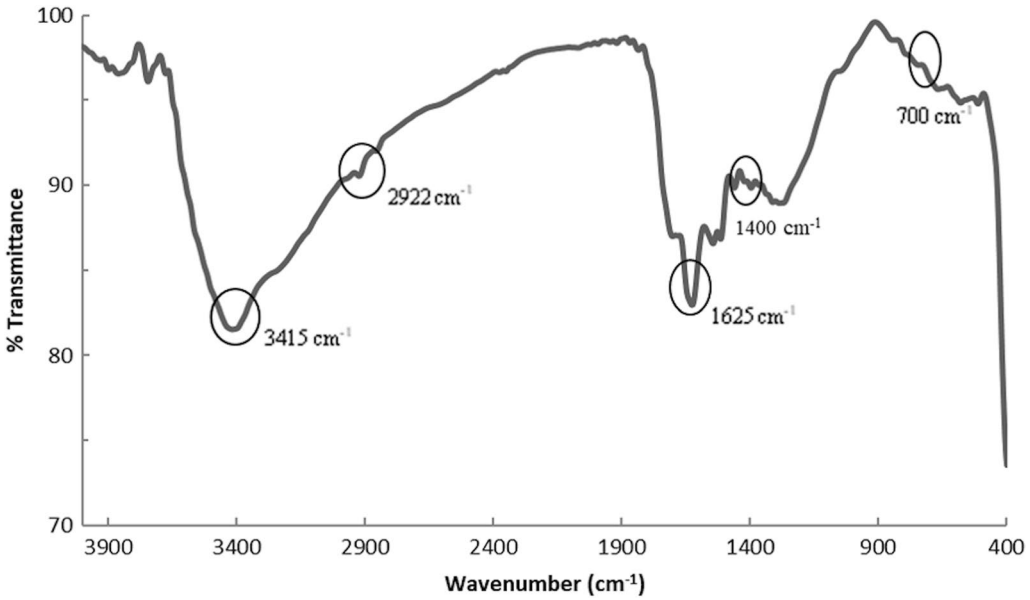

Fig. 2 FTIR spectroscopy of the extracted sepia melanin (SM) powder

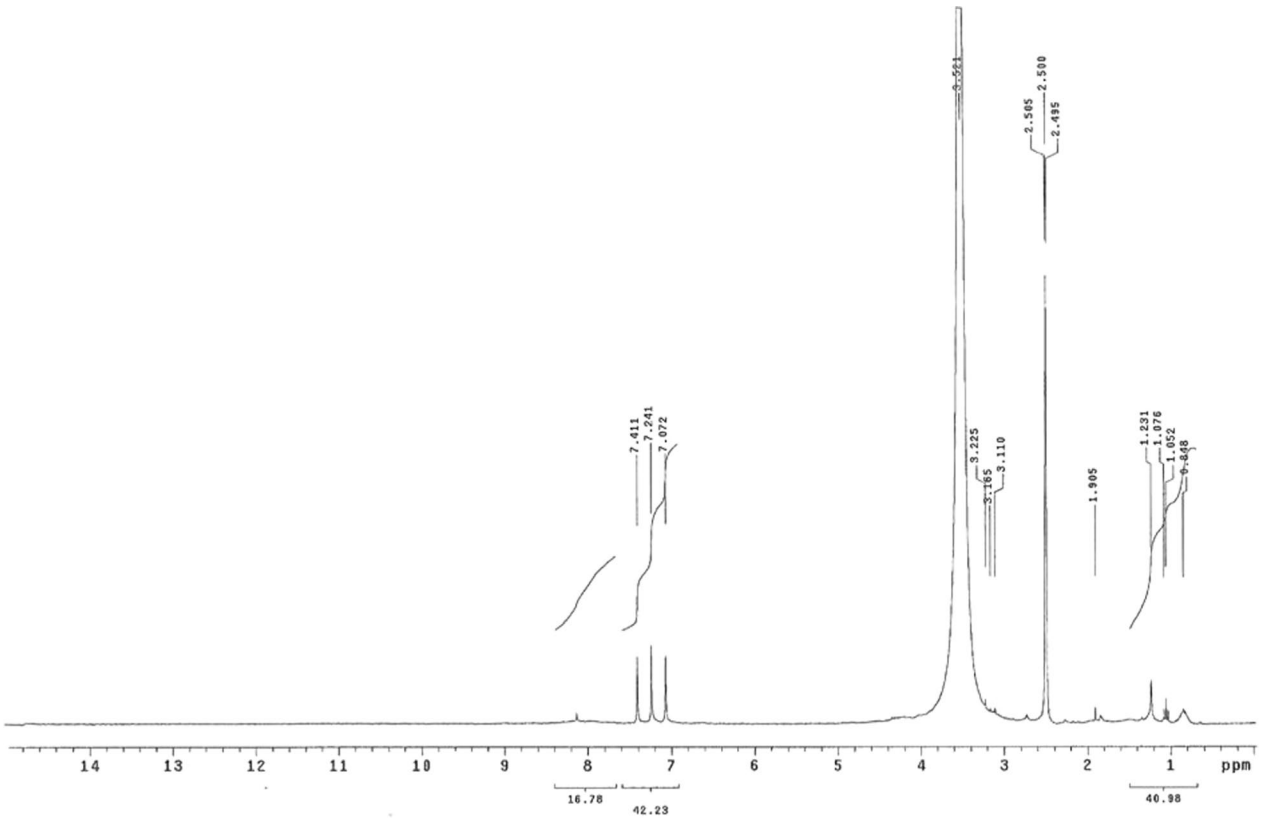

Fig. 3 [1]H-NMR spectrum of the extracted sepia melanin (SM) powder

hydrogens of the indole and/or pyrrole rings of the melanin molecular chain. The observed signals in the absorption region between 3.2 and 4 ppm were caused by the methyl or methylene group attached to nitrogen and/or oxygen atoms. Signals in the region between 1.2 and 2.5 ppm were caused by the presence of the NH group attached to the indole ring. In the aliphatic region, the signals between 0.8 and 1.2 ppm were assigned to the methyl group of alkyl fragments [19, 20].

TGA thermograms and derivative thermogravimetric (DTG) curves (Fig. 4) showed a three steps thermal degradation process. The first endothermal peak at 81 °C can be attributed to the evaporation of weakly bound water. The exothermal peak at 307 °C may be due to the loss of carbon dioxide. The main thermal degradation of the SM occurred at an elevated temperature due to melanin decarboxylation. The residual mass value, after decarboxylation, was approximately 44%. This proves that the extracted SM has high thermal stability which encourages its use in many applications [3, 7, 20, 29].

SEM micrograph (Fig. 5a) demonstrated that the extracted SM appeared as aggregates of spherical nanoparticle-like granules with different sizes ranging from 100 to 200 nm which is in perfect agreement with literature findings [1, 7, 9, 29–31].

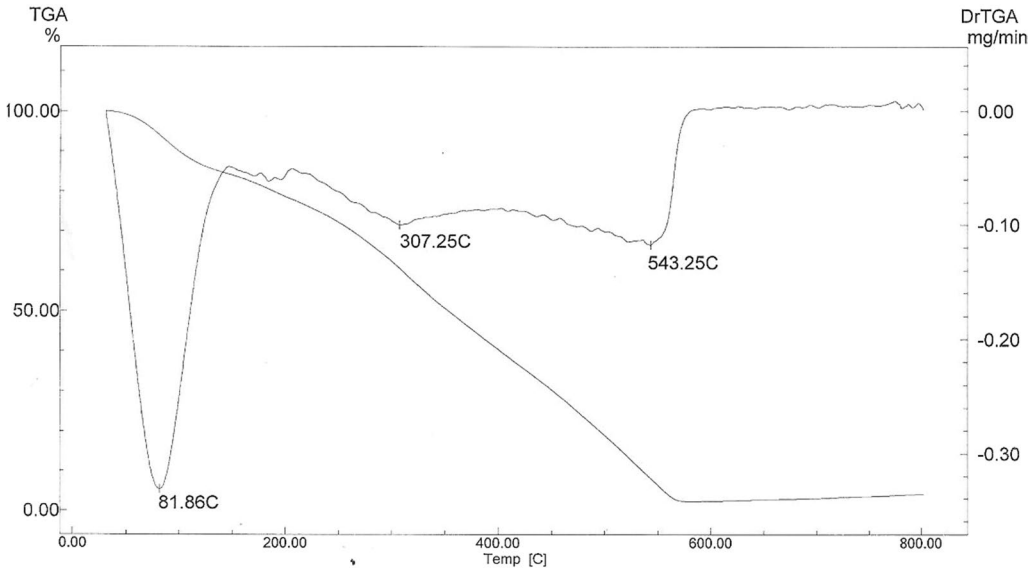

Fig. 4 TGA thermograms and derivative thermogravimetric (DTG) curves of the extracted sepia melanin powder (SM**)**

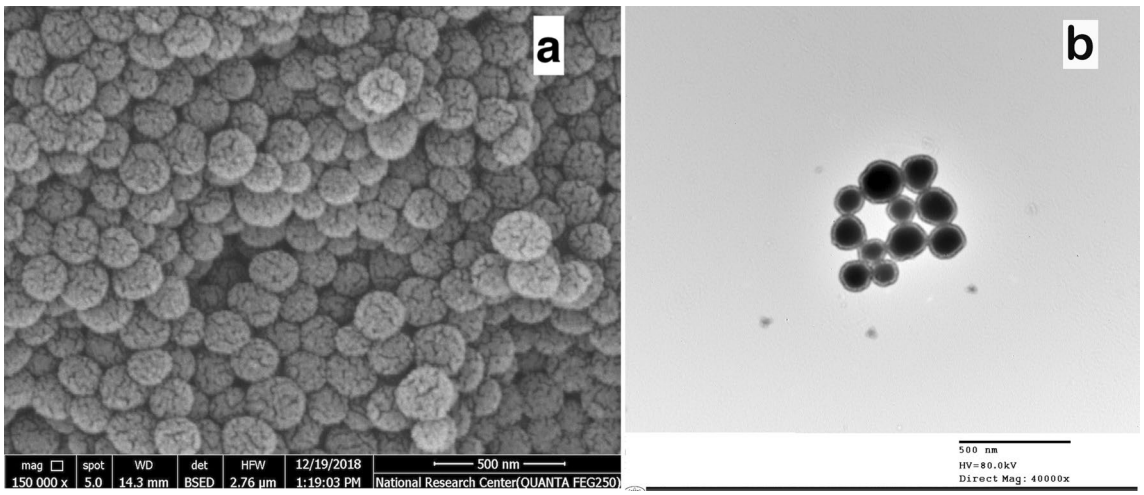

Fig. 5 **a** SEM micrograph of the extracted sepia melanin (SM) powder, **b** TEM micrograph of self-assembled tween-based nanoparticles (SM-NP-Tw)

3.2 Characterization of the prepared SM-NP-Tw

TEM micrograph (Fig. 5b) of SM-NP-Tw showed that they appeared spherical in shape with no aggregates.

The particle size and the zeta potential of SM-NP-Tw, as measured by DLS, were 308 ± 86 nm, and -25 mv, respectively. The surface negative charge imparts stability to the particles via electrostatic repulsion and prevents agglomeration [6]. The polydispersity index was 0.2 indicating good sample homogeneity.

The UV–Vis spectra of the alkaline aqueous solution of SM and SM-NP-Tw (Fig. 6) showed a broad and strong absorption in the NIR region making melanin a promising photothermal agent. An absorption peak at 340 nm is shown, indicating the ability of SM to absorb UV radiation which accounts for the protective effect of melanin against UV hazards [6]. There is a monotonically decreasing broadband absorption curve extending from UV to NIR without a distinct peak in the visible region, which is consistent with many previous reports that studied natural melanin [1, 28, 32].

The temperature of SM-NP-Tw increased gradually during light irradiation at a power density of 1.6 W/cm^2 (Fig. 7). The heating rate of SM-NP-Tw was significantly higher than that of water. After 12 min, the temperature of SM-NP-Tw was 53 ± 3 °C while that of distilled water was 30 ± 2 °C. The heating rate of SM-NP-Tw was found to exhibit a power relationship, as it was the most probable equation (R^2 is 0.94). These results are consistent with Liang et al., who studied the thermal behavior of sepia melanin nanoparticles after IR laser irradiation [29]

There was a nonsignificant change in the particle size, polydispersity index, and zeta potential during the 10 months of storage at 4 °C, as illustrated in Fig. 8. This shows the good colloidal stability of the prepared SM-NP-Tw at 4 °C.

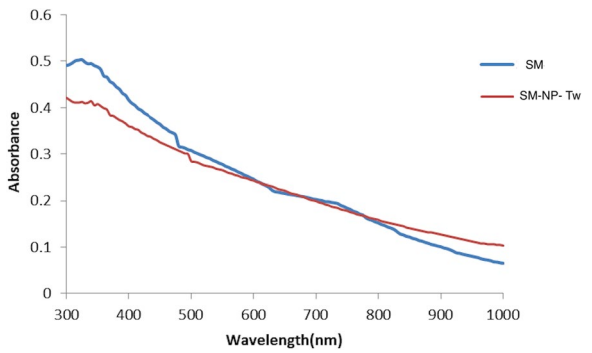

Fig. 6 UV–Visible spectra of Sepia melanin alkaline aqueous solution pH 12 (SM) and self-assembled tween-based nanoparticles (SM-NP-Tw)

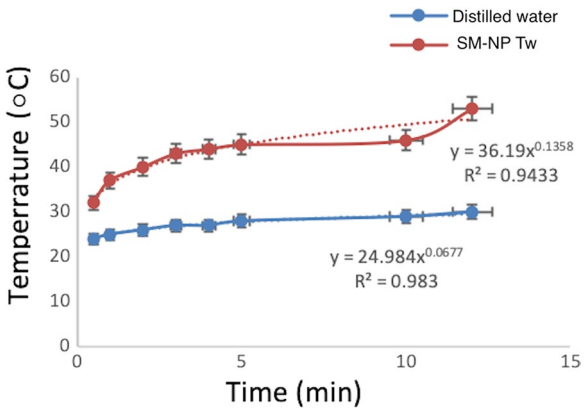

Fig. 7 Photothermal responses of SM-NP-Tw and distilled water upon light irradiation

3.3 In Vitro *release*

The release of the melanin from the prepared SM-NP-Tw was monitored for a period of 13 days under sink conditions. The melanin release profile (Fig. 9) showed a cumulative release ratio of 27%, after 24 h, and 45% after 13 days. The release mechanism of SM-NP-Tw was best fitted with zeroth-order kinetics, the release rate constant (k) was 5.47 h^{-1} and the correlation coefficient (R^2) was 0.99.

3.4 In vitro *dark and photocytotoxicity assay*

Dark and photocytotoxicity of SM-NP-Tw was studied on HepG2 cells at different melanin concentrations (10, 100, and 500 µg/ml). As shown in Fig. 10, in the absence of irradiation, cell viability remained above 70% after incubation for 48 h even at high concentrations. However, after laser irradiation at 660 nm and 820 nm for 10 min, the cell viability decreased significantly ($p < 0.05$). Collectively, the cell viability decreased significantly upon increasing the concentration and upon radiation by IR laser (820 nm).

4 Discussion

In this study, we extracted the natural sepia melanin from the cuttlefish and prepared it in stable, water-dispersible, surfactant-based nanoparticles (SM-NP-Tw). Owing to the high heterogeneity of melanin and its interactable chemical properties, its physiochemical characterization could be a challenging process [30]. The melanin source, extraction, and purification processes greatly impact the shape and chemical structure of melanin [20]. In our work, the structure of SM was confirmed by FTIR and ^{1}H-NMR [33], as the obtained spectra were consistent with that of natural sepia melanin [1, 9]. The functional groups of melanin may vary according to the source of

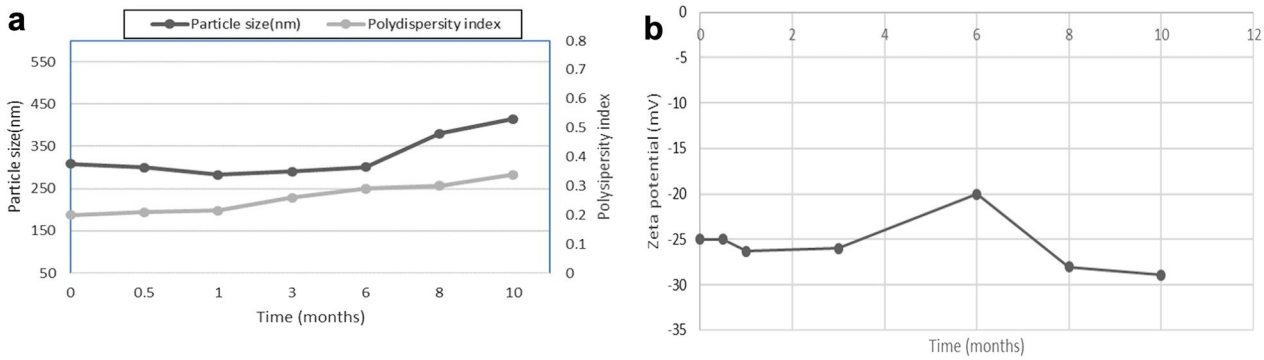

Fig. 8 Stability study of SM-NP-Tw: **a** the change of particle size and polydispersity index by time, **b** the change of zeta potential by time

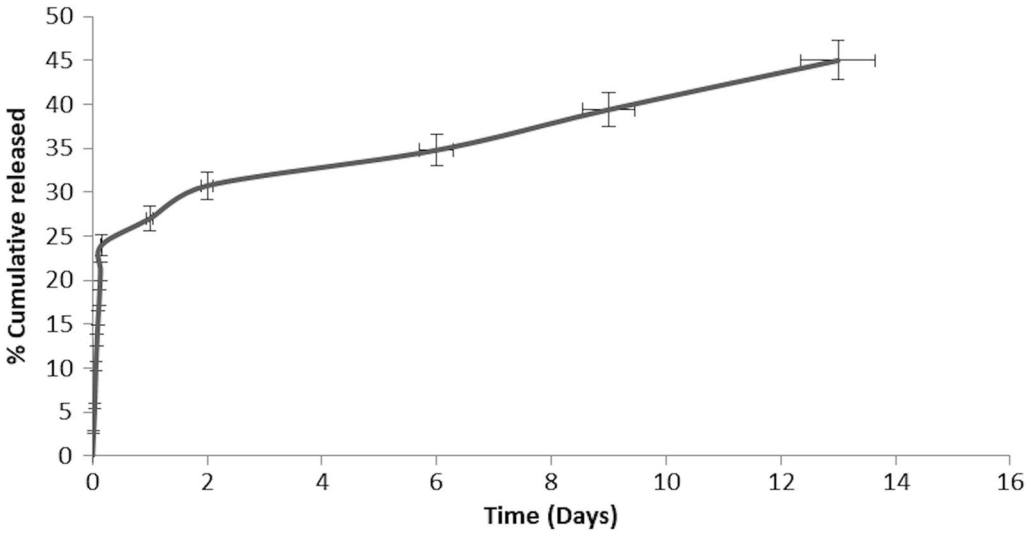

Fig. 9 In vitro release profile of SM from the prepared SM-NP-Tw

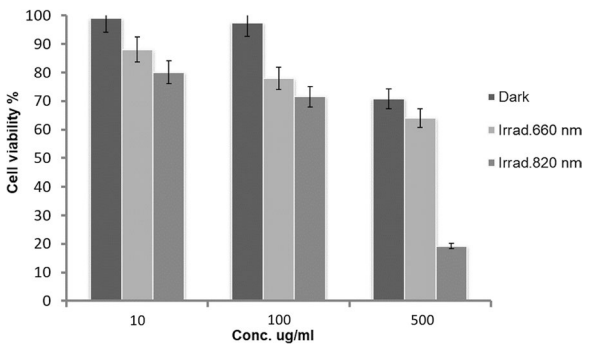

Fig. 10 Dark and photocytotoxicity of SM-NP-Tw at different melanin concentrations

melanin. Correa et al. [34] reported that the strength of signals at 1625 and 1522 cm^{-1} in human and fungal melanin differed from that of synthetic melanin. SEM images showed that the extracted SM were arranged as nanosized spherical aggregates. It has been reported that sepia melanin is composed of monomers arranged as graphite-like blocks that aggregate forming particles with different particle size [35]. Eliato et al. [28] reported that human melanin exhibited an elliptical heterogenous shape, but the sepia melanin had a homogenous spherical nanoparticle-like shape. In the present work, the particle size ranged from 100 to 200 nm which is in perfect agreement with the previous work of Jiang et al. and Mbonyiryivuze et al. [1, 30]. TGA thermogram and DTA curves were used to study the thermal behavior and stability of the extracted SM, as the thermal resistance differs according to the origin of melanin [3, 7, 20]. The results proved high resistance to thermal degradation which indicates that the extracted SM has high thermal stability.

The main problem of the natural melanin nanoparticles that limits their clinical applications is the poor solubility in most of the solvents including physiological fluids. The solubility of the extracted SM powder

was tested in several organic solvents and in an aqueous buffer at different pH values. SM powder was insoluble in all the tested organic solvents. In addition, it was insoluble in aqueous media (HEPES buffer) at pH 7 and was precipitated in acidic pH. It started to disperse upon a gradual increase in pH until complete dispersion occurred at pH 12. Many efforts were exerted to synthesize soluble melanin-like nanoparticles [36–38]. On the other hand, only a few previous studies used the melanin extracted from cuttlefish for cancer photothermal therapy. Deng et al. [39] prepared natural nanoparticles, composed of a mixture of melanin, amino acids, and polysaccharides, extracted from cuttlefish and reported that the extracted nanoparticles exhibited good photothermal conversion activity in vivo. Jiang et al. [1] studied the natural sepia melanin nanoparticles as effective photothermal agents and coated the prepared nanoparticles with red blood cells to improve their accumulation in tumors.

We attempted to solve the problem of melanin insolubility by self-assembling the extracted SM into tween 80-based nanostructures (SM-NP-Tw). Complete dispersion of SM was attained upon using 10% w/v tween 80, which was consistent with Ravichandran et al. [17] who used 10% w/v tween 80 to form micelles loaded by indocyanine green and piperlongumine. Self-assembled Tween 80 micelles were used as drug delivery carriers for many drugs with solubility problems, such as curcumin [18], thymol [40], and other essential oils [41], where they can improve the solubility and bioavailability by encapsulation of the insoluble drugs in the micelles hydrophilic core [16]. The dynamics of micellar solubilization and the factors affecting it were widely investigated [42]. It is primarily based on a decrease in system free energy caused by micelles formation, above the surfactant critical micelle concentration, and a reduction in the contact area between the insoluble particles and the polar solvent [40, 43].

The process of SM-NP-Tw preparation was very simple and cost-effective, and did not need the use of any organic solvents or hazardous materials. SM-NP-Tw showed a broad absorption curve extending from the UV to NIR region, suggesting the use of melanin as a photothermal agent [1, 32]. The photothermal performance of the prepared SM-NP-Tw showed a higher temperature increase when compared to the distilled water which proves that SM-NP-Tw have reasonable photothermal conversion properties and can generate heat upon light irradiation.

Melanin was released from SM-NP-Tw in a controlled manner following zeroth-order kinetics. Our results were consistent with those obtained by Zhang et al. [10] who reported that less than 40% of melanin was released from nanoliposomes during a period of two weeks which is sufficiently long for therapeutic purposes

In the absence of laser, HepG2 cells retained high viability after the treatment with SM-NP-Tw, even at high concentrations, indicating high safety. However, after laser irradiation by either wavelength, 660 nm or 820 nm, the cell viability of HepG2 cells decreased as the concentration increased. The reduction in the cell viability was more significant at 820 nm, indicating that irradiation in the NIR region provides better photocytotoxicity and photothermal activity. These results were consistent with those obtained by Jiang et al., who revealed high photothermal efficacy of SM-NP on lung cancer cells upon the NIR laser radiation [1].

The results of the cell toxicity can be explained by the results of UV–Vis spectroscopy and the photothermal performance, which revealed that melanin can absorb light in a wide range of wavelengths including the NIR region; thus, it can emit sufficient heat to kill the cells through photoheat conversion effects. The main feature of the photothermal activity is the effective conversion of electronic excitation energy to vibrational energy as heat energy dissipation happens from light-excited molecules by internal conversion and non-radiative relaxation of the vibrational energy levels, generating fast heating [44, 45].

Taken together, these results pointed out that sepia melanin nanoparticles self-assembled in tween-based nanostructures have excellent photothermal properties.

5 Conclusion

Natural melanin nanoparticles, prepared from extracted ink of living cuttlefish, are of great interest as photothermal agents due to their natural abundance and low cost. However, many challenges still need to be overcome for their clinical applications. We tried to overcome these challenges by preparing highly soluble self-assembled tween-based melanin nanoparticles. Different techniques were employed for the characterization of the extracted melanin and the prepared nanostructures including SEM, TEM, particle size, zeta potential, FTIR, ^{1}H-NMR, and TGA. The prepared nanoparticles were nanosized with good dispersion and high stability. Moreover, they caused temperature rise in aqueous media after light irradiation and exhibited photothermal cytotoxic effect on the HEPG2 cell lines. Therefore, the tween-based self-assembled melanin nanoparticles can be promising platform for photothermal cancer therapy.

They are suitable for clinical application due to their natural components which can guarantee their biocompatibility and biosafety. Further research should be conducted on different cell lines before proceeding to animal and clinical studies.

Abbreviations

SM	Sepia melanin
PTT	Photothermal therapy
NIR	Near-infrared
SM-NP-Tw	Self-assembled tween 80-based micelles loaded with sepia melanin
FTIR	Fourier transform infrared spectroscopy
^{1}H-NMR	Proton nuclear magnetic resonance
DMSO	Dimethyl sulfoxide before measurement
TGA	Thermogravimetric analysis
SEM	Scanning electron microscope
TEM	Transmission electron microscope
SRB	Sulforhodamine B assay
TCA	Trichloroacetic acid
PI	Polydispersity index

Acknowledgements
Not applicable.

Author contributions
MF and DA designed and planned the work. NME was responsible for carrying out the experimental procedures. All authors have shared equally in the interpretation of the results, writing, and reviewing of the manuscript. All authors read and approved the final manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials
The main data supporting the finding of this study is available in the manuscript. Other detailed data can be available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors report there are no competing interests to declare.

Received: 1 November 2022 Accepted: 22 January 2023
Published online: 30 January 2023

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