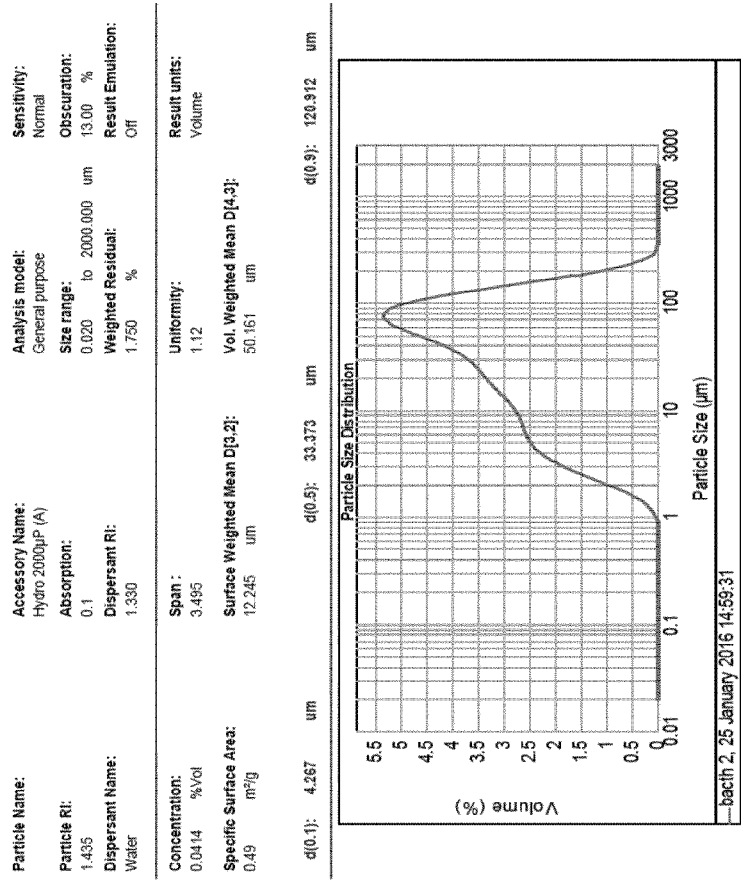
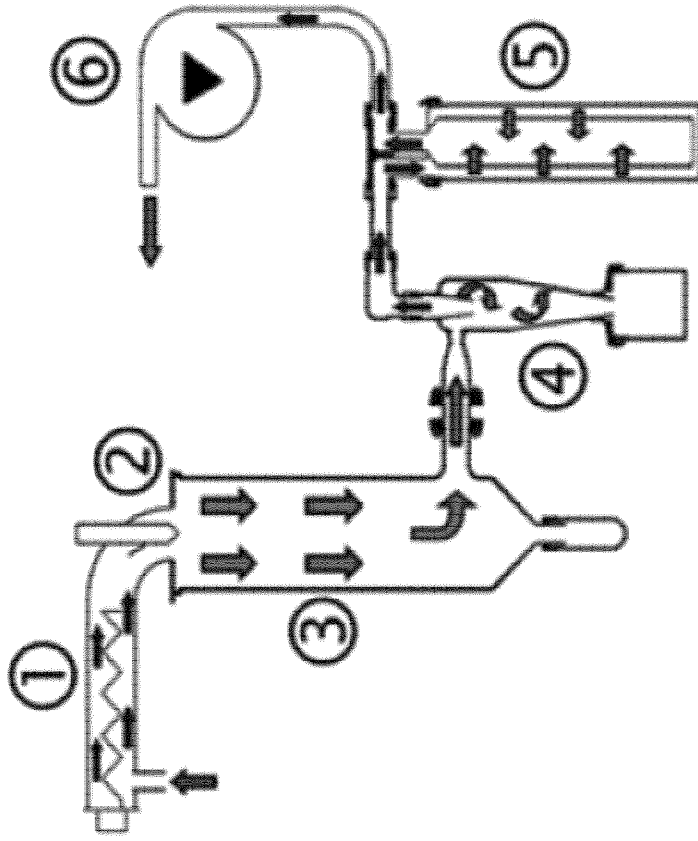


Figure 1. Particle Size Profile of Gel Microbeads



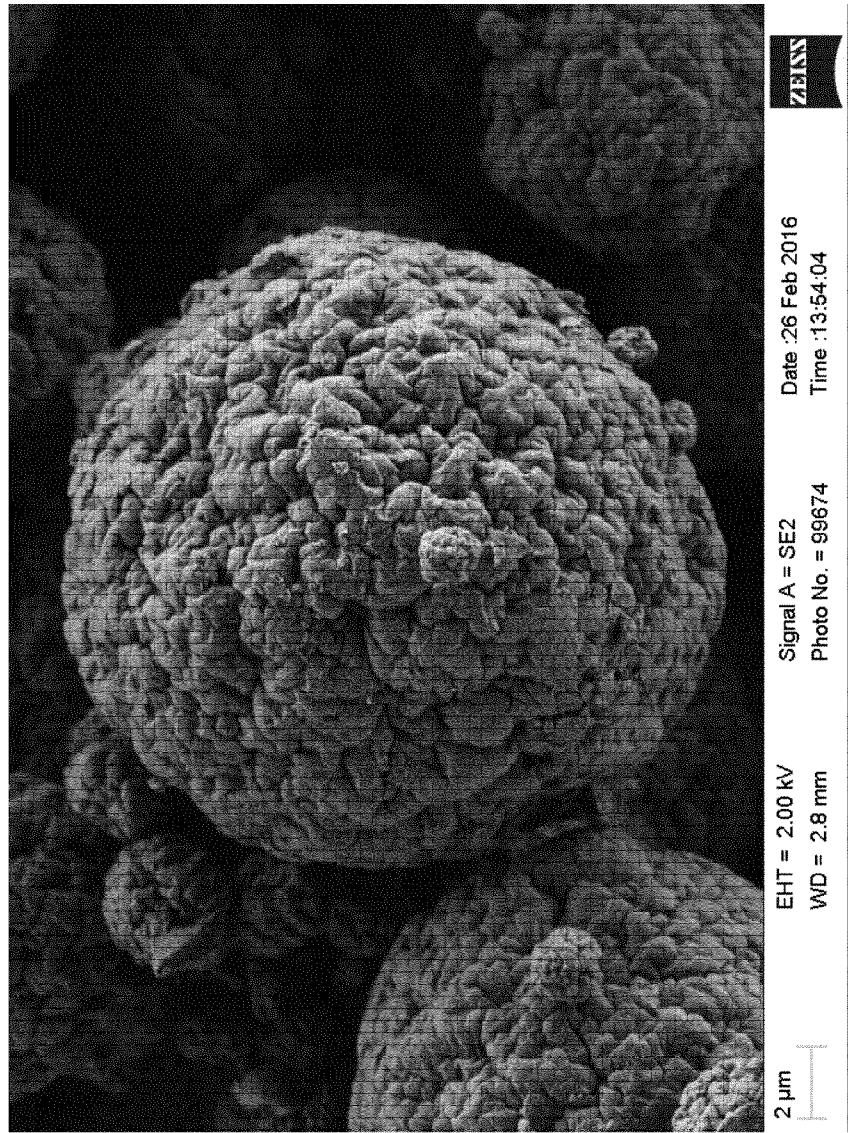
The curing solution (500mM Ferrous Sulfate, 500mM to 5M Sodium Acetate) was placed into and IKA LR-1000 and heated to 40°C. Approximately 500 ml of denatured whey protein solution (10.5%) was added over a 30 second period. Following addition of the whey protein solution and gel formation, the curing solution was agitated with a Turrax rotary-stirrer at 15,000 rpm for 2 minutes and the solution was cured for 60 minutes at low agitation speed (stirrer 100 RPM). The wet particles formed again were very small (Particle Size Results from Malvern shown) showing a bimodal pattern with D50 of 33 microns before tray drying at 80°C..

Figure 2. Schematic of the typical spray drying setup



First the protein solution (up to 10.5%) is prepared as described above, and mixed with the curing solution as described below forming a gel. The gel is subjected to a particle size reduction process using shear forces (e.g. rotar stator or blades) to achieve the desired particle size of gel particle. The gel suspension is then gently agitated for at least 30 minutes before being pumped to a spray drier. In [1], the suspension is heated to between 140°C and 160°C. [2] Then the droplet is formed using a two-fluid nozzle in the Buchi B-290. [3] conductive heat exchange occurs between the drying gas and sample droplets. This removes the excess fluid from the mother liquid and also the fluid within the gel bead. [4] particles are collected using cyclone technology. [5] in the outlet filter there is collection of the finest particles. [6] The drying gas is delivered by aspirator. Schematic from www.buchi.com/products/b-290

Figure 3. Scanning Electron Microscopy of Microbeads



A low magnification SEM showing an image of microbeads of the composition prepared by spray drying. The scanning electron microscopy (SEM) images were recorded on a Zeiss Ultra Plus Field Emission SEM with a Gemini® column (Zeiss). The dry sample beads were placed on a conducting carbon tape without any further preparation or sample coating. Accelerating voltages between 2-3kV was used to overcome the extensive discharge effect

Figure 4. Immediate Release Profile of iron in ST1501

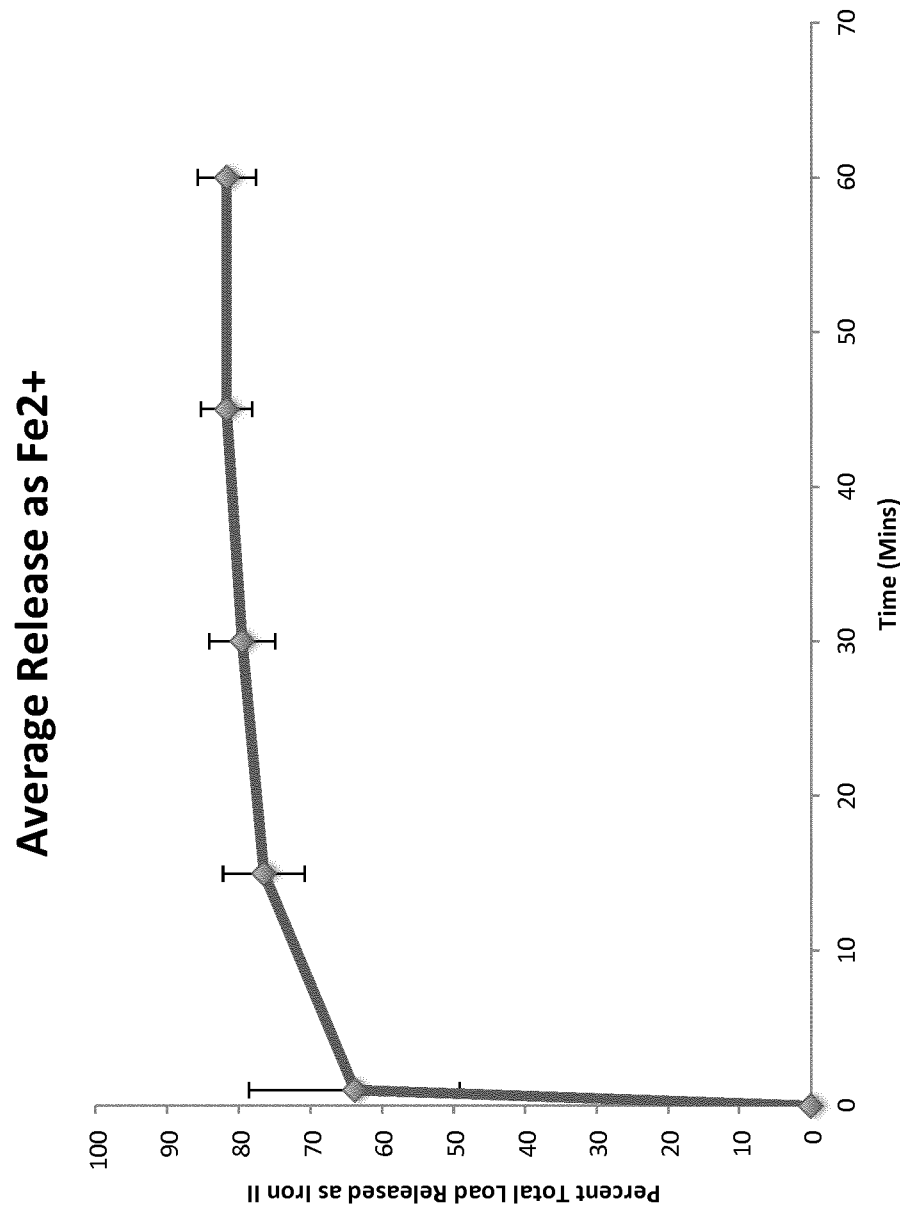


Figure 5. pH profile of compositions of the invention in simulated gastric and intestinal fluid (ST1501, Ferrous Sulfate)

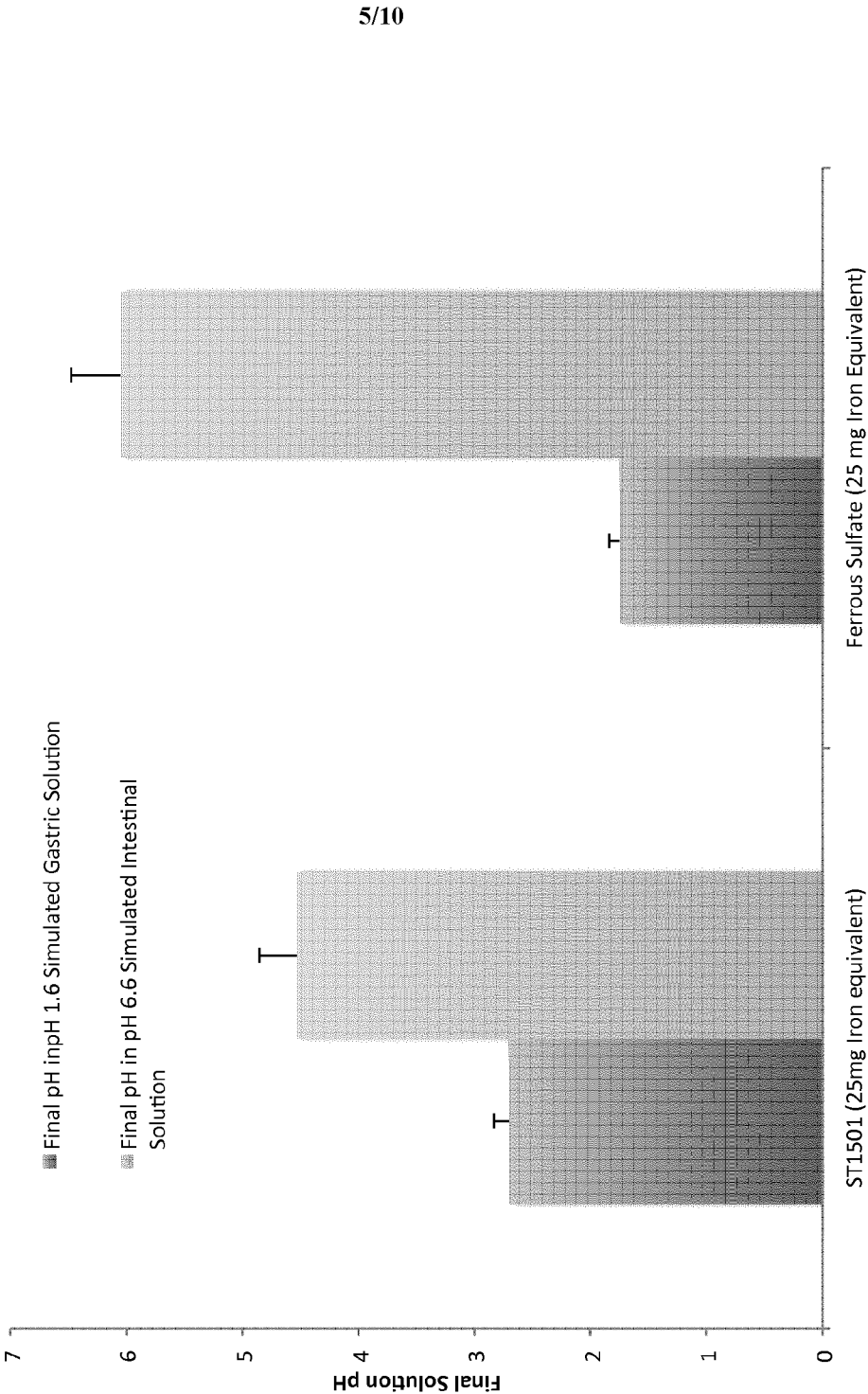


Figure 6 – Serum Iron Trough To Peak Ratio over 2 hours in fasting subjects

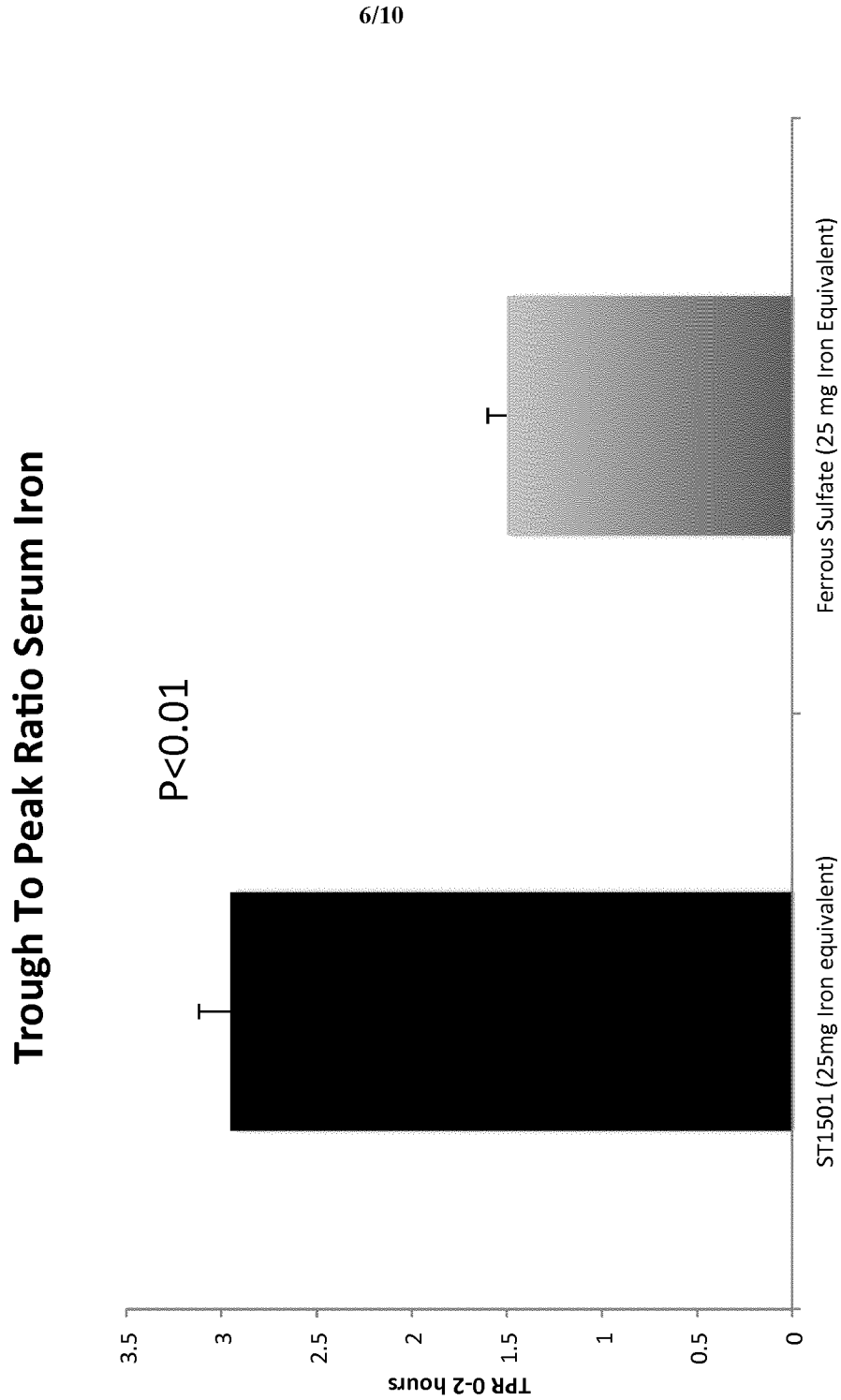
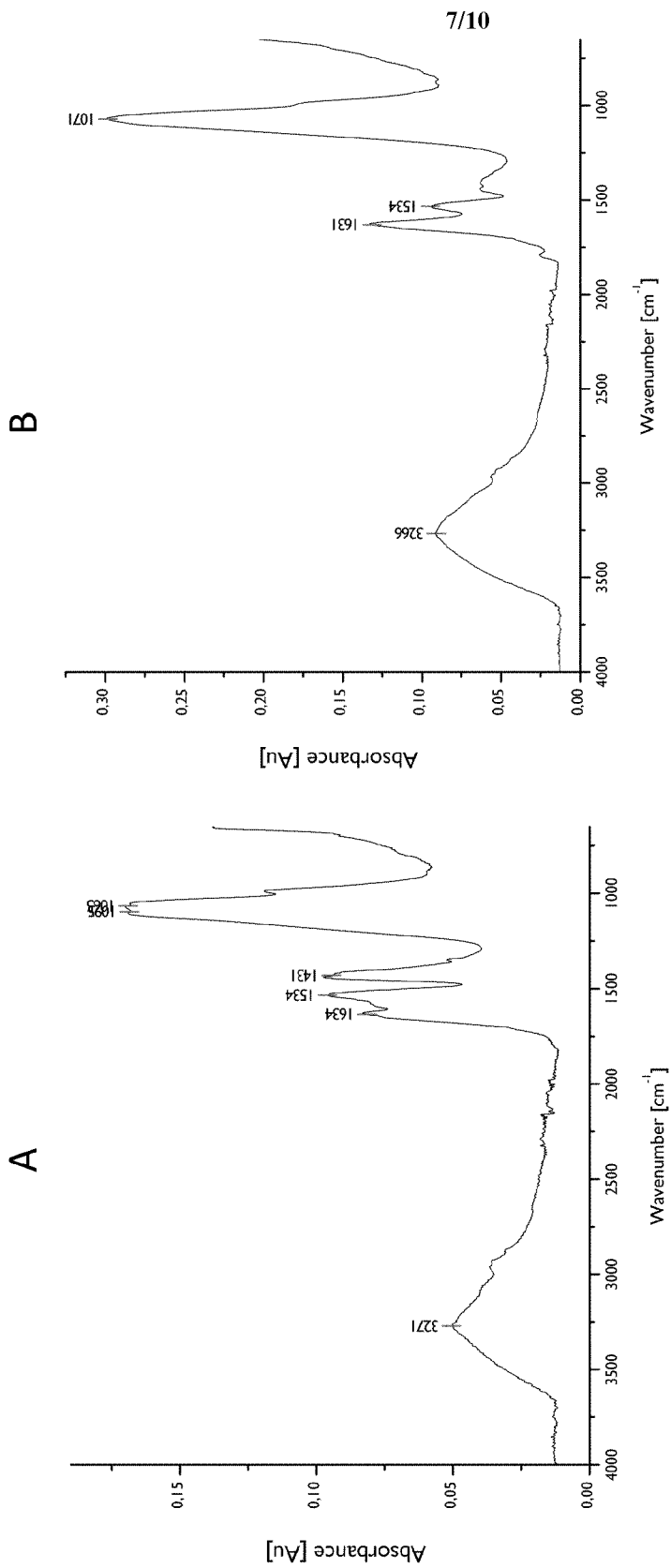
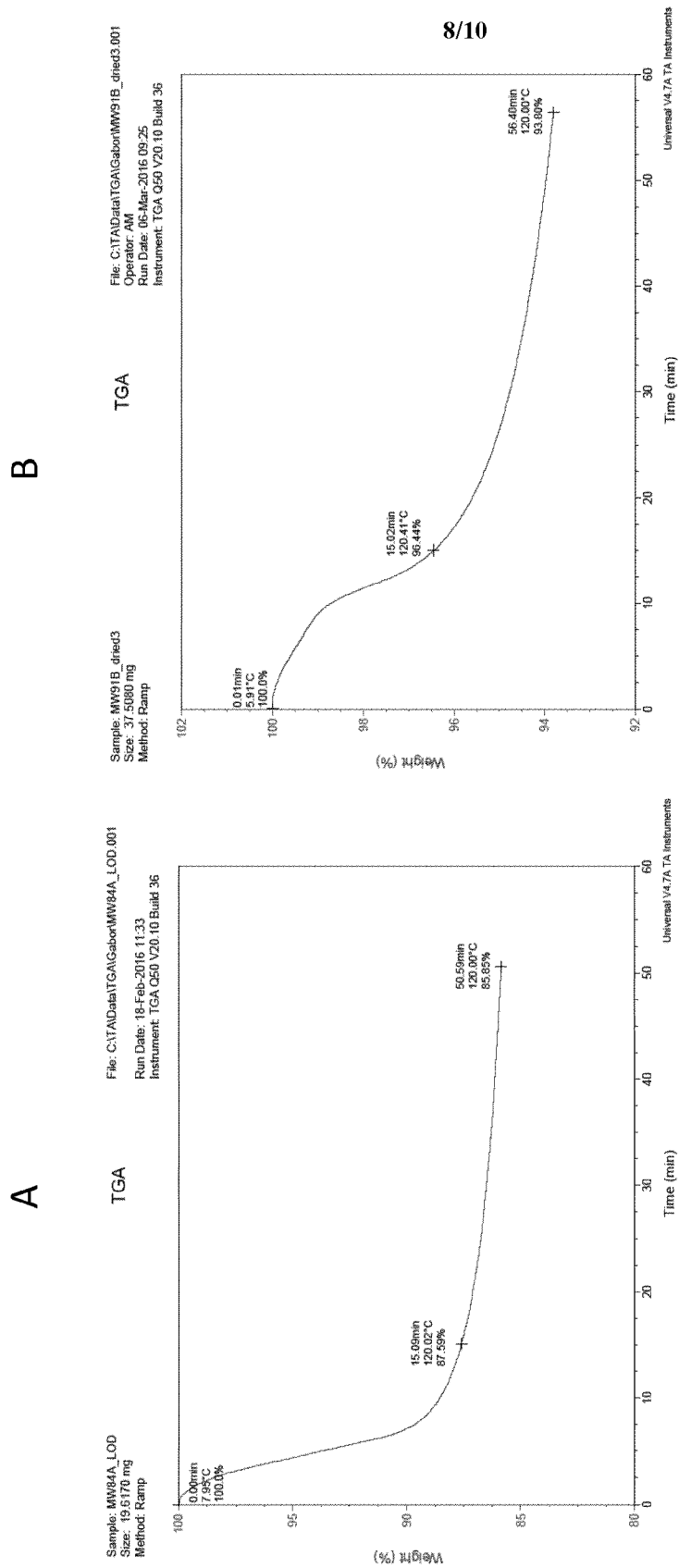


Figure 7 FTIR of microbeads of the composition



FTIRs showing the characteristic presence of characteristic sodium acetate peaks in the microbead of the composition in the region 1560-1410 cm⁻¹ compared to denatured whey protein. Figure 6B depicts FTIR showing the reduced sodium acetate peaks in the composition in the region 1560-1410 cm⁻¹ reflecting a reduced (<3% w/w) sodium acetate composition, a reduced acetate:iron ratio and a reduced acetate:protein ratio.

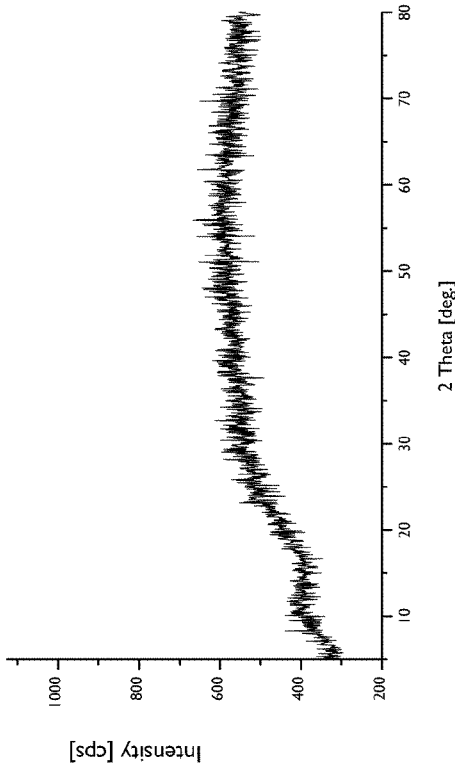
Figure 8 TGA analysis of microbeads of the composition



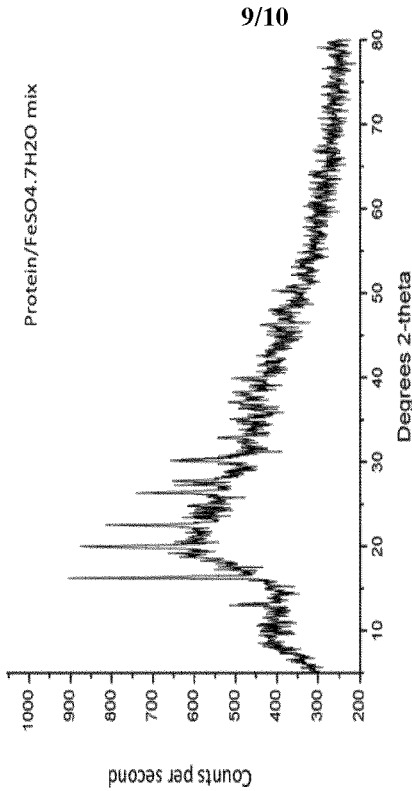
Illustrative thermogravimetric analysis (TGA) of loss on drying of microbeads of the invention following spray drying alone (A) and following spray drying with further drying at 80°C. Weighed, powdered samples (10–15 mg) were analysed in open ceramic pans. For the TGA measurement a TA-Instruments Thermogravimetric Analyzer TGA-Q50 instrument was used with the following temperature program: sample heated to 120°C (10°C/min) and 45 min isothermic at 120°C.

Figure 9 pXRD analysis of microbeads of the composition

A



B



Powder XRD analysis showing a largely amorphous nature of the compositions of the invention [A]. There are no typical PXRD peaks present which are associated with crystalline Iron(II) sulfate. The broad baseline peaks however reflect low level order in the protein structure. Powder XRD pattern [B] depicts the profile of denatured whey protein physically mixed with ferrous sulfate heptahydrate showing evidence of crystallinity.

Figure 10 – Serum Iron Trough To Peak Ratio over 2 hours in fasting subjects

