Supporting Information

Amorphous calcium carbonate based-microparticles for peptide pulmonary delivery

Frederic Tewes[1, 2]*, *Oliviero L. Gobbo*[1], *Carsten Ehrhardt*[1] *and Anne Marie Healy*[1*]

1: School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin 2, Ireland

2: INSERM U 1070, Pôle Biologie-Santé, Université de Poitiers, Faculté de Médecine & Pharmacie, 1 rue Georges Bonnet, 86022 Poitiers Cedex, France

E-mail: ftewes@univ-poitiers.fr; healyam@tcd.ie

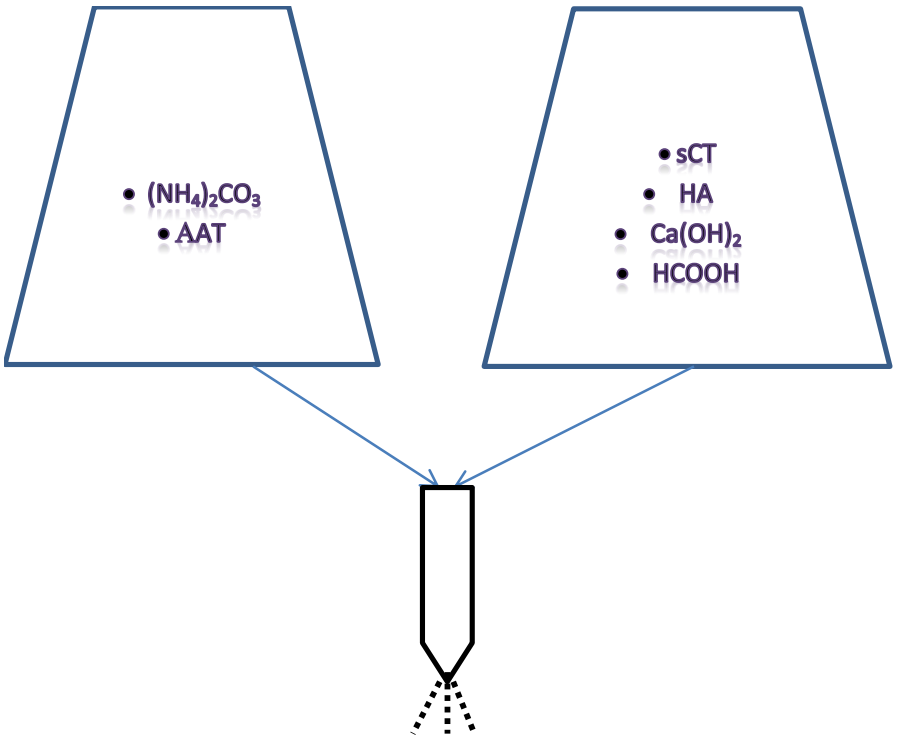

Figure S1: Spray drying process scheme.*Spray drying*: The microparticles were manufactured in a one-step procedure using a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland). Two solutions were prepared separately and mixed during the process in a Y shape tubing connector fed to the spray dryer. One solution was composed of salmon calcitonin acetate salt (sCT) (0, 0.2 or 0.4 g L^{-1}, Polypeptide Laboratories, Sweden) $Ca(OH)_2$ (0.8 g L^{-1}), hyaluronic acid sodium salt from *Streptococcus equii* (0.1 – 0.6 g L^{-1}, Sigma-Aldrich, Dublin, Ireland) and 0.1% v/v of formic acid. The other solution was composed of ammonium carbonate ($(NH_4)_2CO_3$)(1.2 – 6 g L^{-1}) and alpha 1-antitrypsin (AAT) (0 or 0.2 g L^{-1}). sCT and AAT were in separate solutions as their mixing induced the formation of a precipitate. The spray dryer was operated in an open-cycle suctionmode as follows: A 2-fluid nozzle was used to disperse the solution in the same direction as the flow of hot drying air (flowing at 630 L min^{-1}); spraying air nozzle flow rate was 15 L min^{-1}; inlet temperature ranged from 100 to 140 °C; feeding peristaltic pump was set at 30 % for the 2 solutions. These conditions resulted in an outlet temperature ranging from 47 to 70 °C.

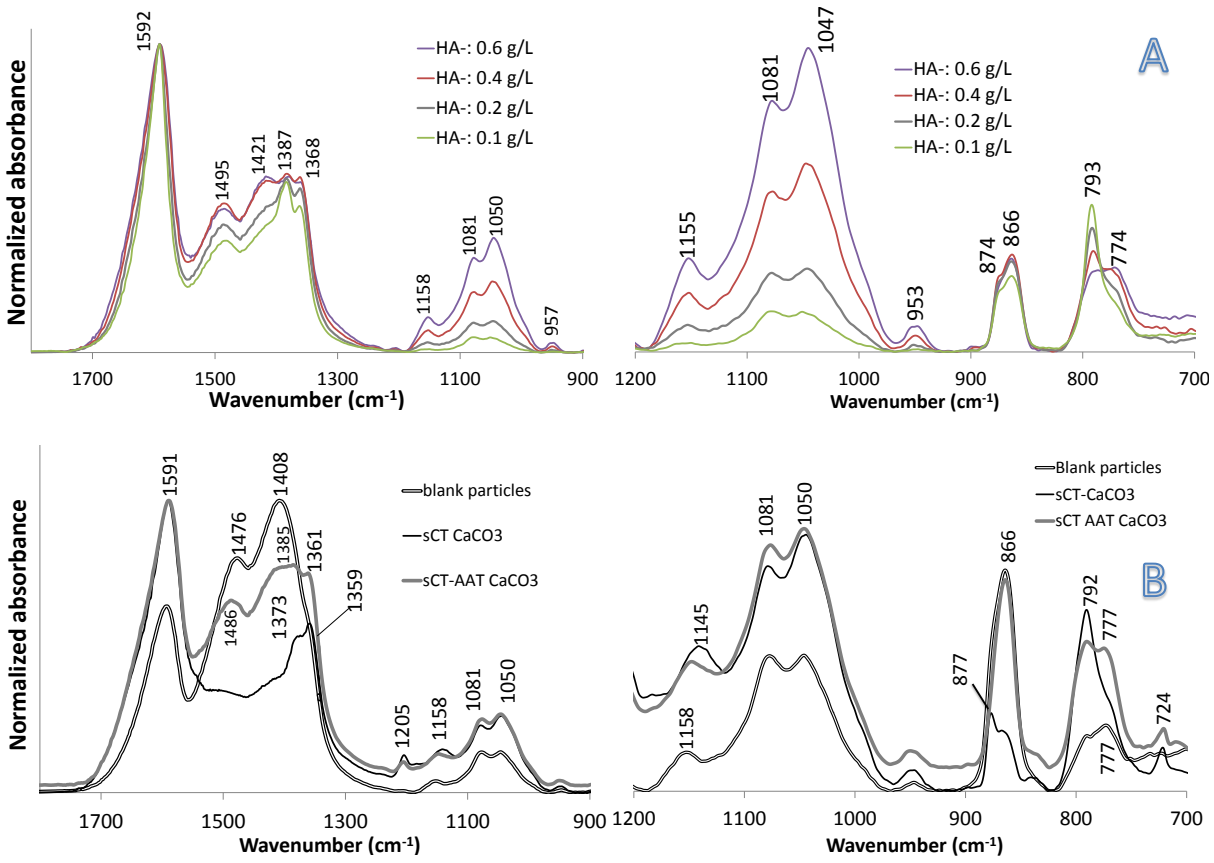

Figure S2: FTIR spectra of Ca/HA composite microparticlesA) Formulated at different concentrations of HA, B) Loaded with sCT and AAT.

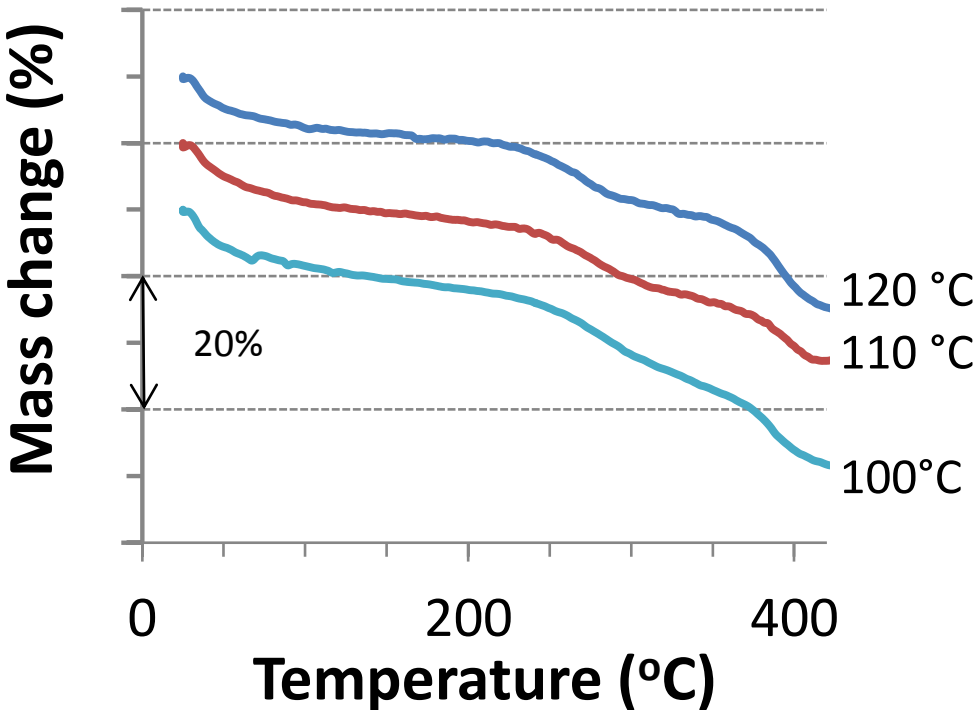

Figure S3: TGA thermograms of Ca/HA composite particles formulated at different spray drying inlet temperatures (T_{inlet}).Thermogravimetric Analysis (TGA) was performed using a Mettler TG 50 (Mettler Toledo, Greifensee, Switzerland). Samples were loaded into 40 µL aluminium open pans and run at a heating rate of 10 ºC min^{-1} from 25 – 420 ºC.

Thermogravimetric analysis of the particles formulated at various temperatures showed a 3-step decrease in mass with the increase in temperature on each thermogram. The first step of 12 wt.% occurred at a temperature in the TGA lower than 200 °C and is assumed tobe related to water release from ACC. The second step of 10 wt.% had an onset temperature of 230 °Cand maybe due to the decomposition of HA. The third step of 10 wt.% had an onset temperature at 360-380 °C and corresponds to the melting/decomposition temperature of calcium formate.

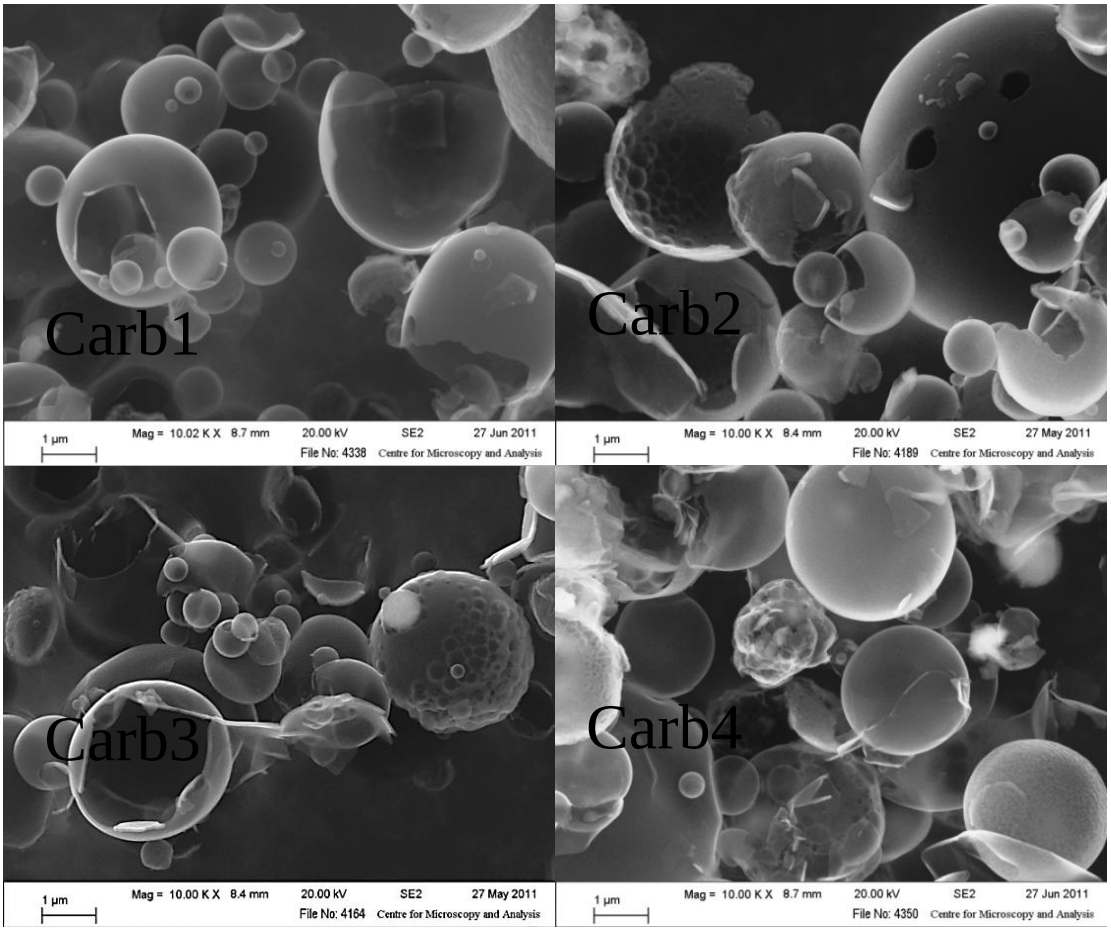

Figure S4: SEM micrographs of composite particles formulated withdifferent concentrations of$(NH_4)_2CO_3$. Carb1 (1.2 g L^{-1}), Carb2 (2.4 g L^{-1}), Carb3 (4.8 g L^{-1}), Carb4 (6 g L^{-1}).

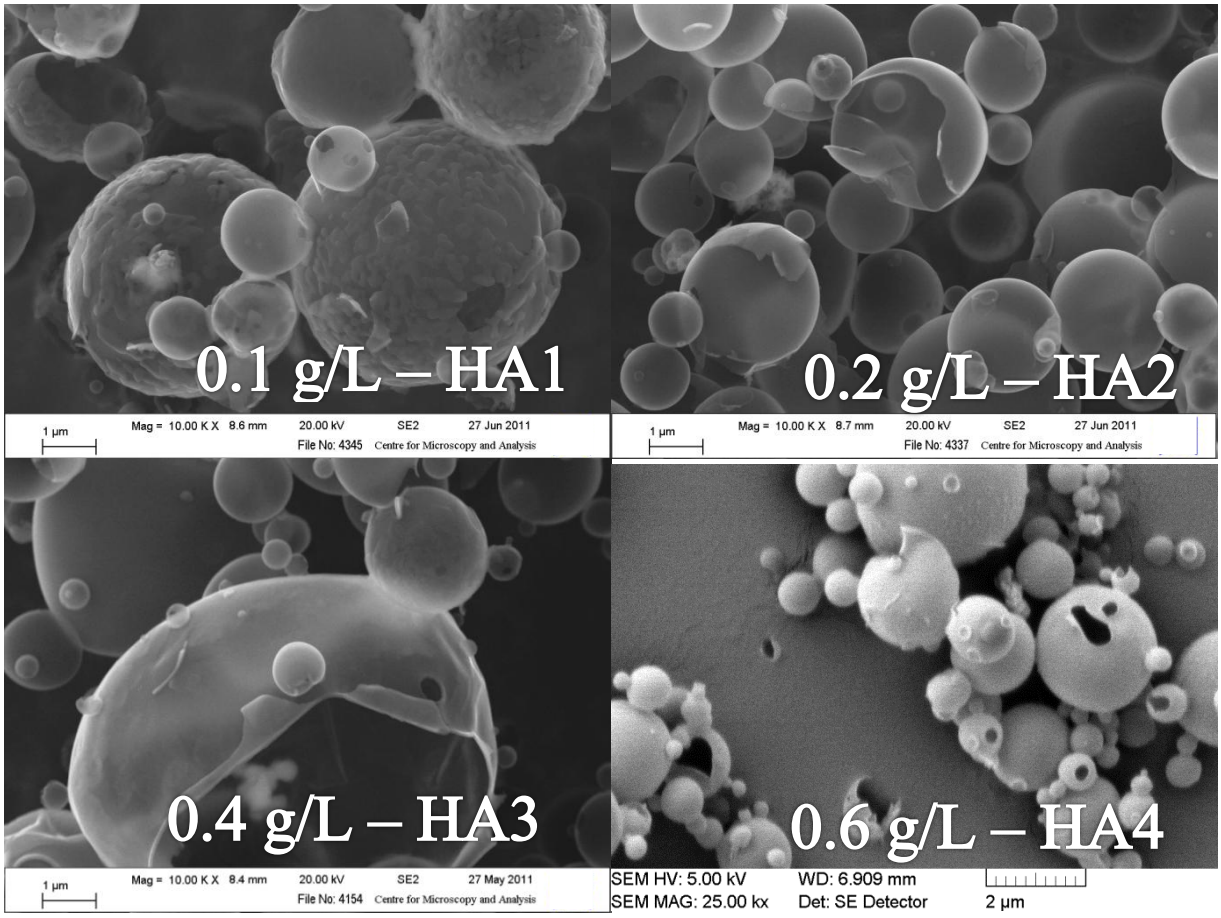

Figure S5: SEM micrographs of Ca/HA composite particles formulated withdifferent concentrationsofHA.

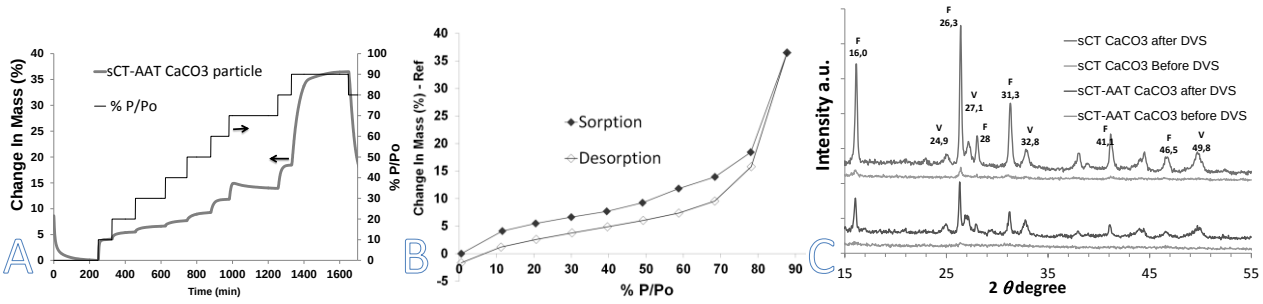

Figure S6: A) sCT-AAT Ca/HA composite particle water sorption versus time profiles obtained at different % relative humidity, B) sCT-AAT Ca/HA particle water sorption-desorption isotherm, C) XRD patterns of sCT-AAT and sCT Ca/HA particles after DVS experiments.

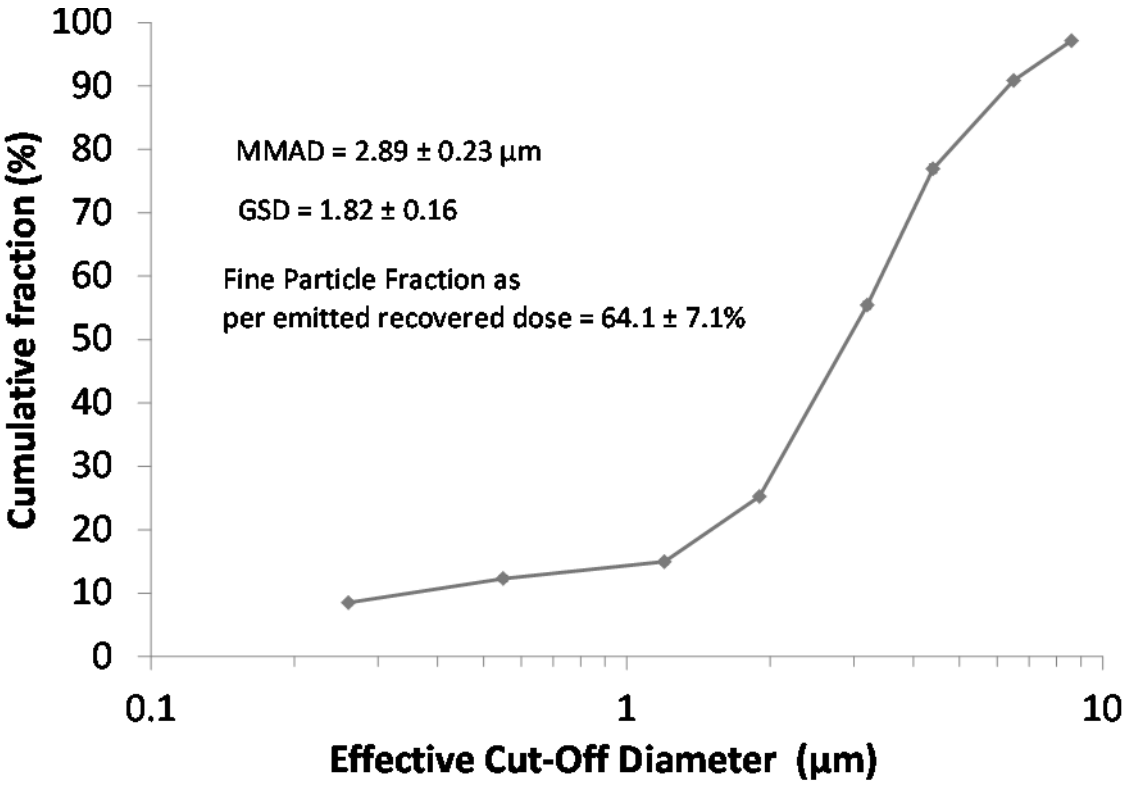

Figure S7: sCT-AAT-loaded microparticles'aerodynamic diameter size distribution.

The aerodynamic diameter (AD) distribution of the particles was measured using an Andersen cascade impactor (ACI). The flow rate was adjusted to 60 L min^{-1} in the powder inhaler (Handihaler®, BoehringerIngelheim) and the time of aspiration was adjusted to obtain 4 L. The inhaler was filled with gelatin n°3 capsule loaded with 20 ± 2 mg of powder (n = 3). After inhaler actuation, particle deposition on the ACI was determined by calcium assay. The amount of particles with AD≤ 5.0 µm, expressed as a percentage of the emitted recovered dose, was considered as the fine particle fraction (FPF). The mass median aerodynamic diameter (MMAD) and FPF were calculated as previously described. (Tewes, F.; Gobbo, O. L.; Amaro, M. I.; Tajber, L.; Corrigan, O. I.; Ehrhardt, C.; Healy, A. M., Evaluation of HPβCD-PEG microparticles for salmon calcitonin administration via pulmonary delivery. **Molecular Pharmaceutics** 2011, 8 (5), 1887-1898)