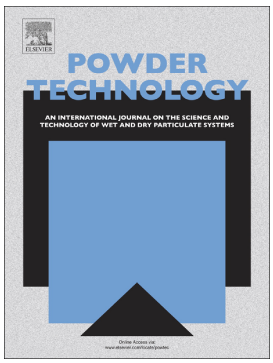
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Water sorption and hydration properties of high protein milk powders are influenced by enzymatic crosslinking and calcium chelation

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Abstract

Calcium chelating agents, such as sodium hexametaphosphate (SHMP), can be added to milk protein (MPC) solutions to aid in rehydration; however, this leads to a concomitant increase in solution viscosity due to micelle swelling/dissociation. Crosslinking casein proteins in MPC using transglutaminase (TGase) can help retain casein micelle structure and maintain low viscosity. This study aimed to determine the water sorption and hydration properties of MPC powders, as influenced by the crosslinking of milk proteins and the addition of SHMP. Crosslinked casein protein (TG-MPC) powders without SHMP addition had improved wettability, water sorption and water diffusion compared to the non-crosslinked control (C-MPC) powder. All powders containing SHMP were found to have increased water sorption compared to control powders without SHMP addition. Powder dissolution data showed that increasing SHMP content increased powder particle size, compared to powders without SHMP addition, indicating increased powder particle swelling due to increased water uptake.

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1. Introduction

Milk protein concentrate (MPC) powders have numerous commercial applications and are used as ingredients in value added dairy products, such as in high protein yogurts, medical nutrition, infant milk formula, dairy-based beverages and muscle building products [1, 2, 3, 4]. MPC is manufactured from skim milk through ultrafiltration using membranes with a molecular weight cut-off of 5 to 10 kDa. Additional water is often added during filtration (i.e., diafiltration) to further remove lactose and minerals, allowing for even higher protein to solids ratios to be achieved. Liquid MPC is usually then heat treated, evaporated and spray dried. Spray dried MPC powders (i.e., >60% w/w, protein) have been shown to have challenges with rehydration with poor wetting and hydration properties making them difficult to incorporate in to finished products [5, 6].

Insolubility of freshly produced MPC powders can be mainly attributed to non-covalent hydrophobic interactions between proteins, due to the exposure of hydrophobic amino acids during the drying process [7]. Casein proteins are highly hydrophobic and are able to assemble through the formation of weak hydrophobic forces and calcium binding, with a number of models proposed for the structure of the casein micelle [8, 9, 10, 11]. Several methods have been employed to improve solubility of MPC powders, with a number of these methods targeting casein micelle integrity through the depletion of calcium from the serum, and consequently, colloidal phases [2, 12]. Casein micelle integrity is dependent on the equilibrium between serum and colloidal minerals, as well as attractive (e.g., hydrophobic interactions) and repulsive (e.g., electrostatic repulsion) forces between proteins resulting in the dissociation of casein micelles and allowing water uptake and subsequent hydration [11, 13, 14, 15]. The addition of calcium chelating salts to milk protein systems changes this electrostatic balance by binding calcium from the serum phase and depletion of calcium from

colloidal calcium phosphate (CCP) nanoclusters by diffusion, depending on the electrostatic strength of the calcium chelator [16, 17, 18, 19].

Aside from powder insolubility, one of the major challenges associated with MPC production is the high viscosity incurred at relatively low solids content (i.e., 20-30%, w/w), limiting the achievable dry matter prior to spray drying, compared to skim milk concentrates which are dried at solids contents of ~50%, w/w, [20, 21, 22]. Increases in MPC viscosity can be attributed to increases in protein-protein interactions during heat treatment and the innate high water holding capacity of casein micelles (~3.3 g g^{-1} of protein) [23]. In addition, calcium chelation can cause further increases in viscosity depending on the strength of the chelator, resulting in dissociation of the casein micelle into multiple protein clusters and reduced turbidity [2, 16, 24, 25]. Therefore, in order to combat high viscosity in MPC systems a previous study by Power et al. [26] crosslinked milk proteins using the enzyme transglutaminase in an attempt to control viscosity during calcium chelation. Transglutaminase creates isopeptide bonds *via* an acyl transfer reaction between the carboxyl group of glutamine residues and the ε-amino group of lysine residues, forming intra-micellar crosslinks [27, 28, 29, 30]. However, the combined effects of crosslinking milk proteins and the addition of SHMP on the subsequent water sorption and hydration properties of MPC powders are unknown. Thus, the objective of this study was to determine if the addition of SHMP could improve powder rehydration properties and if protein crosslinking (to control viscosity) would affect these attributes.

2. Materials and methods

2.1. Materials

Milk protein concentrate (MPC) powder was obtained from a local dairy ingredient manufacturer. The protein, moisture, fat and ash content of this MPC was 81.4% (w/w),

4.30% (w/w), 1.40% (w/w) and 7.8% (w/w), respectively. The lactose content was determined by difference as 5.1% (w/w). Transglutaminase (Activa MP, Ajinomoto enzyme, Germany) was obtained from Healy Group (2nd Avenue, Cookstown Industrial Estate, Tallaght, Co. Dublin). Sodium hexametaphosphate (SHMP) was obtained from Sigma Aldrich limited (product number 68915-31-1).

2.2. Pilot-scale production of milk protein concentrate powders with enzymatic crosslinking and calcium chelator addition

Milk protein concentrate powder was rehydrated (10%, w/w, total solids) at 50°C using a Y-Tron ZC-1 high shear mixer (YTRON, Process Technology GmbH & Co. KG, Handwerkerpark 21, Germany). Following rehydration, the MPC dispersion was cooled to 30°C and divided into a control batch and enzyme modified batch (batch size 16 Kg). Protein crosslinking was carried out by incubating the MPC dispersion with the TGase enzyme (0.5 g/L) at 30°C and stored for 24 h under gentle agitation to allow sufficient crosslinking of casein proteins to occur (TG-MPC) [21]. The control batch (C-MPC) was also treated under the same incubation conditions but without the addition of TGase. Sodium azide (0.02% w/w) was added to prevent microbial growth in both batches. Following incubation, each batch was sub-divided and combined with SHMP to give final chelator concentrations of 0, 2.5, 5, 10 or 25 mM. All MPC dispersions were then stirred for 30 min before being heated to 50°C prior to drying using a single-stage spray dryer equipped with a two fluid nozzle. Inlet and outlet air temperatures were set at 187 and 83°C, respectively. All powders were stored for analysis in aluminium foil bags.

2.3. Particle size measurement of milk protein concentrate powders

The particle size distribution (volume in %) of C-MPC and TG-MPC powders were determined using a Malvern Morphologi G3 (Malvern Instruments, Malvern, UK) image analysis-based particle characterisation system [32].

2.4. Scanning electron microscopy of milk protein concentrate powders

C-MPC and TG-MPC powder samples were attached to double-sided adhesive carbon tabs and mounted on scanning electron microscope (SEM) stubs, after which tabs were coated with chromium (K550X, Emitech, Ashford, UK). Scanning electron microscopy images were collected using a Zeiss Supra 40P field emission SEM (Carl Zeiss SMT Ltd., Cambridge, UK) at 2.00 kV. Representative micrographs were taken at 200×, 500×, 1000×, 5000× and 10000× magnification.

2.5. Capillary rise of water in milk protein concentrate powders

Wettability was determined for C-MPC and TG-MPC powders using a modified version of the Washburn capillary rise method, as described previously by Ji, Cronin, Fitzpatrick, Fenelon and Miao [33] and Ji, Cronin, Fitzpatrick, Maguire, Zhang and Miao [34]. Powders were loaded into a glass cylindrical tube with the bottom covered by a filter. The tube was then held at the surface of a body of distilled water at 20°C and wettability was calculated based on the increase in mass of the powder when weighed after 10 min of absorption. All measurements were repeated five times to insure repeatability.

2.6. Determination of the initial water content of milk protein concentrate powders

The water content of spray dried powders was determined by weighing ~1.0 g of each powder and placing them in a Jeio Tech vacuum oven (Jeio Tech®, Seoul, Korea) at 70°C and

at an absolute pressure of <10 mbar for 24 h. The difference in mass of samples before and after drying (g/100g of dry solids) was defined as the initial water content (IWC).

2.7. Water sorption analysis of milk protein concentrate powders

Water sorption analysis was carried out on vacuum dried C-MPC and TG-MPC powders after storage in desiccators over phosphorous pentoxide (P_2O_5). Each powder was stored in an evacuated desiccator (21°C) for 120 h over saturated solutions of lithium chloride (LiCl), potassium acetate (CH_3COOK), magnesium chloride ($MgCl_2$), potassium carbonate (K_2CO_3), magnesium nitrate ($Mg(NO_3)_2$), sodium nitrite ($NaNO_2$), sodium chloride (NaCl) and potassium chloride (KCl) (Sigma Chemical Co., St. Louise, MO. U.S.A.), which at equilibrium provided water activities (a_w) of 0.11, 0.23, 0.33, 0.44, 0.545, 0.66, 0.76 and 0.85, respectively. Water activity of each powder was measured after storage using a Novasina water activity meter (Labmaster-a_w, Novatron, London, UK). Powders were weighed at intervals of 0, 2, 4, 6, 8, 10, 24, 48, 72 h and then every 24 h up to 240 h [35]. The water content in each system was plotted as a function of time, and the Guggenheim-Anderson-de Boer (GAB) relationship was fitted to data to relate water activity and water content of anhydrous powders (Eq. 1):

$$\frac{m}{m_0} = \frac{Cka_w}{(1- ka_w)(1 - ka_w + Cka_w)} \quad (1)$$

Where m is the water content (g of water/100 g of dry solids), m_0 - the monolayer value of water content, C, k - constants related to energy constant, which can be calculated from m_0.

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2.8. Confocal laser scanning microscopy of milk protein concentrate powders

A Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica Microsystems CMS GmbH, Wetzlar, Germany) was employed for powder particle visualisation. The DPSS 561 nm laser was engaged. C-MPC and TG-MPC powder particles were placed onto a glass slide and labelled using rhodamine dye (0.1%, Rhodamine B; Sigma-Aldrich Co., St. Louis, MO. USA.) in ultrapure water. The confocal images of each system were taken using 20x air and 63x oil immersion objective with numerical aperture of 1.4. The areas of particles were measured using Image Pro Premier 3D software and z-Stacks were obtained in order to generate a 3-D structure of selected particles. Green pseudo-coloured pictures (8-bit), 512x512 pixels in size, were acquired using a zoom factor of 1-3 [36, 37].

2.9. Real time visualisation of liquid phase water diffusion in milk protein concentrate powders

Real time visualisation of liquid phase water diffusion in C-MPC and TG-MPC powders was carried out in accordance with the method described by Maidannyk, Lutjes, Montgomery, McCarthy and Auty [37]. Rhodamine B and PEG 200 were added to anhydrous C-MPC and TG-MPC powders. Images were obtained at fixed time intervals using a Leica TCS SP5 CLSM and particle diameters were detected using the Leica TCS SP5 software in the size range 3-200 μm. The areas of individual powder particles were measured using spherical approximation which allowed for the local effective diffusivity of the liquid phase in individual powder particles to be calculated.

2.10. Bulk diffusion coefficients for milk protein concentrate powders

The effective bulk diffusivity of C-MPC and TG-MPC powders was determined experimentally from water kinetic profiles as per the method outlined by Maidannyk, Lutjes,

Montgomery, McCarthy and Auty [37]. It was assumed that water sorption kinetics are limited only by diffusion. The solution of Fick's second law equation for one-dimensional slab can be obtained as shown in Eq. 2 [38].

$$\Gamma = \frac{(\mathrm{M}-\mathrm{Me})}{(\mathrm{Mo}-\mathrm{Me})} = \frac{8}{(\Pi)^2}\sum_{n=0}^{\infty}(2n+1)^{-2}\exp\frac{Deff(2n+1)^2\,(\Pi)^2 t}{4L^2} \quad (2)$$

Where M_e is equilibrium moisture content, M_o is initial moisture content, M is moisture content at time t and L is thickness of the slab. This equation assumes that the initial moisture content is uniform and that surface moisture and density are constant. The thickness of the sample volume (L) was measured using a numeric micrometer (5 times) and was assumed to be constant for each powder. Eq. 3 has been used to determine the effective or apparent diffusion [39].

$$Deff = -\left(\frac{4L^2}{\Pi^2}\right) * Slope \quad (3)$$

The slope was determined from a plot of LnΓ versus time. When a break in this plot was observed, diffusion coefficients were determined for each part of the curve [40, 41].

2.11. Temperature dependant dissolution of milk protein concentrate powders

Solubility characteristics of C-MPC and TG-MPC powders were analysed using a laser-light diffraction unit (Malvern Mastersizer, Malvern Instruments Ltd, Worcestershire, UK). Dispersions were prepared by adding 7 g of MPC powder to 100 mL of tempered deionised water at 20 or 40°C along with 3 drops of silicon antifoam emulsion. Dispersions were high shear mixed for 30 s using a solubility index mixer (Labinco l295, Labinco B.V, Netherlands). Following mixing, dispersions were transferred to beakers and allowed to rest

for 30 s after which aliquots were added to distilled water re-circulating at 20°C in the dispersion unit (Hydro MV; Malvern Mastersizer) at 1760 rpm. Measurements were performed using particle and dispersant refractive indices of 0.001 and 1.33, respectively. All particle size distribution profiles and mean diameters ($D_{(4,3)}$) presented were carried out in triplicate.

2.12. Data analysis

Water sorption and morphology analyses were performed in triplicate. Confocal microscopy data was obtained using five replicates. Statistical analysis was carried out using one-way analysis of variance (ANOVA), with post-hoc Tukey analysis using SPSS software (SPSS V.18, IBM, New York, US). The level of significance was determined at $P < 0.05$.

3. Results and discussion

3.1. Powder particle size analysis

The powder particle size distribution for C-MPC and TG-MPC are given in Fig. 1. The majority of C-MPC powder particles were in the size range 0-200 µm (Fig. 1A), while the addition of SHMP caused a shift in size distribution towards larger particles with the extent of this shift increasing with increasing SHMP concentration (i.e., 0 to 10 mM)., It is noted that C-MPC dispersions containing 25 mM SHMP developed such high viscosity prior to drying that atomization was not possible and so powder was not produced [42]. TG-MPC without SHMP had a particle size distribution ranging from 0-100 µm (Fig. 1B). TG-MPC powders containing SHMP had a maximum particle size of ~300 µm (Fig. 1B), compared to C-MPC powders which had a maximum particle size well in excess of 300 µm (Fig. 1A).

The increase in powder particle size with increasing SHMP addition level in both C-MPC and TG-MPC may be explained by the increase in viscosity of the MPC feed prior to

spray drying. Power et al. [27] showed that the addition of 5 mM SHMP to MPC systems caused a significant increase in viscosity from 13 to 125 mPa.s. Therefore, if the liquid feed viscosity increases, but the energy supplied to the atomization nozzle remains constant then the atomized droplet size will be greater. The feed viscosity also affects spray pattern with a more viscous liquid typically producing a narrower spray angle. Increased viscosity can also sometimes increase the product flow rate and contribute to the formation of even larger droplets [43]. Murphy, Tobin, Roos and Fenelon [44] found similar results to those presented in the current study, with a significantly larger particle size for infant formula powders produced from a high viscosity concentrate compared to the same system with a low viscosity. Power et al. [26] described the mechanism behind the increased viscosity with increasing SHMP concentration through micelle swelling and dissociation, *via* chelation of calcium in the aqueous phase and potential solubilisation of CCP. Mineral chelation also changes the electrostatic balance in such high protein systems, and therefore impacts protein-protein interactions [2, 26]. SEM images of C-MPC powders showed that increasing the SHMP concentration caused the surface morphology of particles to progressively change from having a shrivelled, collapsed shape to a smoother, spherical particle surface (Supplementary data: Fig. S1A-D). In contrast, TG-MPC powder morphology remained relatively unchanged with increasing SHMP concentration (Supplementary data: Fig. S1E-H).

3.2. Capillary rise and water sorption kinetics

Capillary rise data for C-MPC and TG-MPC powders is shown in Fig. 2. Water absorption in general increased with increasing levels of SHMP in both C-MPC and TG-MPC powders. TG-MPC powders, with and without SHMP, had higher levels of absorbed water than the corresponding C-MPC powders. Water sorbance was almost two fold higher in

C-MPC and TG-MPC powders containing 10 mM SHMP (0.07 and 0.10 g, respectively) compared to C-MPC and TG-MPC powders with no SHMP added (0.04 and 0.05 g, respectively). Ji et al. [34] showed similar results to the current study for non-agglomerated and agglomerated whey protein isolate powders with water absorption levels of 0.05 and 0.07 g, respectively.

Water sorption profiles of C-MPC and TG-MPC powders as a function of relative humidity are shown in Fig. 3A and B, respectively. All powders showed an increase in water content with increasing relative humidity, with no evidence of lactose crystallization occurring, similar to results shown by Hogan and O'Callaghan [45] for MPC powders. Similar to the trend observed for the capillary rise data, all TG-MPC powders were found to contain higher water content than the corresponding control powders at the same relative humidity. At a relative humidity of 85% C-MPC powders containing 10 mM SHMP had a final water content of 21.2 g/100 g compared to 17.3 g/100 g for C-MPC powders without SHMP. While water sorption values for TG-MPC were significantly ($P < 0.05$) higher than C-MPC powders, water content did not significantly increase with increasing SHMP content (Fig. 3B). Water sorption data was modelled using the GAB sorption isotherm model and is shown in Fig. 4. The data showed the increase in water content for TG-MPC powders compared to C-MPC powders, especially at water activity values in the range of 0.1 to 0.5 (Fig. 4A and B). All powders exhibited typical sorption isotherm profiles for non-crystalline high protein powders, with the GAB model having a good fit with the experimental data over the water activity range 0.1 to 0.9.

3.3. Real time visualization of liquid phase water diffusion and effective diffusivity behaviour in milk protein concentrate powders

Diffusion of Rhodamine B dye molecules into the anhydrous powder particles allowed for the visualization of dye penetration into the particle centre, indicative of powder hydration, while preventing particle solubilisation and any major disruption to powder morphology (Supplementary data: Fig. S2). The sorption of water containing fluorescent Rhodamine B dye into, and through, dry powder particles occurred more quickly in TG-MPC powders containing 10 mM SHMP than the corresponding C-MPC powder. Complete dye penetration to the centre of the powder particle occurred after ~65 min for TG-MPC powders but took ~80 min for C-MPC powders (results not shown). From the penetration of dye into the powder particles of known size (i.e., data from Fig. 1A and B) at set time intervals the effective diffusivity of liquid water into individual powder particles could be calculated. The dependence of local diffusion coefficients on particle size is shown in Fig. S3. For both C-MPC (Fig. S3A) and TG-MPC (Supplementary data: Fig. S3B) powders the effective diffusion coefficients increased linearly with increasing particle size. The relationship between dye diffusion and SHMP concentration in the powders is shown in Fig. 5, with a linear relationship established between dye diffusion and SHMP concentration (Fig. 5; R^2 0.89 and 0.98 for C-MPC and TG-MPC powders, respectively). TG-MPC powders had higher effective diffusivity compared to C-MPC powders across all SHMP concentrations (Fig. 6). The increase in water sorption and hydration observed in TG-MPC may be due to a restructuring of the casein molecules in the MPC. Agyare and Damodaran [46] showed that the surface hydrophobicity of whey protein isolate solutions decreased significantly after 30 h of incubation with microbial TGase and reported that the decrease in surface hydrophobicity was due to partial burial or occlusion of hydrophobic cavities or clefts on the protein surface.

3.4. Vapour phase diffusion in milk protein concentrate powders

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The effective moisture (vapour phase) diffusivity of powder was calculated using the method of slopes and the raw data from Fig. 3 [39]. Fig. 6 shows the results of effective diffusivity for C-MPC (Fig. 6A) and TG-MPC (Fig. 6B) powders. All powders showed a trend towards increasing effective diffusivity with increasing SHMP concentration. The effective diffusivity of C-MPC powders containing SHMP were observed to remain relatively constant over the entire a_w range studied (Fig. 6A). TG-MPC powders showed significantly ($P < 0.05$) higher effective diffusivity compared to C-MPC over the entire water activity range studied (Fig. 6). The increased levels of vapour sorption in TG-MPC powders may be due to lower surface hydrophobicity of the casein proteins following TGase treatment. However, studies have shown that the hydrophobicity of proteins after crosslinking using TGase vary depending on the length of time allowed for crosslinking. Liu, Zhang, Liu, Wang, Du and Chen [47] monitored the surface hydrophobicity of wheat gluten during incubation with TGase and showed that the surface hydrophobicity initially increased and then decreased with incubation time. The authors stated that the increase in hydrophobicity may be attributed to the exposure of hydrophobic residues induced by cross-linking and the latter decreases due to the deamidation of gluten mediated by TGase, which led to hydrophilic and ionised carboxylic acid side chains. A previous study by Jaros and Rohm [48] stated that in the absence of primary amines, water molecules can substitute as acyl acceptors causing deamination of glutamine residues and the formation of glutamic acid and ammonia in solution.

3.5. Temperature dependant hydration properties of milk protein concentrate powders

Particle size distribution profiles of C-MPC and TG-MPC powders re-dispersed in water at 20 and 40°C are shown in Fig. 7. Monomodal size distribution profiles were observed for all C-MPC dispersions, with and without SHMP. The size distribution profiles

of C-MPC dispersions at 20°C (Fig. 7A) exhibited increasing particle size with increasing SHMP addition level, with $D_{(4,3)}$ values increasing from 25.1 to 53.8 μm. A similar trend was observed for C-MPC powders dispersed at 40°C with an increase in particles size with increasing SHMP content; however, the $D_{(4,3)}$ values increased from 25.1 to 64.0 μm. This increase in particle size with increasing SHMP concentration could be due to a change in electrostatic interactions within the casein micelle caused by calcium chelation, resulting in micellar swelling and a subsequent increase in powder particle size. McCarthy et al. [17] found that even after 2 h of agitation, MPC dispersions containing SHMP had a bimodal particle size distribution. This is one of the principal characteristics of casein dominant powders which are highly hydrophobic and disperse slowly during the rehydration process, resulting in the irregular dissolution of powder particles.

In Fig. 7C, a bimodal size distribution was observed for TG-MPC powders containing no SHMP dispersed at 20°C, with a primary peak ranging from 4.23 to 119 μm and a smaller secondary peak ranging from 134 to 484 μm. Interestingly, TG-MPC powders containing 5 mM SHMP showed a bimodal size distribution similar to the profile for TG-MPC without SHMP but, with a significantly larger secondary peak (Fig. 7C). However, a monomodal size distribution profile was observed for TG-MPC powders containing 2.5, 10 and 25 mM SHMP, all of which had a larger primary peak than TG-MPC without SHMP. All TG-MPC dispersed at 40°C were monomodal with the exception of the powder containing 5 mM SHMP which remained bimodal but with a small peak ranging from 0.05 to 0.64 μm and a larger peak ranging from 4.89 to 105 μm (Fig. 7D). Particle size $D_{(4,3)}$ values ranged from 29 to 67 μm for TG-MPC dispersions with 0 to 25 mM SHMP (results not shown). In general, the addition of SHMP to liquid MPC prior to drying caused significant changes to water sorption and hydration properties even after only 30 s of high shear mixing.

4. Conclusion

This study has shown that the hydration of milk protein concentrates can be improved by mineral chelation and that measures can be implemented to off-set issues with viscosity during processing. The addition of SHMP improved water sorption and hydration of MPC powders. Crosslinking of casein proteins helped maintain a lower powder particle size during atomization compared to non-crosslinked samples when dried in the presence of SHMP. Crosslinking using TGase also improved powder hydration and water sorption even without SHMP addition, when compared to the corresponding non-crosslinked sample. Overall, crosslinking casein proteins in MPC powders had positive effects on water sorption and hydration in addition to decreasing concentrate viscosity during the drying process.

Future work on the effects of storage conditions on crosslinked protein powders may be of interest, as previous work by Mimouni et al. [49] stated that protein-protein interactions occur between micelles during storage, leading to the formation of a monolayer skin on the surface of powder particles composed of packed closely packed casein micelles which prevents rapid dissolution. However, crosslinking of casein proteins could hinder casein-casein surface interactions during storage, as crosslinked casein molecules may have less mobility to reorganise hydrophobic groups towards the powder particle surface.

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure legends

Fig. 1. Powder particle size distribution for anhydrous control (A) and crosslinked (B) casein protein powders, containing 0 (—), 2.5 (---), 5 (···), 10 (−·−·) and 25 mM (−··−) sodium hexametaphosphate.

Fig. 2. Water absorbance through capillary rise for control (■) and crosslinked (□) milk protein powders containing 0, 2.5, 5, 10 and 25 mM sodium hexametaphosphate.

Fig. 3. Water sorption kinetics for control (A) and crosslinked (B) milk protein concentrate powders at 0 (i), 2.5 (ii), 5 (iii), 10 (iv) and 25 mM (v) sodium hexametaphosphate (SHMP) addition, measured at relative humidity values of 11 (□), 23 (■), 33 (○), 44 (●), 55.5 (Δ), 65 (▲), 76 (◊) and 85% (♦) over 120 h at 21 ± 2°C ,.

Fig. 4. Guggenheim-Anderson-de Boer (GAB) water sorption isotherms for control (A) and crosslinked (B) casein protein concentrate 0 (−), 2.5 (····), 5 (---) and 10 mM (−−) sodium hexametaphosphate addition and experimental data points at 0 (■), 2.5 (♦), 5 (▲) and 10 mM (●), measured at 21 ± 2 °C .

Fig. 5. Effective diffusivity of control (♦) and crosslinked (■) casein protein concentrate powders measured as a function of sodium hexametaphosphate concentration. Symbols are experimental data obtained by confocal laser scanning microscopy (CLSM).

Fig. 6. Effective diffusivity of control (A) and crosslinked (B) milk protein concentrate powders measured as a function of water activity at concentrations of 0 (■), 2.5 (□), 5(■) and 10 (■) mM sodium hexametaphosphate.

Fig. 7. Particle size distribution profiles of control protein powders dispersed at 24 (A) and 40°C (B) and crosslinked milk protein concentrate powders dispersed at 24 (C) and 40°C (D)

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at sodium hexametaphosphate concentrations of 0 (—), 2.5 (···), 5 (---)10 (−·−·) and 25 mM (−··−).

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Highlights

- Calcium chelation improved water sorption properties of milk protein powders
- Transglutaminase enzymatic crosslinking can be used to reduce dispersion viscosity
- Calcium chelation increased powder particle size
- Crosslinking improved powder rehydration and water sorption properties

Graphical abstract

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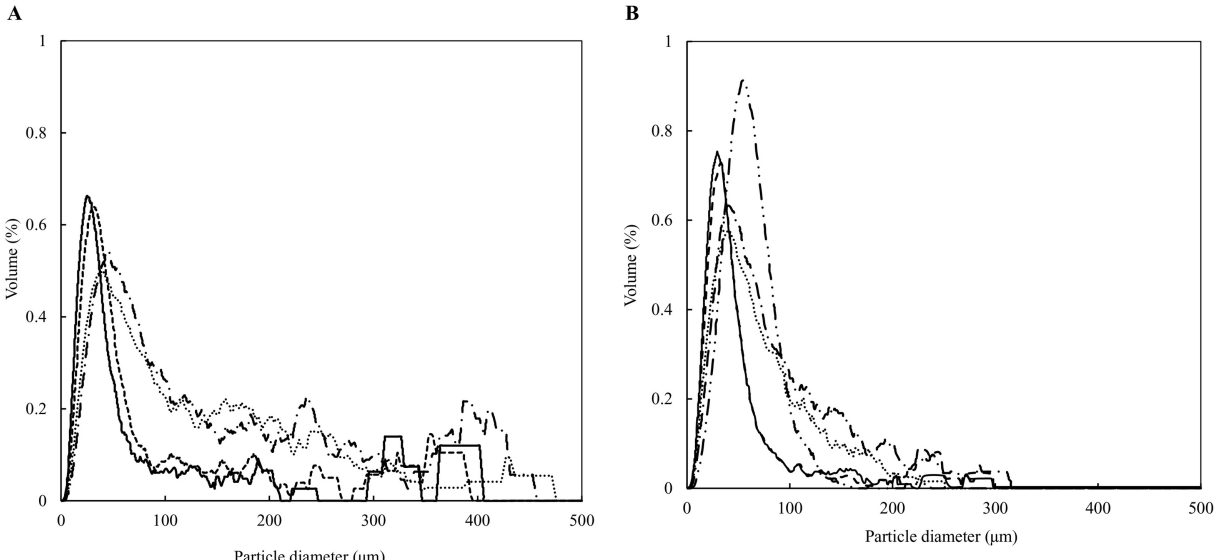

A
B
Volume (%)
Particle diameter (μm)
0
0.2
0.4
0.6
0.8
1
0
100
200
300
400
500

Figure 1

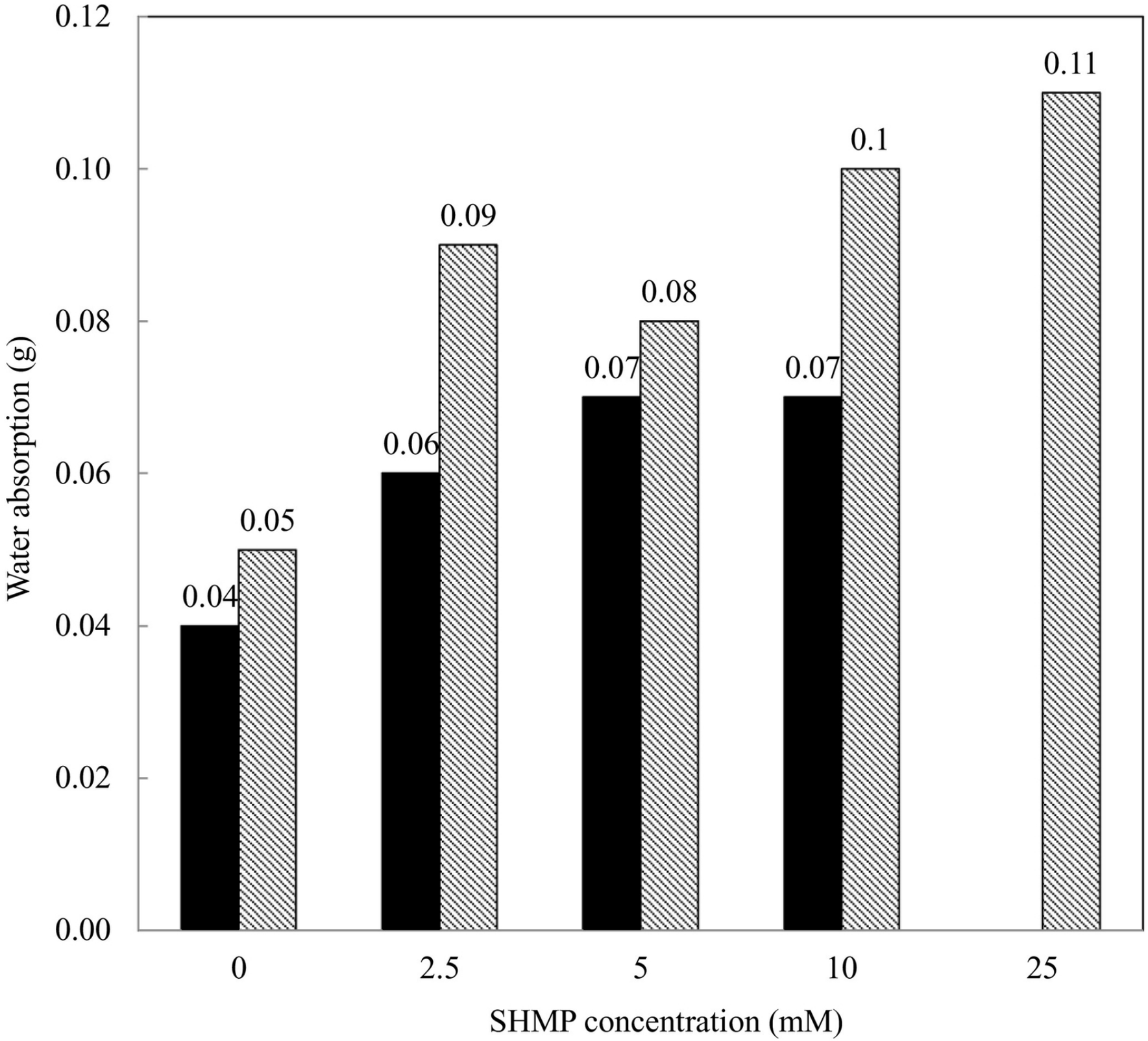

0.12
0.10
0.08
0.06
0.04
0.02
0.00
Water absorption (g)
0.04
0.05
0.06
0.09
0.07
0.08
0.07
0.1
0.11
0
2.5
5
10
25
SHMP concentration (mM)

Figure 2

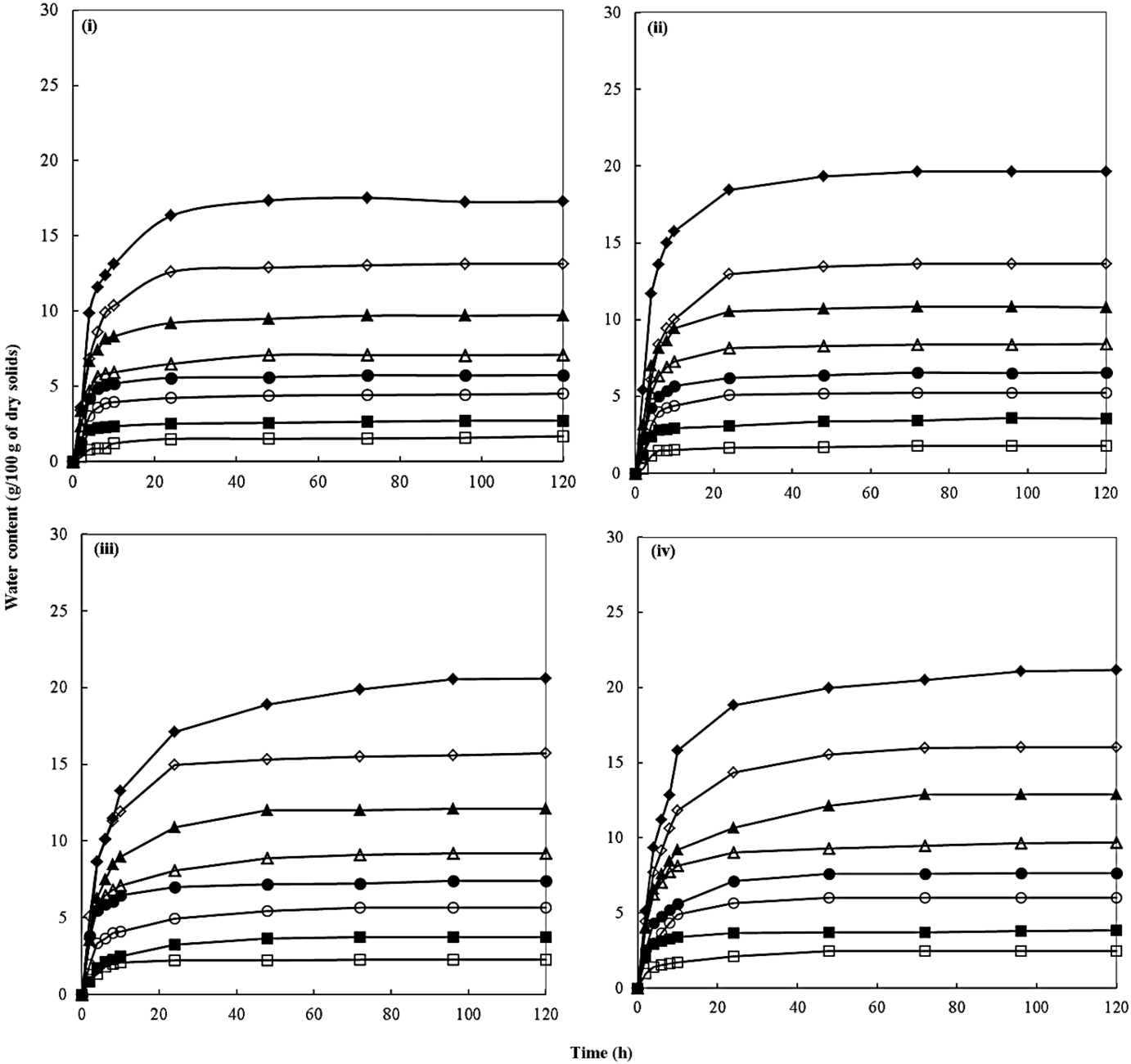

(i)
(ii)
(iii)
(iv)
Water content (g/100 g of dry solids)
Time (h)
0
5
10
15
20
25
30
0
20
40
60
80
100
120

Figure 3A

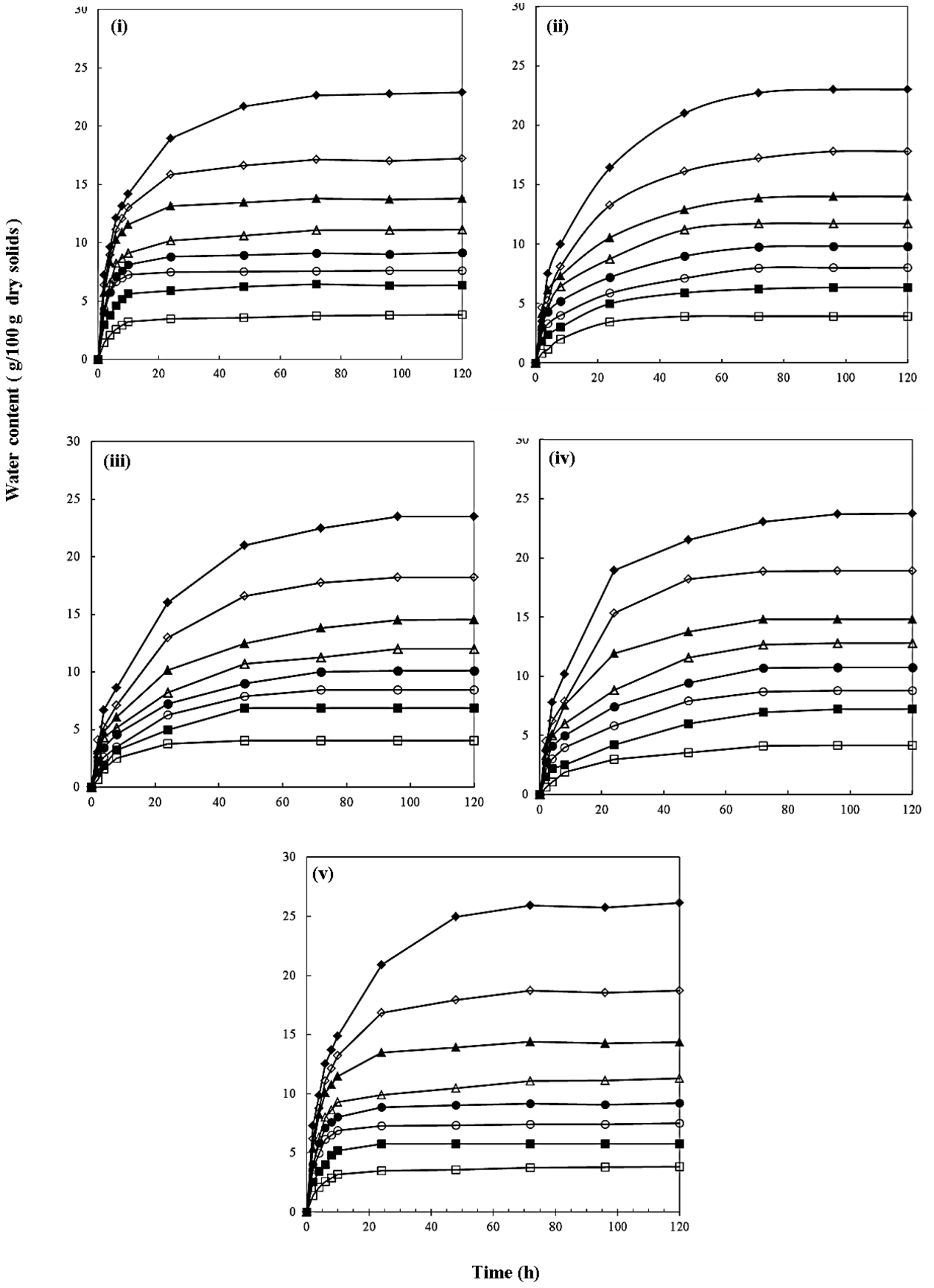


Figure 3B

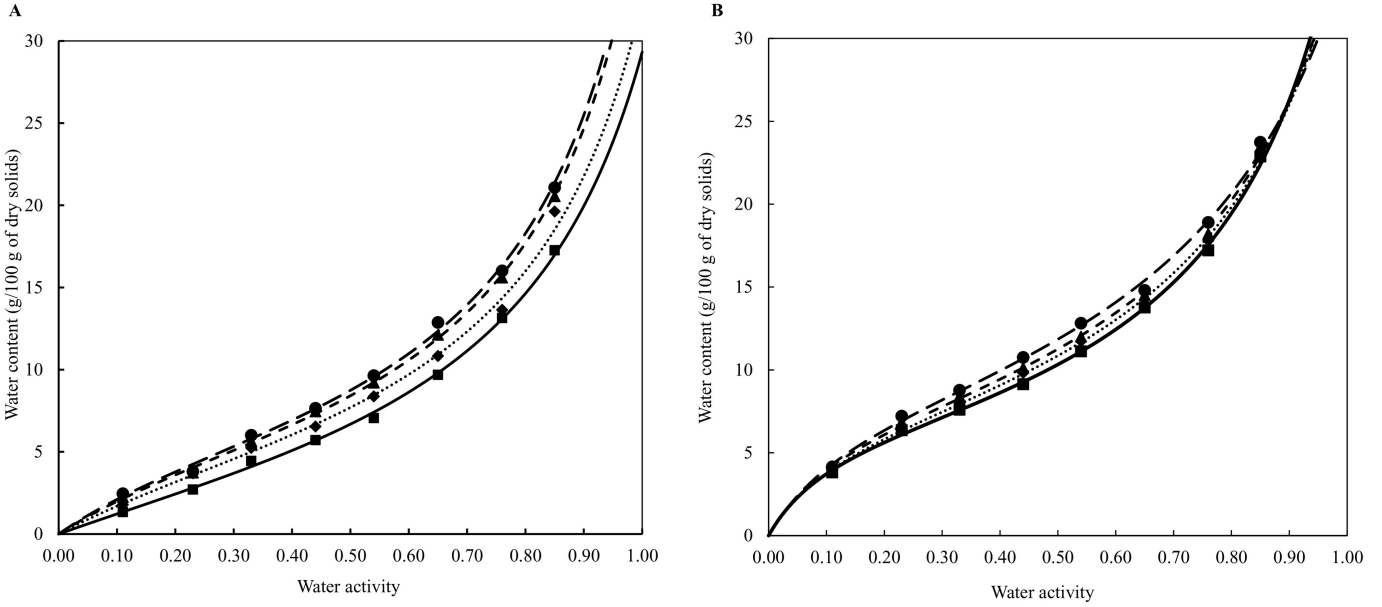

A
B
Water content (g/100 g of dry solids)
Water activity
0
5
10
15
20
25
30
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00

Figure 4

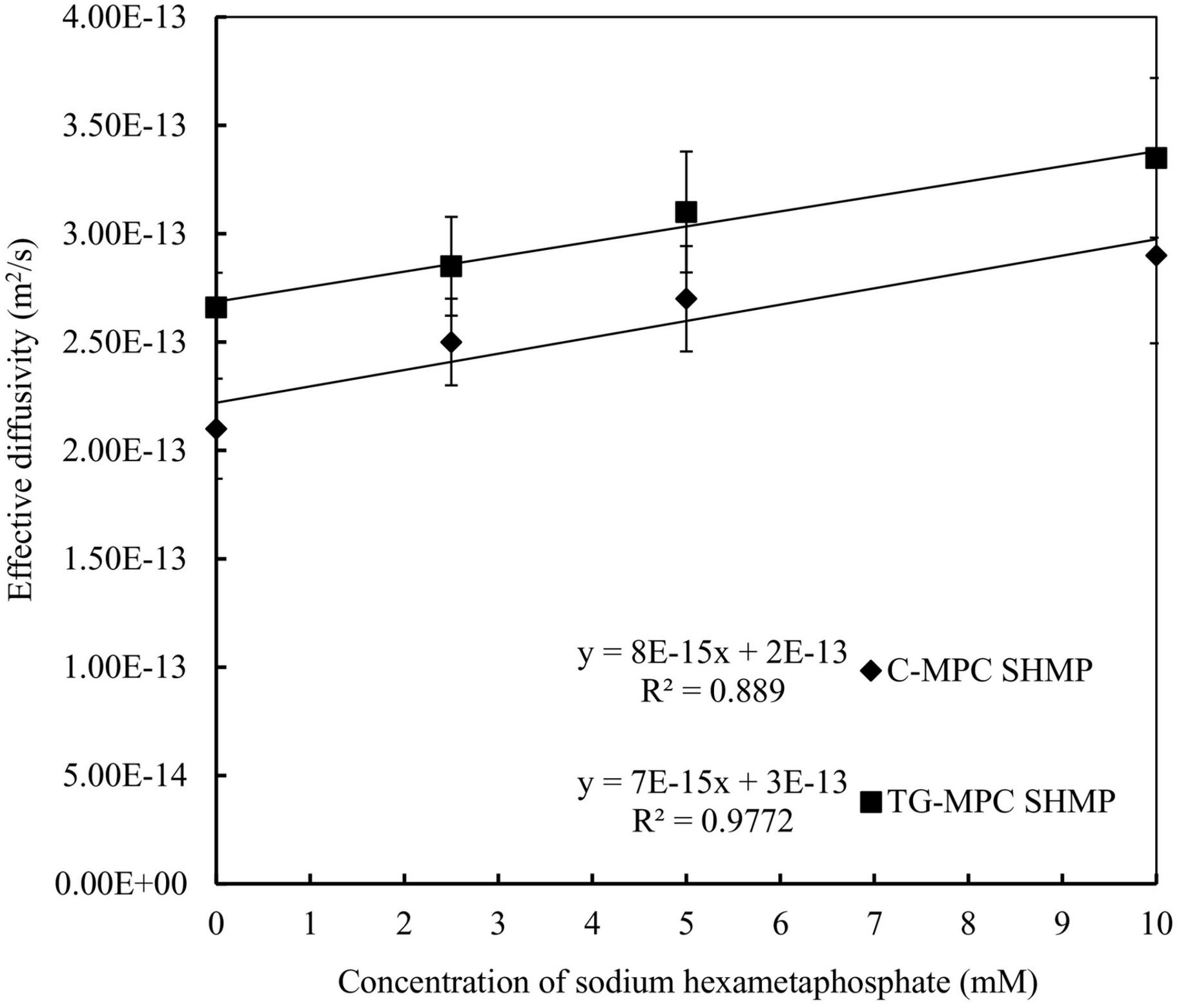

4.00E-13
3.50E-13
3.00E-13
2.50E-13
2.00E-13
1.50E-13
1.00E-13
5.00E-14
0.00E+00
Effective diffusivity (m²/s)
y = 8E-15x + 2E-13
R² = 0.889
C-MPC SHMP
y = 7E-15x + 3E-13
R² = 0.9772
TG-MPC SHMP
0
1
2
3
4
5
6
7
8
9
10
Concentration of sodium hexametaphosphate (mM)

Figure 5

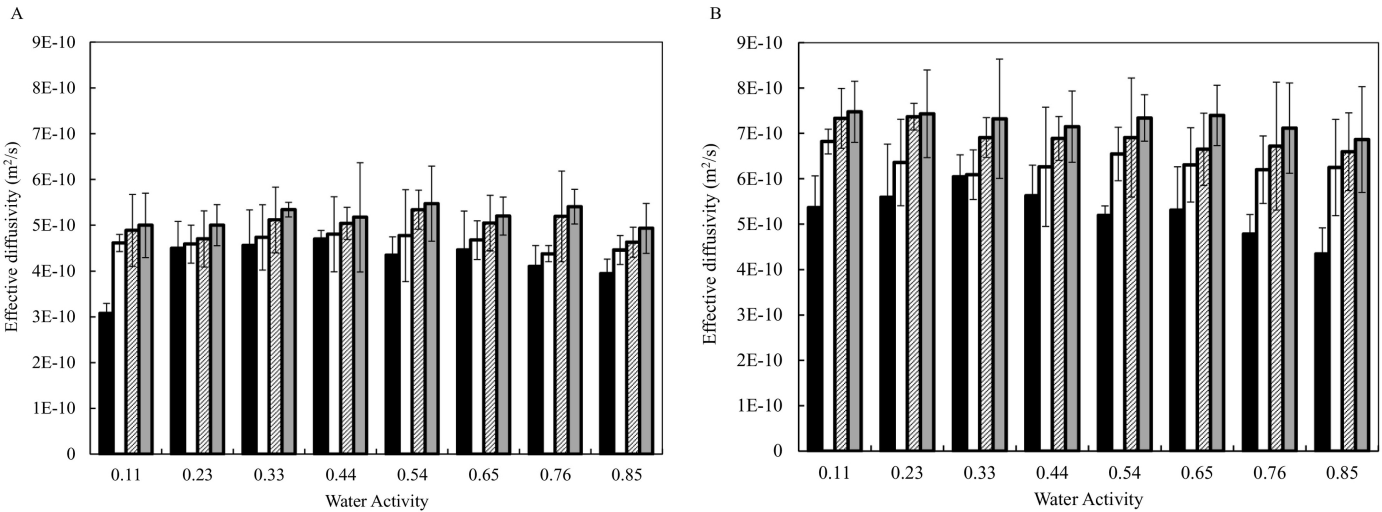

A
B
9E-10
8E-10
7E-10
6E-10
5E-10
4E-10
3E-10
2E-10
1E-10
0
Effective diffusivity (m²/s)
0.11
0.23
0.33
0.44
0.54
0.65
0.76
0.85
Water Activity

Figure 6

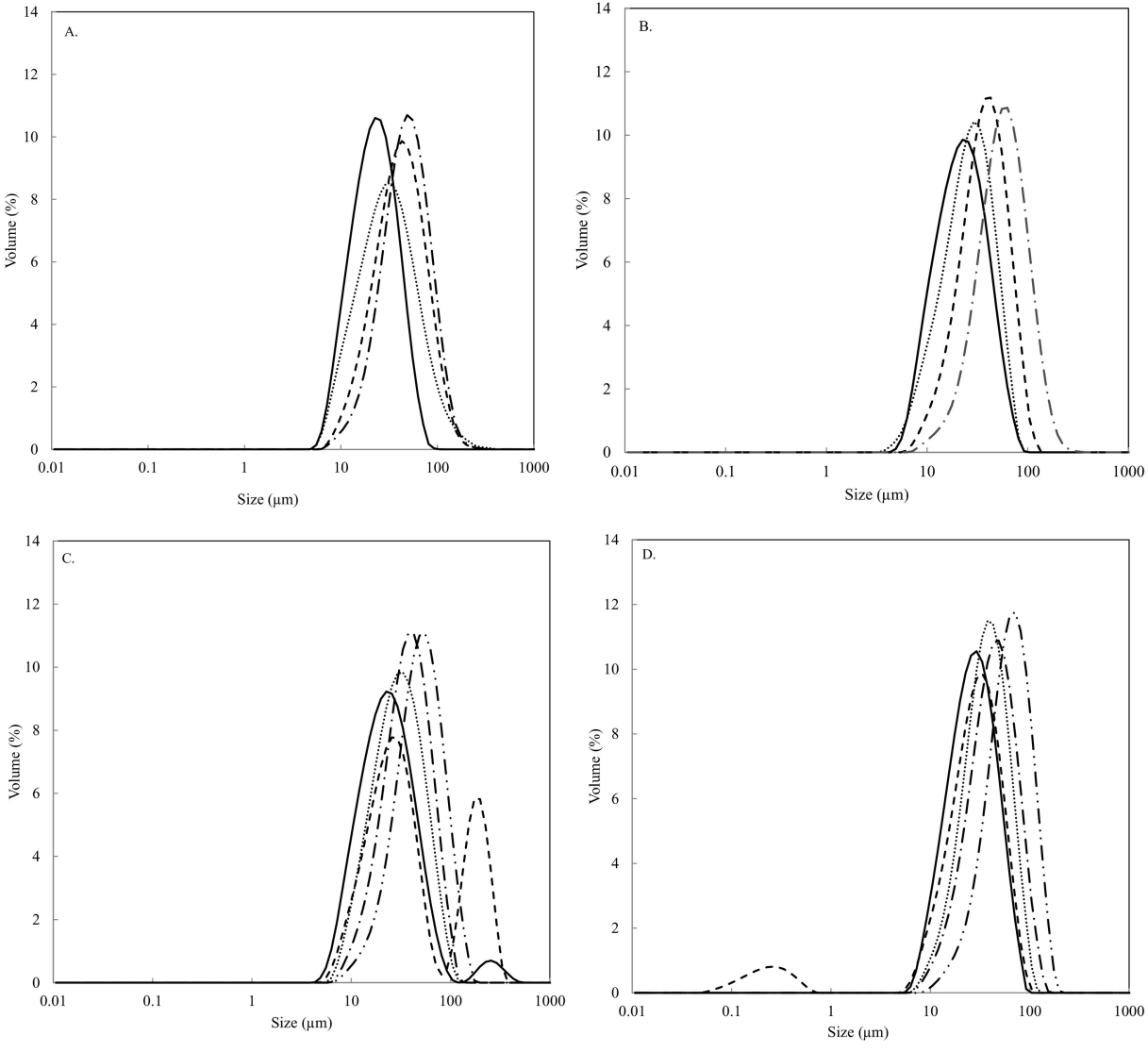


Figure 7