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

A thesis presented in partial fulfilment of the requirements for the degree of

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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 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 <sub>blank</sub>           |
| nHA in milk | nHA <sub>milk</sub>            |
| mg          | Milligram(s)                   |
| min         | Minute(s)                      |
| ml          | Millilitre(s)                  |
| mM          | Millimolar (mmol.L-1)          |
| mmol        | Millimole(s)                   |
| mol         | Mole(s)                        |
| Milk blank  | Milk <sub>blank</sub>          |
| 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                |

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**Appendix 2** Determination of dissolution (%) of nHA in nHA<sub>blank</sub>.

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