Electronic Supplementary Information For

Surface Engineering of Bismuth Nanocrystals to Counter Dissolution

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1. Synthetic Details.

1.1. General Information

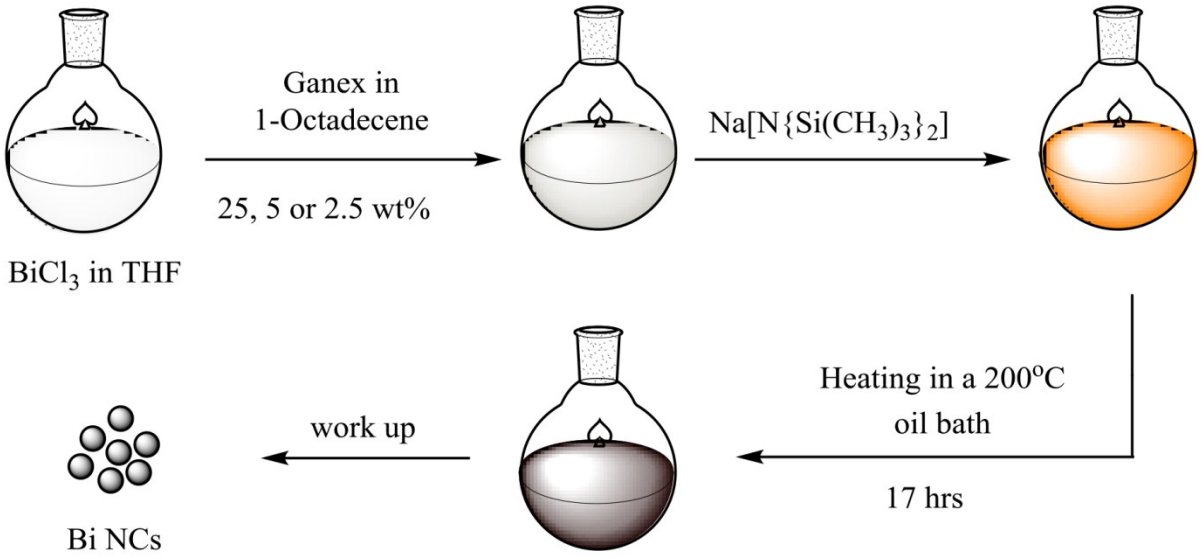
All reactions, unless otherwise stated, were performed with oven-dry glassware under an atmosphere of dry nitrogen. PHD-co-PVP copolymer [poly(1-hexadecene-co-1-vinylpyrrolidinone), Trade name Ganex™ V-216, or simply *Ganex*; 8 oz. Product code 828318, Lot number 0001793300] was obtained as a generous gift from Ashland Specialty Ingredients. Poly(*DL*-Lactic-co-Glycolic Acid) (or *PLGA*), [LG 50:50, *ester terminated* (nominal), Inherent viscosity range 0.95 – 1.20 dL/g in HFIP, 20.0 g) was purchased from LACTEL Absorbable Polymers, DURECT Corporation, AL, USA and stored at -20 °C prior to use. Dry tetrahydrofuran was directly obtained from a solvent purification system. All other reagents and solvents were obtained from commercial suppliers and used without further purification. For nanoparticle formulation, tip sonication was performed using a QSonica microtip sonicator probe with a tip diameter of 3 mm. at varying amplitudes. Centrifugation to isolate nanoparticles from reaction mixture was performed on a Sorvall LYNX 4000 Superspeed centrifuge.

1.1.1. Synthesis of Ganex coated Bismuth Nanocrystals (BiG NCs).

Prior to use, a solution of varying concentrations of Ganex in 1-Octadecene (Technical grade, 90%) (w/w) was prepared and stored under molecular sieves (3 Å, pellets, 1.6 mm) for at least a week, with periodic shaking to maintain homogeneity. For our purpose, 2.5, 5 and 25 wt% solution of Ganex in 1-Octadecene were prepared and stored as described.

Typical Procedure: In a 100 ml, three neck round bottom flask, fitted with a septa and an inlet and outlet for dry nitrogen, Bismuth(III) chloride, $BiCl_3$ (99.99 %, 1 eqv., 1.126 mmol) was taken and dry tetrahydrofuran (2.5 mL) was added to it. The contents were stirred to make a homogenous suspension under a dry nitrogen blanket and a solution of Ganex in 1-Octadecene

(25, 5 or 2.5 wt%, 25 g) was added to obtain a white suspension. After stirring for 5 min. at RT, Sodium bis(trimethylsilyl)amide, $Na[N\{Si(CH_3)_3\}_2]$ (95%, 6.5 eqv., 7.362 mmol) was added in one portion to the reaction mixture and the contents were stirred at RT for 5 min. The initial white suspension now turned orange-red in color and at this instant the flask was transferred to an oil bath maintained at 200 °C. An instant color change to brown was observed and the resulting reaction mixture was heated at this temperature for 17 hours under a dry nitrogen atmosphere. After 17 hours, the reaction mixture was allowed to cool down to RT. Isolation of the Bi nanocrystals (NCs) from the reaction mixture was condition to the amount of precursor Ganex used during the reaction and is detailed as follows. BiG NCs obtained from 25, 5 and 2.5 wt% Ganex precursors are termed as BiG-25, BiG-5 and BiG-2.5 NCs respectively for easy referral.



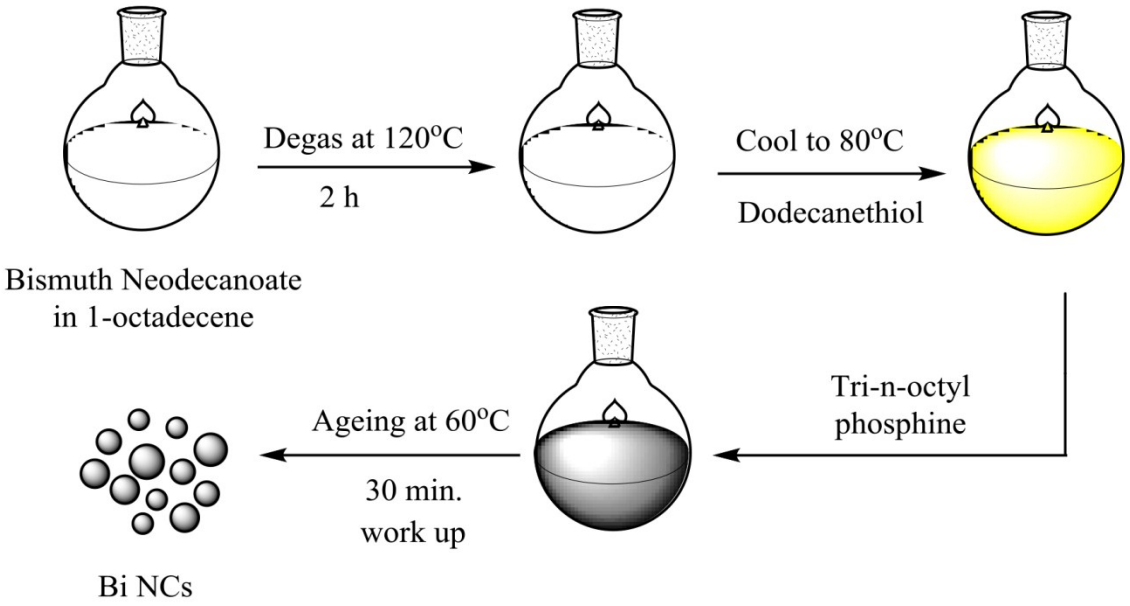
Scheme S1: Synthesis of Bi Nanocrystals using Ganex (Method A).

For 25 wt% Ganex: The reaction mixture was dissolved in toluene (1:1) and methanol (4x the volume of toluene used) was added. The resulting suspension was centrifuged at 18,000 rpm for 10 min and the supernatant was kept aside (not thrown as it contains BiG-25 NCs). The procedure was repeated multiple times by dissolving the residue obtained after centrifugation in toluene and adding methanol (1:4) to it, followed by centrifugation to isolate a brown oil (Inset, Figure S1). Numerous attempts at washing this oil using a wide array of solvents such as toluene, petroleum ether, hexane, methanol, ethanol, acetone, dichloromethane and their subsequent mixtures were unsuccessful. With each subsequent wash using centrifugation, there was considerable product loss in the supernatant layer (Inset, Figure S2).

For 5 and 2.5 wt% Ganex: The reaction mixture was dissolved in toluene (1:1) and methanol (4x the volume of toluene used) was added. The resulting suspension was centrifuged at 18,000 rpm

for 10 min and the supernatant was discarded. Finally, the isolated nanocrystals were suspended in a mixture of hexane and methanol (1:1) and centrifuged again at 15,000 rpm for 10 min to isolate clean bismuth nanocrystals as a black powder. TEM images for the BiG-5 NCs are given in Figure S4 with a picture of the as synthesized NCs shown in the inset. Corresponding TEM images and a picture of the as synthesized BiG-2.5 NCs are shown in Figure S6.

1.1.2. Synthesis of Non-Ganex Coated Bismuth Nanocrystals (Bi NCs).



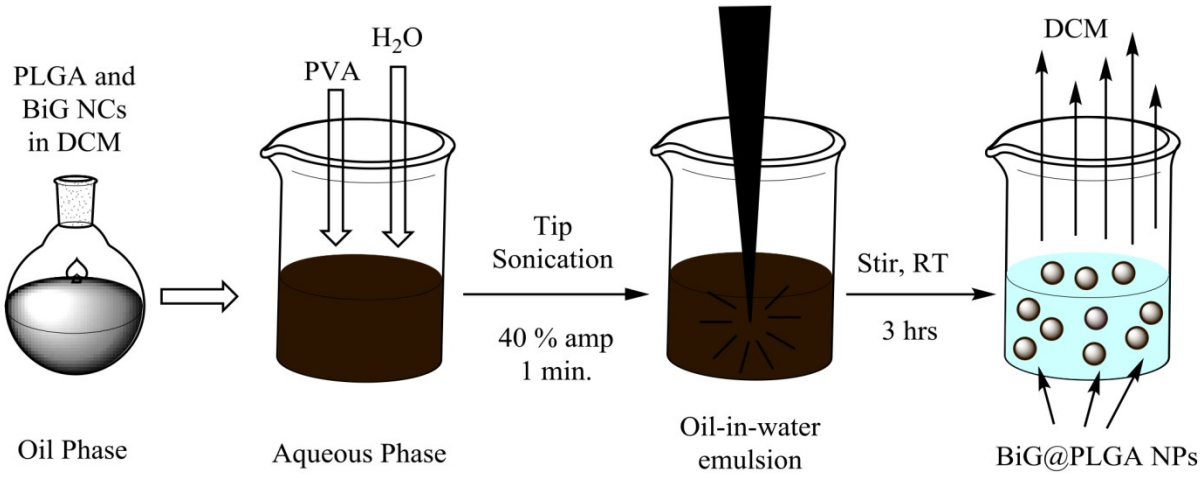
Scheme S2: Synthesis of Bi Nanocrystals without using Ganex (Method B).

Typical Procedure: In a 100 ml, three neck round bottom flask, fitted with a septa and an inlet and outlet for dry nitrogen, Bismuth neodecanoate, $Bi[COOC(CH_3)_2CH_2(CH_2)_4CH_3]_3$, (Technical grade, 1 eqv., 4.98 mmol) was taken and 1-Octadecene (Technical Grade, 90%, 25 mL) was added to it. The reaction vessel was fitted with a Kjeldahl flask on the inlet side and a receiving flask on the outlet side and the contents were stirred to make a homogenous colorless solution and subsequently heated at 120 °C under a dry nitrogen blanket for 2 hours to remove water and oxygen. Next, the reaction mixture was cooled down to 82-85 °C and maintained at this temperature for 10 min, following which 1-Dodecanethiol (≥ 98%, 1 eqv., 5.01 mmol) was added in one portion to it. The reaction instantly changed color from colorless to yellow and the contents were stirred at 82-85 °C for 5 min. To this solution was then added Tri-n-octylphosphine (Technical grade, 90%, 2.2 eqv., 2.24 mmol) in one portion. An instant color change from yellow to black was observed and the reaction mixture was transferred to a water bath maintained at 60 °C and stirred at this temperature for 30 min. Finally, the contents were allowed to cool down to room temperature.

For isolation of the as synthesized Bi NCs, a solution of hexane and ethanol (1:1) was added to the reaction mixture to precipitate the nanocrystals. Next, the resulting suspension was centrifuged at 12,000 rpm for 10 min and the supernatant was discarded. The residue so obtained was again dispersed in a mixture of hexane and ethanol (1:1) by sonication and the nanocrystals were isolated by centrifugation. This process was repeated until a clear supernatant was obtained (3 times). The as synthesized Bi NCs were finally isolated as a black powder (inset Figure S7) and dried in a heated vacuum oven prior to use.

It is important to mention that the Bi NCs made using method B were inhomogeneous in size as is evident from the TEM images (Figure S7). The Bi NC size was critically influenced by the final ageing temperature and the relative amounts of Dodecanethiol and Tri-n-octylphosphine used in the reaction.

1.1.3. Synthesis of PLGA Nanoparticles Encapsulating BiG NCs (BiG@PLGA NPs).

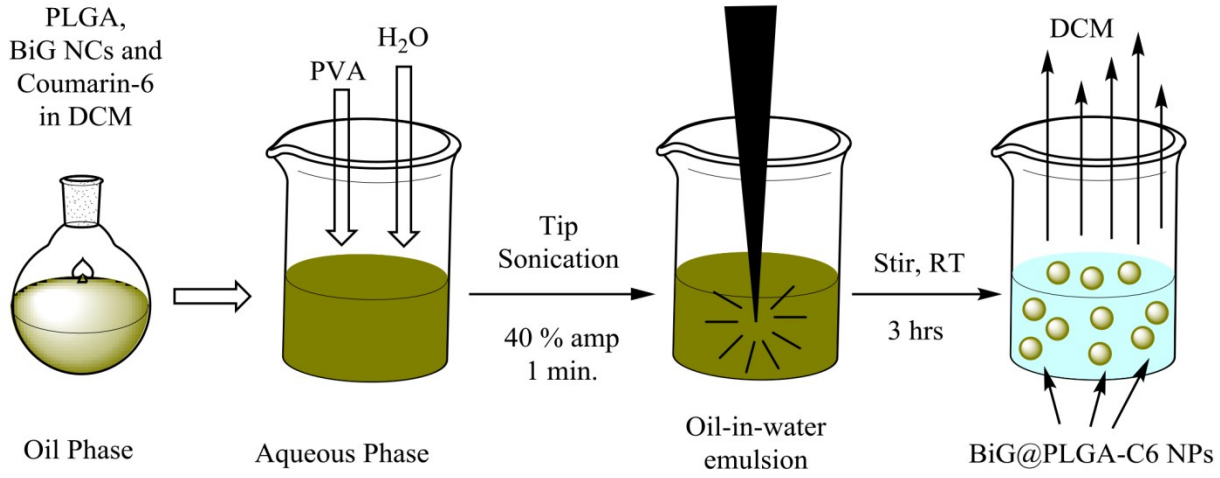


Scheme S3A: Synthesis of Non-Fluorescent BiG@PLGA NPs, (Method C).

Prior to encapsulation in PLGA, the BiG NCs (both BiG-5 and BiG-2.5 variants) were suspended in Dichloromethane (DCM). The resulting black suspension (40 mg BiG NCs per mL DCM) was sonicated for 20 min. with periodic vortex to form a homogenous suspension of BiG NCs in DCM. This suspension was further utilized for NP formulation. A stock solution of 2% Poly(vinyl alcohol) (PVA) in DI water (w/w) was prepared by dissolving 4.0 g PVA (22 kDa, 88% hydrolyzed) in 900 mL DI water by continuous stirring at 50 °C for 2 hrs. Once a clear solution was obtained with no visible residue, the volume of the solution was increased to 1000 mL by adding DI water to it. This final solution was allowed to cool down to RT, filtered (using a coarse filter paper) and stored at 4 °C prior to use. A stock solution of PLGA in DCM was prepared (20 mg PLGA polymer per mL DCM) and stored at sub-zero temperatures.

Typical Procedure: In a 15 mL falcon tube, 2% PVA (2 mL) was taken. In a separate 2 mL micro centrifuge tube, 0.5 mL of the BiG NC suspension in DCM was mixed with 0.5 mL PLGA stock solution in DCM (20 mg BiG NC : 10 mg PLGA polymer). The resulting black suspension was sonicated for 5 min. with periodic vortex to make it homogenous. This solution was next added dropwise to the 2% aqueous PVA solution in the falcon tube with rigorous and continuous vortex. Once addition was complete, the resulting brown suspension was tip sonicated at 40% amplitude for 20 sec. and then transferred to an ice bath for 10 sec. This process of tip sonication, followed by rapid cooling in an ice bath was repeated six times. After the final cycle, the brown suspension was added to 25 mL 2% PVA and the resulting reaction mixture was stirred at RT for 3 h to remove DCM, resulting in particle hardening and consequent NP formulation. After 3 h, the NPs were isolated by centrifugation at 18,000 rpm for 10 min. The brown NPs so obtained were cleaned again by repeated dispersion in aqueous media and centrifugation to isolate the NPs, until the supernatant was clear (3 times).

For Coumarin-6 (C6) loaded BiG@PLGA NPs: The reaction sequence for the fluorescent, Coumarin-6 loaded variant of the BiG@PLGA NPs is shown in Scheme S3B and is very much identical to the one previously discussed. The only variation lies in adding a freshly prepared solution of Coumarin-6 in DCM (250 µL, 2 mg Coumarin-6 per mL DCM) in the oil phase, together with BiG NCs and PLGA polymer. The rest of the steps were exactly identical. In order to protect the reaction from light, all the reaction beakers and falcon tubes were covered with Al foil. The work up for this reaction was also identical to the non-fluorescent BiG@PLGA NPs.



Scheme S3B: Synthesis of Fluorescent, Coumarin-6 loaded BiG@PLGA-C6 NPs (Method C).

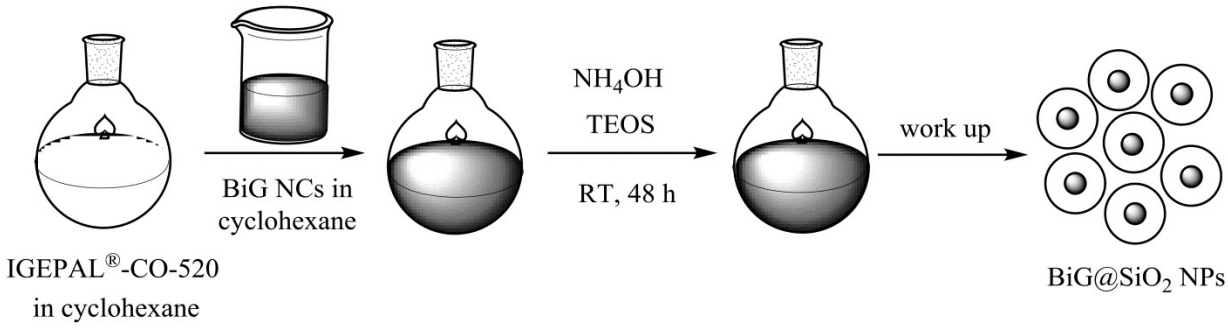
In all, four different types of BiG@PLGA NPs were prepared, viz., BiG-2.5@PLGA, BiG-2.5@PLGA-C6 (with Coumarin-6 tag), BiG-5@PLGA and BiG-5@PLGA-C6 (with Coumarin-6 tag). These have been characterized by TEM, SEM, EDS, IR and TGA. As shown below by the

TEM images, a more homogenous and efficient packing of NCs in PLGA was observed for the BiG-5 variant (Figure S15, S17), as compared to the BiG-2.5 NCs (Figure S10, S12).

1.1.4. Silica Coating of BiG NCs to form BiG@SiO2 NPs

Prior to silica coating, the BiG NCs (both BiG-5 and BiG-2.5 variants) were suspended in Cyclohexane. The resulting black suspension (10 mg BiG NCs per mL cyclohexane) was sonicated for 20 min. with periodic vortex to form a homogenous suspension of BiG NCs in DCM. This suspension was utilized for silica encapsulation.

Typical Procedure: In a 125 mL one neck round bottom flask, IGEPAL®-CO-520 (3.6 g) was taken and Cyclohexane (≥ 99%, 60 mL) was added to it. The flask was stoppered, vortexed and put under sonication to ensure complete mixing of the contents. To this clear solution 5 mL of the previously made BiG NC solution was added (50 mg BiG NCs in 5 mL cyclohexane) and the black colored suspension so obtained was sonicated for 20 minutes so that a homogenous suspension of BiG NCs in IGEPAL®-CO-520 was obtained. To this stirred reaction mixture, ammonium hydroxide solution (NH_4OH, 28-30 % NH_3 basis, 500 µL) was added and the contents were stirred at RT. After 5 min. Tetraethyl orthosilicate (TEOS, 99.999 % trace metal basis, 375 µL) was added to the reaction mixture and the final suspension was stirred at RT for 48 h. After 48 h, the BiG@SiO2 NPs were precipitated by adding excess hexane to the reaction mixture and collected by centrifugation at 18,000 rpm for 10 min. The brown NPs so obtained were cleaned again by repeated dispersion in hexane and centrifugation to isolate the NPs, until the supernatant was clear (2 times).



Scheme S4: Reaction sequence for silica coating of BiG NCs, (Method D).

The above detailed procedure worked perfectly for the BiG-5 NCs and resulted in an efficient silica coating to generate core shell BiG-5@SiO2 NPs as shown by the TEM images (Figure S20). However, this technique did not work with the BiG-2.5 NCs and incomplete encapsulation in silica was observed. As is evident from the TEM images shown in Figure S22, limited silica coating of the BiG-2.5 NCs, with a large population of empty SiO2 NPs and some uncoated, bare Bi NCs were obtained. Variations in the ratio of various reactants used did not improve the

encapsulation efficiency. As a result, only the BiG-5@SiO2 NPs were used for further experiments in current work.

2. Characterization Details.

2.1. General Information-Physicochemical Characterization.

The Bi nanocrystals and nanoparticles were characterized using a variety of techniques. The surface morphology was determined using Scanning Electron Microscopy (SEM; Carl Zeiss Auriga Cross Bean FBI-SEM, Carl Zeiss, Germany). Encapsulation of Bi nanocrystals within PLGA and silica was observed using Transmission Electron Microscopy (TEM; JEOL, 2200FS, JEOL, USA). The Bi nanocrystal size was determined by analyzing corresponding TEM images using Image J. software. For each batch, 200 nanocrystals were analyzed in triplicates with images taken from different portions of a TEM grid. Fourier Transform Infrared (FTIR) spectroscopy was performed on a Mattson Genesis 3025 FTIR spectrometer and used to verify the surface coating of Ganex and dodecanethiol on the Bi nanocrystals as well as to ascertain the presence of PLGA, Ganex and Coumarin-6 in the different Bi nanoparticle formulations. Energy Dispersive Spectroscopy (EDS; TEM-EDS, JEOL, 2200 FS, JEOL, USA) was used to confirm the presence of Bi and SiO2 in the nanoparticles. For EDS, INCA software program was employed to carry out analysis of samples prepared on a TEM grid. Thermogravimetric Analysis (TGA; TGA 500, TA instruments, USA) was performed to estimate the overall Bi content in the nanoparticles by heating at a rate of 30 °C from ambient temperatures to 700 °C.

Sample Preparation for TEM: To carry out TEM analysis, square mesh, carbon support film on copper grids (CF300-Cu, 300 mesh, standard thickness, Electron Microscopy Sciences, USA) were used. For nanocrystals, a homogenous, semi-transparent suspension in hexane was prepared and 10 μL was dropped on the grid. The suspension was allowed to stand for 2 minutes to allow for the crystals to settle down, following which the residual solvent was blown off and the grid was air dried prior to imaging on the electron microscope. For nanoparticles, a homogenous aqueous suspension was prepared and 10 μL was gently placed on the grid. The particles were allowed to settle down over a period of 30 minutes and the residual aqueous drop was absorbed using a kimwipe. The resulting grid was air dried prior to imaging.

Sample Preparation for SEM: For SEM analysis, freeze dried nanoparticles were mounted on SEM holders using a double-sided conductive carbon tapes. The solid sample was put on the taped SEM sample holder and pressed lightly using a spatula to seat the particles. The holder was then gently tapped to remove any loose particles, thus forming a thin uniform layer of particles. Finally, the sample was sputtered to form a 1.0 nm thick tungsten layer on top prior to analysis.

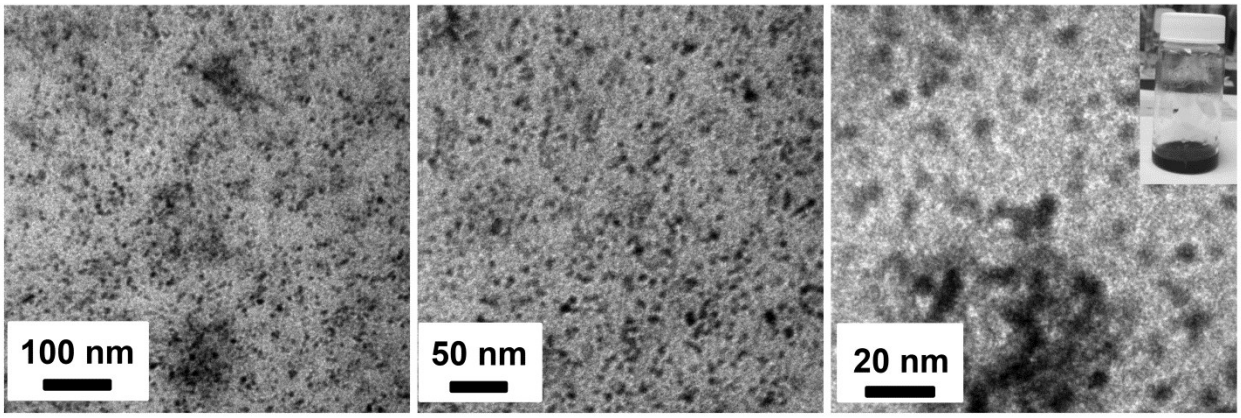


Figure S1: TEM images of Bismuth Nanocrystals (thick oil) made using 25 wt% Ganex (BiG-25). The thick Ganex coat on the NCs can be easily ascertained from the images. The inset picture shows the brown oil obtained during the reaction.

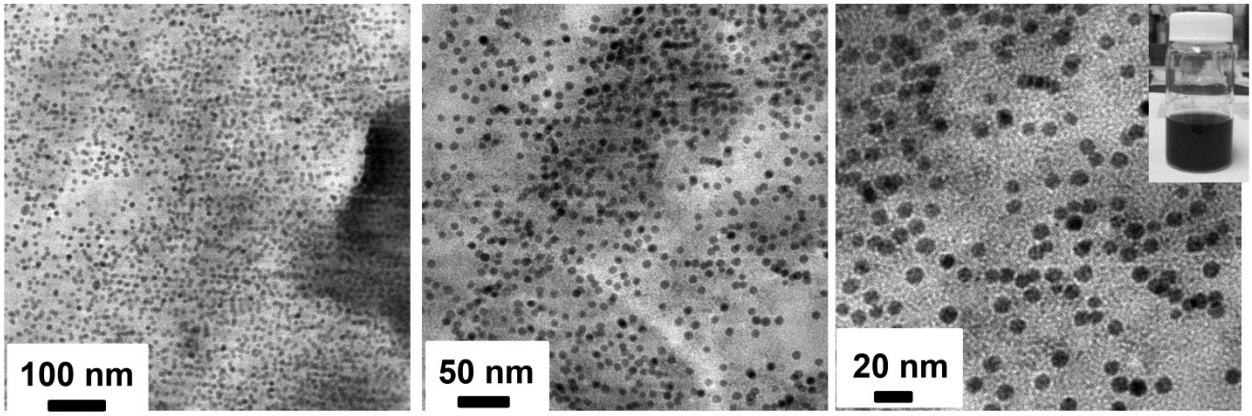


Figure S2: TEM images of Bismuth Nanocrystals (supernatant from the centrifuge) made using 25 wt% Ganex (BiG-25). BiG-25 NCs are still covered in a thin Ganex film. The inset picture shows the brown supernatant obtained during centrifugation.

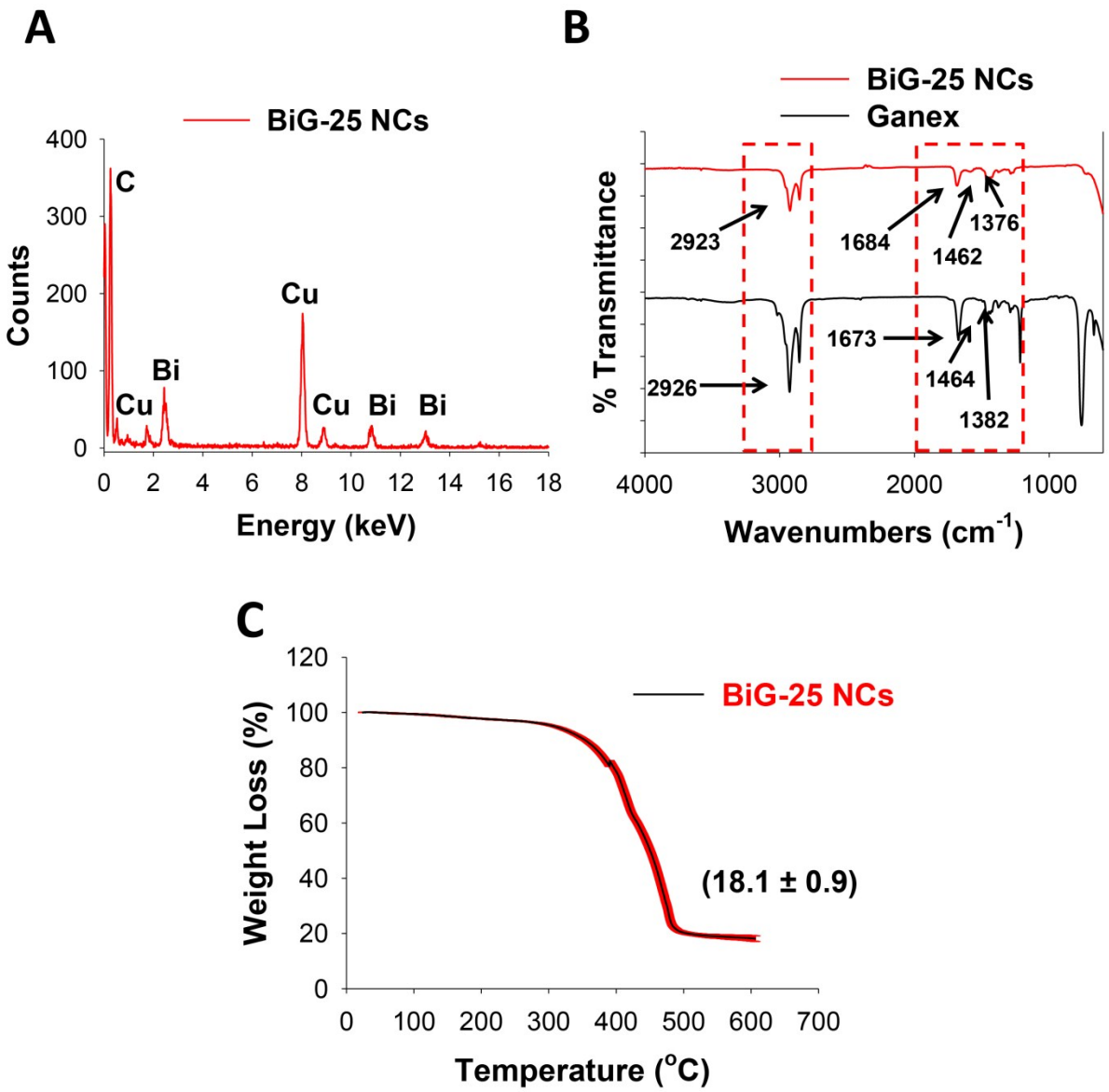


Figure S3: Characterization of BiG-25 NCs using (A)EDS; (B)FTIR and (C)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for TEM imaging. The FTIR spectra compares BiG-25 NCs and Ganex, clearly showing the presence of a Ganex surface layer on the as synthesized BiG-25 NCs. The area enclosed within the dotted box represents regions of similar transmittance in the FTIR spectra; prominent common peaks are pointed out. The TGA analysis determines the % weight loss in three different BiG-25 NC samples and gives an idea of the overall extent of Ganex surface coating on the NCs. Values within the parentheses in Figure C represent the mean residual Bi content and the mean standard deviation.

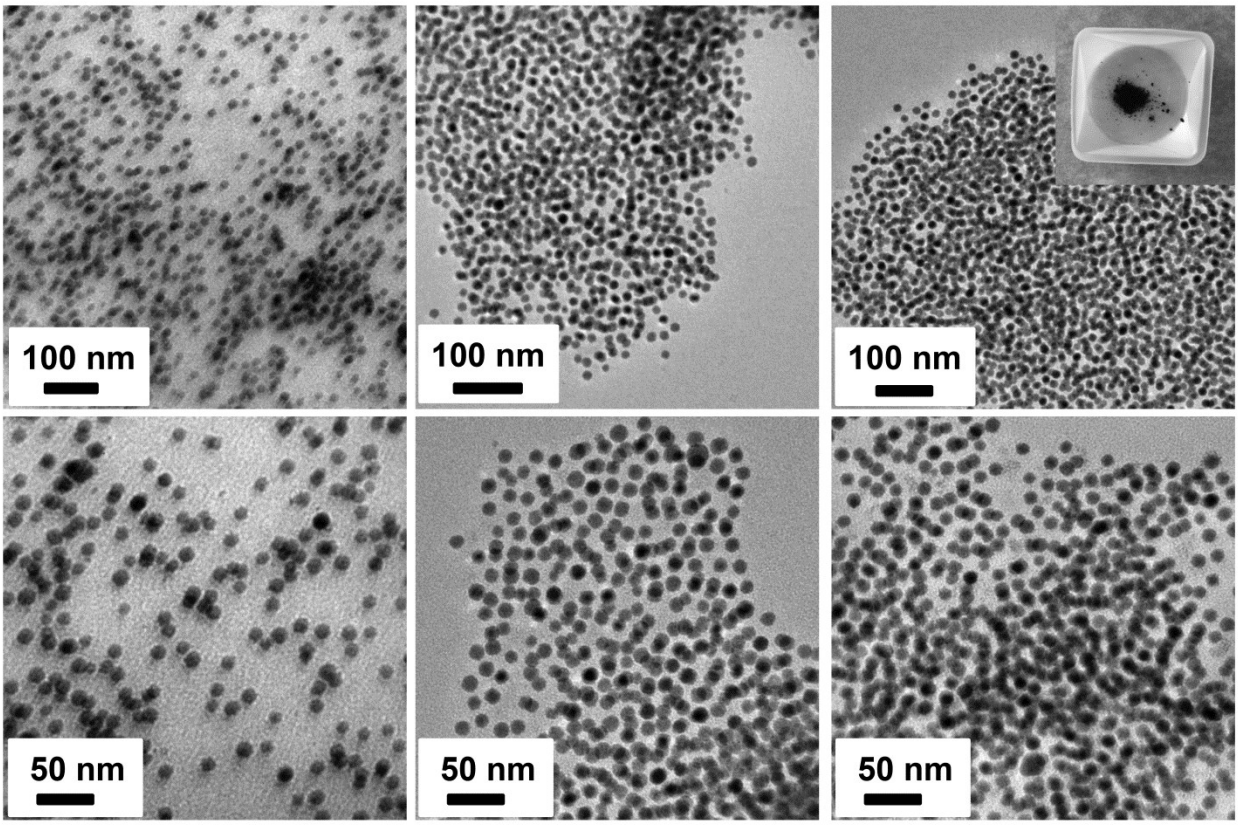


Figure S4: TEM images of Bismuth Nanocrystals made using 5 wt% Ganex (BiG-5). The left, center and right panels correspond to BiG-5 NCs obtained from three different batches and show excellent homogeneity in size and morphology. Inset shows a picture of the BiG-5 NCs obtained during a typical synthesis.

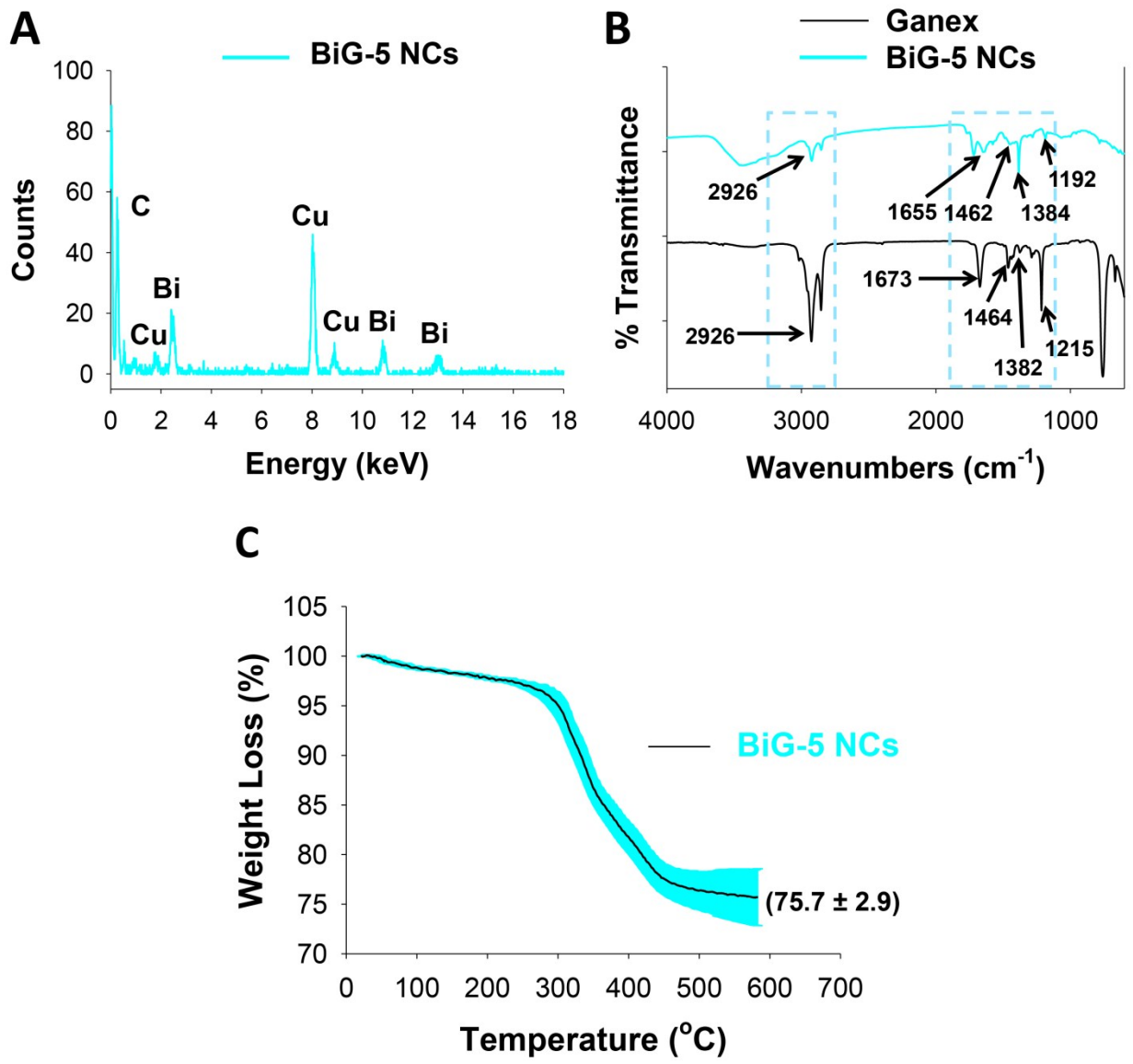


Figure S5: Characterization of BiG-5 NCs using (A)EDS; (B)FTIR and (C)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for TEM imaging. The FTIR spectra compares BiG-5 NCs and Ganex, clearly showing the presence of a Ganex surface layer on the as synthesized BiG-5 NCs. The area enclosed within the dotted box represents regions of similar transmittance in the FTIR spectra; prominent common peaks are pointed out. The TGA analysis determines the % weight loss in three different BiG-5 NC samples and gives an idea of the overall extent of Ganex surface coating on the NCs. Values within the parentheses in Figure C represent the mean residual Bi content and the mean standard deviation.

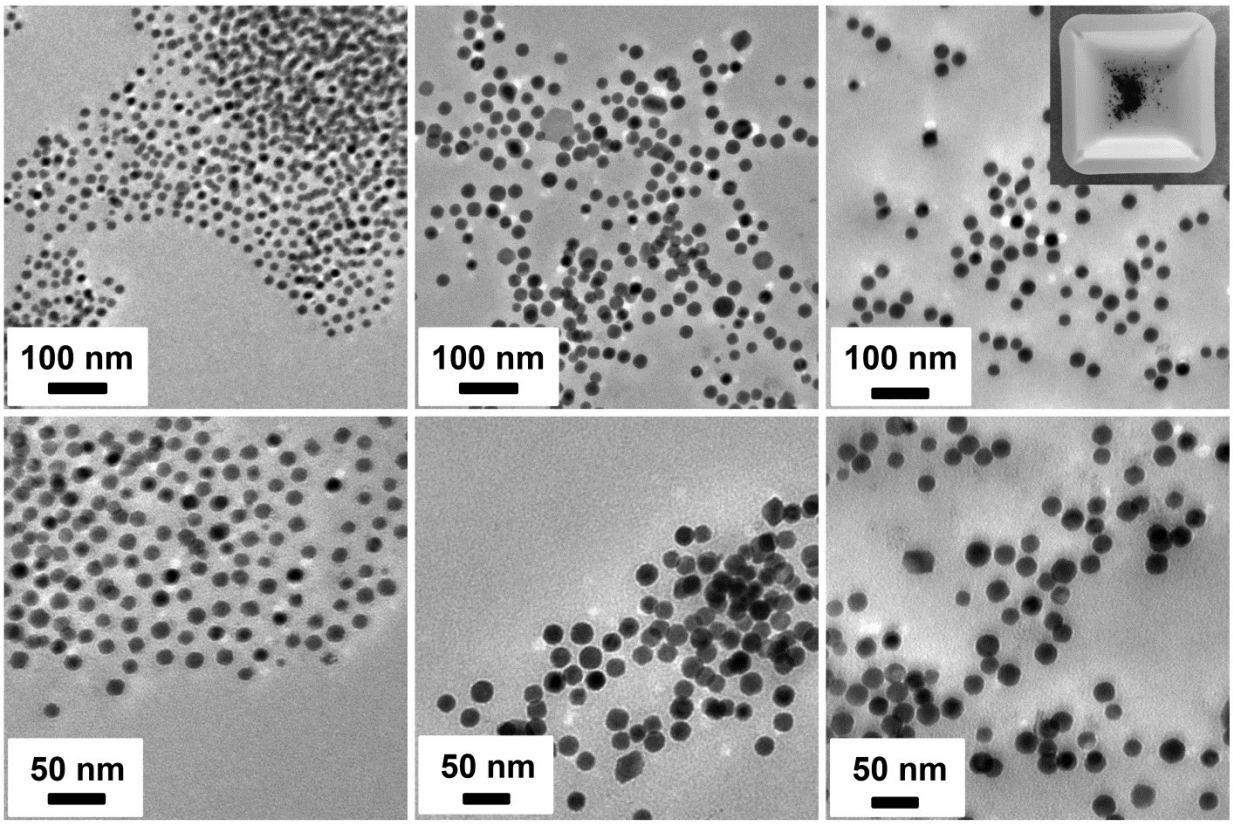


Figure S6: TEM images of Bismuth Nanocrystals made using 2.5 wt% Ganex (BiG-2.5). The left, center and right panels correspond to BiG-2.5 NCs obtained from three different batches and show some inhomogeneity in size. Inset shows a picture of the BiG-2.5 NCs as obtained.

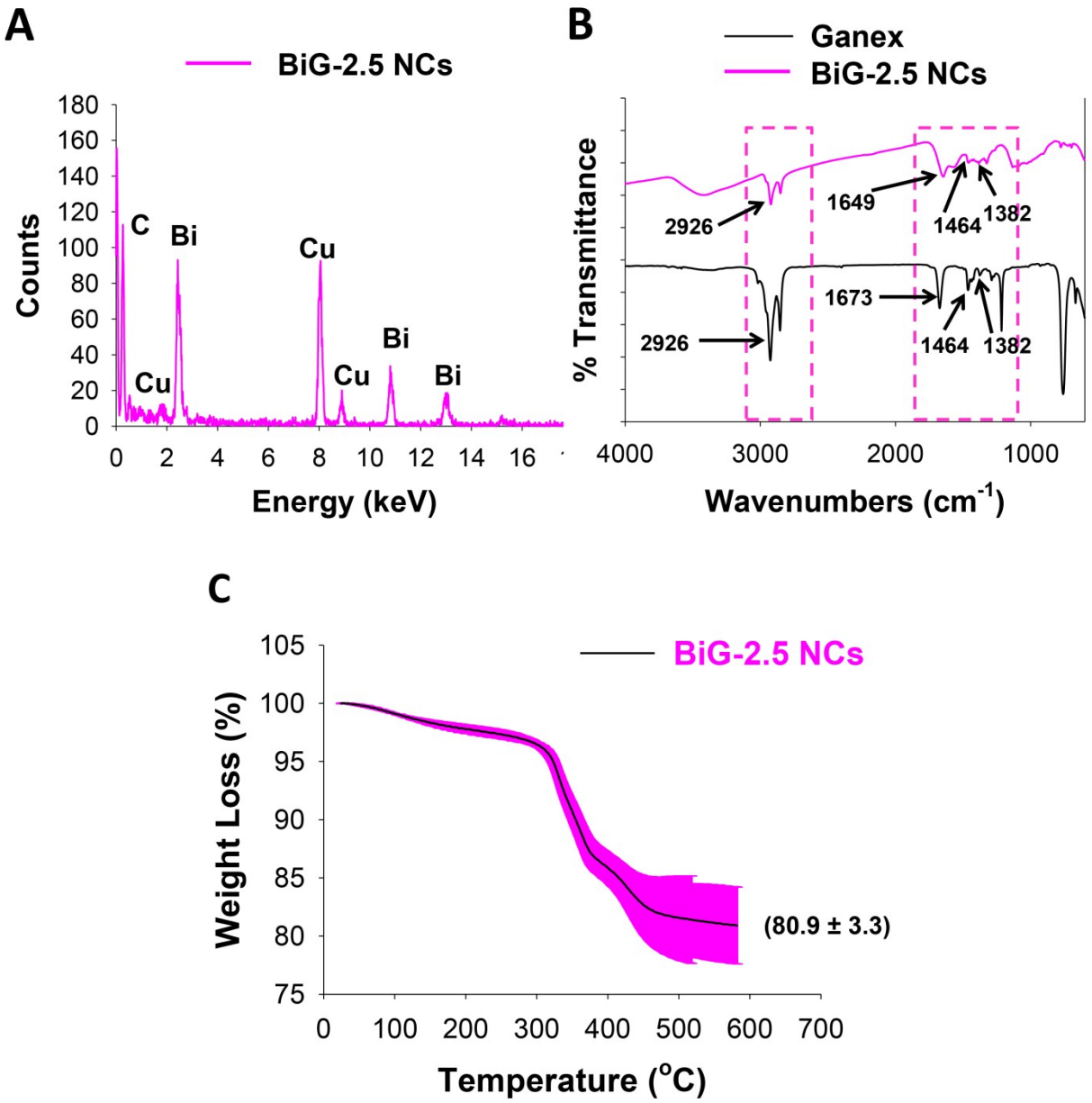


Figure S7: Characterization of BiG-2.5 NCs using (A)EDS; (B)FTIR and (C)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The FTIR spectra compares BiG-2.5 NCs and Ganex, clearly showing the presence of a Ganex surface layer on the as synthesized BiG-2.5 NCs. The area enclosed within the dotted box represents regions of similar transmittance in the FTIR spectra; prominent peaks that are similar are mentioned. The TGA analysis determines the % weight loss in three different BiG-2.5 NC samples and gives an idea of the overall extent of Ganex surface coating on the NCs. Values within the parentheses in Figure C represent mean residual Bi content and the corresponding mean standard deviation.

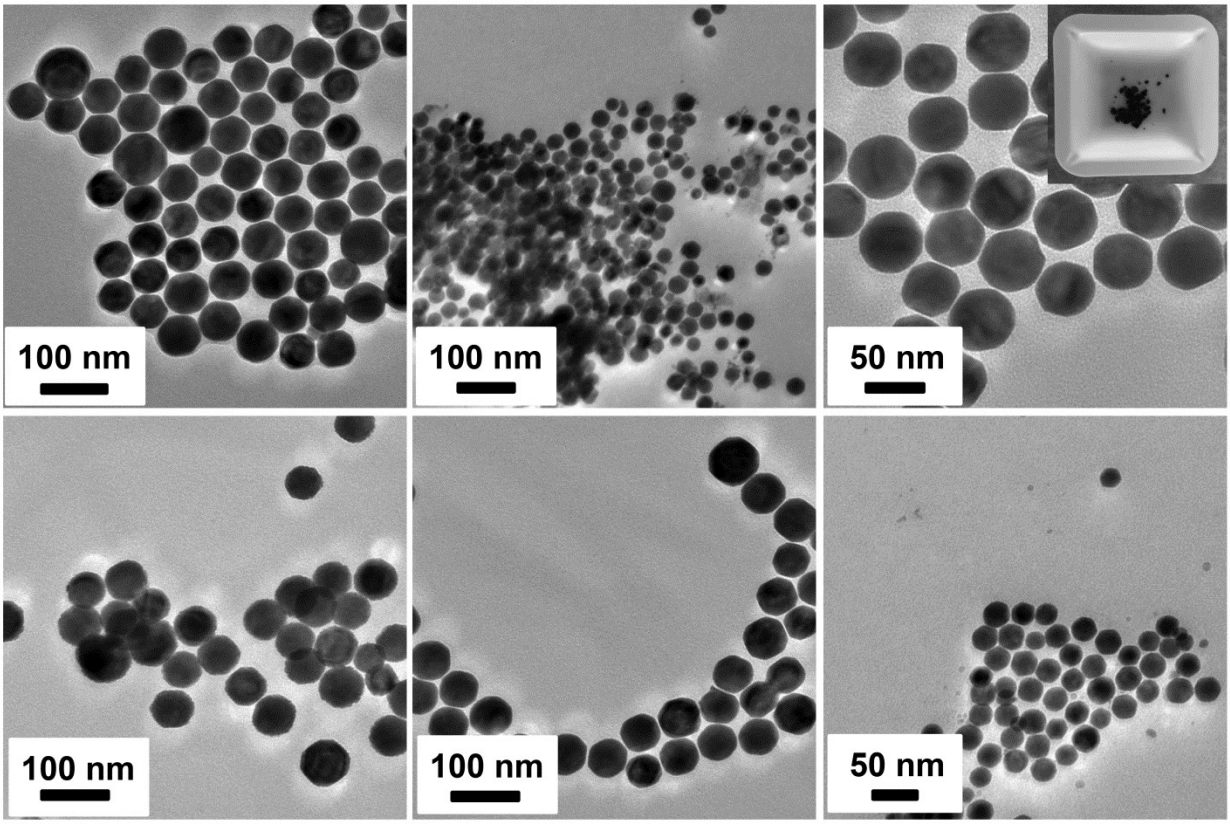


Figure S8: TEM images of Bismuth Nanocrystals made using Method B (Bi NCs). All the individual images are from separate reactions, each set up under exactly identical conditions. The variability in size is very evident from the images and clearly Method B provides very little control on size among different reaction batches, which is non-ideal. The figure in the inset is a picture of the as synthesized Bi NCs.

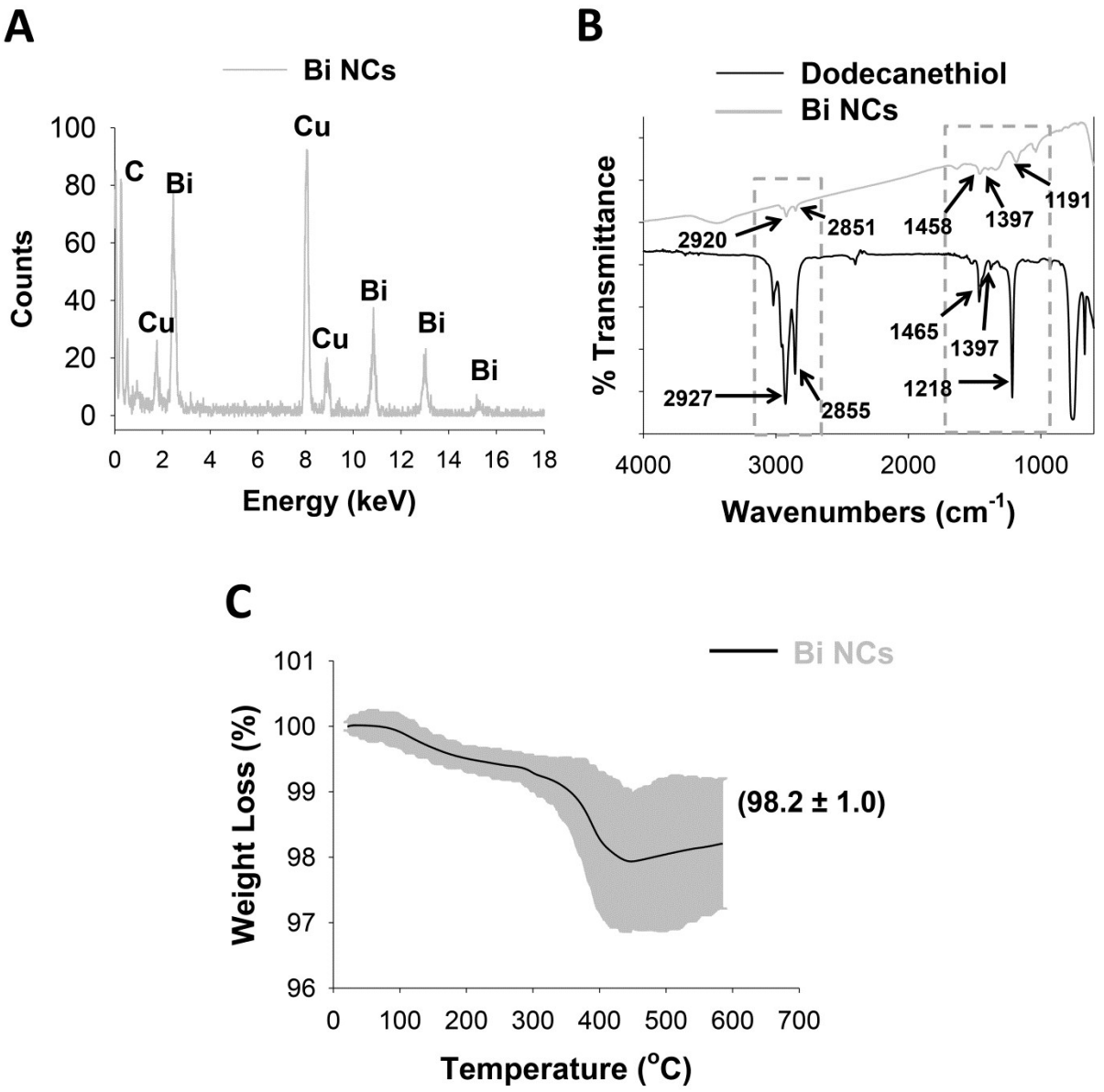


Figure S9: Characterization of Bi NCs using (A)EDS; (B)FTIR and (C)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The FTIR spectra compares Bi NCs and dodecanethiol, clearly showing the presence of a Dodecanethiol surface layer on the as synthesized Bi NCs. The area enclosed by the dotted box represents regions of similar transmittance in the FTIR spectra; prominent peaks that have similar transmittance find mention. The TGA analysis determines the % weight loss in three different Bi NC samples and gives an idea of the overall extent of Dodecanethiol surface coating on the NCs. Values within the parentheses in Figure C represent residual mean Bi content and the mean standard deviation.

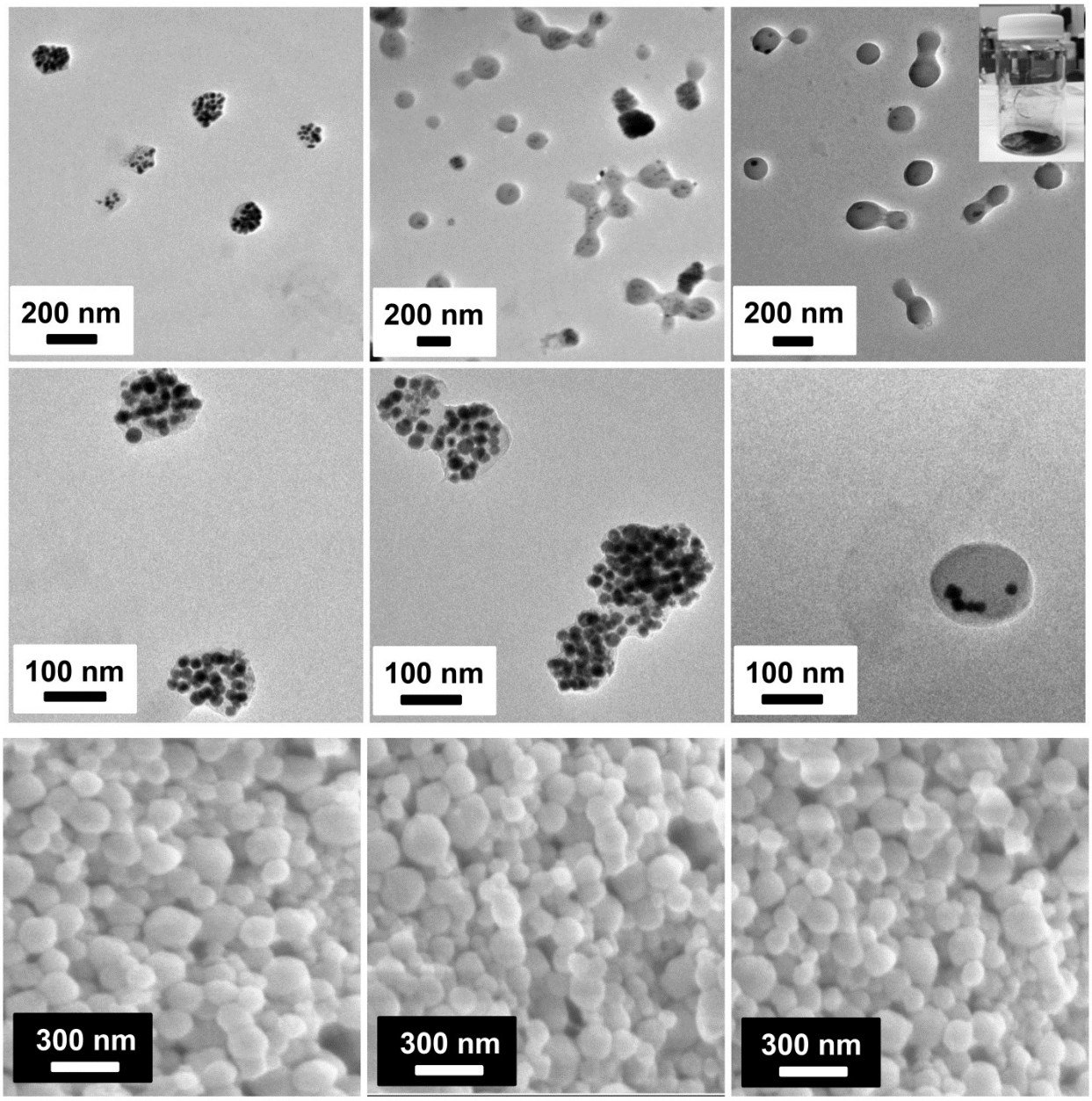


Figure S10: TEM (top and center panel) and SEM images (bottom panel) of BiG-2.5@PLGA NPs. The left, center and right panels correspond to BiG-2.5@PLGA NPs obtained from three different batches that quite clearly show the inhomogeneity in size and the inefficiency in packing of Bi NCs within the PLGA polymer. Inset shows a picture of the as prepared BiG-2.5@PLGA NPs.

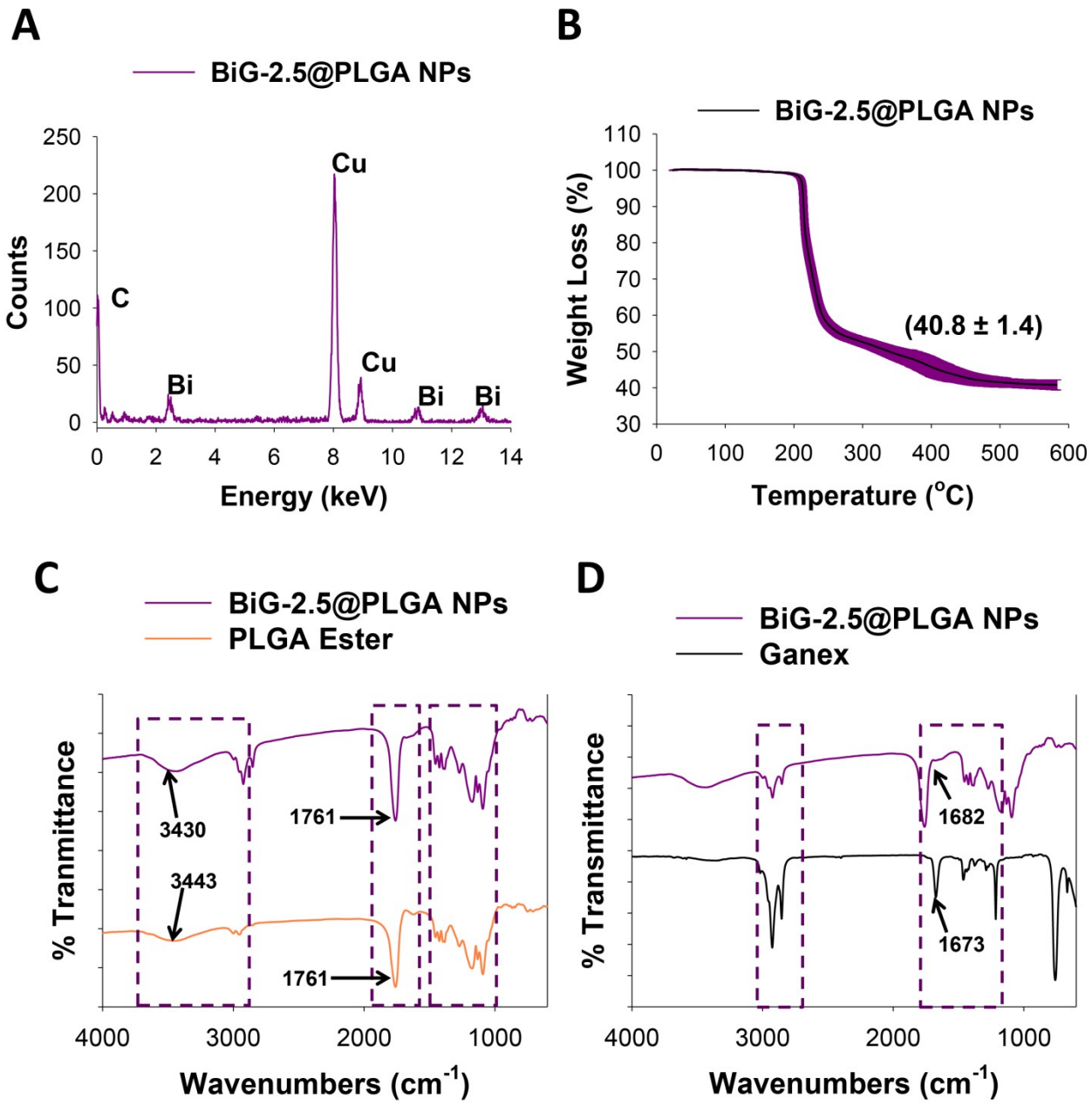


Figure S11: Characterization of BiG-2.5@PLGA NPs using (A)EDS; (B)TGA and (C), (D) FTIR. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The TGA analysis determines the % weight loss in three different BiG-2.5@PLGA NPs samples and gives an idea of the overall extent of Bi encapsulation in PLGA polymer together with residual Ganex surface coating on the NCs. Values within the parentheses in Figure B represent residual mean Bi content and the mean standard deviation. The FTIR spectra (C) compares BiG-2.5@PLGA NPs and PLGA-Ester polymer, clearly showing an intact peak at 1761 cm^{-1} for C=O ester stretch in both the starting

polymer as well as the final NP; spectra (D) compares and NP FTIR with that of Ganex and confirms presence of Ganex surface coat on the BiG-2.5@PLGA NPs.

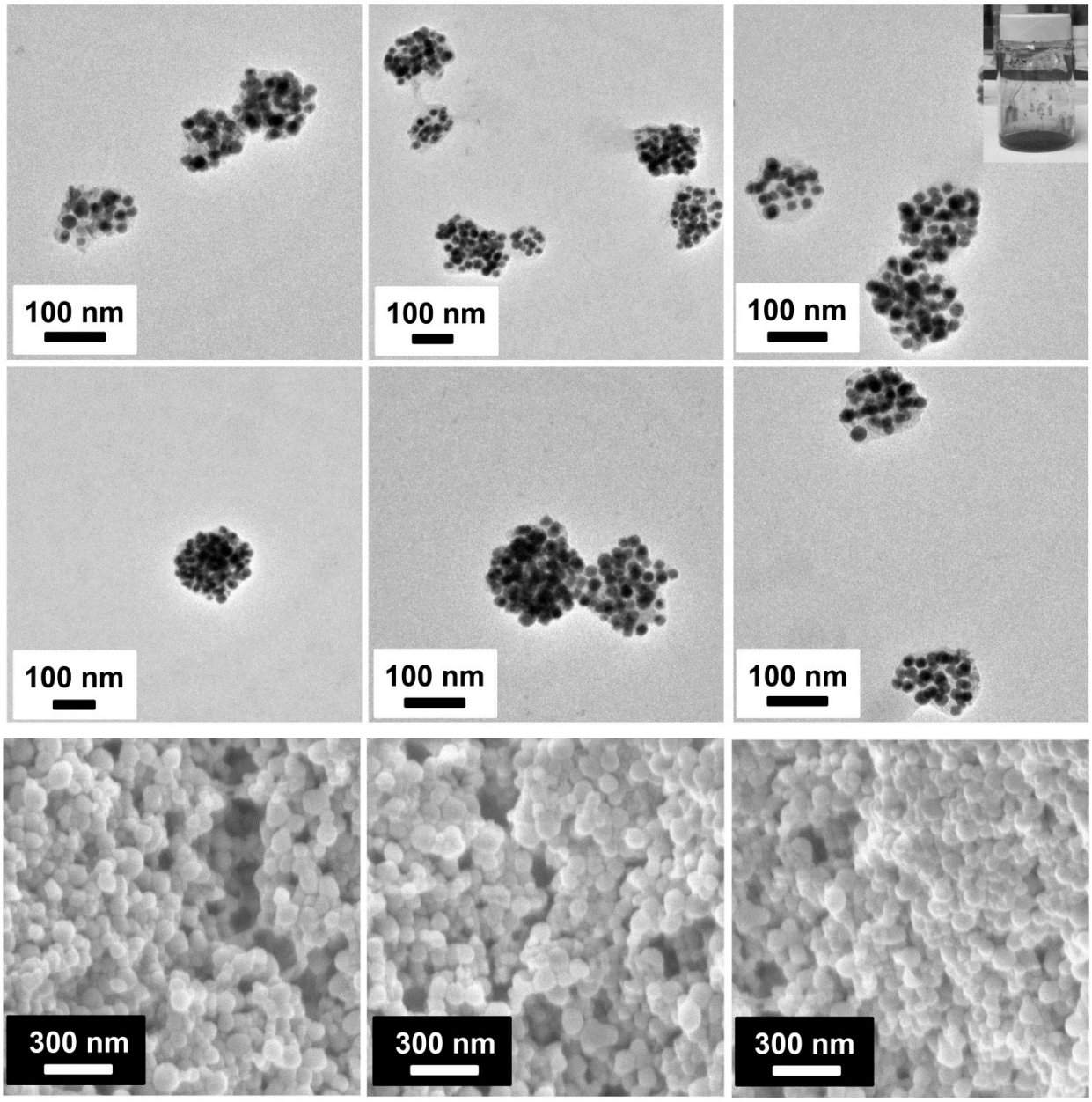


Figure S12: TEM (top and center panel) and SEM images (bottom panel) of BiG-2.5@PLGA-C6 NPs. The left, center and right panels correspond to BiG-2.5@PLGA NPs obtained from three different batches that quite clearly show the inhomogeneity in size and the inefficiency in packing of Bi NCs within the PLGA polymer. Inset shows a picture of the as prepared BiG-2.5@PLGA-C6 NPs.

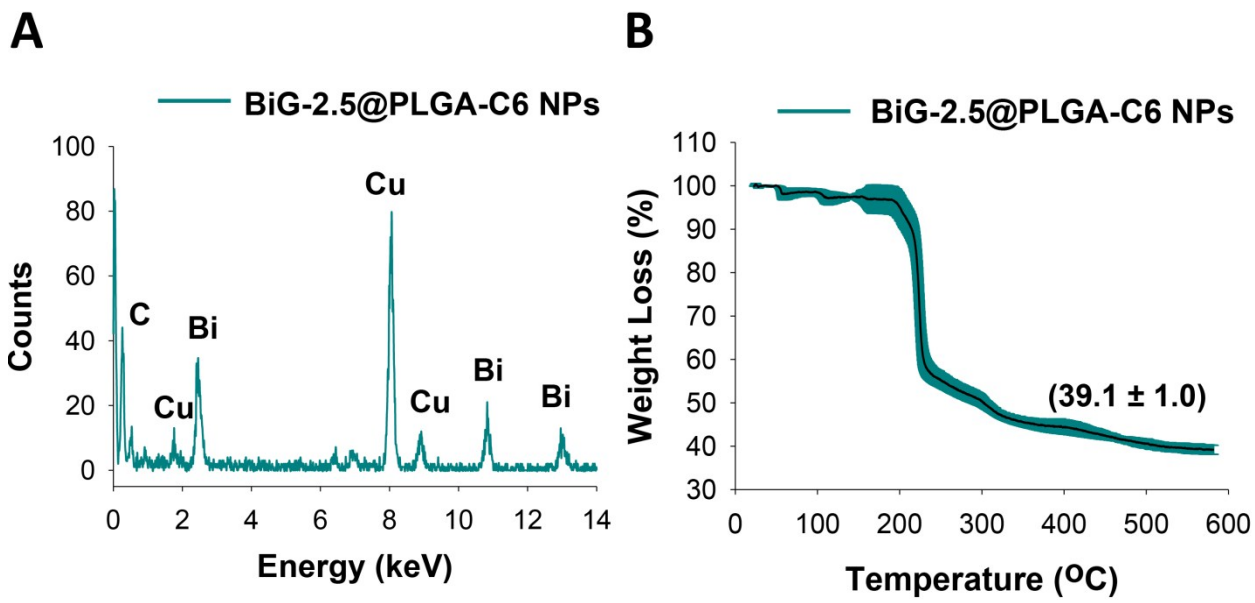


Figure S13: Characterization of BiG-2.5@PLGA-C6 NPs using (A)EDS and (B)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The TGA analysis determines the % weight loss in three different BiG-2.5@PLGA-C6 NPs samples and gives an idea of the overall extent of Bi encapsulation in PLGA polymer together with residual Ganex surface coating on the NCs. Values within the parentheses in Figure B represent residual mean Bi content and the mean standard deviation.

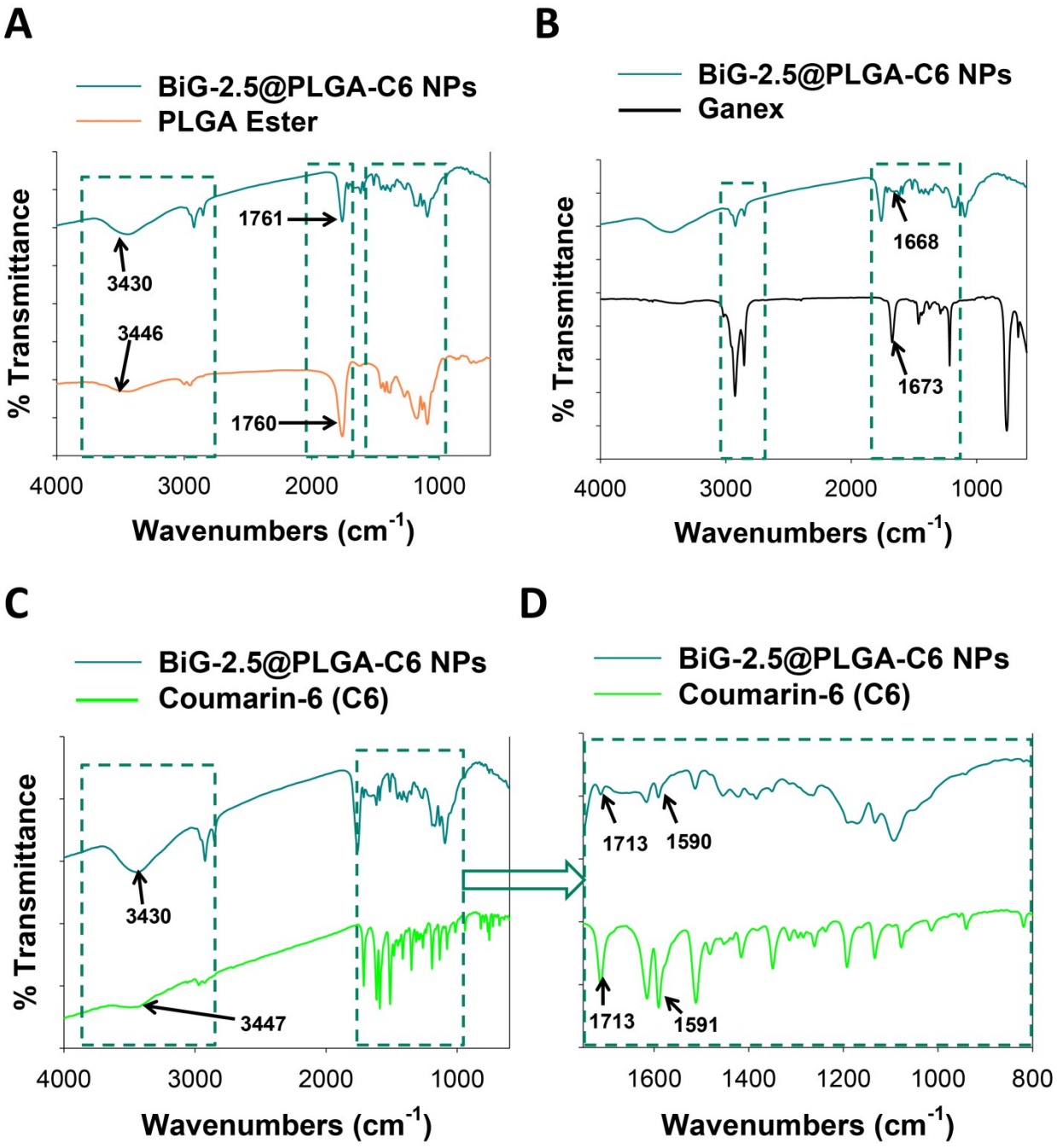


Figure S14: Characterization of BiG-2.5@PLGA-C6 NPs using FTIR. (A) compares the FTIR spectra of BiG-2.5@PLGA-C6 NPs and PLGA-Ester polymer, clearly showing an intact peak at 1761 cm^{-1} for C=O ester stretch in both the starting polymer as well as the final NP; spectra (B) compares and NP FTIR with that of Ganex and shows a persistent presence of Ganex surface coat on the BiG-2.5@PLGA-C6 NPs. Spectra (C) compares the FTIR spectra of coumarin-6 (C6) and ascertains its presence in the NP construct. Figure (D) is an expanded view of a common region within the two spectra, with prominent common peaks being mentioned.

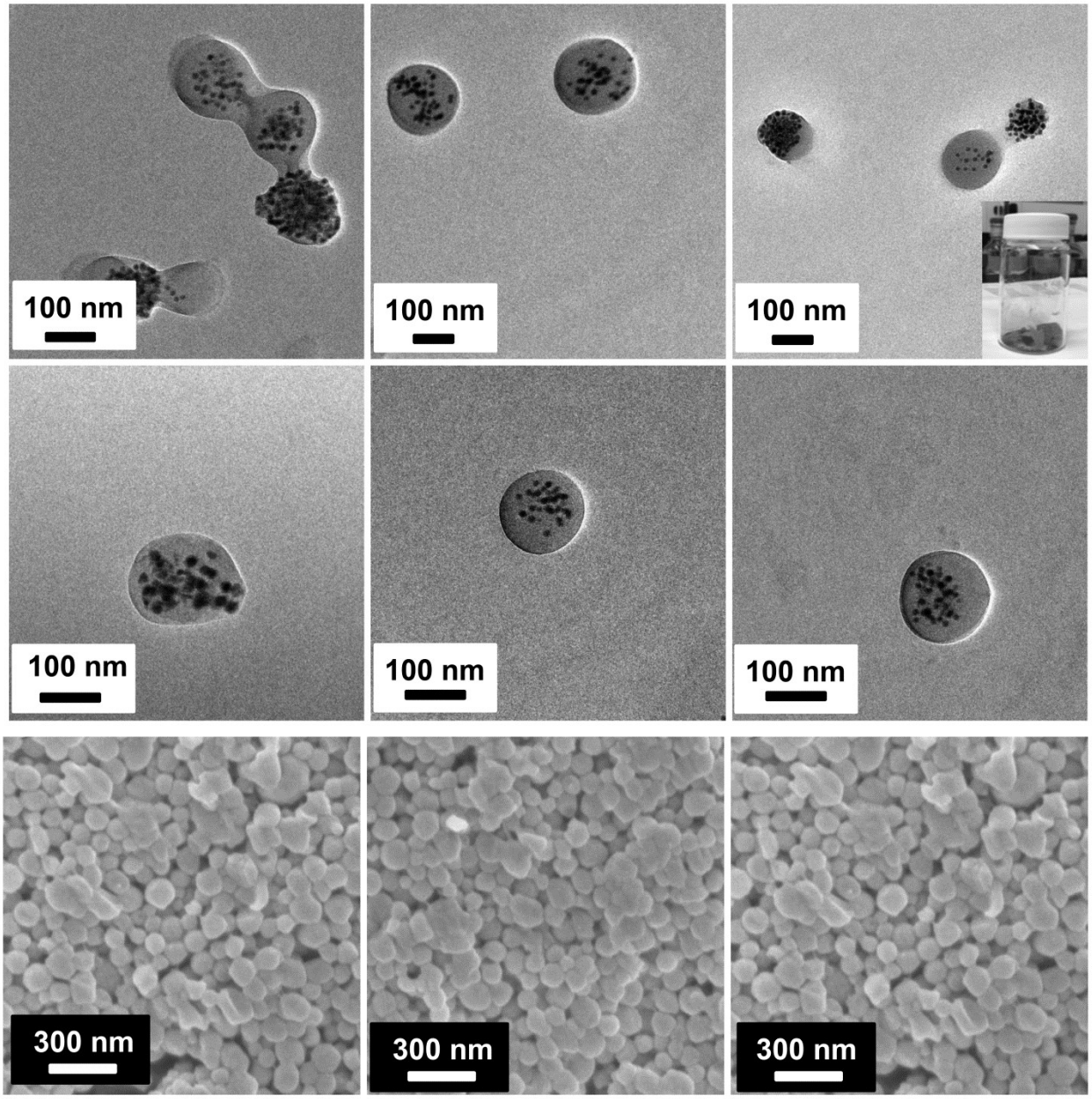


Figure S15: TEM (top and center panel) and SEM images (bottom panel) of BiG-5@PLGA NPs. The left, center and right panels correspond to BiG-2.5@PLGA NPs obtained from three different batches that quite clearly show the uniformity in size and the efficiency in packing of Bi NCs within the PLGA polymer. Inset shows a picture of the as prepared BiG-5@PLGA NPs.

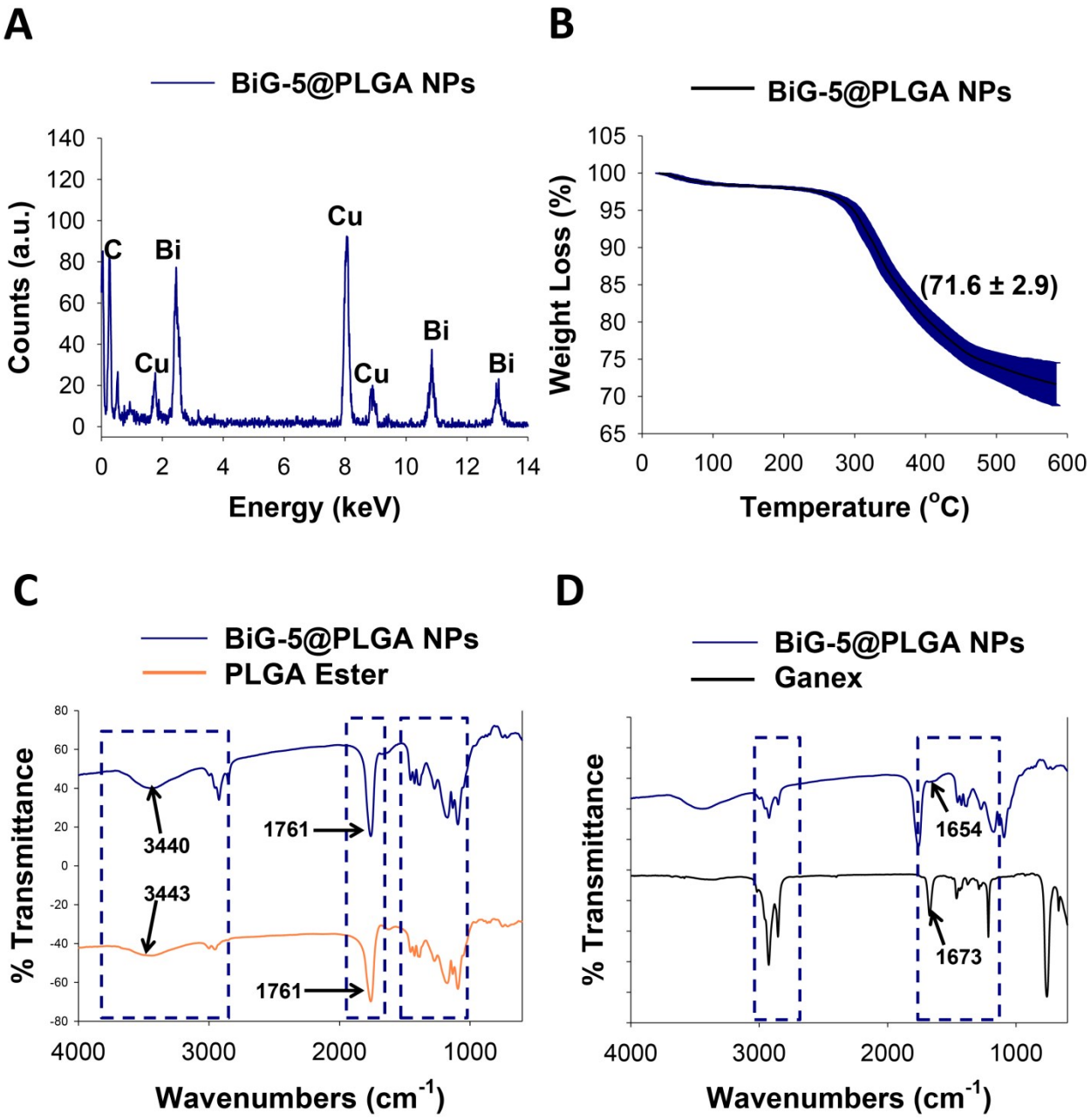


Figure S16: Characterization of BiG-5@PLGA NPs using (A)EDS; (B)TGA and (C), (D) FTIR. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The TGA analysis determines the % weight loss in three different BiG-2.5@PLGA NPs samples and gives an idea of the overall extent of Bi encapsulation in PLGA polymer together with residual Ganex surface coating on the NCs. Values within the parentheses in Figure B represent residual mean Bi content and the mean standard deviation. The FTIR spectra (C) compares BiG-5@PLGA NPs and PLGA-Ester polymerd, clearly showing an intact peak at 1761 cm-1 for C=O ester stretch in both the starting

polymer as well as the final NP; spectra (D) compares and NP FTIR with that of Ganex and shows a persistent presence of Ganex surface coat on the BiG-5@PLGA NPs.

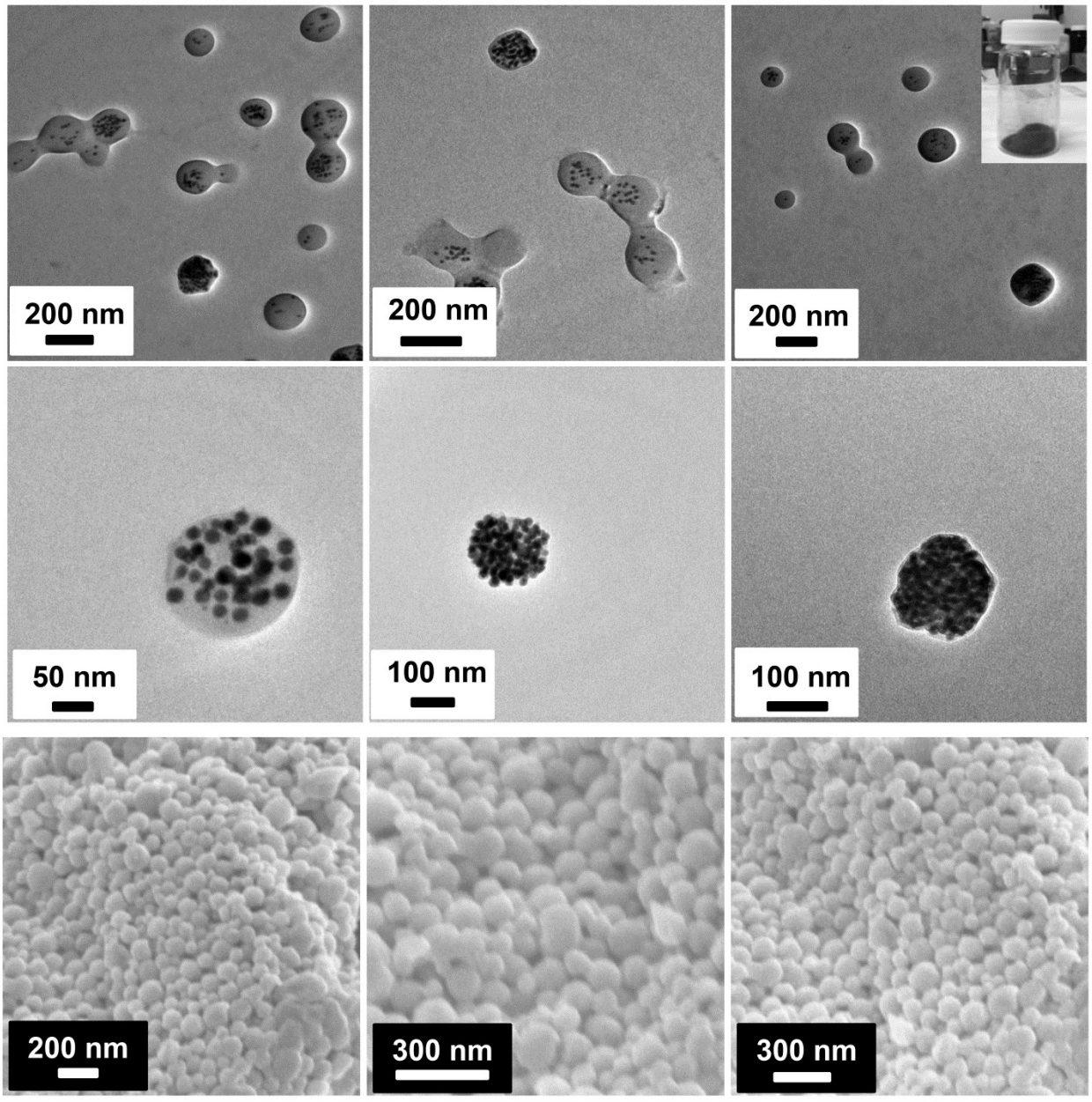


Figure S17: TEM (top and center panel) and SEM images (bottom panel) of BiG-5@PLGA-C6 NPs. The left, center and right panels correspond to BiG-2.5@PLGA NPs obtained from three different batches that quite clearly show the uniformity in size and the efficiency in packing of Bi NCs within the PLGA polymer. Inset shows a picture of the as prepared BiG-5@PLGA-C6 NPs.

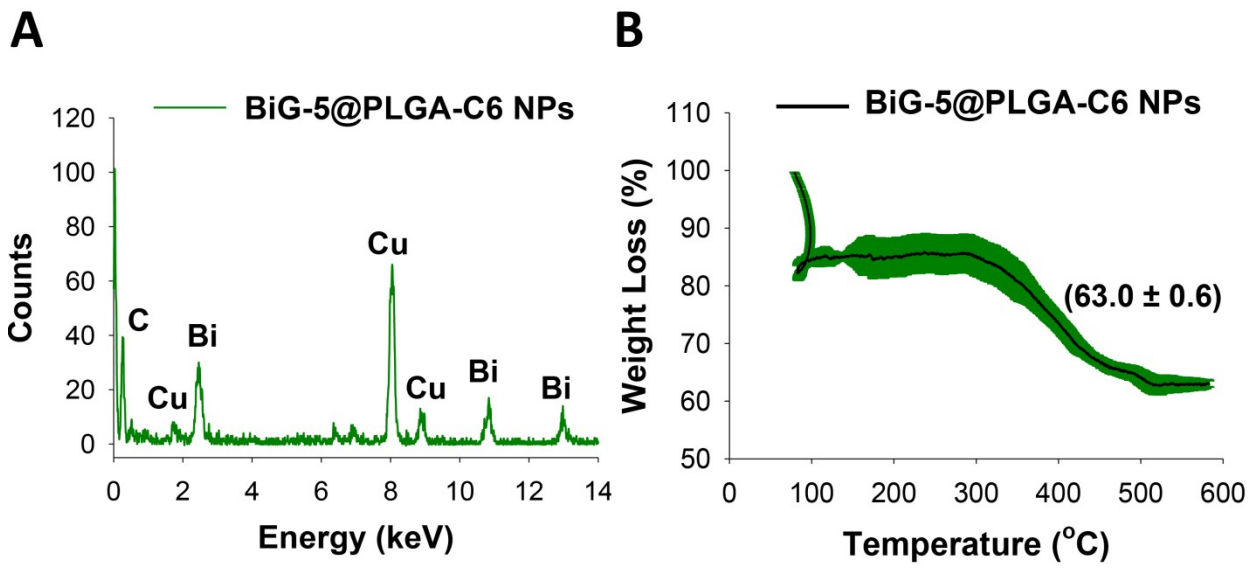


Figure S18: Characterization of BiG-5@PLGA-C6 NPs using (A)EDS and (B)TGA. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. The TGA analysis determines the % weight loss in three different BiG-5@PLGA-C6 NPs samples and gives an idea of the overall extent of Bi encapsulation in PLGA polymer together with residual Ganex surface coating on the NCs. Values within the parentheses in Figure B represent residual mean Bi content and the mean standard deviation.

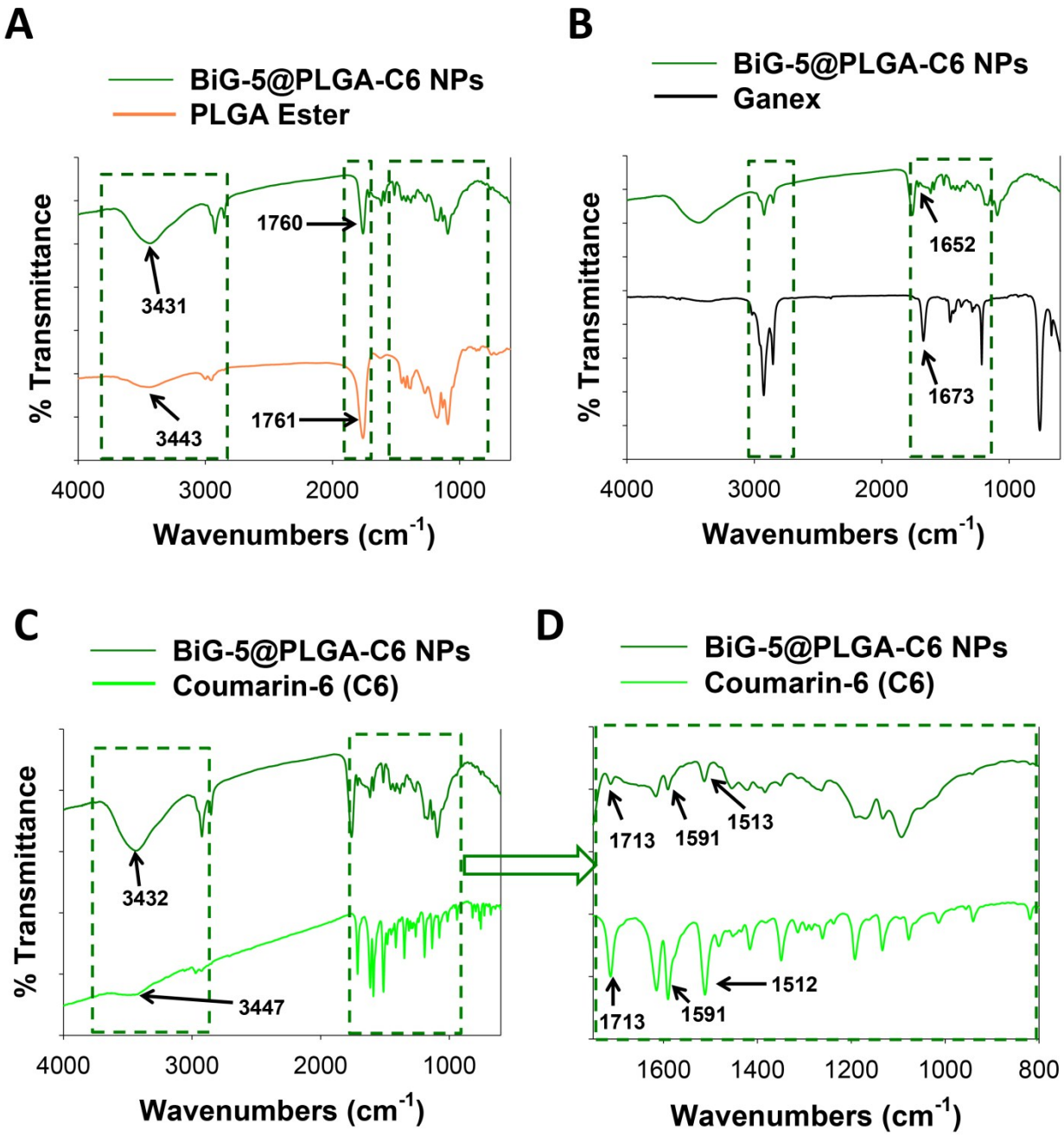


Figure S19: Characterization of BiG-5@PLGA-C6 NPs using FTIR. (A) compares the FTIR spectra of BiG-5@PLGA-C6 NPs and PLGA-Ester polymer, clearly showing an intact peak at 1761 cm^{-1} for C=O ester stretch in both the starting polymer as well as the final NP; spectra (B) compares and NP FTIR with that of Ganex and shows a persistent presence of Ganex surface coat on the BiG-5@PLGA-C6 NPs. Spectra (C) compares the FTIR spectra of coumarin-6 (C6) and ascertains its presence in the NP construct. Figure (D) is an expanded view of a common region within the two spectra, with prominent common peaks being mentioned.

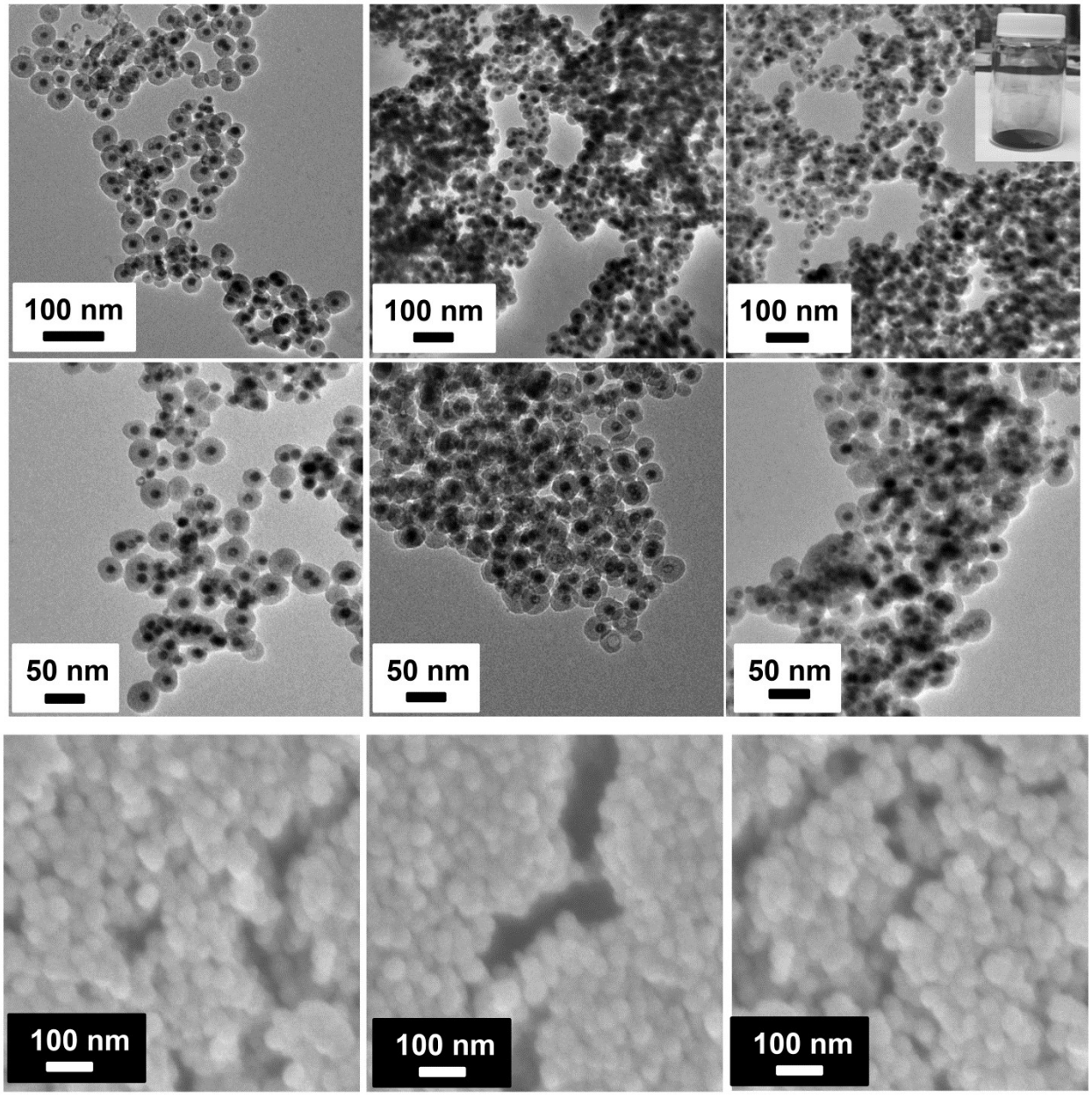


Figure S20: TEM (top and center panel) and SEM images (bottom panel) of BiG-5@SiO2 NPs. The left, center and right panels correspond to BiG-5@SiO2 NPs obtained from three different batches that quite clearly show the uniformity in size and the efficient silica encapsulation of individual Bi NCs.. Inset shows a picture of the as prepared BiG-5@SiO2 NPs.

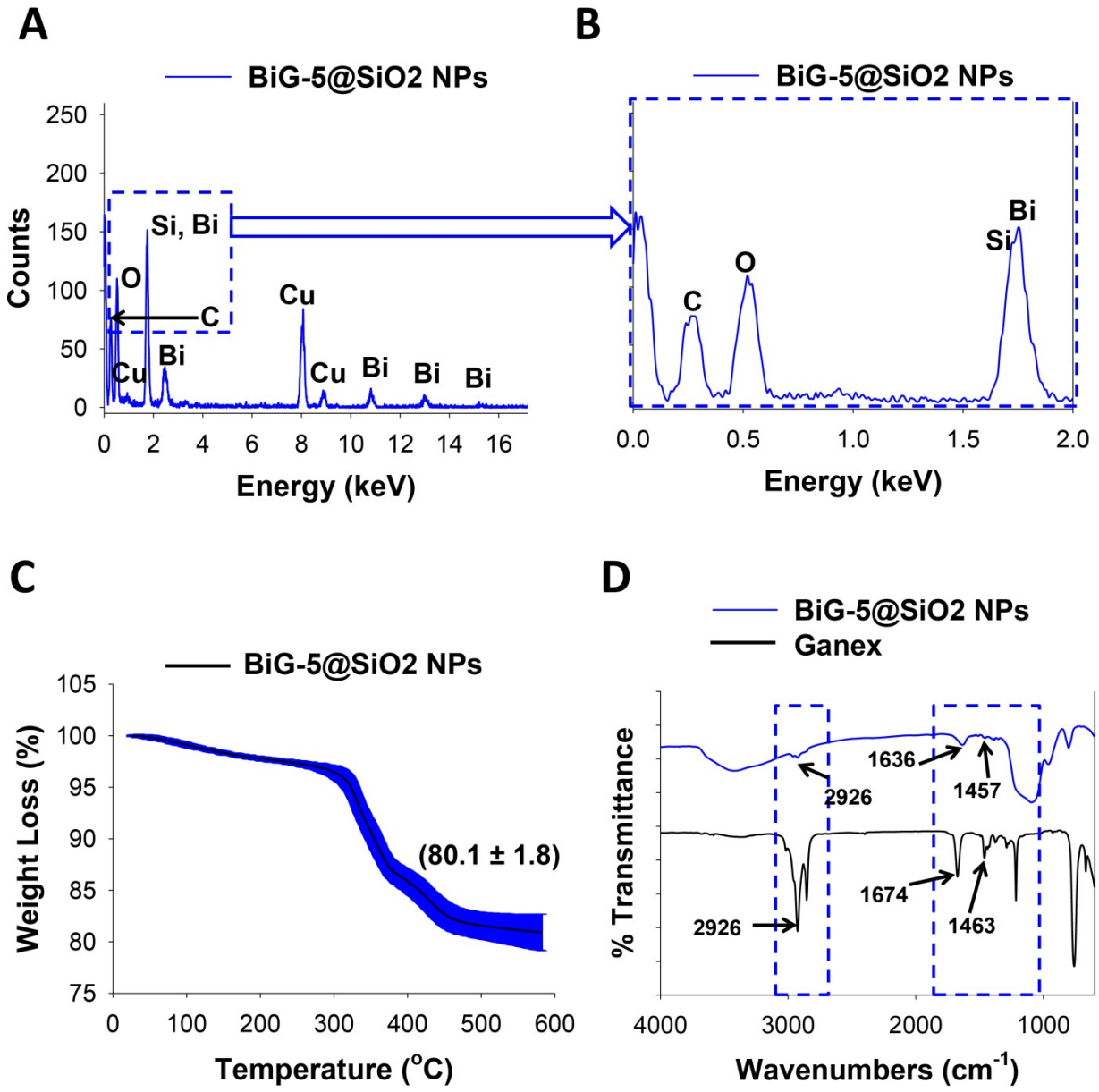


Figure S21: Characterization of BiG-5@SiO2 NPs using (A), (B) EDS; (C)TGA and (D) FTIR. The peaks for carbon (C) and copper (Cu) in the EDS spectra are attributed to the grid mesh used as a sample holder for imaging. Figure B shows an expanded view of a section of the EDS spectra in Figure A that ascertains the presence of an additional Si and O peak in the sample, further confirming a SiO2 encapsulation. The TGA analysis determines the % weight loss in three different BiG-5@SiO2 NPs samples and gives an idea of the overall extent of Bi encapsulation in SiO2 together with residual Ganex surface coating on the NCs. Values within the parentheses in Figure C represent residual mean Bi content and the mean standard deviation. The FTIR spectra (D) compares BiG-5@SiO2 NPs and Ganex, clearly showing the presence of a Ganex surface coat even after silica coating. Further common peaks are mentioned in Figure (D).

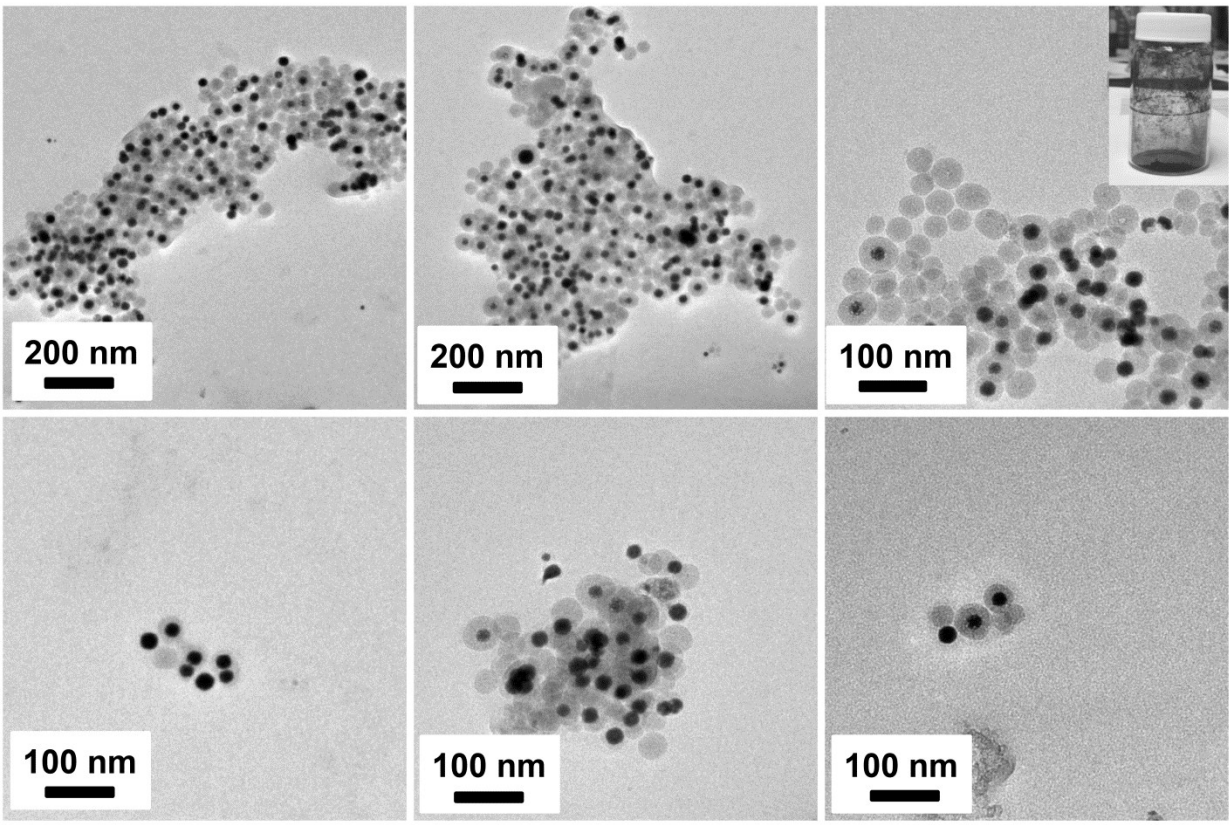


Figure S22: TEM images of BiG-2.5@SiO2 NPs. All the individual images are from separate reactions, each set up under exactly identical conditions. The variability in size, encapsulation efficiency is very evident from the images and clearly this method provides very little control on size and morphology of the silica coating process. Additionally, a number of empty silica particles are also formed as seen from the images. The figure in the inset is a picture of the as synthesized BiG-2.5@SiO2 NPs.

2.2. Nanoparticle characterization using DLS

Dynamic Light Scattering (DLS) for various Bi nanoparticle formulations was carried out using a Zetasizer instrument (Malvern, USA) to determine the hydrodynamic radii (D_h, nm), polydispersity index (PDI) and zeta potential (ζ, mV). Nanoparticle solutions with concentration 0.1 mg per mL in DI water were prepared and analyzed in triplicates. The results have been presented in Table S1. As is evident from the data, a smaller size distribution for the BiG-5@PLGA and BiG-5@SiO2 NPs (110-120 nm) was realized as compared to the BiG-2.5@PLGA NPs. Again, on addition of a coumarin-6 tag, the size of the resulting NPs was found to decrease, irrespective of the BiG NC type being used. This decrease in size was however, much more prominent with the BiG-5@PLGA NPs as compared to the BiG-2.5 NP variant.

Table S1: Hydrodynamic radii (D_h, nm), polydispersity index (PDI) and zeta potential (ζ, mV) of different BiG@PLGA and BiG@SiO2 Nanoparticles (NPs).

NP type	D_h (nm)	PDI	ζ (mV)
BiG-2.5@PLGA	294.0±5.1	0.31±0.01	-8.06±0.43
BiG-2.5@PLGA-C6	248.7±1.7	0.24±0.02	-9.21±0.39
BiG-5@PLGA	138.6±5.1	0.22±0.03	-12.07±0.38
BiG-5@PLGA-C6	117.6±4.1	0.18±0.03	-13.83±0.55
BiG5@SiO2	115.3±3.5	0.14±0.03	-13.27±0.40

3. Dissolution Study

3.1. General Information:

To analyze the dissolution of Bi NCs, Bi@PLGA and Bi@SiO2 NPs in lysosomal media, an *in vitro* dissolution study was carried out. In a typical experimental set up, 5 mg of each variety of NCs and NPs were taken in two 2 mL centrifuge vials and suspended separately in 1 mL each of PBS (Phosphate Buffer Saline, pH 7.2) and sodium citrate (NaCit, 50 mM, pH 5.5). The resulting suspensions were transferred to a rotor maintained in an oven at 37 °C. After various time points (1 h, 4 h, 8 h, 20 h, 24 h, 48 h, Day 3, 4, 5, 7, 8, 9, 10, 11, 14, 21 and 28) the tubes were centrifuged and the supernatant was collected. The residues were re-suspended in the respective media and the experiment was continued. After 4 weeks, the supernatant liquid was evaporated and the residue so obtained was digested by adding 1 mL conc. nitric acid and leaving the suspension so obtained till a clear yellow solution was obtained (48 h). After 48 h, each sample was diluted to a final concentration of 2% nitric acid and the Bi content in the digested samples was analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Varian 710-ES, ICP-OES instrument. Each sample was analyzed in triplicate and each study was repeated thrice. The results from the ICP-OES experiment (Figure 3 and 4) clearly demonstrate the effective reduction in Bi dissolution as seen with the Ganex coated Bi NCs, an effect that is translated as these unique BiG NCs are encapsulated within the FDA approved PLGA polymer as well as SiO2.

4. Cellular Studies.

4.1. Cellular Uptake in Raw 264.7

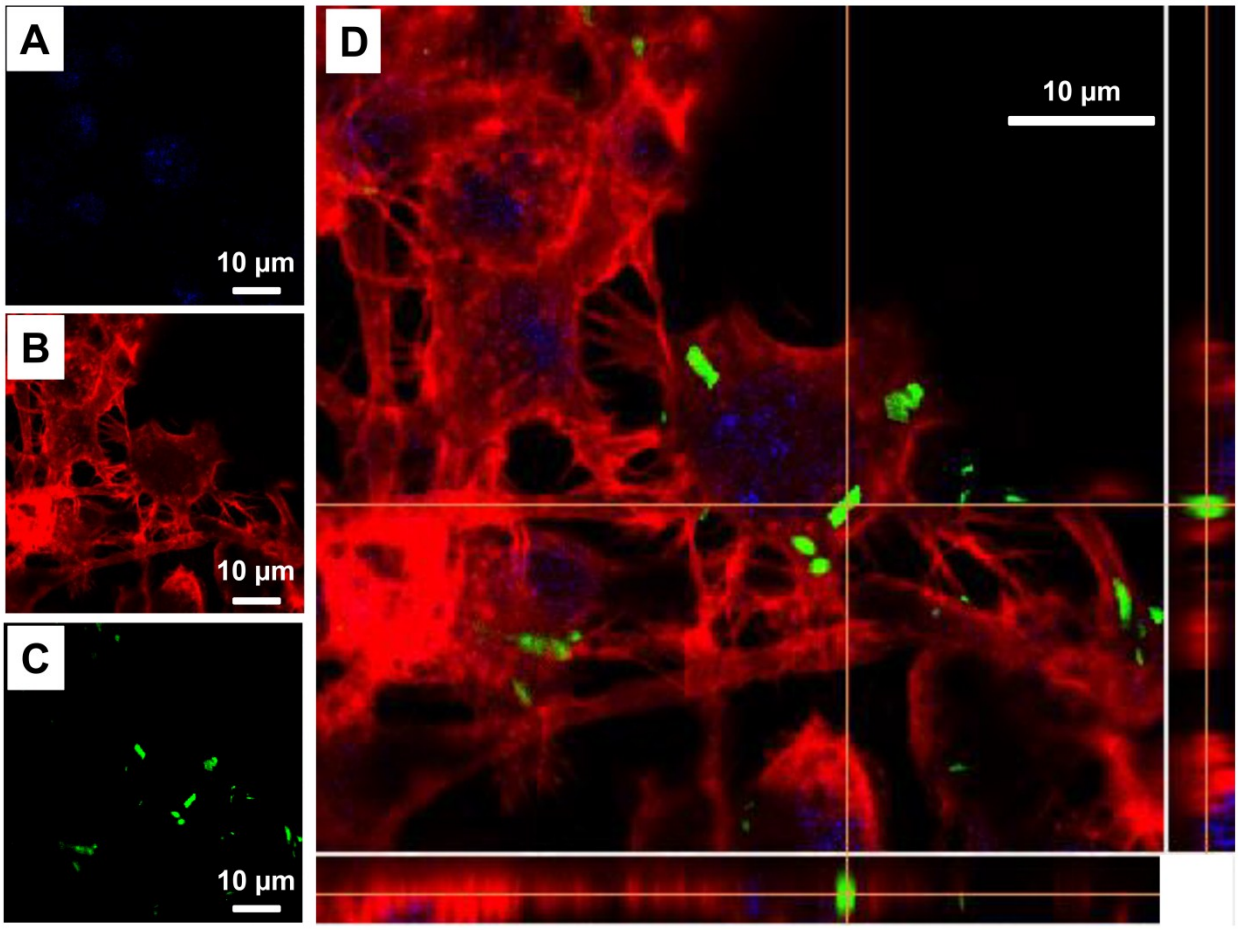


Figure S23: Confocal microscopy images for the cellular uptake of BiG-2.5@PLGA-C6 NPs at a concentration of 0.1 mg per mL after 3 h incubation with Raw 264.7 macrophage cells. (A) Nuclei stained by DAPI; (B) Cytoskeleton dyed by phalloidin; (C) NPs loaded with Coumarin-6 (C6) and (D) all the channels merged.

A suspension of Raw 264.7 cells (100,000 cells per well, 1 mL) in DMEM media supplemented with 10% FBS and 1% antibiotic Pen Strep was seeded in 4-well chamber slides (LabTek® II CC^{2TM} Chamber Slide™, Nunc., USA) and incubated for 24 h (37 °C, 5% CO_2). After 24 h, the supernatant cell media was aspirated and NP suspensions containing BiG-5@PLGA-C6 and BiG-2.5@PLGA-C6 NPs in DMEM media (0.1 mg/mL, 0.5 mL) were added and the slides were incubated (37 °C, 5% CO_2) for 2 h. Afterwards, supernatant media was removed and the cells were washed with PBS (pH 7.2, 0.5 mL) thrice. Next, the cells were fixed using a freshly prepared paraformaldehyde solution (4% w/v in PBS, pH 7.2, 250 µL, 10 minutes), washed again thrice with PBS (pH 7.2, 0.5 mL) and permeabilized with Triton X-100 (0.2% in PBS, pH

7.2, 250 µL, 5 minutes). Next, the cells were again washed three times with PBS (pH 7.2, 0.5 mL), stained with phalloidin (150 µL, Alexa Fluor® 647 phalloidin, Life Technologies, USA, 20 minutes); washed again with PBS (pH 7.2, 0.5 mL) and dried in air for 30 minutes. Finally, the bottom chamber slide was carefully taken apart from the its adjacent cover and a thin stream of the assembling solution containing DAPI was added to each of the chambers of the slide. Lastly, the slides were covered with a glass cover slip and sealed using nail polish. A negative control (just DMEM cell media) was also performed as a standard. The slides as such were viewed using a Nikon A1Rsi confocal laser scanning microscope equipped with a 100x oil-immersion objective (Nikon) that used for the recording the images and controlled by Nikon Elements imaging software (NIS).

As shown in Fig. 5a-d in the main paper and Figure S23 above, both the BiG-5@PLGA and BiG-2.5@PLGA NPs respectively were highly uptaken by the Raw 264.7 macrophage cells at a concentration of 0.1 mg per mL. Again, the uptake was higher for the BiG-5@PLGA NPs as compared to the BiG-2.5 variant.

4.2. Cellular Viability using Raw 264.7

To test the cytocompatibility of various Bi nanoparticle formulations, MTT assay using Raw 264.7 macrophage cells was performed. Briefly, Raw 264.7 cells (100 µL, 200, 000 cells per mL) suspended in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotic Pen Strep (Penicillin Streptomycin) were seeded in multiple 96-well plates and incubated for 24 h (37 °C, 5% CO2). Next, 100 µL each of BiG-2.5@PLGA, BiG-5@PLGA and BiG-5@SiO2 NP suspension in DMEM media were added to the respective wells in a range of concentrations (0.0001 to 1 mg per mL Bi concentration) and the plates were incubated for another 24 h. For the 48 h and 72 h experiment, corresponding plates with identical settings and content were incubated for 48 h and 72 h respectively. After incubation for the desired period, supernatant media from the wells were aspirated out and each well was washed with 100 µL PBS (Phosphate Buffer Saline, pH 7.2) thrice, following which 100 µL of fresh DMEM media was added to each well. The final MTT assay was done as described by the manufacturer's protocol.

The cell viability *vs.* concentration plot for the 24 h experiment is shown in Figure 5e in the main paper while the plots for the 48 h and 72 h experiment are shown in Figure S24A and S24B respectively. The higher biocompatibility (upto 0.1 mg/mL) obtained for both the BiG-5@PLGA and BiG-5@SiO2 NPs at the 24 h point is retained at both the 48 h and 72 h timepoints, with no acute toxicity observed during prolonger incubation for upto 72 h. For the BiG-2.5@PLGA NPs, the cell viability is considerably decreased over longer time points as compared to the 24 h incubation period. This clearly suggests that the limited Bi dissolution from BiG-5@PLGA and BiG-5@SiO2 NPs results in higher cell viability as compared to the BiG-2.5@PLGA NPs that have a rapid Bi dissolution rate in lysosomes.

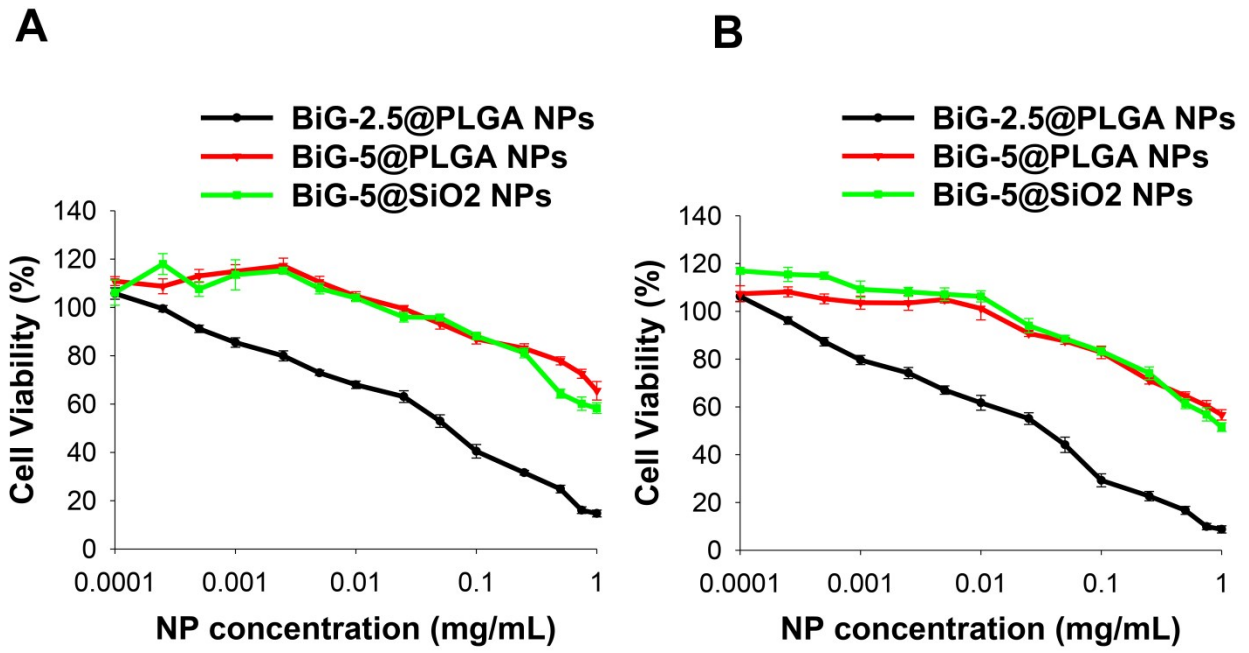


Figure S24: Cell viability (%) over A) 48 h and B) 72 h for different BiG NPs in Raw 264.7 macrophage cells.

5. µCT Experiments.

5.1. General Information

µCT phantom imaging was performed using a GE eXplore Locus µCT scanner (GE, Waukesha, Wisconsin) with a tube voltage of 80 kV and 450 µA. Samples (in suspension) of BiG-5@PLGA and BiG-5@SiO2 NPs were prepared in 500 µL of 0.5% agarose at 20, 40, 60 and 80 mM Bi concentrations. For comparison, similar samples for iodine at 20, 40, 60 and 80 mM concentration were prepared in 500 µL of 0.5% agarose solution using the clinically used CT contrast agent Iopamidol (Trade name ISOVUE®-300, Iopamidol Injection 61%, 30% organically bound iodine, Bracco Diagnostics Inc., Princeton, NJ, USA). For baseline corrections, 500 µL of 0.5% agarose without any Bi or iodine in it (0 mM Bi or I) was also prepared.

Typically, weighed out proportions of Bi NPs and ISOVUE-300 were taken in 500 µL centrifuge tubes and specified amounts of a hot 0.5% agarose stock solution were added to them. The contents were immediately put to vortex and set in an ice bath so that a hardened gel was obtained. These were next analyzed for CT contrast using the µCT scanner and the corresponding rate of attenuation was measured. The enhanced contrast profile obtained for both the BiG-5@PLGA (Figure 5f) and BiG-5@SiO2 NPs (Figure S25A) as compared to the

clinically used CT contrast agent ISOVUE-300, signifies the importance of our design and efficient packing of individual Bi NCs within a PLGA or SiO2 envelope. The contrast is obvious at a very low concentration of 20 mM Bi as compared to that obtained for a similar 20 mM I concentration for ISOVUE-300. Further, a linear relationship between X-ray attenuation and Bi concentration is observed with a slope greater than that obtained for the clinically used ISOVUE-300 as shown in Figure S25B.

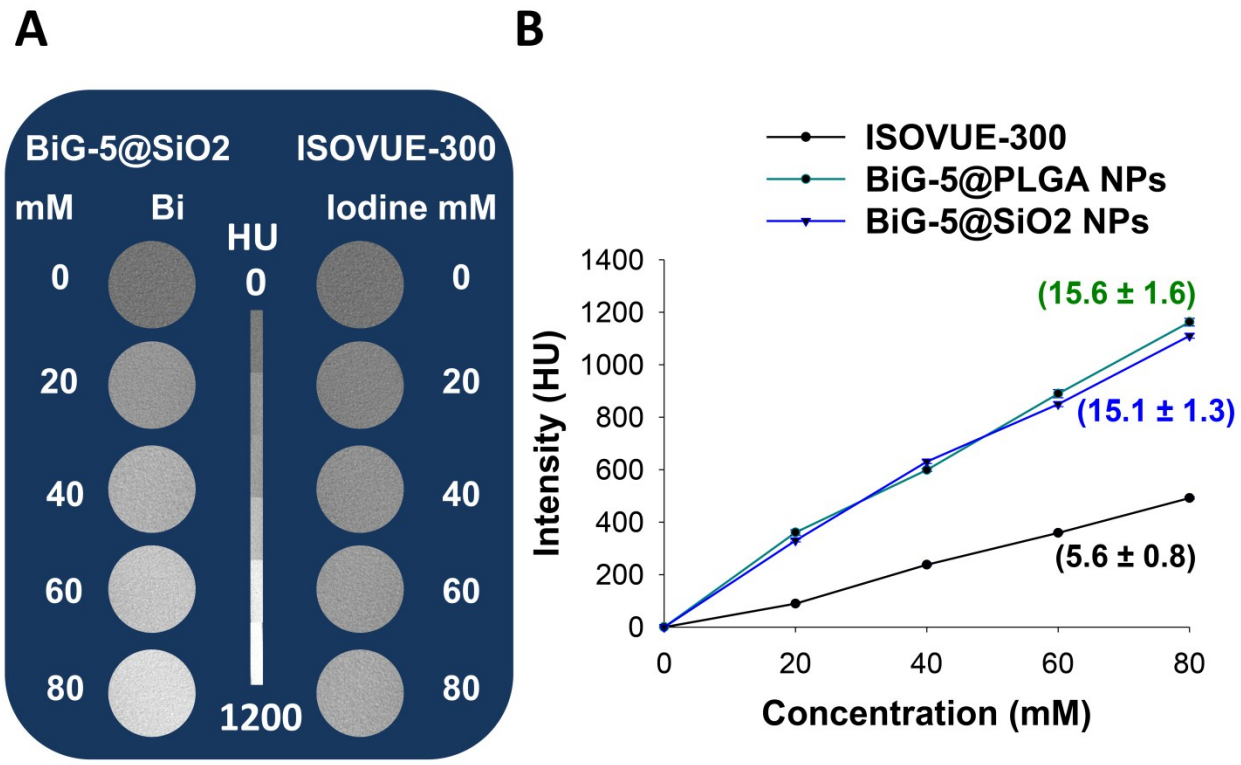


Figure S25: (A) µCT phantom images of BiG-5@SiO2 NPs and ISOVUE-300 samples prepared in 0.5% agarose gel. (B) Graph of X-ray attenuation *vs.* concentration for Bi in BiG-5@PLGA and BiG-5@SiO2 and Iodine in ISOVUE-300 measured using the µCT scanner operating at 80 kV. The X-ray attenuation curve for Bi is similar for both the NP formulations and is much higher than that observed for ISOVUE-300. Attenuation values in terms of HU per mM of Bi and Iodine are given within the parentheses in Figure (B).