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Fate of Hydroxyapatite Nano particles during *In Vitro* Gastrointestinal Digestion

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Abstract

There is an increasing change in population demographics towards an aging population in the world, which had led to the availability of various commercial nutritionally supplemented products. Hydroxyapatite (HA), with chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is an insoluble calcium salt used for calcium supplementation because of its similarity to the minerals found in human bone and teeth. The insoluble calcium salts are preferred over the soluble ones because of their high heat stability during milk processing under high heat treatment. However, the drawback of insoluble calcium salts is the tendency to sediment during storage resulting in unfavourable gritty texture. Thus, reduction in particle sizes into micron to nano-size improves the dispersion of these insoluble salts. However, the application of nano-sized particles in food products have raised concerns from both the regulatory organizations and consumers on the implications related to both the environmental and health safety aspects. Thus, the objective of the study is to determine the digestion behaviour of nano-sized needle/rod shaped HA (nHA) when added into skim milk during *in vitro* gastrointestinal digestion. Determination of calcium such as soluble and ionic calcium was conducted to determine the dissolution of nHA. The structural changes and the crystallographic changes of nHA were determined using electron microscopy and x-ray diffraction techniques. The results of *in vitro* gastric digestion showed presence of undissolved nHA particles even after 240 min of gastric and 120 min of intestinal digestion when examined under TEM, while the XRD analysis detected the presence of crystalline nHA in the first 120 min of gastric digestion. Thus, the possible mechanisms leading to the incomplete dissolution of nHA under acidic conditions of the stomach are discussed subsequently.

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List of figures

Figure 2.1 Application of nanotechnology in food.	6
Figure 2.2 Mechanisms of NMs uptake in the GI tract adapted from (Yada et al., 2014).	12
Figure 2.3 Crystalline structure of Hydroxyapatite adapted from (Rivera-Munoz, 2011).	17
Figure 2.4 The structure of HA showing the columnar Ca and screw Ca forming a triangular tunnel adapted from (Sakae et al., 2015).	17
Figure 2.5 Solubility isotherm calculation of Calcium phosphate at 37°C, 0.15 M NaCl adapted from (Ehrlich et al., 2009).....	28
Figure 2.6 Schematic representation of tripototic equilibrium in a sytem adapted form (Lynn & Bonfield, 2005).....	29
Figure 2.7 Different models adapted from (Ehrlich et al., 2009).	30
Figure 2.8 Adsorbed layer of calcium ions of the HA surface. The calcium ions are assumed as a hard disk of radius $2L_D$ from (Mafe et al., 1992).....	31
Figure 2.9 Mineral equilibrium in milk representing the serum and micellar phase (Gaucheron, 2005).	38
Figure 2.10 (a) Casein submicelle model (HA thesis) (b) Casein nano cluster model from (Holt, 1992) (c) Dual binding model from (Horne, 1998) (d) Casein micelle structure with calcium phosphate nanoclusters (grey) with attached caseins (red) and κ -casein (green) on the surface. In the interior of the micelle the “hydrophobically bound” is the mobile β -casein (blue) inside the water channels adapted from (Dalglish, 2011).	41
Figure 2.11 Overview of digestion process.	52
Figure 2.12 Structured clot formation during gastric digestion; top row clot obtained from unheated milk and bottom row clot obtained from heated milk from (Ye et al., 2016b).....	61
Figure 3.1 Schematic representation of sequence of gastrointestinal digestion.....	66
Figure 3.2 Sample preparation for TEM analysis.	69

Figure 4.1 pH profile (mean \pm SD) as a function of gastric digestion time for different digesta samples.....	74
Figure 4.2 Ionic calcium concentration (mM) in the three different gastric digesta samples.....	75
Figure 4.3 Soluble calcium concentration of nHA _{milk} and milk _{blank}	77
Figure 4.4 Ionic calcium vs. soluble calcium at each digestion sampling time for nHA _{milk} digesta samples.....	78
Figure 4.5 Dissolution (%) of nHA _{milk} as a function of digestion time in SGF.....	80
Figure 4.6 TEM micrographs of reference nHA powder (1% suspension solution) @ 105kx magnification.	82
Figure 4.7: TEM micrographs of nHA _{blank} digesta at different digestion times, red arrows indicate the presence of undissolved nHA @26500x magnification.	84
Figure 4.8: TEM micrographs of nHA _{blank} digesta at different digestion times, demonstrates the presence of undissolved nHA @105Kx magnification.	85
Figure 4.9: TEM micrographs of nHA _{milk} digesta at different digestion times, red circles and arrows indicate the presence of undissolved nHA @26500x.	86
Figure 4.10: TEM micrographs of nHA _{milk} digesta at different digestion times, demonstrates the presence of undissolved nHA @105Kx magnification.	87
Figure 4.11 TEM micrographs showing dissociation of nHA aggregates marked with red circles (A) nHA _{blank} digesta at 60 min and 120 min of digestion time and (B) nHA _{milk} digesta at 60 min and 120 min of digestion time @105kx magnification.	88
Figure 4.12 TEM micrographs after 120 min of intestinal digestion (A) nHA _{blank} and (B) nHA _{milk} represented by (i) Red circles represent nHA particles @ 26500x magnification and (ii) 105kx magnification.	89
Figure 4.13: XRD pattern from pellets collected from nHA _{blank} at different gastrointestinal digestion times.....	91

Figure 4.14: XRD pattern from pellets collected from nHA _{milk} at different gastrointestinal digestion times.....	92
Figure 4.15: XRD pattern from pellets in milk _{blank} at different gastrointestinal digestion times.....	93
Figure 4.16 Schematic representation of dissolution of nHA by the action of acid during gastric digestion, leading to critical size effect.	99
Figure 4.17 Schematic diagram showing the formation of calcium rich layer on nHA surface during gastric digestion inhibiting nHA dissolution.....	99
Figure 4.18 Schematic diagram showing binding of milk proteins on nHA surface and entrapment of nHA inside the clot.	104

List of tables

Table 2.1 Analytical techniques available for the characterization of NMs and its relevance to nano toxicity.	8
Table 2.2 Summary of different HA production techniques.....	23
Table 2.3 Mineral composition of milk adapted from (Lucey & Horne, 2009). ...	35
Table 2.4: Salt partition in cow's milk from (Gaucheron, 2005).....	36
Table 2.5 shows the RDA of calcium as developed by Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies.	49
Table 3.1 Skim milk composition as derived from the packaging label.	63
Table 3.2 Digesta sample sampling points for different measurements.....	67
Table 4.1 Ionic and soluble calcium concentration after 120 min of intestinal digestion.....	79
Table 4.3: EDS results of freeze-dried digesta pellet from nHA _{milk} samples at different gastric digestion times.....	94

List of abbreviations

°C	Degree(s) Celsius
%	Percent
CCP	Colloidal calcium phosphate
CaP	Calcium phosphate
EDS	Energy dispersive spectroscopy
g	gram(s)
GI	Gastrointestinal
h	hour(s)
HA	Hydroxyapatite
HGS	Human gastric simulator
L	Litre(s)
nHA	Nano hydroxyapatite particles
nHA blank	nHA _{blank}
nHA in milk	nHA _{milk}
mg	Milligram(s)
min	Minute(s)
ml	Millilitre(s)
mM	Millimolar (mmol.L ⁻¹)
mmol	Millimole(s)
mol	Mole(s)
Milk blank	Milk _{blank}
nm	Nanometre(s)
NMs	Nano materials
NPs	Nano particles
RDA	Recommended dietary allowance

SBF	Simulated biological fluid
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SM	Skim milk
TEM	Transmission electron microscopy
WP	Whey protein
WPI	Whey protein isolate
XRD	X-ray diffraction

List of appendices

Appendix 1 Calculation for addition of nHA in skim milk

Appendix 2 Determination of dissolution (%) of nHA in nHA_{blank}.

Appendix 3 Determination of dissolution (%) of nHA in nHA_{milk}.

Table of contents

Abstract	i
Acknowledgement	ii
List of figures	iii
List of tables	vi
List of abbreviations	vii
List of appendices	ix
Table of contents	x
Chapter 1 Introduction.....	1
Chapter 2 Literature review.....	3
2.1 Nanotechnology	3
2.1.1 Nanotechnology in food	4
2.1.2 Challenges and implications of nanotechnology	6
2.1.2.1 Characterization and detection of nanomaterial	7
2.1.2.2 Exposure routes of nanomaterial	10
2.1.2.3 Nano toxicity.....	12
2.2 Hydroxyapatite (HA).....	16
2.2.1 Production and physiochemical properties of hydroxyapatite nanoparticles (nHA)	18
2.2.2 Applications of HA	24
2.2.3 Crystal growth and dissolution/solubility of HA.....	24
2.3 Milk calcium and its significance.....	34
2.3.1 Calcium in milk.....	34
2.3.2 Significance of calcium during processing.....	44
2.3.3 Calcium fortification in milk	46
2.4 Gastrointestinal digestion	51
2.4.1 Physiology of digestive tract.....	51
2.4.2 Digestion Models	55
2.4.3 Studies on milk <i>in vitro</i> digestion	59
2.4.4 Correlation between <i>in vivo</i> and <i>in vitro</i> digestion	61
2.5 Conclusion.....	62
Chapter 3 Materials and methods	63
3.1 Materials	63

3.2 Digestion sample preparation.....	64
3.3. Simulated gastric and intestinal preparation	64
3.3.1 Simulated gastric fluid (SGF).....	64
3.3.2 Simulated intestinal fluid (SIF).....	65
3.4 <i>In vitro</i> gastrointestinal digestion	65
3.4.1 Sampling procedure and sample preparation.....	66
3.5 pH measurement.....	68
3.6 Characterization and identification of HA in digesta.....	68
3.6.1 Electron microscopy	68
3.6.2 X ray diffraction (XRD)	69
3.6.3 Energy dispersive spectroscopy (EDS).....	70
3.7 Calcium analysis.....	71
3.7.1 Ionic Calcium	71
3.7.2 Soluble calcium.....	71
3.8 nHA dissolution (%) calculation	72
3.8.1 Dissolution (%) of nHA in nHA _{blank}	72
3.8.2 Dissolution (%) of nHA in nHA _{milk}	72
3.9 Statistical analysis.....	73
Chapter 4 Results and discussion.....	74
4.1 Gastrointestinal digestion results	74
4.1.1 pH profile	74
4.1.2 Calcium results	75
4.1.3 Determination of the dissolution rate of nHA.....	80
4.1.4 Detection and identification of undissolved nHA during gastrointestinal digestion.....	81
4.2 Discussion	95
4.2.1 Dissolution of nHA in nHA _{blank}	95
4.2.2 Dissolution of nHA in nHA _{milk}	100
4.2.4 Detection and identification of nHA in gastro intestinal digesta	105
4.2.5 Intestinal digestion.....	106
Chapter 5 Conclusion.....	107
5.1 Recommendations and future research	109
References	109
Appendices	129
Appendix 1:	129

Appendix 2:	130
Appendix 3:	131