Nanodispersed UV blockers in skin-friendly silica vesicles with superior UV-attenuating efficiency

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Supporting Information

Experimental Section

Chemicals and skin samples

$EO_{39}BO_{47}EO_{39}$ [commercial name B50-6600, EO is poly(ethylene oxide) and BO is poly(butylene oxide)] was purchased from Dow Company. Tetraethyl orthosilicate (TEOS, ≥98%), ammonium hydroxide solution (28%), (3-aminopropyl)triethoxysilane (APTES), fluorescein-5-isothiocyanate (FITC) were purchased from Sigma-Aldrich. 2,2'-methylene-bis-(6-(2*H*-benzotriazole-2-yl))-4-(1,1,3,3-tetramethyl-butyl)-phenol (MBBT) was received from Tokyo Chemical Industry (TCI). *Ex vivo* pig skin samples were excised from pig ears sourced from Highchester Pty Ltd, Australia. All the other reagents were of analytical reagent grade.

Synthesis of silica vesicles

In the synthesis of silica vesicles with uniform size of 50 nm (denoted as SV-50), 0.5 g of $EO_{39}BO_{47}EO_{39}$ and 0.852 g of Na_2SO_4 (0.20 M) were dissolved in a flask with 30 g of pH = 4.7 NaAc-HAc buffer solution ([NaAc] = [HAc] = 0.40 M) to form a homogenous solution under stirring at 10 °C. 3.33 g of TEOS was then added to the above flask with continuously stirring for 24 h and the reaction solution was hydrothermally treated at 100 °C for another 24 h. After hydrothermal treatment, the precipitates were filtered, repeatedly washed with deionized water to remove the added salts, and then dried in air. The final product was obtained by calcination of the precipitates at 550 °C for 5 h.

Synthesis of Stöber spheres

In order to synthesis dense silica nanoparticles with the same particle size as SV-50, 50 nm Stöber spheres was synthesized using Stöber method (denoted as SS-50).[1] 50 ml of ethanol, 3.8 ml of deionized water and 2 ml of 28% ammonium hydroxide solution were mixed in one flask at 70 °C. 2.8 ml of TEOS was then added to the above solution under vigorous stirring for 1 h. The as-synthesized nanoparticles were collected by high speed centrifugation (20000 rpm, 15 min), washing with ethanol and deionized water and drying at 50 °C overnight.

Amino and FITC functionalization of nanoparticles

In the amino- modification process, 1.5 g of SV-50 or SS-50 were added to two flasks, respectively. Each flask was added 60 ml of toluene and stirred for 6 h before adding 1.0 ml APTES. After stirring at 110 °C for 12 h, the nanoparticles were centrifugated, extensively washed with toluene and ethanol, and dried in a fume-hood at room temperature. Since the thiocyanate group of FITC is highly aminoreactive, free amino- moieties were utilized for labeling nanoparticles with FITC. In a typical experiment, 20 mg of powdered nanoparticles were dispersed in 3 ml of deionized water, mixed with 5ml of FITC ethanol solution (0.3 mg ml^{-1}), and stirred in the dark at room temperature for 6 h. The particles were centrifuged and washed with ethanol for three times until the supernatants were colorless. The FITC labelled nanoparticles were re suspended in water in 1 mg ml^{-1} for confocal microscopy observations after applying in skin samples. In order to compare the amount of FITC labelled to silica nanoparticles, 1 mg of each FITC labelled silica nanoparticles was dissolved in 1 ml of NaOH (0.6 M) solution overnight to dissolve the silica. The FITC amount was calculated from the absorbance of as above solutions measured in a UV-Vis plate reader (Tecan M200 Pro) at a wavelength of 490 nm.

Characterizations of silica nanoparticles

The morphology of silica nanoparticles was observed by field-emission scanning electron microscope (FE-SEM) and transmission electron microscopy (TEM). The FE-SEM images of SV-50 (Figure 2A) and SS-50 (Figure 2C) were obtained with a JEOL 7800F operated at 1 kV by the gentle beam method with a UED in-column detector. For FE-SEM observation, the samples were prepared by dispersing the powder

nanoparticles in ethanol, after which they were dropped to the aluminum foil pieces, dried in air and attached to conductive carbon film on SEM stubs. The TEM images were obtained with a JEOL 2100 operated at 200 kV. For preparation of the TEM samples, the powder nanoparticle-ethanol dispersion was dropped and dried on carbon film on a Cu grid. Dynamic light scattering (DLS) and ζ potential measurements were carried out at 25 °C using a Zetasizer Nano-ZS from Malvern Instruments. The samples were dispersed in deionized water by ultrasonication before analysis.

Skin safety evaluation in *ex vivo* pig skin model

Pig ear skin sourced from a local abattoir was used as skin model for safety evaluation. The pig ears were washed with soap, thoroughly rinsed by water and stored at −20 °C until required. The pig ears were thawed and then gently shaved to remove excessive hair, then rinsed and pat dried. The ventral side of the ear was then separated from the cartilage using a scalpel. The excised skin was pinned down on a cork-board maintaining slight tension across the surface. The FITC labelled silica nanoparticles were dispersed in water with a concentration of 1 mg cm^{-3}. Silica nanoparticle-water suspensions were applied to the excised skin samples at a concentration of 2 mg cm^{-2}. The skin was incubated at 37 °C for 2 h. The skin samples were then swabbed, rinsed under water for 30 seconds then patted dry. Biopsies (6 mm) were taken of the treated areas and imaged.The tissue samples were analysed *en face* using confocal laser scanning microscopy (710META, Zeiss, Germany). Ten regions were imaged for n = 1 per silica nanoparticle sample. Nanoparticles were excited at 488 nm and reflectance was used to visualize the surface of the skin at 633 nm. The samples were optically sectioned at 1 μm intervals from the surface down to a depth of approximately 100 μm. Following image acquisition, the confocal image stacks were analysed using Image J Software (NIH, USA). The nanoparticle pixel intensity was quantified using the raw integrated density per layer within the stack for each sample.

Encapsulation of MBBT

A rotary evaporation method[2, 3] was utilized for encapsulation of MBBT into the silica vesicles. In the procedure, 0.12, 0.15 or 0.20 g of SV-50 after calcination were added to 100 ml of MBBT–ethyl acetate solution (1.20 g L^{-1}), with a MBBT:SV-50 feeding ratio of 1:1, 0.8:1 and 0.6:1, respectively (denoted as MBBT-SV-X, X is the ratio of MBBT:SV). The mixture was removed into a long cylindrical flask attached to a rotary evaporator (BUCHI R-210) and evaporated at 50 °C in a vacuum system in dark with a residual pressure of 225 Torr until all solvent had been removed. In comparison, similar procedure had been carried out with MBBT-ethyl acetate solution only.

Characterization of MBBT-SV-X

FE-SEM images of the MBBT-SV-X compared to pure SV-50 and MBBT after rotary evaporation were observed using a JEOL JSM 6300F operated at 15 kV. For FE-SEM observation, the powder samples were adhered onto the conductive carbon film which is attached on SEM stubs and coated with platinum. Wide angle X-ray diffraction (WA-XRD) patterns of the materials were recorded on a German Bruker D8 X-ray diffractometer with Ni-filtered Cu Kα Radiation. A Metter Toledo GC200 thermogravimetric analysis (TGA) station was used for the loading amount and differential scanning calorimetry (DSC) study at a heating rate of 2 °C min^{-1}. Nitrogen adsorption-desorption isotherms were measured at -196 °C by using a Micromeritics Tristar II system, before which the samples were degassed at 100 °C overnight on a vacuum line. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure (P/P_0) of 0.99, and the Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. Fourier transform infrared (FTIR) spectra were collected on a ThermoNicolet Nexus 6700 FTIR spectrometer equipped with a Diamond ATR (attenuated total reflection) Crystal. For each spectrum, 64 scans were collected at resolution of 4 cm^{-1} over the range 450-4000 cm^{-1}.

UV-vis spectroscopy

To test the UV-Vis transmittance of MBBT-SV-0.6 and pure MBBT after rotary evaporation in aqueous suspensions, the powder samples were suspended in water in ultrasonic bath for 10 minutes, which are compared to pure MBBT dissolved in organic solvent of ethyl acetate. The concentrations of MBBT in all suspension/solution were kept as 0.004 % (w/v). In another group of experiment, MBBT-SV-0.6 was treated with Na_2CO_3 solution to remove silica. In this process, 10 mg MBBT-SV-0.6 was added into 10 mL of Na_2CO_3 solution (0.6 M). The mixture was suspended in ultrasonic bath for 10 minutes and shaken at room temperature for at least 12 h. The above solution was then neutralized to pH 7 using HCl solution (0.6 M) and diluted to a final MBBT concentration of 0.004 % (w/v) with MilliQ water.

UV-vis transmittance spectra were measured with a Shimadzu UV-2450 double beam spectrophotometer. In order to measure the direct transmittance, the MBBT suspension/solution and the corresponding reference (pure water, ethyl acetate or neutralized and diluted Na_2CO_3 solution) were filled into quartz cells with d = 1 cm pathlength, which were then placed into the respective beams of the device. Measurements were performed in a range of 280-400 nm with a spectral resolution of 0.5 nm. The direct transmittance of 0.007 % (w/v) silica vesicle suspension in water was also tested. Equation S2 was used to calculate the direct extinction spectra from the direct transmittance, which were contributed by three fractions: absorbance, forward scattering and backward scattering of the UV blockers.[4]

The diffuse transmittances of MBBT-SV-0.6 suspension and MBBT in ethyl acetate solution were also measured to evaluate the forward scattering properties using the same instrument. An integration sphere accessory was fixed to the instrument in order to collect the direct transmitted light and also the light scattered in forward direction. Sample and reference cells were placed in the respective light beams at their entrance into the integration sphere, while $BaSO_4$ pressings were mounted into the sample and reference windows opposite thereof. The diffuse extinction spectra were calculated using Equation S3 from diffuse transmittance which had only absorbance and backward scattering contributions. By subtracting extinction spectra from direct and diffuse transmittance, the fraction of forward scattering was also calculated.[4]

Formulation preparation and *in vitro* sun protection factors

In order to evaluate the protection factors of MBBT encapsulated in SV-50 in sunscreen application, a water-based formulation containing MBBT-SV-0.6 was prepared. The formulation recipe is listed in Table S3. In a typical process, MBBT-SV-0.6 was suspended in MilliQ water in ultrasonic bath with different concentrations. 1 % w/w triethanolamine was also added into the water suspension to form the water phase. Cetyl alcohol, stearic acid and glycerin (2.0, 4.0 and 2.0 % w/w, respectively) were mixed at 85 °C to form a liquid oil phase. The water phase and the oil phase were kept at 85 °C separately, and then mixed at the same temperature, vigorously stirred with a glass rod by hand till the mixture cooled to room temperature with a homogeneous creamy appearance. The final MBBT concentration is controlled as 1, 2, 4 and 8 % w/w.

The *in vitro* sun protection factors were calculated from diffuse transmittance of formulation films.[5, 6] In order to test the diffuse transmittance, formulations were applied on a 50×10×1 mm quartz plate, and the formulation film thickness is 26 microns simulating the thickness of applied sunscreen on skin. The quartz plate with the formulation film was placed in the UV-vis spectrophotometer with an integration sphere for characterizations. The *in vitro* sun protection factor (SPF) value was calculated from diffuse transmittance spectra in a wavelength range of 290-400 nm according to Equation S4. Data points were selected from diffuse transmittance spectrum every 0.5 nm (d_λ = 0.5 nm).

Table S1. Structural information of silica nanoparticles.

Sample Name	Paritcle size (nm)	ζ potential (mV)	ζ potential after amino modification (mV)	$\rho_{apparent}$ (g cm^{-3})	FITC density (mg/mg Silica)	N (10^5 μm^{-2})
SV-50	45.9±3.0	-16.6±0.5	17.6±0.5	1.01	0.052	3.90
SS-50	52.3±2.2	-32.8±0.6	20.3±0.7	1.70	0.049	1.57

Particle size: the particle size of nanoparticles is a statistical value measured from TEM images of over 50 particles; $\rho_{apparent}$: apparent density of nanoparticles; N: calculated particle number applied on *ex vivo* pig skin in Figure 2 and Figure S3 from apparent density.

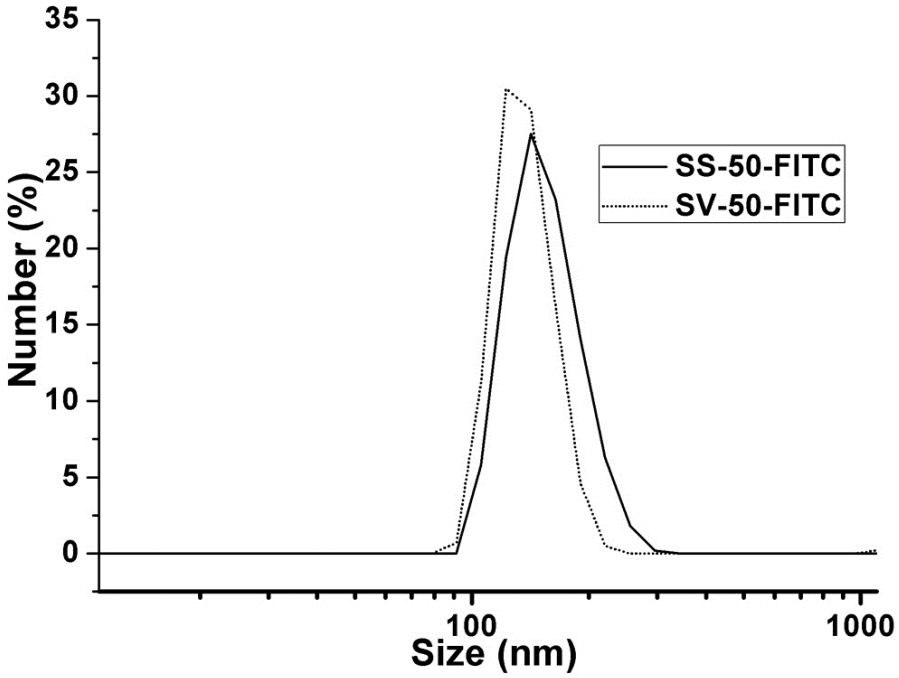

Figure S1. Particle size distribution curves of SS-50-FITC (solid) and SV-50-FITC (dash) by DLS.

Calculation of apparent density of nanoparticles

The density of dense silica nanoparticles (SS-50) is considered to be equal to the density of amorphous silica (ρ_{Silica} = 1.70 g cm^{-3}).[7] The apparent density of SV-50 is estimated using a simplified hollow spherical model with dense wall, which is considered with the density of amorphous silica (ρ_{Silica}). The schematic image of SV-50 (Fig. S1 right) shows a similiar particle radius (R = 23.0 nm) to SS-50 and a cavity radius of r = 17.0 nm. As a result, the apparent density of silica vesicles can be calculated using Equation S1. The result of calculation is summarized in Table S1. This calculation ignores the existence of porosity within the walls which is hard to estimate the exact volume. Therefore, the actual apparent density of SV-50 will be further lower if wall porosity is considered.

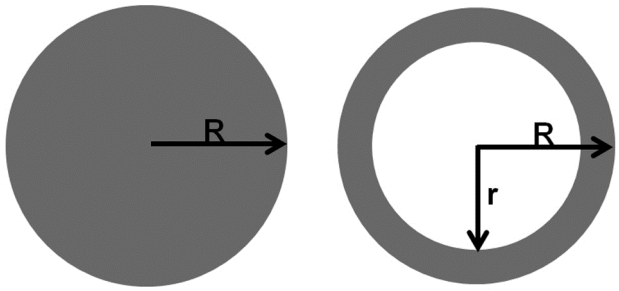

Figure S2. Schematic image for silica vesicles SV-50 (right) with cavity radius of r and particle radius R which is the same with SS-50 (left). The apparent density of SV-50 is calculated using Equation S1.

$$\rho_{apparent} = \frac{\rho_{Silica}(R^3 - r^3)}{R^3} \qquad \text{(Equation S1)}$$

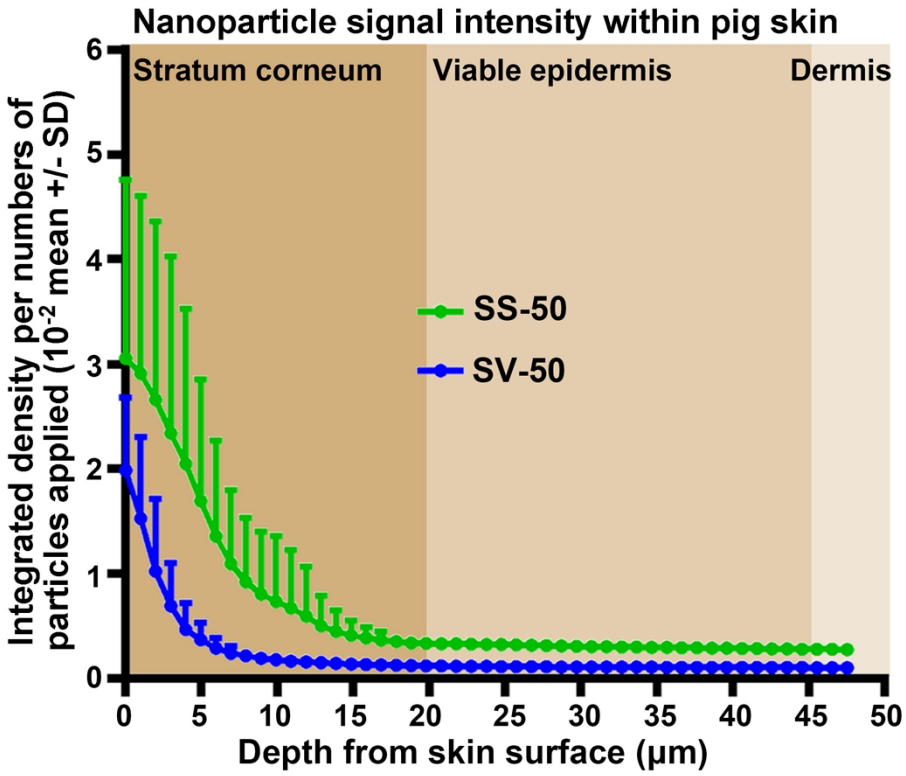

Figure S3. Distribution profile of FITC fluorescence integrated density from nanoparticles within *ex vivo* pig skin as a function of skin depth, normalized by numbers of applied nanoparticles.

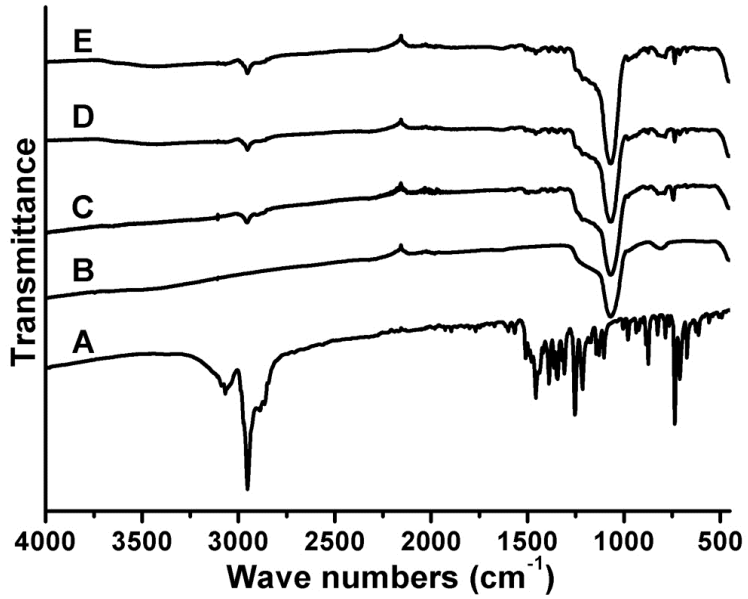

Figure S4. FTIR spectra of (A) pure MBBT, (B) SV-50 after calcination, (C) MBBT-SV-0.6, (D) MBBT-SV-0.8 and (E) MBBT-SV-1.0.

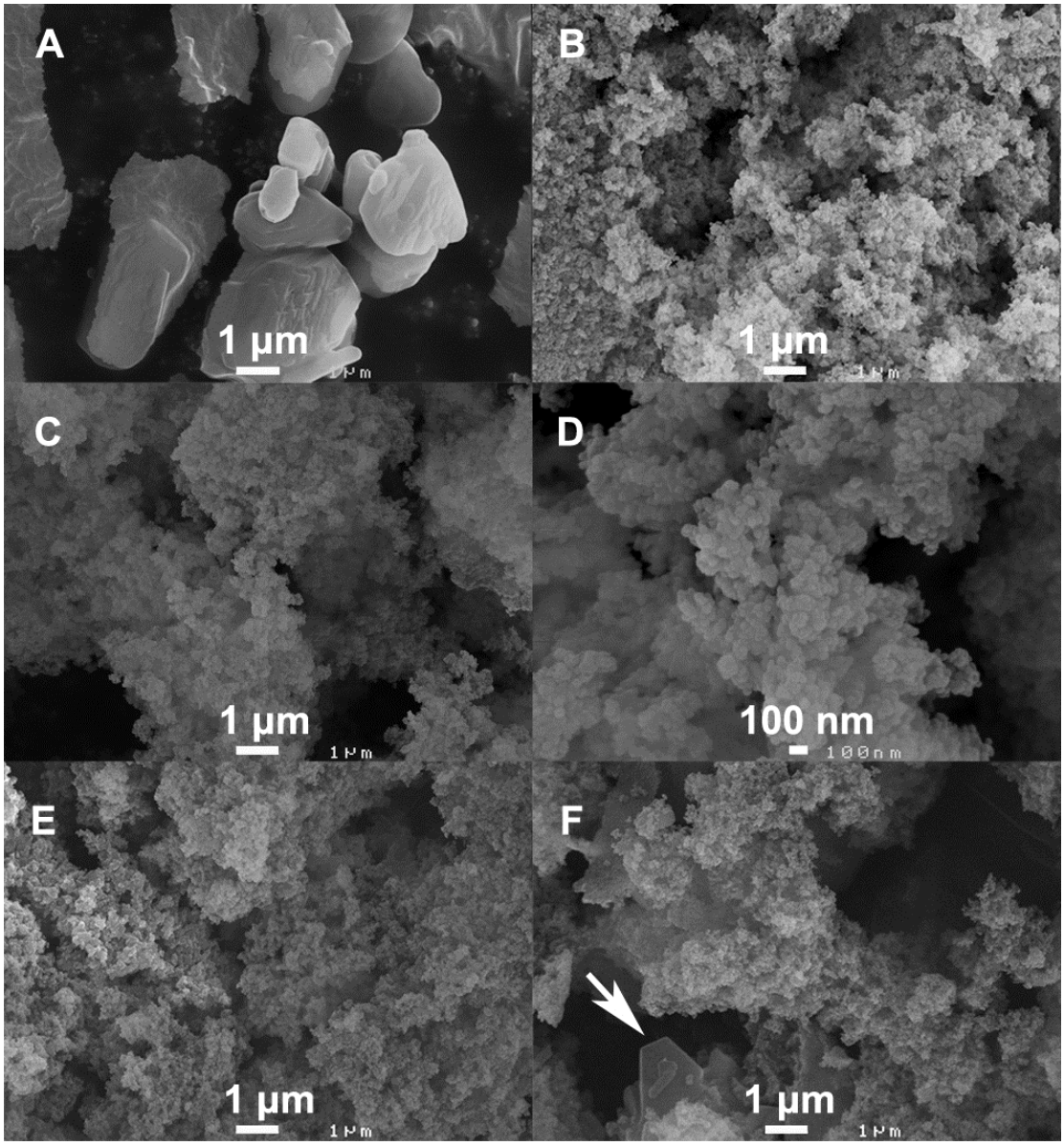

Figure S5. FE-SEM images of (A) pure MBBT, (B) SV-50 after calcination, sample MBBT-SV-0.6 in low (C) and high (D) magnification, (E) sample MBBT-SV-0.8 and (F) MBBT-SV-1.0.

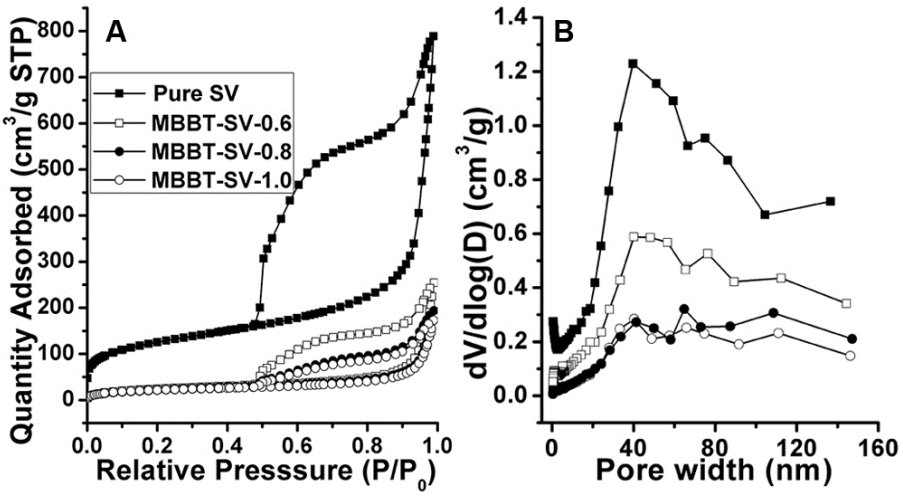

Figure S6. (A) Nitrogen sorption isotherm plots and (B) BJH pore size distribution curves calculated from the adsorption branch of SV-50 before and after encapsulated with MBBT.

Table S2. Structural information from N_2 sorption and TGA results.

Sample Name	V_p (cm^3 g^{-1})	S_{BET} (m^2 g^{-1})	Weight loss (%)	ζ potential (mV)
SV-50	1.22	431	1.70	-16.6
MBBT-SV-0.6	0.39	65	38.3	-0.53
MBBT-SV-0.8	0.30	77	44.9	-0.51
MBBT-SV-1.0	0.26	71	50.8	0.02

V_p: total pore volume; S_{BET}: BET surface area.

The nitrogen sorption isotherms of SV-50 show type VI isotherm and H3 hysteresis loop with steep capillary condensation at P/P_0 of ~0.9. All MBBT-SV-X show similar isotherms with much lower quantity adsorbed at the highest relative pressure. After encapsulation the total pore volume of SV-50 decreases from 1.22 cm^3 g^{-1} to 0.26-0.39 cm^3 g^{-1} for MBBT-SV-X, indicating the mesopores of SV-50 starts to be occupied by MBBT. The Brunauer–Emmett–Teller (BET) surface area also decreases from 431 m^2 g^{-1} to 65-77 m^2 g^{-1}. Considering the pore volume contributed by the packing void is 0.35 cm^3 g^{-1}, the mesopores volume of SV-50 is estimated to be 0.87 cm^3 g^{-1}. As a result, the theoretical loading capacity to MBBT is 0.87 g/1.0 g SVs where the density of organic molecules of MBBT is estimated for ~ 1 g cm^{-3}.

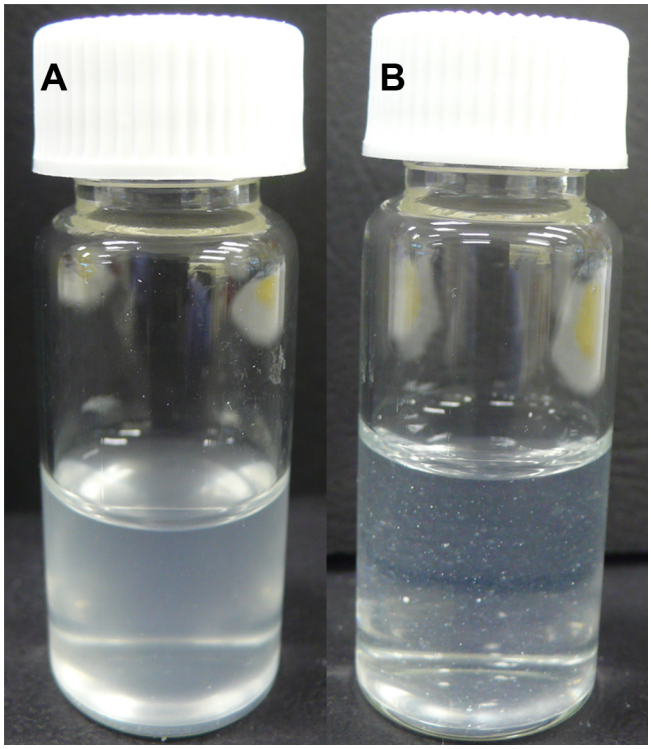

Figure S7. Digital photos of suspensions of MBBT-SV-0.6 in water with MBBT concentration of 0.004% w/v, before (A) and after (B) removal of silica by 0.6 M Na_2CO_3 solution.

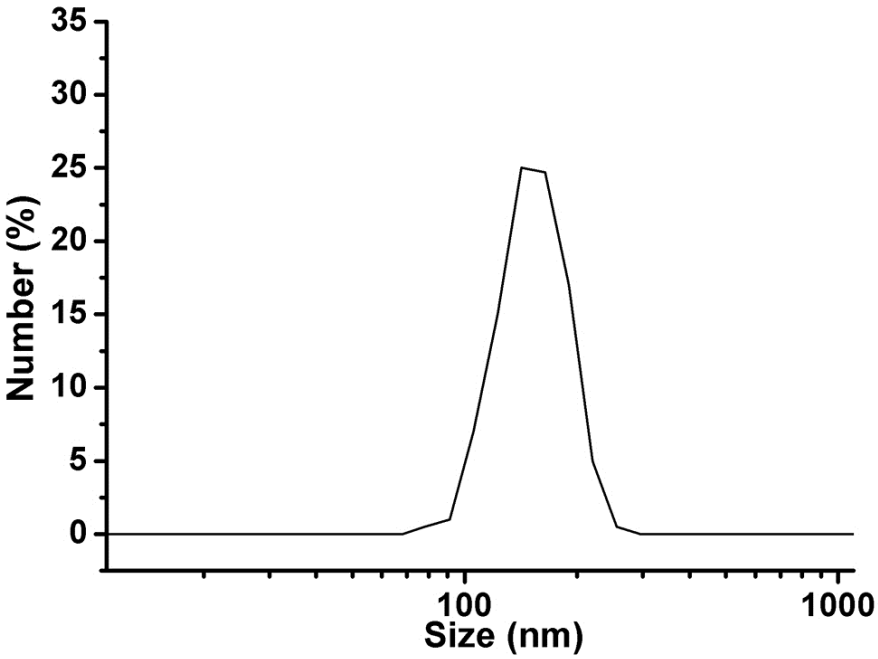

Figure S8. Particle size distribution curve of MBBT-SV-0.6 in aqueous dispersion by DLS.

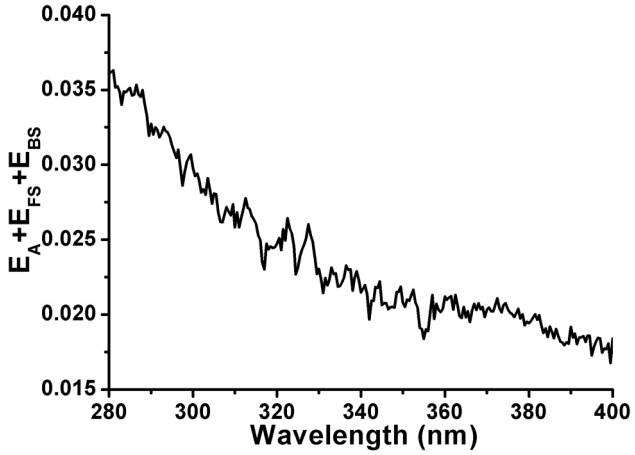

Figure S9. Extinction spectra calculated from direct UV-Vis transmittance of 0.007% SV water suspension.

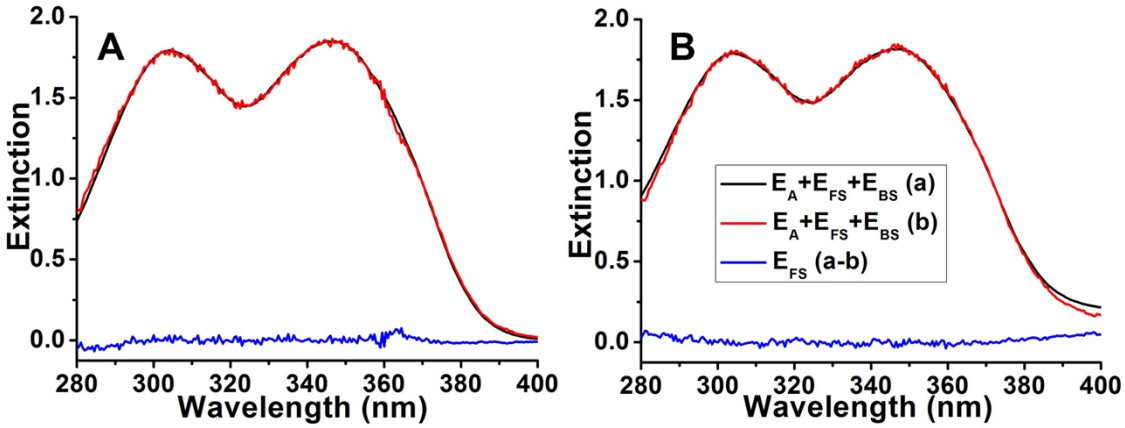

Figure S10. Extinction spectra calculated from direct UV-Vis transmittance (black), diffuse UV-Vis transmittance (red) and their difference (blue), for (A) MBBT in ethyl acetate solution, (B) MBBT-SV-0.6 suspension with 0.004% MBBT.

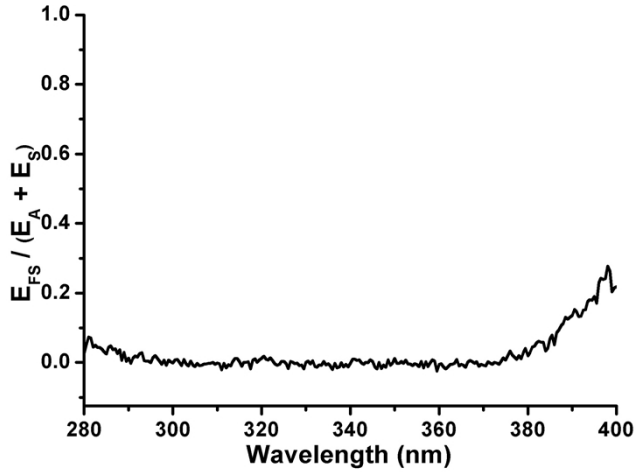

Figure S11. Fraction of forward scattering of MBBT-SV-0.6 water suspension with 0.004% MBBT.

$$\log \frac{1}{T_{direct}} = E_A + E_{FS} + E_{BS} \quad \text{(Equation S2)}$$

T_{direct}: Direct transmittance of MBBT solution/suspension, tested without integration sphere accessory; E_A: extinction contributed by absorption of MBBT; E_{FS}: extinction contributed by forward scattering of MBBT; E_{BS}: extinction contributed by back scattering of MBBT.

$$\log \frac{1}{T_{diffuse}} = E_A + E_{BS} \quad \text{(Equation S3)}$$

$T_{diffuse}$: Diffuse transmittance of MBBT solution/suspension, tested with an integration sphere accessory; E_A: extinction contributed by absorption of MBBT; E_{BS}: extinction contributed by back scattering of MBBT.

$$SPF = \frac{\sum_{290}^{400} E_\lambda I_\lambda d_\lambda}{\sum_{290}^{400} E_\lambda I_\lambda T_\lambda d_\lambda} \quad \text{(Equation S4)}$$

E_λ: erythema action spectrum; I_λ: the spectral irradiance of sunlight expected for a clear sky at noon in midsummer for a latitude of 40°N (solar altitude 70°) at wavelength λ and T is measured transmittance of the sunscreen layer at wavelength.

Table S3. Formulation recipe.

Phase	Ingredient	MBBT weight ratio 1 (% w/w)	MBBT weight ratio 2 (% w/w)	MBBT weight ratio 4 (% w/w)	MBBT weight ratio 8 (% w/w)
Oil phase	Cetyl alcohol	2.0	2.0	2.0	2.0
	Stearic acid	4.0	4.0	4.0	4.0
	Glycerin	2.0	2.0	2.0	2.0
Water phase	Triethanolamine	1.0	1.0	1.0	1.0
	Milli Q water	88.3	85.7	80.3	69.7
	MBBT-SV-0.6	2.7	5.3	10.7	21.3

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