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Barriers to adoption of biogenic carbonates in the food, pharmaceutical & supplement sectors

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ABSTRACT

There is an increasing demand for environmentally sustainable sourcing of ingredients for the food, pharmaceutical and supplements industries. In the case of calcium carbonate (E170) as by-products from the egg and shellfish processing industries these have the potential to be sustainably sourced. In addition to their green credentials, biogenic carbonates have intrinsic benefits in terms of their chemical composition, such as a low heavy metal burden. However, their biogenic origin can potentially lead to manufacturing issues such as higher levels of co-mineralising components and the organic templates of their natural production. This contribution identifies the regulatory barriers to the adoption of biogenic eggshell calcium carbonate by assessing materials from biological sources along with commercial precipitated and ground carbonates against current regulatory standards.

1. Introduction

Calcium carbonate is an important ingredient or additive in the food, pharmaceutical and supplement sectors due to its numerous functions beyond acting as a source of calcium; these include acidity regulation, colouring and use as an anti-caking agent [1]. The traditional source of $CaCO_3$ is from the mining of carbonate rich geological mineral (or GCC) deposits. The use of its calcined derivative, 'lime' or calcium oxide has been estimated at 350 million/t per annum in 2017, with the majority of global production originating in China [2]. However, dietary and pharmaceutical applications of $CaCO_3$ utilise only a fraction of $CaCO_3$ production. A percentage of GCC undergoes additional processing to yield precipitated $CaCO_3$ (PCC) [3]. The precipitation process allows much finer control over crystal size than is available naturally and, most importantly in the food and healthcare sectors, a much more chemically refined product [3]. None the less, both GCC and PCC are generally recognized as safe by the U.S. food and drug administration (FDA) [3,4].

Many geological sources of $CaCO_3$ are sedimentary rocks that have a biological origin. Further, biogenic $CaCO_3$ (BCC) is a major constituent of the shells of avian eggs and molluscs and forms the skeletons of marine organisms such as corals [5]. For commercial and ecological reasons, there are several compelling arguments for the use of BCC rather than relying on geological mineral sources. Firstly, BCC is derived directly from a renewable source and recovery is not dependant

on extensive mining. Secondly, BCCs have the advantage that they are generally low in heavy metals, which can be a problem for carbonate containing products [6]. Finally, BCCs represents an example of waste valorisation, being by-products of existing commercial processes e.g. the egg and shellfish processing industries. Waste valorisation is an activity that is gaining considerable importance in the global economy [7,8].

While BCCs represent potentially attractive alternatives to geological sources, there are a number of potential issues to overcome for BCCs to be used as an additive/ingredient in the food, pharmaceutical and supplement sectors. Within these sectors, standards have been developed concerning what constitutes an acceptable 'grade' of $CaCO_3$ with respect to permissible levels of impurities. In the food industry the $CaCO_3$ standard is 170 (E170 in the EU) and defined in the Food Chemical Codex (FCC) by the FDA in the U.S.A. [1,9]. For the pharmaceutical industry, $CaCO_3$ is defined in pharmacopeia published nationally (e.g. European Pharmacopeia [Ph. Eur.] and United States Pharmacopeia [USP]) [10,11]. The supplements sector adopts the standards of both the food and pharmaceutical industries; Table 1 presents a summary of the specifications to be met for the $CaCO_3$ to meet a given standard.

BCC has been applied to calcium formulations in the laboratory to improve pharmaceutical availability [13]. However little attention has been paid to the regulatory hurdles of adopting BCC against current regulatory standards. While BCC may be desirable from a chemical,

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Table 1
Comparison of CaCO₃ specifications between different regulatory bodies including the Food Chemical Codex (FCC) (limestone [11] and CaCO₃ [9]), E170 [1], European Pharmacopeia (Ph. Eur.) [10] and U.S. Pharmacopeia (USP) [12].

Test	Limestone FCC	FCC	E170	Ph. Eur.	USP
Identity	PASS	PASS	PASS	PASS	PASS
Assay (%)	> 94.0	> 98.0	> 98.0–100.0	> 98.5–100.5	> 98.0–100.5
Loss on drying (%)	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
Acid-insoluble substances (%)	< 3.5	< 0.2	< 0.2	< 0.2	< 0.2
Free alkali (%)	–	–	< 0.05	–	–
Magnesium & alkali salts (%)	< 1.0	< 1.0	< 1.0	< 1.5	< 1.0
Fluoride (mg/kg)	< 0.005%	< 0.005%	< 50 mg/kg	–	< 0.005%
Antimony (mg/kg)	–	–	< 100	–	–
Barium (mg/kg)	–	–	< 100	PASS	PASS
Copper (mg/kg)	–	–	< 100	–	–
Chromium (mg/kg)	–	–	< 100	–	–
Zinc (mg/kg)	–	–	< 100	–	–
Arsenic (mg/kg)	< 3.0	< 3.0	< 3.0	< 4.0	–
Cadmium (mg/kg)	–	–	< 1.0	–	–
Lead (mg/kg)	< 10.0	< 10.0	< 3.0	< 20	< 3.0
Mercury (mg/kg)	< 0.5	–	–	–	< 0.5
Chlorides (mg/kg)	–	–	–	< 330	–
Sulphates (%)	–	–	–	< 0.25	–
Iron (mg/kg)	–	–	–	< 200 mg/kg	< 0.1%

ecological and economic point of view, prospective products produced or containing BCC must comply with the same standards as traditional GCC and PCC material. Important considerations for CaCO₃ materials being CaCO₃ purity (low in organic and inorganic impurities) while obtaining a low heavy metal content [1,9–12].

In this contribution the composition of a variety of CaCO₃ from geological, synthetic and biological sources are tested using methods recommended by current regulatory monographs. Additional characterisation techniques such as X-ray diffraction (XRD), infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and thermal analysis (TGA) have been performed to interpret the outcomes of the tests performed according to the regulatory monographs. XRD and FTIR were selected as they are routinely used to characterise the composition of inorganic and organic materials and compliment monograph tests for specific impurities which do not focus on the specific identity of the impurity [14]. TGA was selected due to its ability to determine organic and inorganic content of composite materials based on mass loss at a given temperature, the loss of CO_2 during calcination of CaCO₃ can be useful to estimate purity [15]. Scanning electron microscopy provides morphological information about the different sources and allows elemental composition to be surveyed by energy dispersive spectroscopy (see supplementary information) [16]. The results of these analyses are compared against the current legislation for CaCO₃ and highlight the challenges faced for the adoption of BCC in the food, pharmaceutical & supplement sectors.

2. Materials and methods

2.1. Materials

Commercial CaCO₃ samples including two agglomerated PCC for tabletting (ACC1, 2) and two PCC (PCC1, 2) all of E170 grade were obtained from UK suppliers. Two samples were derived from valorised eggshell (BCC1, 2), BCC1 of E170 grade was obtained from an E.U. supplier, BCC2 was ungraded and obtained from a UK supplier. A food grade above shore marine coral carbonate was obtained from the U.S.A. (GCC1). A CaCO₃ standard which met Ph. Eur., BP, USP, FCC and E170 and a CaCO₃ 99 + % ACS low in alkali metals were supplied by Sigma-Aldrich Company Ltd. (Gillingham, UK). Acetic acid (Fisher Chemical, glacial), ammonium hydroxide (Fisher Chemical, 35%), ethanol (Fisher Chemical, absolute), hydrochloric acid (Fisher Chemical, 37%), nitric acid (Fisher Chemical, 70%), oxalic acid dihydrate (Acros Organics, > 99%), sodium hydroxide (Acros Organics, 98.5%),

sulphuric acid (Fisher Chemical, > 95%) was supplied by Fisher Scientific UK (Loughborough, UK). calcium sulphate dihydrate (> 99%), calconcarboxylic acid (for complexometry), chloride standard solution (for ion-selective electrodes), ethylenediaminetetraacetic acid disodium salt dihydrate (99.0–101.0%), fluoride standard solution (for ion-selective electrodes), methyl red (Ph. Eur.), phenolphthalein (2% in ethanol), silver nitrate (> 99%) and sodium citrate (> 99%) was supplied by Sigma-Aldrich Company Ltd. (Gillingham, UK).

2.2. Monograph methods

2.2.1. Moisture content by loss on drying

In triplicate samples (~1.0 g) were dried at 200 ± 10 °C to constant weight using an AAF 11/3 ashing furnace (Carbolite Gero, Hope Valley, UK). The result is reported as an average with the standard deviation of 3 replicates.

2.2.2. Determination of acid insoluble substances content

As BCC samples may contain residual organic content, acid insoluble substances were determined using a method based on the Ph. Eur. monograph, which does not rely on ignition to estimate insoluble content [10,12]. Briefly, ~5 g of sample was dissolved in 80 mL of 12% acetic acid. When effervescence ceased, the sample was boiled for 2 min and once cool diluted to 100 mL with 12% acetic acid and passed through a sintered-glass (Pyrex™, grade 2) Gooch crucible. The residue obtained was washed 4 times with 5 mL of freshly boiled water, dried at 100–105 °C for 1 h, and weighed, the residue calculated as a percentage of the initial sample mass. The result is reported as an average with the standard deviation of 5 replicates.

2.2.3. Calcium carbonate assay

The assay was based on the Ph. Eur. monograph [10], though only minor differences (e.g. choice of indicator) were noted between different monograph methods [1,10–12]. A 3 mL aliquot of 3 M HCl and 20 mL of water was used to dissolve 0.150 g of sample. The solution was boiled for 2 min, cooled and diluted with 200 mL of 18 MΩ H_2O. A complexometric titration for calcium was conducted by adding 6 mL of 10 M NaOH and 0.1 mL of 15 mg/mL of calconcarboxylic acid triturate. This solution was titrated with 0.1 M sodium edetate until the endpoint was reached (colour change from violet to blue) and the amount of CaCO₃ determined on the basis that 1.0 mL of 0.1 M sodium edetate is equivalent to 10.01 mg of CaCO₃. The result is reported as an average with the standard deviation of 8 replicates.

2.2.4. Determination of magnesium and alkali salts content

The assay was based on the E170 method [1]. Around 1.0 g of sample was added to 40 mL of 18 MΩ H$_2$O, 5 mL of hydrochloric acid was added, and the solution boiled for 1 min. Rapidly 40 mL of oxalic acid (6.3 w/v dihydrate) was added and the solution stirred vigorously until precipitation was established. Immediately 2 drops of methyl red (0.1 g in 100 mL EtOH) indicator was added, then ammonia (400 mL of 28% ammonium solution in 1 L H$_2$O) was added dropwise, until the mixture was just alkaline. The mixture was transferred to a 100 mL cylinder, diluted to 100 mL with water and allowed to stand overnight before the precipitate free supernatant was filtered with a MINISART® NML 0.2 μm syringe filter. To 50 mL of the filtrate, 0.5 mL of sulphuric acid was added and the mixture evaporated on a hot plate to a small volume in a porcelain crucible. The crucible was then heated to dryness over a free flame until any residual ammonium salts had been volatilized. The residue was ignited at 600 °C to constant weight. The result is reported as an average with the standard deviation of 5 replicates.

2.2.5. Determination of fluoride by ion selective electrode

The assay was based on the E170 method [1]. The sample (~1.0 g) was transferred into a 150 mL glass beaker to which was added 10 mL of water and while stirring continuously 20 mL of 1 M HCl to dissolve the sample. The sample was boiled for 1 min, transferred to a 250 mL plastic beaker cooled on ice. A 15 mL aliquot of 1 M sodium citrate followed by 10 mL of 0.2 M disodium edetate was added and the pH adjusted to 5.5 ± 0.1 with 1 M HCl or NaOH, then made up to 100 mL. A 50 mL aliquot was transferred into a 125 mL plastic beaker and the potential of the solution was measured with an Orion™ fluoride ion selective electrode (Thermo Fisher Scientific, Paisley, UK) and MeterLab PHM240 meter (Radiometer UK Ltd., Crawley, UK). The fluoride content was determined from a standard curve (1.0–15.0 μg F$^-$) made from a 5 μg F$^-$ per mL standard, each standard also containing 5 mL of 1 M HCl, 10 mL of 1 M sodium citrate and 10 mL of 0.2 M disodium EDTA and made up to 100 mL. The result is reported as an average with the standard deviation of 8 replicates.

2.2.6. Limit testing for free alkali, chloride and sulphate

To 3 g of sample, 30 mL of freshly boiled and cooled water was stirred for 3 min and filtered. To 20 mL of the filtrate 2 drops of phenolphthalein (0.2 g in 60 mL 90% EtOH and diluted to 100 mL in H$_2$O). To pass the indicator would decolour once 0.2 mL of 0.1 M HCl was added. For chloride, a 2 mL aliquot of the filtrate from the acid insolubles test was diluted to 15 mL with 18 MΩ H$_2$O. Nitric acid (14%) and 1.7% silver nitrate solutions were added (1 mL each) and the solution mixed. To pass the resulting opalescence of the samples was observed to be no greater than a standard containing 330 ppm Cl$^-$ (from a 0.1 M standard solution). For barium, 10 mL the filtrate from the acid insolubles test was added to 10 mL of saturated calcium sulphate. After 15 min opalescence was compared to a mixture of 10 mL of solution S and 10 mL of 18 MΩ H$_2$O. To pass, the opalescence of the sample must not be greater than a sample of 18 MΩ H$_2$O alone.

2.2.7. Elemental content by induction coupled plasma – optical emission spectroscopy (ICP-OES) and mass spectrometry (ICP-MS)

Tests in triplicate for Ba, Cr, Cu, Fe, Mg, P and Zn were performed using an Optima 2100 DV ICP-OES (Perkin Elmer, Seer Green, UK) calibrated using TraCERT® standards (Sigma Aldrich, Gillingham, UK). Weighed samples (~0.2 g) were dissolved in 70% nitric acid, after effervesce ceased the sample was boiled for 1 min and allowed to cool before being filtered and made up to 50 mL with 18 MΩ H$_2$O. Element concentrations were calculated from emission intensity of the samples at given wavelengths (233.527 nm [Ba], 267.76 nm [Cr], 327.393 nm [Cu], 238.204 nm [Fe], 285.213 nm [Mg], 213.617 nm [P] and 206.2 nm [Zn]) in comparison to a standard curve constructed for each element stock diluted in 1% nitric acid. Tests for As, Cd, Pb and Sb were performed by ICP-MS, which was performed externally by Edinburgh

Innovations Ltd. (Edinburgh, UK). Sample preparation was performed as for ICP-OES analysis described above. The calculated LOD for each instrument for each element is given in supplementary table S1.

2.3. Additional characterisation methods

2.3.1. Morphological studies by scanning electron microscopy

Samples were mounted onto aluminium stubs using adhesive carbon tape, both supplied by TAAB Laboratories Equipment Ltd. (Aldermaston, UK). A 5.0 nm layer of gold was applied using a Q150R ES sputter coater (Quorum Technologies, Laughton, UK) equipped with a crystal microbalance to monitor the rate of gold deposition. Micrographs of coated samples were obtained using a JEOL 7100F scanning electron micrograph operating at an accelerating voltage of 10.0 kV in secondary electron mode.

2.3.2. Crystallography by powder X-ray diffraction

Diffractograms were collected using a PANalytical Xpert PRO (Royston, UK) equipped with a PW3064 spinner stage and a PW3050/60 goniometer. Raw scans were collected with a 0.017 step between 5.0 and 90.0°2θ. Diffractograms were background corrected using an automated algorithm using smoothed input with 10 point blending and 20 point granularity factors [17]. The data was smoothed with a quantic polynomial function, omitting peaks and using a 23 data point convolution range. The processed diffractograms were matched against the PDF-2 2015 release RDB library for identification, using a minimum 2nd derivative algorithm and a minimum significance of 5, 0.0°2θ and 9.98°2θ minimum and maximum tip-width respectively and a base width of 15.0°2θ.

2.3.3. Chemical composition by Fourier transform infrared (FTIR) spectroscopy

Spectra were collected using an iS50 FTIR spectrometer (Thermo Fisher Scientific, Paisley, UK) equipped with a golden gate diamond ATR accessory (Specac, Orpington, UK). Spectra were collected between 400 and 4000 cm^{-1}, in total 32 spectra at a resolution of 2.0 cm^{-1} were accumulated. These spectra were collected and processed in Omnic 9 using an advanced ATR correction assuming an incidence angle of 45° and a sample refractive index of 1.6.

2.3.4. Organic content and purity by thermogravimetric analysis (TGA)

Carbonate samples in triplicate were heated in a stream of air at a rate of 10 °C/min from 30 °C to 900 °C with a 10 min hold at 30 and 900 °C. Mass loss over this range was recorded using a TGA/DSC DSC3+ (Mettler-Toledo Ltd., Beaumont Leys, UK) instrument. Thermograms were processed using STARe ver. 16.0 and were converted to percentage loss of total mass from which DTGA curves were calculated, integration of these curves was used to determine mass loss over specified temperature ranges.

3. Results

Analysis of the composition of the different CaCO$_3$ materials together with a CaCO$_3$ standard was performed as per the methods outlined in the monographs. These results are presented in Table 2 as a mean value with standard deviation. For several tests methodological variation was noted between monographs, the testing methodology adopted is detailed in the corresponding methodology section.

All samples passed the identity tests for CaCO$_3$, being insoluble in water, effervescing in acids and testing positive for Ca. Several monographs state limit tests for specific anions such as chloride and sulphate, no sample failed these. CaCO$_3$ is readily dried at 200 °C and does not have a particularly strong affinity for water, unlike its chloride and nitrate salts. These results correlated well with the analysis of the powder XRD patterns obtained, which matched library diffractograms for CaCO$_3$ Fig. 1. Four of the six CaCO$_3$ (ACC1, 2 and BCC1, 2) fell

Table 2

Analysis of different commercial calcium carbonates including agglomerated (ACC), precipitated (PCC), biogenic (BCC) and above shore (GCC) samples and an E170 standard (Sigma) as per monograph tests, data represented as the mean with error represented as standard deviation. Also presented is calculated $MgCO_3$ content (based on induction coupled plasma – optical emission spectroscopy (ICP-OES) Mg), purity (derivative thermogravimetric analysis (DTGA) 500–850 °C) and losses (TGA 210–500 °C) of different commercial calcium carbonates, with data represented as a wt%. Particle size (μm) as measured from scanning electron microscopy (SEM) micrographs.

| Test	Sigma	ACC1	ACC2	PCC1	PCC2	BCC1	BCC2	GCC1
Identity	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
Assay (%)	98.63 ± 0.22	96.67 ± 0.34	96.1 ± 0.55	99.02 ± 0.44	99.54 ± 0.44	95.6 ± 0.43	95.24 ± 0.47	98.94 ± 0.72
Loss on drying (%)	0.26 ± 0.03	0.77 ± 0.01	1.72 ± 0.12	0.21 ± 0.02	0.18 ± 0.01	0.59 ± 0.04	0.33 ± 0.01	0.07 ± 0.01
Acid-insoluble substances (%)	0.01 ± 0.01	0.1 ± 0.02	0.11 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	1.19 ± 0.09	0.39 ± 0.04	0.42 ± 0.1
Free alkali (%)	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	< 0.05	< 0.05
Magnesium & alkali salts (%)	0.66 ± 0.1	1.11 ± 0.09	1.2 ± 0.11	1.29 ± 0.17	1.07 ± 0.14	3.2 ± 0.26	2.13 ± 0.44	2.22 ± 0.22
Fluoride (mg/kg)	13.44 ± 1.23	6.37 ± 0.18	6.03 ± 0.36	17.06 ± 0.83	16.02 ± 1.31	4.99 ± 0.63	3.75 ± 0.28	36.74 ± 0.27
Chlorides (mg/kg)	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
Sulphides (%)	PASS	PASS	PASS	PASS	PASS	PASS	PASS	PASS
Est. $MgCO_3$ by ICP-OES (%)	0.767	0.903	0.885	0.539	0.570	1.346	1.511	0.812
Purity by DTGA (%)	95.74 ± 0.54	97.26 ± 0.21	95.67 ± 2.13	94.69 ± 0.15	93.45 ± 0.15	94.91 ± 0.34	97.26 ± 0.04	98.92 ± 0.08
Loss by TGA 200–500 °C (%)	0.12 ± 0.02	1.49 ± 0.28	1.59 ± 0.2	0.09 ± 0.15	0.14 ± 0.11	0.37 ± 0.1	0.45 ± 0.05	0.34 ± 0.11
Particle size by SEM (μm)	1.55 ± 0.54	2.37 ± 2.43	2.28 ± 3.99	1.43 ± 1.0	2.4 ± 2.75	2.37 ± 1.69	9.43 ± 7.75	3.03 ± 1.94

under the required 98.0–98.5% minimum assay target. For the agglomerated $CaCO_3$ ACC1, 2 this discrepancy was attributed to the declared presence of ~5.0–10% agglomerant (which FTIR suggests to be some form of dextrin – see Fig. S1), which are common additives for many $CaCO_3$ formulations [18]. The low purity for BCC1, 2 can be attributed to a variety of factors such as acid insoluble substances and other impurities as discussed below.

Acid insoluble substances were higher than the required 0.2% in the case of BCC1, 2. Dextrin being soluble unlike certain organic residues (e.g. protein from eggshell membrane), was not detected by this test. Notably GCC1 failed for acid insoluble substances but passed the assay test, this was attributed to the presence of inorganic components (Fig. S3), with minimal losses being detected between 200 and 500 °C by TGA, Fig. 3C. Magnesium and alkali salts (Mg&A) was passed only by the PCC standard (Table 2), though all ACC and PCC were borderline with the higher limit of the test and would be compliant with Ph. Eur. (Table 1). The BCC and the GCC were higher than all the other samples tested, this trend correlated with ICP-OES data obtained on Mg content, Table 3.

All samples achieved the limit for fluoride with GCC and PCC having

notably higher fluoride levels than BCC, consistently above 10.0 mg/kg. The eggshell BCC demonstrated the lowest fluoride, with BCC2 consistently demonstrating < 5.0 mg/kg, contrasting sharply with above shoreline coral (GCC1), which returned the highest fluoride levels of all samples tested. Only BCC2 failed the required free alkali limit of the E170 specification. All the samples tested were shown to be compliant for trace metal impurities by ICP-OES, and ICP-MS (Table 3). Metals levels for Zn and Ba were comparable to studies of the shell of *G. gallus*, with Fe and Cu being slightly lower in our materials [19]. Additional tests for P and Mg were performed by ICP-OES, as these elements were suspected as potential impurities, specifically for samples with high Mg &A content. Mg levels tended to be higher for samples with higher Mg& A results, when calculated as $MgCO_3$ (Table 2), the $MgCO_3$ content was calculated to be a major constituent, > 1.0% w/w of the BCC specifically.

Further to the monograph assays, additional analysis were performed to identify the impurities present and interpret the results of the monograph assays. XRD diffractograms are shown in Fig. 1, 1A and show matches to the reference spectra for calcite as was expected for materials where $CaCO_3$ was believed to be the dominant component.
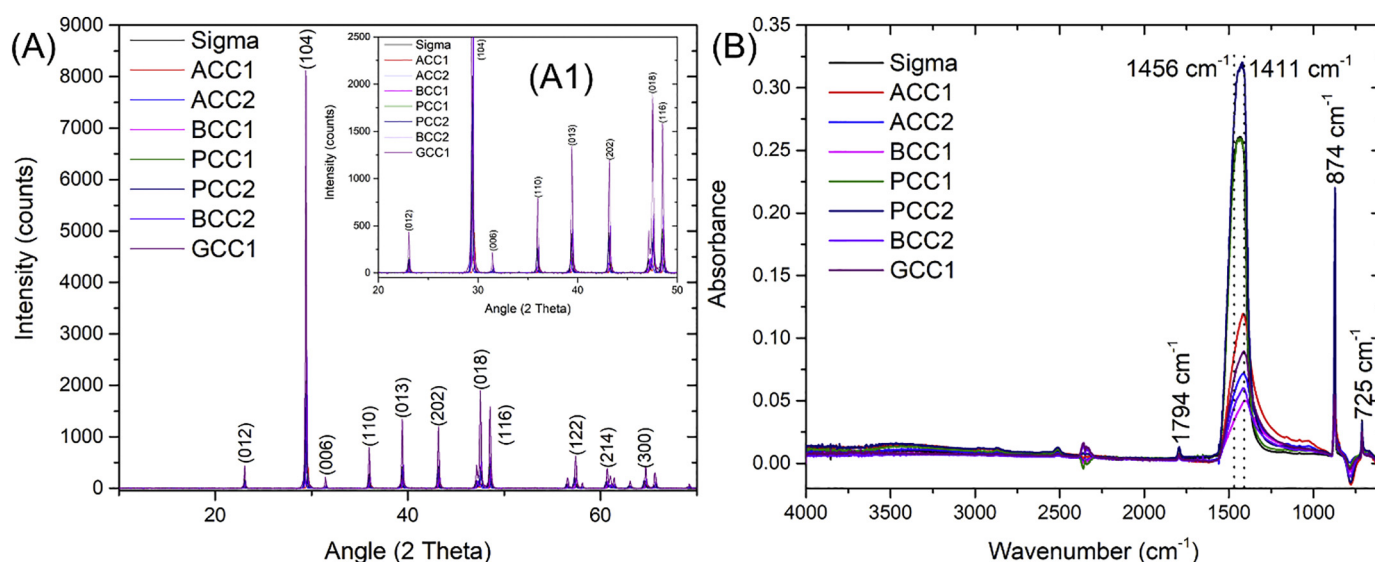
Fig. 1. X-ray diffractograms (A) of agglomerated (ACC), precipitated (PCC), biogenic (BCC) and above shore (GCC) $CaCO_3$ samples and an E170 standard (Sigma). Inset diffractogram A1 shows the region 20–50 2θ of diffractogram A. The diffractograms obtained for all the samples show the characteristic diffraction pattern for calcite. Attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR) spectra (B) of ACC, PCC, BCC and GCC carbonate samples and an E170 standard (Sigma).

Table 3
Metal content (mg/kg) of different $CaCO_3$ including agglomerated (ACC), precipitated (PCC), biogenic (BCC) and above shore (GCC) samples and an E170 standard (Sigma) by induction coupled plasma – optical emission spectroscopy (ICP-OES) (Ba, Cr, Cu, Fe & Zn) and induction coupled plasma – mass spectroscopy (ICP-MS) (As, Cd, Sb & Pb), data represented as the mean with error represented as relative standard deviation. ND stands for no detection.

Element (mg/kg)	Sigma	ACC1	ACC2	PCC1	PCC2	BCC1	BCC2	GCC1
Barium	8.11 ± 0.45	1.96 ± 15.47	1.74 ± 3.43	0.98 ± 5.7	2.74 ± 3.5	10.73 ± 0.51	16.24 ± 2.78	1.33 ± 7.65
Chromium	0.51 ± 5.3	0.09 ± 63.54	0.05 ± 78.0	0.16 ± 10.98	0.18 ± 20.41	0.22 ± 29.18	2.15 ± 4.1	3.25 ± 11.82
Copper	4.76 ± 28.81	1.89 ± 25.56	0.91 ± 24.99	0.64 ± 29.53	0.27 ± 37.56	3.76 ± 35.73	0.39 ± 39.11	ND
Iron	135.67 ± 1.49	63.07 ± 3.64	58.81 ± 4.05	132.17 ± 1.0	113.26 ± 0.47	12.02 ± 15.18	11.12 ± 5.86	107.45 ± 9.69
Magnesium	2212.02 ± 0.78	2603.64 ± 0.8	2551.81 ± 3.07	1554.88 ± 1.17	1643.18 ± 3.33	3880.96 ± 2.1	4354.75 ± 3.01	2341.62 ± 13.35
Phosphorous	68.56 ± 0.55	12.61 ± 4.6	12.51 ± 6.58	61.93 ± 0.61	58.17 ± 0.24	829.35 ± 0.13	1067.46 ± 2.18	191.12 ± 11.12
Zinc	1.92 ± 15.17	1.35 ± 7.47	0.91 ± 16.09	3.94 ± 2.27	3.61 ± 1.21	11.79 ± 20.04	4.91 ± 26.43	0.64 ± 18.84
Antimony	0.02 ± 8.51	0.04 ± 4.90	0.05 ± 3.31	0.01 ± 1.15	0.03 ± 6.07	0.03 ± 10.54	0.01 ± 13.21	0.04 ± 3.92
Arsenic	2.94 ± 12.77	2.60 ± 33.37	2.35 ± 20.96	1.87 ± 20.22	2.67 ± 10.04	2.80 ± 15.01	3.20 ± 11.22	2.85 ± 15.51
Cadmium	0.10 ± 7.59	0.05 ± 2.47	0.05 ± 10.91	0.03 ± 0.37	0.43 ± 1.27	0.38 ± 9.67	0.04 ± 8.46	0.23 ± 2.46
Lead	0.22 ± 3.41	0.26 ± 1.96	0.27 ± 0.85	0.11 ± 1.54	0.49 ± 0.82	0.27 ± 0.76	0.52 ± 2.70	0.83 ± 0.43

ATR-FTIR spectra (Fig. 1B) for all samples demonstrated peaks characteristic for $CaCO_3$ (calcite). All major symmetric and asymmetric vibrational modes (ν1-4) for calcite were observed [20]. Minor contributions to the spectra were noted in the OH (3000–3750 cm^{-1}) and CH (2800–3000 cm^{-1}) regions, considering the known organic content (Table 2) for specific samples such as ACC2 and BCC1 very little spectral evidence was obtained using this method. The intensity of the major vibrational modes of $CaCO_3$ however correlated well with the purity of the material obtained by titration (Table 2).

As expected thermograms of the $CaCO_3$ samples were dominated by the decomposition of $CaCO_3$ to CaO and CO_2 at > 550 °C. In Table 2 is shown $CaCO_3$ purity determined by the amount of CO_2 evolved between 500 and 850 °C. Precipitated samples (including the standard) was found to vary significantly from the assay values determined by complexometric titration, Table 2. When a further standard was used (Sigma, 99 + % ACS low in alkali metals) the expected response was produced (refer to Supplementary Fig. S2), which suggests that these impurities may be inorganic in nature such as other alkali metal carbonates. Major impurities by TGA were shown to be lost between ~200–600 °C, for ACC1, 2 a peak in the DTG curves was present ~300 °C (*, Fig. 3A) which was taken to represent the decomposition of the agglomerant. For PCC1, 2 very similar thermograms to the standard were observed (Fig. 3B). A broad range of losses were observed for BCC1, 2 attributed to the known components of eggshell, organic residues and the decomposition of inorganic impurities such as $MgCO_3$ [21], Fig. 3C, D. For GCC1 fewer losses between ~200–600 °C were detected than the standard, suggesting any impurities observed are purely inorganic.

Particle size and morphology was examined by scanning electron microscopy, Fig. 2. The particle size and morphology varied significantly for each sample, PCC1, 2 and the standard displayed angular needle like crystallites with features smaller than 1 μm, characteristic of precipitated $CaCO_3$ [22]. AGG1, 2, having undergone agglomeration, exhibited larger and more irregular structures. The BCC displayed distinct but more irregular morphology with larger particle sizes and broader particle size distributions, with particles up to many tens of micron in size. Both retained many features (e.g. pores, refer to arrows Fig. 2 BCC1, 2) characteristic of biogenic eggshell $CaCO_3$ [23]. These features were lacking from the GCC1 above shoreline coral sample, which otherwise shared the larger particle size and broader particle size distributions of BCC1, 2, Table 2.

4. Discussion

Our results illustrate the strengths and weaknesses of different sources of $CaCO_3$. Many attributes were readily attained by all the samples, including the BCC, though there were notable differences. Although all samples met the fluoride specification, GCC and PCC had notably higher fluoride levels than BCC. Fluoride in the environment is known to adsorb to $CaCO_3$, forming fluorite, the PCC product likely acting as a sink for fluorine impurities during processing [24]. Regarding BCC, the eggshell naturally acts as a barrier to fluoride uptake with concentrations averaging 0.18 mg/kg reported for egg yolk and white [25]. Fluoride results obtained were notably lower than those determined by others for eggshell [26], which may be attributed to the treatments used in processing and environmental conditions encountered during laying. The highest fluoride levels obtained by others would not be expected to exceed the regulatory limit [26]. For marine carbonates such as corals fluoride content has been correlated with $[CO_3^{2-}]$ in seawater, with the carbonate containing ~500–1300 mg/kg [27,28]. This content would be in excess of all $CaCO_3$ specifications which specify fluoride limits, the coral sample here was above shore and the lower fluoride content may be explained by mechanisms such as leaching [29].

The content of most metals including heavy metals was low in the BCC and though heavy metals are a notable problem for mineral [30,31] and specifically calcium dietary and medicinal preparations [6] all samples tested readily passed the monograph limits. BCC was notably lower in iron than the other carbonates tested, but was found to be higher in barium, though well under regulatory limits. Barium is a notable component of the eggshells of terrestrial birds, like other biomineralisation mainstays such as strontium, the elements having a similar chemistry but lower abundance to calcium [19]. Reported iron values vary considerably in eggshell [32] while no specific role for trace transition metals is known for eggshell calcification, marine GCC from oyster are also noted to have higher levels of Fe than eggshell but not in excess of regulatory limits [33]. Though not a concern in this study some of the values reported would exceed regulatory limits (e.g. Ph. Eur) [32].

The main challenges to BCC in meeting the $CaCO_3$ regulatory specifications came from a variety of additional impurities; in specific cases, this influenced the assay content of the $CaCO_3$ sufficiently to fail the assay. Processing of $CaCO_3$ may involve washes with caustic agents or in the case of PCC manufacture from a hydroxide [3], which may result in high residual free alkali; one standard (E170) specifies limits for this directly [1]. For BCC, free alkali is not present in quantity unless introduced as part of the manufacturing process, and would readily be removed by washing.

Magnesium and alkali salts testing poses a particular challenge for BCC and carbonates in general. Specifically regarding Mg, this can be present as a carbonate in significant quantities in biomineralised carbonates [33,34]. During biomineralisation, Mg (an essential element present in quantity in living organisms) is known to influence the process of biomineralisation in avian and marine shells. In avians such as *G. gallus*, Mg has an inhibitory effect on calcite deposition, with Mg content increasing until the termination of crystal growth [35]. For marine BCC, of which the majority are produced in the pelagic and shallow benthic zones, aragonite and 'high' Mg calcite (> 4.0 mol%)
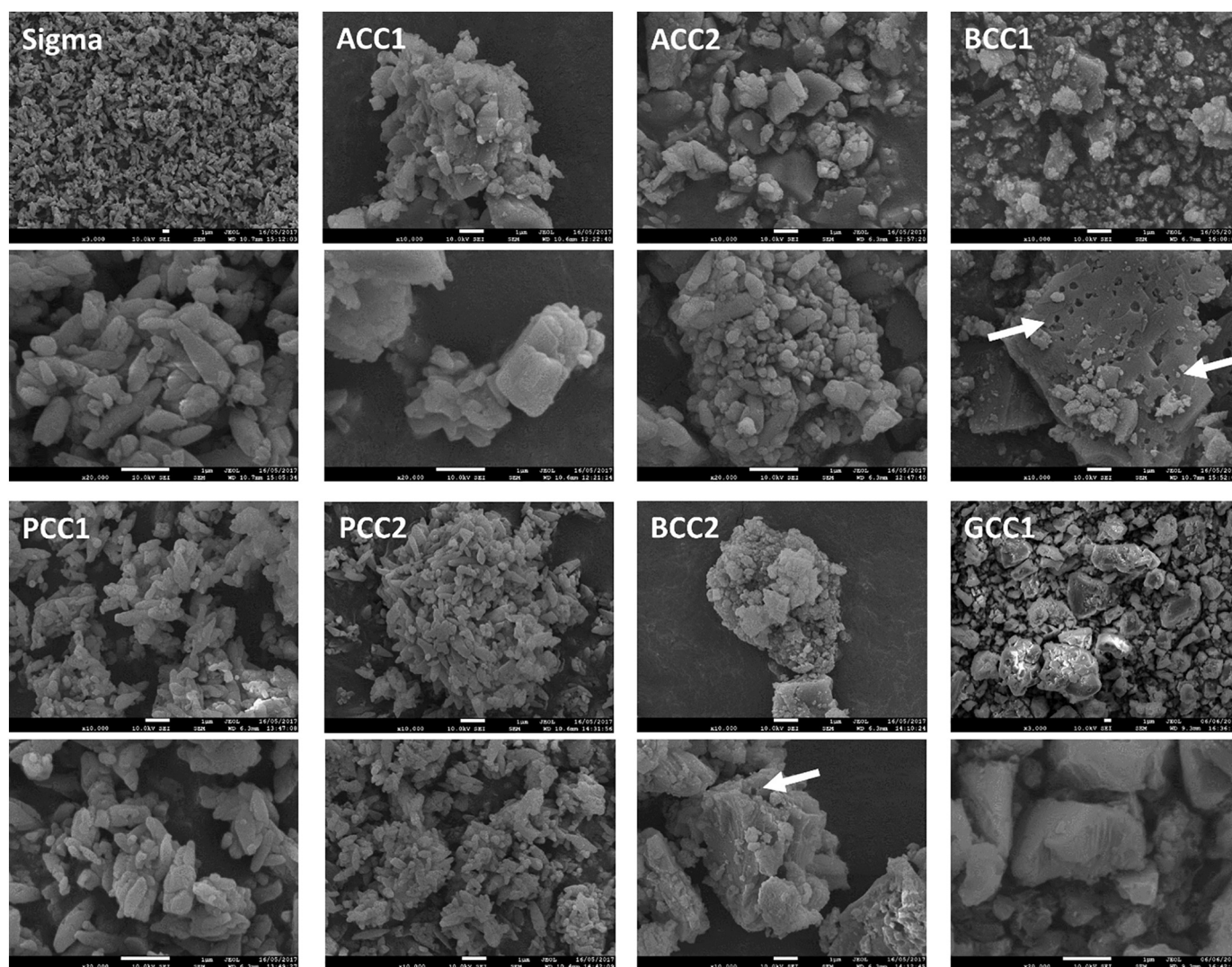
Fig. 2. Secondary electron micrographs of agglomerated (ACC), precipitated (PCC), biogenic (BCC) and above shore (GCC) carbonate samples and an E170 standard (Sigma). Angular crystallites of PCC are easily distinguishable as are the structures of BCC, which are indicative of their origin such as pores within eggshell palisades (arrows on BBC1, 2). All scale bars represent 1.0 μm.

are noted to dominate which would be significantly above the limits for Mg&A [33,36]. The range for eggshell magnesium (5–3.5 and ~28 mg/g depending on origin) as reported by others is noted to fall outside the limits for magnesium and alkali salts [34], especially as magnesium is measured as a salt rather than as elemental magnesium in this assay. For all BCC the Mg present is highly entrained and impossible to remove without dissolution and re-precipitation of the mineral.

For BCC, acid insoluble substances is a difficult characteristic to assess, the outcome is specific to the test methodology employed and these vary considerably [37]. For tests, which do not rely on ignition but filtration or sedimentation, residual organics are present in BCC in sufficient quantity to fail the specification. As with Mg, organics are difficult to remove entirely without complete dissolution of the mineral. The biological component of the carbonate can pose further issues in that there is the additional risk of immune response. Several of the BCC are sourced from organisms, which would qualify as a requirement for the source to be included on labelling such as hen's eggs and oyster shell [38]. The allergy issue is difficult to address for waste valorisation processes due to the lack of clarity regarding acceptable levels for different allergens [39]. Clearly, consumer safety and awareness must come first and for industrial plants, processing allergenic co-products such as avian and marine shells, declaration of the appropriate allergy labelling for any products would be vital.

Finally, it is worth noting while GCCs are generally considered acceptable to different faiths and lifestyles such as vegetarianism and veganism, the case is more complicated for BCCs. BCC originating from molluscs would pose concerns for certain faiths and vegetarians, *G. gallus* eggshell carbonates may be considered acceptable, however all practical sources of BCC would be unacceptable for vegans.

Clearly the source of the material and processing practices contribute to determine the compliance of a $CaCO_3$ with a given regulatory standard and suitability for a given application be it foods, supplements or pharmaceutical products. While the justifications for using BCC remain compelling, [7,8,13] there are important considerations for prospective processes aiming to use BCC as the raw material. The first consideration for processing is an efficient means for the removal of non-calcium alkali earth metal carbonates that are intrinsic to BCC's [33–36]. Second, efficient removal of residual organic matter from BCC, further work on determining safe levels and processing methods for removal/inactivation of potentially immunologically active contaminants such as protein appears essential [39]. All of the above must be achieved using methods which do not increase the heavy metal burden of the resulting product.
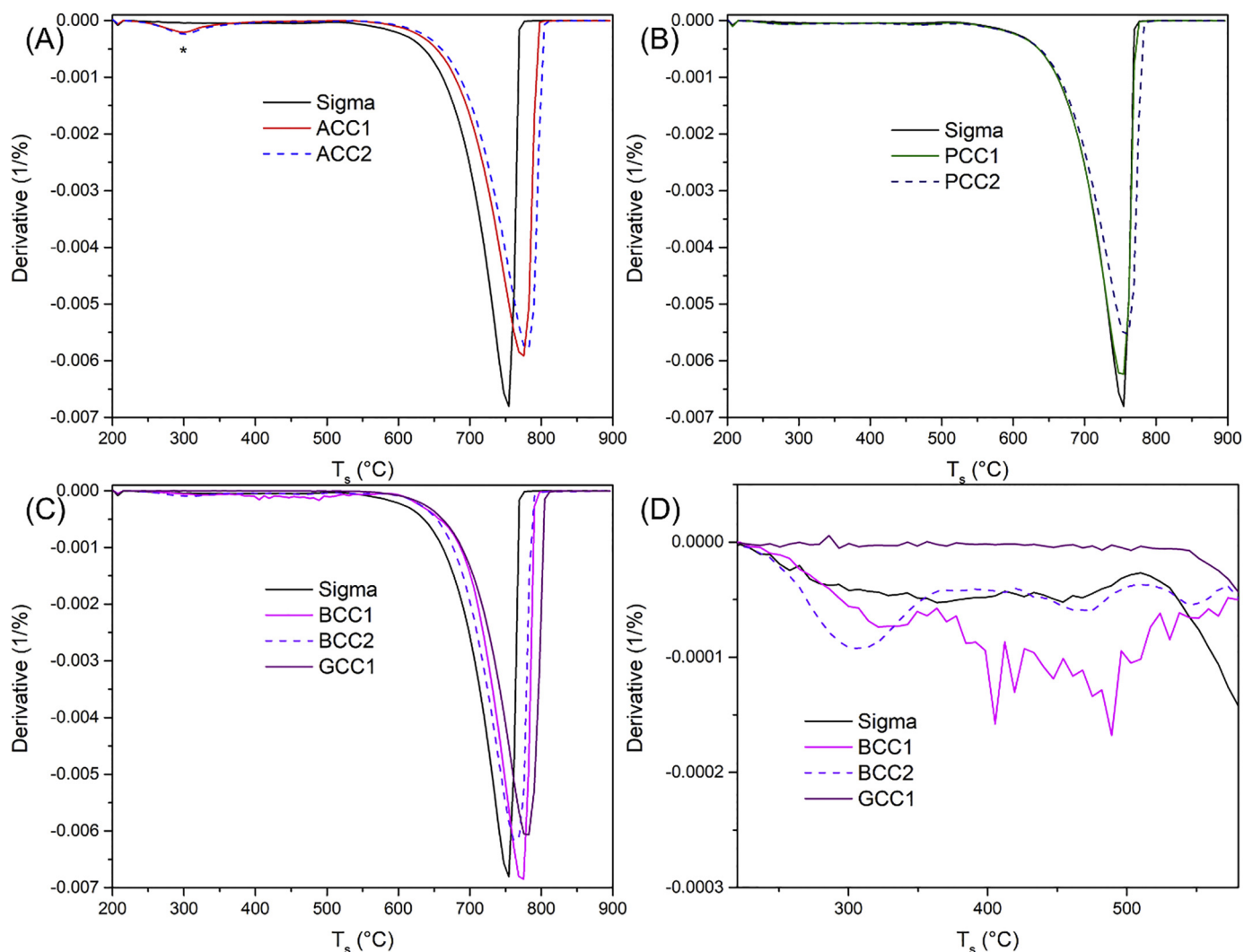
Fig. 3. Representative 1st order derivatives of thermograms taken for (A) agglomerated (ACC) and an E170 standard (Sigma), (B) precipitated (PCC), (C) biogenic (BCC) and above shore (GCC) carbonate samples. For all samples, the principal event is the calcination of $CaCO_3$ to CaO and CO_2. The region below 600 °C of the profile where the combustion of organic components begins in (C) is shown in detail (D).

5. Conclusions

Biogenic $CaCO_3$ presents several chemical challenges to adoption by the pharmaceutical, food and supplement markets, specifically concerning magnesium content, the potential of residual organic matrix but also allergenicity, ethics and depending on the source, an issue of ecological sustainability. As feedstock for precipitated $CaCO_3$, biological $CaCO_3$ are no different to traditional $CaCO_3$ sources. The principal question for the market is if the advantages of biogenic $CaCO_3$, specifically concerning their renewable and sustainable sourcing is worth the additional processing cost required for harmony with existing regulatory specifications. For regulatory bodies, the question of modification of regulatory limits concerning magnesium and organic content, in addition to overall purity to ease access for biogenic, more environmentally sustainable $CaCO_3$ would be possible without compromising consumer safety.

Conflict of interest statement

The authors declare that an aim of the research funding acknowledged was to study the potential of obtaining $CaCO_3$ from eggshells at a grade suitable for the food, pharmaceutical & supplement sectors. This work was performed with a commercial partner with which the authors

institution have a shared intellectual property agreement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nfs.2019.05.002.

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