Novel whey protein isolate nanocarriers for oral micronutrient delivery

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Novel whey protein isolate nanocarriers for oral micronutrient delivery

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Introduction

Iron deficiency is the most prevalent nutritional disorders worldwide (WHO).

Ferrous sulphate (FeSO4) is the most common iron supplement/fortificant, however, it causes gastrointestinal (GI) side effects and has a poor sensory profile. Encapsulation approaches are used to overcome this, but poor absorption is a limitation.

Milk proteins are generally inexpensive, with an established safety and biocompatibility profile and are widely used in the food industry for their nutritional and functional properties.

Whey proteins isolate (WPI) is obtained by processing and filtration of milk whey (Fig. 1.). It has a high protein content (97.5%) and is virtually lactose, carbohydrate and fat free.

WPI is readily available and biocompatible, and also has physiochemical properties such as self assembly. These characteristics make it a promising material for encapsulation and formulation of bioactive compounds.

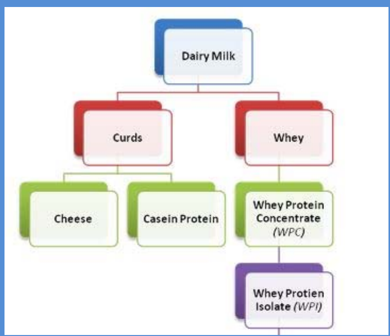

Fig. 1. Separation of dairy milk into protein fractions

We describe for the first time preparation and characterisation of novel WPI composite nanocarriers for oral iron formulation and delivery.

Methods

WPI nanocarriers encapsulating FeSO4 (WPI-NC) were prepared using cold homogenisation method. The mucoadhesive polysaccharide chitosan (CHI) was added to the aqueous phase to prepare chitosan coated nanocarriers (WPI-CHI-NC).

Nanocarrier physiochemical characteristics were assessed by particle size, zeta potential and morphological analysis. Iron uptake from formulations was compared by caco-2 cell uptake experiments using simulated GI fluid, with intracellular ferritin protein as a measure of iron absorption and pure FeSO4 as reference.

Potential toxic effects of nanocarrier formulations on caco-2 cells were assessed by carrying out the colourimetric MTT assay incubating caco-2 cell monolayers with formulations diluted at a final iron concentrations of 20, 50 and 100 µM (and equivalent volumes of corresponding blank iron-free nanocarriers).

FeSO4 was used as a reference standard for quantitaive iron absorption experiments. Equivalent amounts of iron (20 µM) from each formulation was added to caco-2 cells cultured in six-well plates (n = 6 per sample) for 2 hours, the estimated physiological transit time through the duodenum, and cells harvested after 24 hours.

Intracellular ferritin concentration was determined by ELISA. Ferritin concentrations were then standardised against total protein concentration and ng ferritin/mg protein considered an indice of iron absorption in Caco-2 cells. Data is presented as mean ± SEM and difference between samples was analysed by ANOVA followed by Tukey's post-hoc test using the PRISM software package.

Results

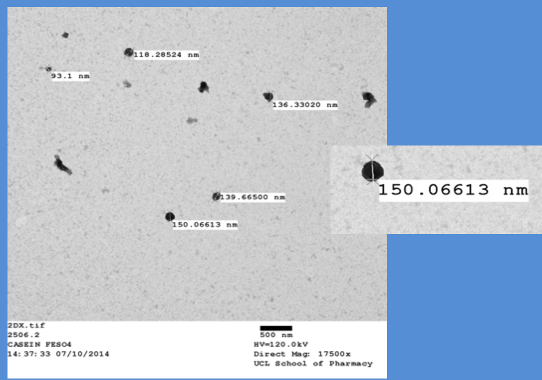

Fig. 2. Particle size and morphological analysis was carried out by scanning electron microscopy as well as using a Malvern Zetasizer. All formulations were found to be within a submicron size range (113.76 ± 7 nm – 125.92 ± 12 nm) favourable for intestinal permeability. SEM analysis was in agreement with Zetasizer measurements, with nanocarrier particle size observed to be of nanoscale dimensions.

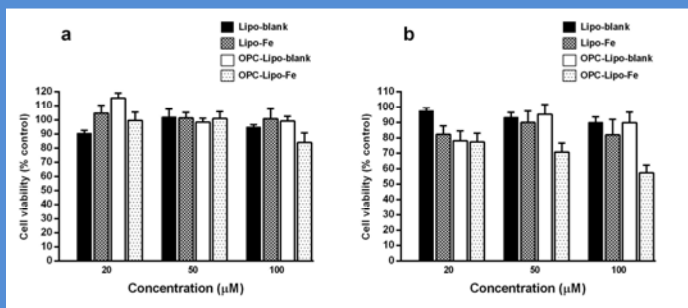

Fig. 3. Caco-2 cell viability as assessed by MTT assay following 48 hour (a) and 72 hour (b) incubation with nanocarrier formulations containing increasing drug concentrations (n=6). Results demonstrate that the nanocarrier formulations did not exert any significant toxic effects upon caco-2 cell viability.

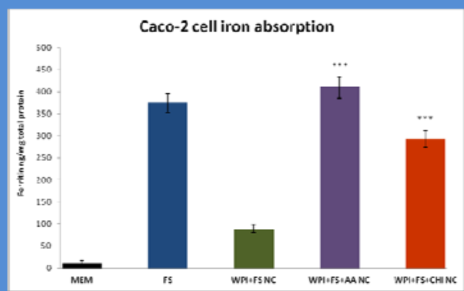

Fig. 4. Quantitative iron uptake from the nanocarrier formulations was compared by carrying out caco-2 cell uptake experiments with intracellular ferritin protein as a measure of iron absorption. Caco-2 iron absorption from WPI-NC (88.96 ± 8.9 ng/mg) was only 23% of FeSO4 control (374.61 ± 22.03 ng/mg). However, chitosan inclusion significantly increased cellular iron absorption, with WPI-CHI-NC iron absorption (410.1ng/mg ± 24.77) 365.9 % higher than WPI-NC and 9.6 % higher than FeSO4 control ($P \leq 0.05$).

Summary

- **Low level iron absorption from WPI-NC composed of WPI alone may potentially be attributed to poor nanocarrier integrity in the GI microenvironment.**
- **Chitosan inclusion leads to formation of robust hybrid protein-polysaccharide nanocarriers possessing greater membrane permeability resulting in high cellular iron delivery.**
- **Our results demonstrate the potential of WPI as a novel biomaterial for formulation of nanocarriers for micronutrient delivery.**

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