Barium Titanate Nanoparticles for Biomarker Applications

O Matar[1], O M Posada[2], N S Hondow[1], C Wälti[3], M Saunders[4], C A Murray[5], R M D Brydson[1], S J Milne[1] and A P Brown[1]

1. Institute for Materials Research, School of Chemical and Process Engineering,*
2. Leeds Insitute of Cardiovascular and Metabolic Medicine, School of Medicine,*
3. Bioelectronics, School of Electronic and Electrical Engineering,*
* University of Leeds, LS2 9JT, UK.
4. Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, Perth, WA, Australia.
5. Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot, OX11 0DE, UK.

sm09om@leeds.ac.uk

Abstract. A tetragonal crystal structure is required for barium titanate nanoparticles to exhibit the nonlinear optical effect of second harmonic light generation (SHG) for use as a biomarker when illuminated by a near-infrared source. Here we use synchrotron XRD to elucidate the tetragonal phase of commercially purchased tetragonal, cubic and hydrothermally prepared barium titanate ($BaTiO_3$) nanoparticles by peak fitting with reference patterns. The local phase of individual nanoparticles is determined by STEM electron energy loss spectroscopy (EELS), measuring the core-loss O K-edge and the Ti L_3-edge energy separation of the t_{2g}, e_g peaks. The results show a change in energy separation between the t_{2g} and e_g peak from the surface and core of the particles, suggesting an intraparticle phase mixture of the barium titanate nanoparticles. HAADF-STEM and bright field TEM-EDX show cellular uptake of the hydrothermally prepared $BaTiO_3$ nanoparticles, highlighting the potential for application as biomarkers.

1. Introduction

Nanoparticles with a non-centrosymmetric crystal structure exhibit second harmonic generation (SHG) of light when illuminated with a femtosecond pulsed near-infrared laser. Such nanoparticles can be used as optical biomarkers to circumvent drawbacks with fluorescent proteins and semiconductor quantum dots, such as photobleaching and fluorescent intermittency (blinking) [1]. Barium titanate ($BaTiO_3$) with a tetragonal crystal structure is known for its ferroelectric properties due to the net dipole moment caused by the offset titanium atom in the oxygen octahedron. However, the reduction of barium titanate particle size is assumed to stabilise the paraelectric (cubic) phase [2].

The size dependence of the tetragonal/cubic phase transition of $BaTiO_3$ has long been debated, and the favoured size dependent transformation mechanism suggests a tetragonal particle core with a cubic shell that dominates the bulk at sufficiently small (< 100 nm) particle size [3]. A recent study has suggested that ferroelectric dipoles are present in all barium titanate nanoparticles regardless of size, and the density of these is a function of particle morphology; they suggest that spherical nanoparticles have fewer ferroelectric dipoles in comparison to faceted nanoparticles of similar size [4].

The BaTiO$_3$ crystal structure can be investigated by X-Ray or electron diffraction. Synchrotron X-Ray diffraction provides a bulk measurement that suffers from crystallite size line broadening in the diffraction pattern, making phase determination difficult. Resolving the phase of BaTiO$_3$ is important when utilizing the nanoparticles as biomarkers that produce second harmonic generation (SHG). Scanning transmission electron microscopy (STEM) however, can incorporate electron energy loss spectroscopy (EELS), a highly spatially resolved analytical technique that can indirectly determine the local phase of nanoparticles by producing characteristic core-loss edges specific to the phase of BaTiO$_3$. Moon *et al*. have suggested that the Ti L_3-edge in BaTiO$_3$ varies in t_{2g}-e_g separation between 2.36 eV for the tetragonal and 1.94 eV for cubic phases [5]; on this basis they report BaTiO$_3$ nanoparticles exhibit a cubic core and tetragonal shell in contrast to Asiaie *et al*. [3]. Other studies report that a broadening of the leading two peaks at the O K-edge of BaTiO$_3$ reflects O $2p$-Ti $3d$ hybridization related to tetragonal distortion [6]. A further study of cubic SrTiO$_3$ reports that a reduction in t_{2g} and e_g splitting and broadening of the Ti L_3-edge is related to surface reconstruction and distortion from the cubic symmetry of TiO$_6$ octahedra [7].

This work utilizes synchrotron XRD to measure the bulk phase and STEM-EEL spectroscopy to resolve the local phase of barium titanate nanoparticles. SHG images of the nanoparticles together with TEM images of cellular uptake highlight the feasibility of using BaTiO$_3$ as biomarkers.

2. Experimental

Barium titanate nanoparticles were synthesized via the hydrothermal method at 150 $^{\circ}$C for 72 hours [8]. Commercial tetragonal and cubic phase powders were purchased from Sigma Aldrich (Dorset, UK). Synchrotron powder diffraction was conducted at Diamond Light Source (Oxford, UK) Beamline I11 [9], under the supervision of the co-author stated. The wavelength of the incident beam (λ= 0.82563 Å) was calibrated using a high quality Si standard powder (SRM640c). Peak fitting was conducted using X'Pert HighScore plus software using tetragonal ICDD reference file: 04-013-5890 and cubic ICDD: 01-078-4475 model patterns.

TEM was conducted on two electron microscopes; an FEI Tecnai F20 FEG-TEM operating at 200 kV and fitted with a Gatan Orius SC600A CCD camera and an Oxford Instruments X-Max SD EDX detector, and a non-corrected FEI Titan G2 80-200 operated at 200 kV fitted with a Gatan Ultrascan 2k x 2k CCD camera with a Gatan Enfinium ER EEL spectrometer.

SHG images were taken on an Olympus confocal laser scanning microscope (Fluoview FV 1000) modified for multiphoton microscopy. Using a Ti: Sapphire laser source at 200 mW centered at 820 nm. The laser is pulsed at 80 MHz with a pulse width of 250 fs at the sample. The signal is detected in epi-geometry with a band pass filter centered at 410 nm (± 5 nm).

A549 lung epithelial cells were treated with BaTiO$_3$ nanoparticles (100 µg/ml) in serum free media for a 24 hour period and harvested. The samples were then prepared for electron microscopy by fixation, dehydration, resin embedding and thin sectioning for TEM.

3. Results and Discussion

3.1. Synchrotron XRD. The (002/200) peak from the synchrotron data was fitted to estimate the tetragonal phase fraction for all barium titanate samples. The peak fitting was conducted only on the (002/200) peaks due to the presence of minor phases of titanium rich BaTi$_2$O$_5$ or hydrated potassium hydroxide. The experimental (002/200) peaks (red) are shown in Figure 1 with the peak fitting analysis overlaid (shaded area) and the cross hairs indicate the fitting of the tetragonal (purple) and cubic (blue) reference data.
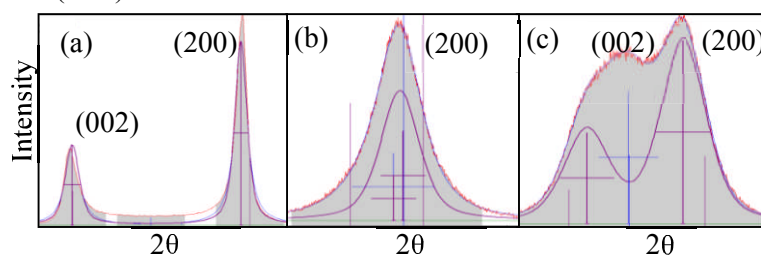

Figure 1. Synchrotron XRD plots of BaTiO$_3$: (a) commercial tetragonal, (b) commercial cubic and (c) hydrothermally prepared BaTiO$_3$.

Peak fitting estimates the tetragonal phase present in (a) commercially purchased tetragonal $BaTiO_3$ to be ~ 94 % tetragonal, (b) commercial cubic $BaTiO_3$ to be ~ 45 % tetragonal and (c) for hydrothermal $BaTiO_3$ synthesised in-house to be ~ 65 % tetragonal.

3.2. Second harmonic generation (SHG). Figure 2 shows that commercial 'tetragonal', 'cubic' and hydrothermal $BaTiO_3$ nanoparticles all produce second harmonic generation of light when illuminated by a near-infrared source. This suggests that the synchrotron XRD analysis is correct in estimating a mixture of cubic and tetragonal phases, as tetragonal is the active phase required for SHG. It is not clear however if this mixture of phases is distributed between particles (*inter*-particle) or within particles (*intra*-particle). Although correlative SHG to SEM imaging suggests the latter (data not shown here).
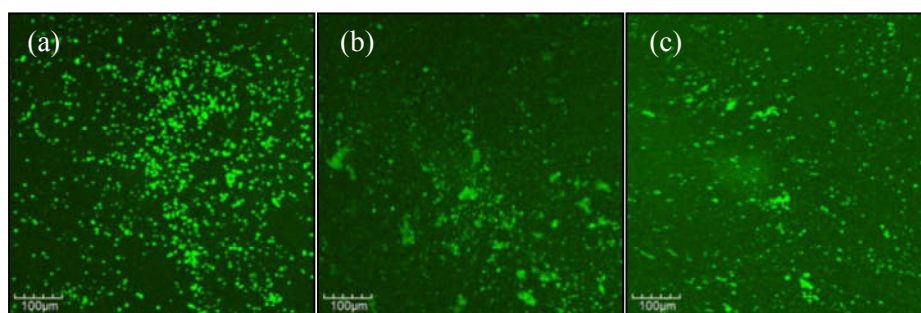

Figure 2. Second harmonic signals of commercially purchased tetragonal (a) and cubic (b) nanoparticles, plus (c) hydrothermally prepared $BaTiO_3$ nanoparticles.

3.3. Scanning transmission electron microscopy and EEL spectroscopy. High angle annular dark field (HAADF)-STEM imaging of $BaTiO_3$ nanocrystals with EEL spectroscopic linescans can indicate the crystallographic phases of $BaTiO_3$ nanoparticles at the particle scale. This technique has been used to investigate the phase content across individual particles at a 2 nm step size. Figure 3 (a) is a HAADF-STEM image of a hydrothermally prepared $BaTiO_3$ nanoparticle. Figure 3 (b) & (c) show the energy separation between the t_{2g}, e_g peaks of the Ti L_3-edges from the core and surface of the particle and the t_{2g}-e_g peak separation of the Ti L_3-edge across the profile of the particle. Figure 3 (d) is the O K-edges from the core and surface of the particle.
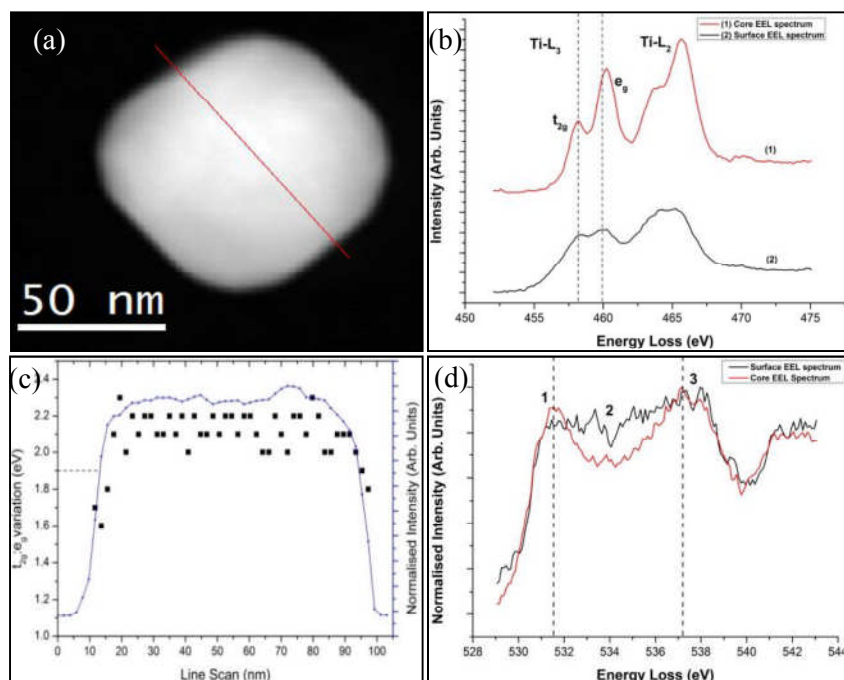

Figure 3. (a) HAADF-STEM image of a hydrothermal $BaTiO_3$ nanoparticle with the line scan location drawn across the particle. (b) Difference between the Ti L_3-edge t_{2g} and e_g peaks at the surface and core of the particle. (c) Energy separation of the Ti L_3-edge t_{2g} and e_g peaks measured across the particle at a 2 nm step size. Dotted line is the HAADF intensity profile. (d) O K-edge of particle surface and core. Features (1 & 2) are O 2p-Ti 3d states split into t_{2g} and e_g, (3) is O 2p-Ba 5d states.

Figure 3 shows a broadening of both the Ti L_3-edge and the O K-edge at the surface of a $BaTiO_3$ nanoparticle which may indicate distortion of TiO_6 octahedra and could possibly suggest a tetragonal shell and cubic core in agreement with the findings of Moon *et al.* [5]. However the t_{2g}-e_g splitting at the Ti L_3-edge is reduced at the surface, which based on the data of Moon would suggest the opposite (i.e. a tetragonal core and a cubic shell) in agreement with [3] – although in absolute terms the splitting are low for both phases. Further work is in progress; however whichever explanation is correct, the results do indicate that there is an intraparticle variation in structure within the nanoparticles with a core volume fraction of ca. 80-85 % not dissimilar to the findings of synchrotron XRD, i.e. this intraparticle phase/structure variation could account for SHG exhibited by the nanoparticles.

Although the phase of a $BaTiO_3$ nanoparticle is important for second harmonic generation of light, if the nanoparticles are to be used as biomarkers; they need to show uptake by cells; Figure 4.
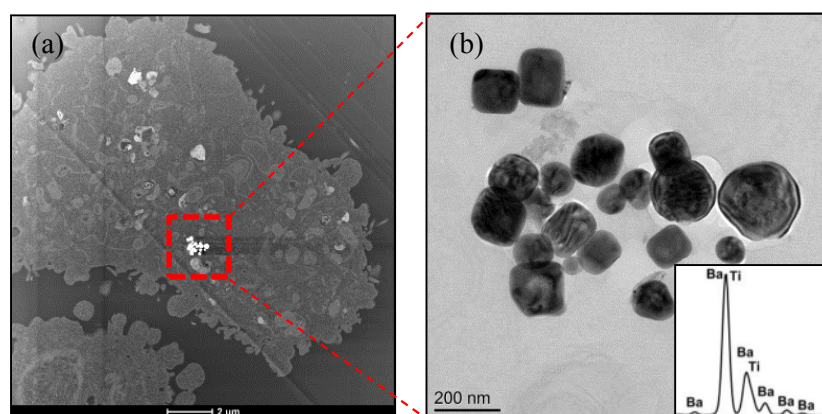

Figure 4. (a) HAADF-STEM image of a thin cell section with an internalized agglomerate of barium titanate nanoparticles. (b) Bright field TEM of the agglomerated nanoparticles from (a), with the associated EDX spectrum revealing barium and titanium inset.

4. Conclusion

Peak fitting of synchrotron XRD suggests that commercial 'tetragonal' and 'cubic', and hydrothermally prepared barium titanate nanoparticles all contain mixtures of tetragonal and cubic phases. Evidence that a tetragonal phase is present in all samples is shown by single and agglomerated particles emitting second harmonic light.

STEM-EELS linescans across $BaTiO_3$ nanoparticles show that there is intraparticle variation in the phase or structure with evidence for some form of surface reconstruction which could account for the phase fractions identified by synchrotron XRD and the presence of SHG.

HAADF-STEM and bright field TEM-EDX show cellular uptake of the hydrothermally prepared $BaTiO_3$ nanoparticles, highlighting the potential for application as biomarkers.

References

[1]   Rogov A, Mugnier Y and Bonacina L 2015 *J. Opt* **17** 33001
[2]   Frey M H and Payne D A 1996 *Phys. Rev. B* **54** 3158
[3]   Asiaie R, Zhu W D, Akbar S and Duta P K 1996 *Chem. Mater* **8** 226
[4]   Polking M J, Han M G, Yourdkhani A, Petkov V, Kisielowski C F, Volkov V V, Zhu Y, Caruntu G, Alivisatos P and Ramesh R 2012 *Nat. Mater* **11** 700
[5]   Moon S M, Wang X, and Cho N H 2013 *Microsc. Microanal* **19** 123
[6]   Bugnet M, Radtke G and Botton G A 2013 *Phys. Rev. B* **88** 201107
[7]   Zhu G, Radtke G and Botton G A 2012 *Nature* **490** 384
[8]   Eckert J O, Hung-Houston C C, Gersten B L, Lencka M M and Riman R E 1996 *J. Am. Ceram. Soc* **79** 2929
[9]   Thompson S P, Parker J E, Potter J, Hill T P, Birt A, Cobb T M, Yuan F and Tang C C 2009 *Rev. Sci. Intrum* **80** 075107