

## Thermo-sensitive Poly(DEGMMA-*co*-MEA) Microgels: Synthesis, Characterization and Interfacial Interaction with Adsorbed Protein Layer\*

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**Abstract** The novel microgels, poly[di(ethylene glycol) methyl ether methacrylate-*co*-2-methoxyethyl acrylate] poly(DEGMMA-*co*-MEA) microgels, were synthesized. The poly(DEGMMA-*co*-MEA) microgels were thermo-sensitive and exhibited a volume phase transitive temperature (VPTT) of 14–22 °C. The incorporation of hydrophobic comonomer MEA shifted the VPTT of poly(DEGMMA-*co*-MEA) microgels to lower temperatures. The interfacial interaction of poly(DEGMMA-*co*-MEA) microgels and three model proteins, namely fibrinogen, bovine serum albumin and lysozyme, was investigated by quartz crystal microbalance (QCM). An injection sequence of “microgel-after-protein” was then established for the real-time study of the interaction of proteins and the microgels at their swollen and collapsed states by using QCM technique. The results indicated that the interfacial interaction of poly(DEGMMA-*co*-MEA) microgels and adsorbed protein layers was mainly determined by the electrostatic interaction. Because poly(DEGMMA-*co*-MEA) microgels were negatively charged in Tris-HCl buffer solution (pH = 7.4), the microgels did not adsorb on negatively charged fibrinogen and bovine serum albumin layers but strongly adsorbed on positively charged lysozyme layer. Stronger interaction between lysozyme and the microgels at collapsed state (*i.e.* at 37 °C) was observed. Furthermore, the incorporation of MEA might weaken the interaction between poly(DEGMMA-*co*-MEA) microgels and proteins.

**Keywords:** QCM; Poly(DEGMMA-*co*-MEA) microgels; Thermosensitive; Proteins; Interfacial interaction.

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## INTRODUCTION

Microgels are colloidal particles with sizes of 1–1000 nm, which have three dimensional cross-linking networks and could not dissolve but swell and well disperse in aqueous solution. The first monodisperse microgel system was poly(*N*-isopropylacrylamide) (PNIPAm) microgels reported in 1986 by Pelton and Chibante<sup>[1]</sup>. After that, many efforts were devoted to develop new microgels and investigate the structure, physical properties and potential applications of the obtained microgels<sup>[2–16]</sup>. With different monomers and comonomers, the microgels could respond to various external stimuli, like temperature, pH, ionic strength or others<sup>[2, 17]</sup>. Microgels have nanometer sizes and might also well disperse in buffer solutions. The swelling-deswelling of microgels might be

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regulated by using external stimuli as required. The cross-linked networks of the microgels could be utilized for loading drugs. Furthermore, the microgels loaded with drugs in buffer solution might be injected to the targeted position where drug-controlled-release was required. Due to the above mentioned properties, microgels have great potential as drug delivery and drug-controlled release systems, which have attracted much attention<sup>[18–21]</sup>.

Before the microgels are employed as drug-controlled release system in real application, there are at least three fundamental issues, which need to be systematically investigated: (1) the biocompatibility of the microgels; (2) the loading and release properties of microgels toward the targeted drugs; (3) the interaction of proteins and microgels. Once the microgels were injected into biological body, inflammation might occur due to the rejection of organisms if the microgels were bio-incompatible. When the microgels were in contact with the blood of organisms, the proteins in the blood might interact with the microgels, leading to the conformation change of proteins, which might probably cause severe malfunction of organisms. The biocompatibility, drug-loading and releasing properties of microgels have been investigated for various microgel systems, like PNIPAm-related microgels and poly(*N*-vinylpyrrolidinone) (PNVP) microgels<sup>[11, 18–22]</sup>. However, the investigation on the interaction between microgels and proteins is less reported. Investigating and understanding the interaction of microgels and proteins have fundamental and practical importance for the real application of microgels in biomedical fields. The interactions between proteins and microgels are very complicated because the interactions may come from Van der Waals force, hydrogen bonds, hydrophobic interactions, and electrostatic interactions, *etc.* Up to date, most studies were mainly focused on the uptake of proteins into the microgels with opposite charges<sup>[23–26]</sup>. For instance, Eichenbaum *et al.* studied the uptake of some proteins by poly(methacrylic acid-co-acrylic acid) microgels with sizes of 4–10  $\mu\text{m}$ <sup>[27]</sup>. Johansson *et al.* investigated the interaction between positively charged lysozyme and negatively charged poly(NIPAm-co-acrylic acid) microgels in aqueous solutions at neutral pH and found that the maximum uptake at 10 mmol/L ionic strength was 2.4 g lysozyme per gram dry gel<sup>[25]</sup>.

In the present work, we reported the synthesis, characterization of a novel thermo-sensitive microgel system and its interfacial interaction with adsorbed protein layer. The microgels based on di(ethylene glycol) methyl ether methacrylate (DEGMA) and 2-methoxyethyl acrylate (MEA) were synthesized by using emulsion polymerization. Poly(2-methoxyethyl acrylate) (PMEA) is a hydrophobic material and has been approved by U.S. Food and Drug Administration (FDA) as the biocompatible drug carrier. PMEA also has excellent blood compatibility<sup>[28]</sup>. Poly[di(ethylene glycol) methyl ether methacrylate] (PDEGMA) is thermo-sensitive and exhibits a low critical solution temperature (LCST) at 22–26 °C, which was thought to have bright biomedical application potential<sup>[29]</sup>. Therefore, poly(DEGMA-co-MEA) microgels might have potential application in bio-medical fields. Since the proteins in body fluid and blood are very complicated, three model proteins were chosen here for studying their interactions with poly(DEGMA-co-MEA) microgels. They were fibrinogen (FIB), bovine serum albumin (BSA) and lysozyme (LYS). Fibrinogen is an extracellular protein with an elongated shape with dimension of 45 nm  $\times$  9 nm  $\times$  6 nm and isoelectric point (pI) of 5.5 and is important in the context of both blood/surface interactions<sup>[30, 31]</sup>. Albumin is a globular, extracellular protein with dimension of 8 nm  $\times$  8.7 nm  $\times$  6 nm and pI of 4.7 and is the most abundant protein in blood<sup>[32]</sup>. Lysozyme is a small and “hard” protein with dimension of 3 nm  $\times$  3 nm  $\times$  4.5 nm and pI of 11.1 and is part of the innate immune system<sup>[33]</sup>. Quartz crystal microbalance (QCM) is highly sensitive and could detect the trace mass changes in nanogram range, which is adsorbed onto the electrode surface of the quartz crystal in air or in liquid. QCM is widely used in various research fields, including the measurement of film thickness, kinetics of adsorption process, surface and interface interactions, *etc.*<sup>[34–41]</sup>. Here, the QCM technique was used to *in situ* investigate the interfacial interaction of the adsorbed protein layers and poly(DEGMA-co-MEA) microgels in their swollen and collapsed states. We focused here on the interfacial interactions between the proteins and microgels but not on the uptake of proteins in the microgels. The preliminary results obtained here might be helpful for the understanding of the interfacial interaction mechanisms between microgels and proteins with different charges.

## EXPERIMENTAL

### *Materials and Equipments*

Di(ethylene glycol) methyl ether methacrylate (DEGMMA, 97%) and fibrinogen (FIB, from bovine, fraction I) were purchased from Tokyo Chemical Industry Co., Ltd. Ethylene glycol dimethacrylate (EGDMA, 99%), sodium dodecyl sulfate (SDS, 99%) and bovine serum albumin (BSA, 98%) were obtained from J&K Chemical. potassium persulfate (KPS) and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sinopharm Chemical Reagent Co., Ltd. 2-Methoxyethyl acrylate (MEA, 98%, Acros Organics) and lysozyme (LYS, min. 100000 units/mg, from chicken egg white, Serva) were used as received. All reagents were used as received. 0.01 mol/L Tris-HCl buffer solutions were prepared by dissolving 1.21 g Tris in 1 L deionized water and then adjusting the pH to 7.4 with HCl aqueous solution at 25 °C.

### *Preparation of Thermo-sensitive Poly(DEGMMA-co-MEA) Microgels*

The thermo-sensitive poly(DEGMMA-co-MEA) microgels were prepared *via* conventional emulsion polymerization. Briefly, 610 mg DEGMMA, 18 mg EGDMA, 10 mg SDS and preset amounts of MEA, *i.e.* 0 mg, 105 mg, 210 mg and 420 mg, respectively, were added into a three-necked flask with 48 mL deionized water (Table S1). Oxygen was eliminated by bubbling nitrogen through the mixture solution for 15 min at 70 °C under vigorous stirring. Afterward, 2 mL of KPS aqueous solution (5 mg/mL) were added into the mixture solution to initiate the copolymerization. The reaction was then preceded at 70 °C for 6 h. The resultant microgels were purified by centrifugation at 13000 r/min for 1 h. Poly(DEGMMA-co-MEA) microgels prepared with different feeding molar fractions of MEA were coded as M0, M20, M33 and M50, respectively.

### *Characterization of Thermo-sensitive Poly(DEGMMA-co-MEA) Microgels*

The morphologies of poly(DEGMMA-co-MEA) microgels were investigated by a transmission electron microscope (TEM, JEOL JEM-1200) operating at an acceleration voltage of 60 kV. The TEM samples were prepared by dip-coating with Formvar-coated copper grids into the microgel suspensions. The solvent was gently adsorbed away by a filter paper. The grids were dried in air at room temperature before observation. The size and thermo-sensitive properties of poly(DEGMMA-co-MEA) microgels were characterized by dynamic light scattering (DLS) at scattering angle of 90° using a ZetaPlus Particle Size Analyzer (Brookhaven Instruments Corporation).

### *Interaction of Poly(DEGMMA-co-MEA) Microgels and Model Proteins by QCM*

For studying the interactions between poly(DEGMMA-co-MEA) microgels and model proteins, the freeze-dried microgels and three proteins, *i.e.* FIB, BSA and LYS, were dissolved in 0.01 mol/L Tris-HCl buffer solution (pH 7.4), respectively, to give a solution concentration of 1 mg/mL.

The interactions between poly(DEGMMA-co-MEA) microgels and the three proteins were first *in situ* studied by QCM. All measurements were performed by using an IQCM (Resonant Probe GmbH, Germany). AT-cut quartz crystals with a fundamental resonant frequency  $f_0$  of 5 MHz and gold electrodes were purchased from Hangzhou Dongwei Inc., which were vacuum packed. The gold surface of cleaned quartz crystal was hydrophobic with a static water contact angle of  $\sim(101^\circ \pm 3^\circ)^{[40]}$ . The cleaned quartz crystal was unpacked and installed in the QCM's liquid cell, of which the temperature was regulated by a heating controller. The resonance behavior of bare quartz crystal in air was first recorded and 0.01 mol/L Tris-HCl buffer solutions were then pumped into the liquid cell at a flow rate of 50  $\mu\text{L}/\text{min}$  until a stable baseline was established. Two different ways were employed to investigate the interaction between poly(DEGMMA-co-MEA) microgels and the model proteins. One way was named as "protein-after-microgel". In this case, the Tris-HCl buffer solution was first replaced with poly(DEGMMA-co-MEA) microgel solution. After reaching the adsorption equilibrium of microgels, the microgel solution was then replaced with the protein solution. After reaching adsorption equilibrium of protein, the quartz crystal was rinsed by replacing the protein solution with Tris-HCl buffer solution. The other way was with the inverse sequence, *i.e.* "microgel-after-protein", of which the model proteins were first adsorbed onto the quartz crystal and poly(DEGMMA-co-MEA) microgels were then interacted with

the adsorbed protein layer. After reaching adsorption equilibrium of microgels, the quartz crystal was rinsed with Tris-HCl buffer solution. The QCM data were acquired at 3<sup>rd</sup> (15 MHz) overtone and the frequency and half-band-half bandwidth shifts were recorded as  $\Delta f_3/3$  and  $\Delta I_3/3$ , respectively, because of the best energy trapping at the 3<sup>rd</sup> overtone<sup>[42]</sup>. The QCM experiments were conducted at temperatures of  $(12 \pm 0.1)^\circ\text{C}$  and  $(37 \pm 0.1)^\circ\text{C}$ , respectively, in order to investigate the interaction between the proteins and poly(DEGMMA-co-MEA) microgels at swollen and collapsed states. Especially,  $37^\circ\text{C}$  was the physiological temperature that the microgels may mostly experience in the real applications as drug-controlled release systems.

## RESULTS AND DISCUSSION

### *Morphology and Thermo-sensitive Property of Poly(DEGMMA-co-MEA) Microgels*

The obtained poly(DEGMMA-co-MEA) microgels were very stable in aqueous solutions. The use of SDS was necessary due to the hydrophobic nature of MEA. Note that the concentration of SDS in the reaction solution was far below its critical micelle concentration (CMC). Figure 1 shows the representative TEM images of poly(DEGMMA-co-MEA) microgels with various feed molar ratios of MEA. The microgels were spherical in shape with a relatively narrow size distribution. The average diameters of the microgels with sample codes of M0, M20, M33, and M50 were  $(146 \pm 30)$  nm,  $(127 \pm 11)$  nm,  $(119 \pm 21)$  nm, and  $(60 \pm 11)$  nm, respectively, as calculated from the TEM images.

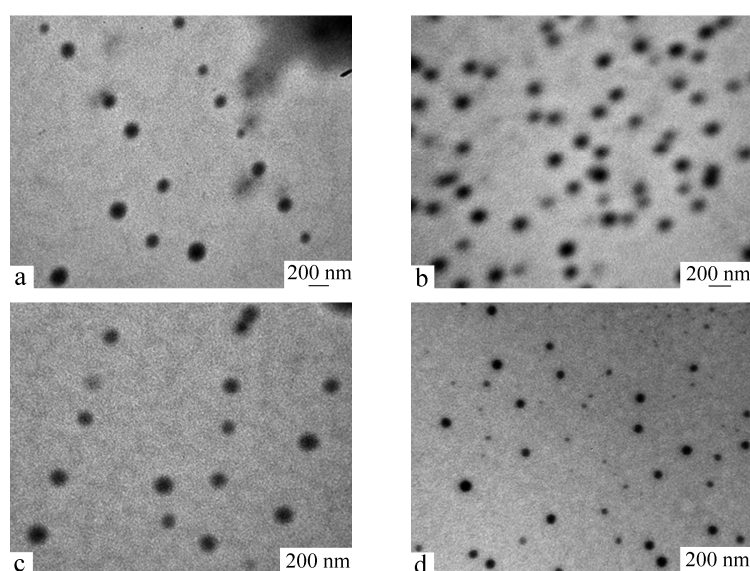


**Fig. 1** TEM images of poly(DEGMMA-co-MEA) microgels: (a) M0, (b) M20, (c) M33 and (d) M50

Figure 2 shows the hydrodynamic diameters of poly(DEGMMA-co-MEA) microgels as a function of temperature. The poly(DEGMMA-co-MEA) microgels exhibited thermo-sensitive behavior. The hydrodynamic diameter of poly(DEGMMA-co-MEA) microgels decreased with increasing temperature and finally reached a plateau value at temperatures above the volume phase transition temperature (VPTT) (Fig. 2a). After differentiating the data sets of Fig. 2(a), the VPTTs of M0, M20, M33 and M50 microgels were determined to be about  $22^\circ\text{C}$ ,  $20^\circ\text{C}$ ,  $18^\circ\text{C}$  and  $14^\circ\text{C}$ , respectively (Fig. 2b). These results indicated that the incorporation of hydrophobic comonomer MEA shifted the VPTT of poly(DEGMMA-co-MEA) microgels to lower temperatures.

The stability and thermo-sensitivity of presentative poly(DEGMMA-co-MEA) microgels with sample code of M20 in Tris-HCl buffer solution were also studied. The M20 microgels were very stable in Tris-HCl buffer solution and exhibited similar thermo-sensitive behavior and hydrodynamic diameter with those in aqueous solution. (Fig. S1, supporting information).

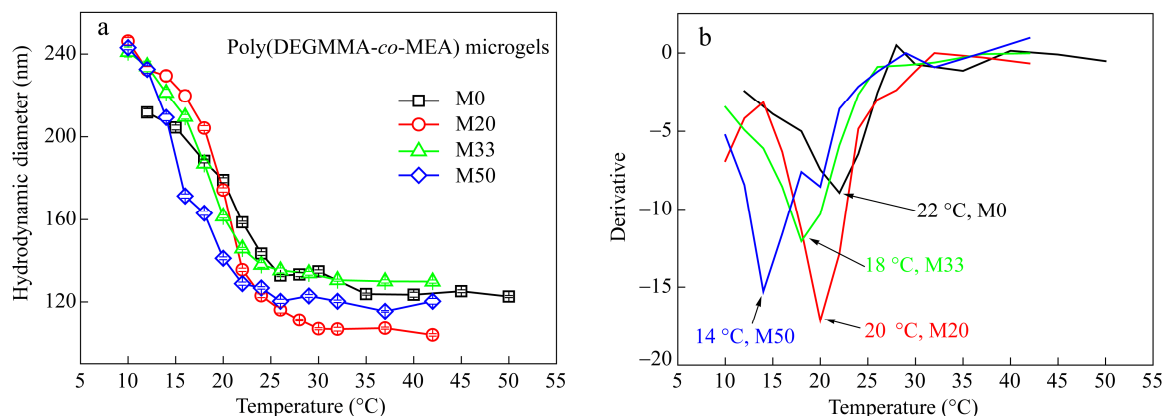


**Fig. 2** (a) Hydrodynamic diameters of poly(DEGMMA-co-MEA) microgels as a function of measuring temperature; (b) The derivative of data shown in (a)

### Interaction of Poly(DEGMMA-co-MEA) Microgels and Proteins by QCM

Quartz crystal microbalance (QCM) was utilized to study the interaction between poly(DEGMMA-co-MEA) microgels and the model proteins, *i.e.* FIB, BSA and LYS. Firstly, the effects of injection sequence of microgels and FIB on the QCM results were investigated in order to give a reliable understanding of the interaction between poly(DEGMMA-co-MEA) microgels and the model protein, as mentioned in the experimental section. Figure 3 shows the frequency shift ( $\Delta f_3/3$ ) of quartz crystal for the interaction of microgels and FIB at 12 °C and 37 °C with the injection way of “protein-after-microgel”. Note that the viscosity effects of microgel and protein suspensions on the frequency shift of quartz crystal were negligible because the concentration of the microgel and protein suspensions were very dilute, *i.e.* 1 mg/mL and their viscosities were close to that of 0.01 mol/L Tris-HCl buffer solution. It was clearly observed that the frequency decreased quickly with the injection of microgels and reached adsorption equilibrium in 20 min. The adsorption of microgels on the gold surface of quartz crystal was more significant in their swollen states at 12 °C. For the adsorption of M20 microgels at 12 °C,  $\Delta f_3/3$  of -52 Hz was observed (Fig. 3a). Followed the injection of FIB, the frequency decreased quickly again and reached adsorption equilibrium in ~30 min. More FIB were adsorbed at 37 °C. Especially, the injection of FIB led to  $\Delta f_3/3$  of -32 Hz for the collapsed state of M20 microgels at 37 °C (Fig. 3b). Such decrease of frequency seemed to indicate the strong interaction between poly(DEGMMA-co-MEA) microgels and FIB, leading to the adsorption of FIB on the adsorbed microgels.

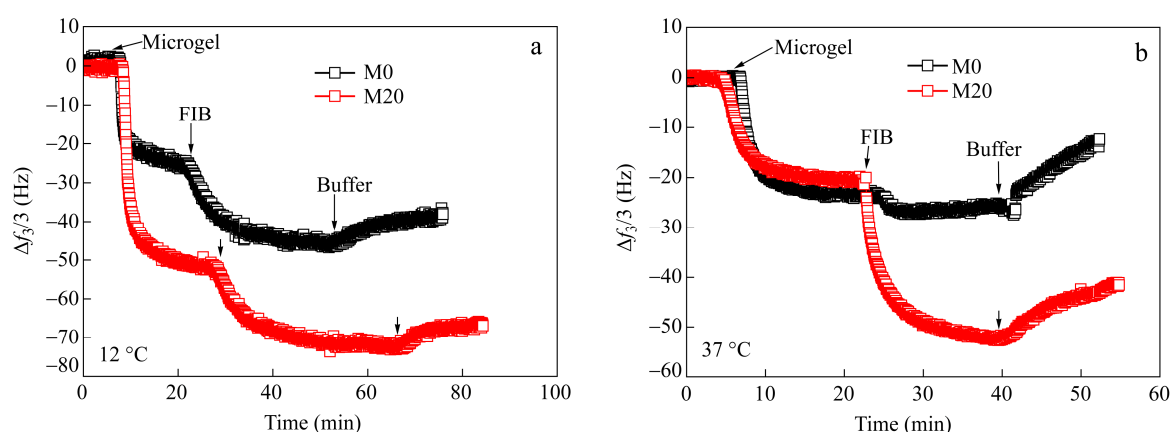


**Fig. 3** Frequency shift ( $\Delta f_3/3$ ) of quartz crystal at 3<sup>rd</sup> overtone for the interaction of poly(DEGMMA-co-MEA) microgels and FIB as a function of time at (a) 12 °C and (b) 37 °C. The injection sequence was “protein-after-microgel”.

However, with the injection sequence of “microgel-after-protein”, different results were obtained. Figure 4 shows the frequency shift ( $\Delta f_3/3$ ) of quartz crystal for the interaction of microgels and FIB with the injection way of “microgel-after-protein” at 12 °C and 37 °C. The injection of FIB led to the fast decrease of frequency, suggesting the strong adsorption of FIB on the gold surface of quartz crystal. The adsorption of FIB reached adsorption equilibrium in ~20 min and led to  $\Delta f_3/3$  of about -74 Hz and -84 Hz at 12 °C and 37 °C, respectively. Interestingly, with the subsequent injection of poly(DEGMMA-co-MEA) microgels, no frequency decrease was observed. Instead, the frequency slightly increased with the injection of microgels. It might be deduced from Fig. 4 that there was not strong interaction between poly(DEGMMA-co-MEA) microgels and FIB, which contradicted with the results of Fig. 3.

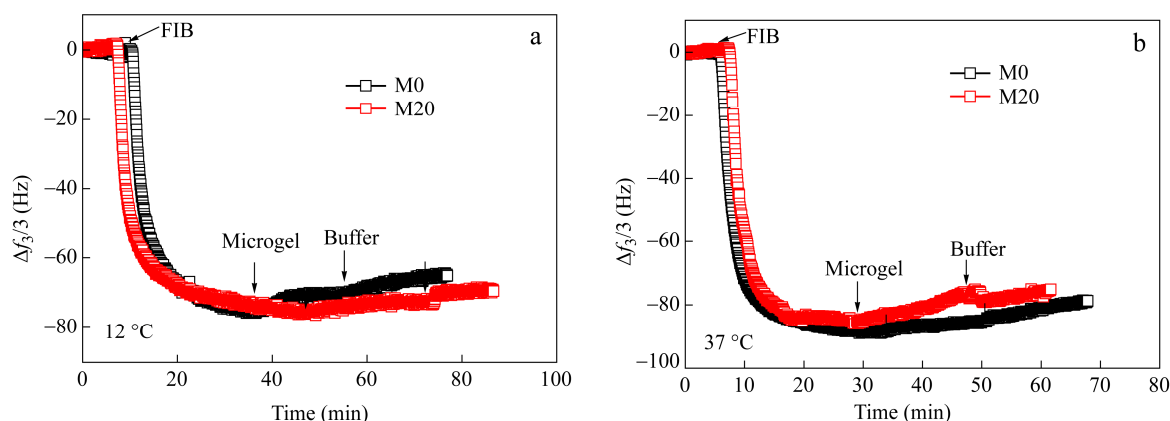


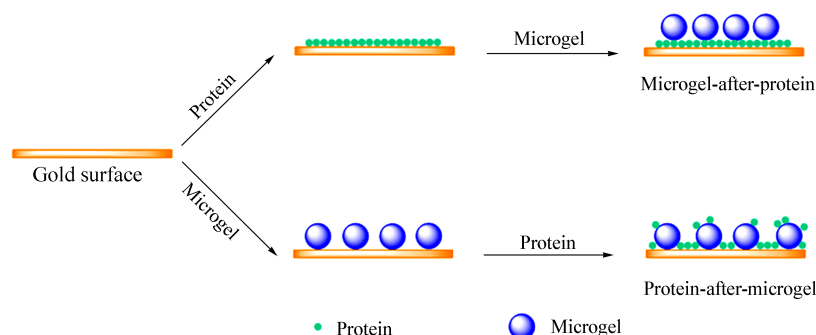
**Fig. 4** Frequency shift ( $\Delta f_3/3$ ) of quartz crystal at 3<sup>rd</sup> overtone for the interaction of poly(DEGMMA-co-MEA) microgels and FIB as a function of time at (a) 12 °C and (b) 37 °C. The injection sequence was “microgel-after-protein”.

In fact, the frequency shift shown in Fig. 3 did not tell us the truth about the interaction between poly(DEGMMA-co-MEA) microgels and FIB. The size of poly(DEGMMA-co-MEA) microgels in Tris-HCl buffer solution was much larger than that of FIB. For example, the hydrodynamic diameters of M20 microgels in Tris-HCl buffer solution were 239 nm and 113 nm at 12 °C and 37 °C, respectively. Whereas, FIB has a dimension of 45 nm × 9 nm × 6 nm<sup>[30, 31]</sup>. From Fig. 3(a), a frequency shift ( $\Delta f_3/3$ ) of -52 Hz was observed for the adsorption of M20 microgels on gold surface of quartz crystal at 12 °C. If assuming the density of microgel was about 1.0 g/cm<sup>3</sup>, the thickness of adsorbed microgels could be estimated by Sauerbrey equation<sup>[43]</sup>:

$$\frac{\Delta f}{f_0} = \frac{-2f}{Z_q} \times m_f = \frac{-2f}{Z_q} \times \rho_f d_f \quad (1)$$

where  $\Delta f$  is frequency shift,  $f = n f_0$  is the frequency with the overtone order  $n$ ,  $m_f$  is the areal mass density of the film,  $\rho_f$  is the density of the film, and  $Z_q = 8.8 \times 10^6$  kg/m<sup>2</sup>s is the acoustic impedance of crystalline quartz. The thickness of adsorbed M20 microgel layer at 12 °C was then calculated to be ~9 nm from  $\Delta f_3/3$  of -52 Hz, which was far smaller than the size of M20 microgels in Tris-HCl buffer (239 nm). In other word, the adsorbed microgels only covered part of the gold surface, as shown in Scheme 1. Most parts of the gold surface were still exposed to the protein when FIB was subsequently injected. The small FIB protein could be then adsorbed not only on the adsorbed microgel surfaces but also on the uncovered gold surface, leading to the apparently large decrease of frequency (cf. Scheme 1). Furthermore, even for the case that the microgels formed a dense packing adsorbed layer on gold surface, the protein could still be adsorbed onto the voids among the microgels because the volume of protein was far smaller than those of the microgels. From the frequency shift ( $\Delta f_3/3$ ) of -32 Hz after injection of FIB at 37 °C (Fig. 3b), a thickness of adsorbed FIB layer was estimated by Eq. (1) to be ~5.5 nm, which was close to the dimension of FIB, *i.e.* 45 nm × 9 nm × 6 nm. The above analyses strongly

suggested that the injection way of “protein-after-microgel” was not suitable for the study of interaction between poly(DEGMMA-*co*-MEA) microgels and protein.



**Scheme 1** Schematic illustration of the adsorption of proteins and microgels on the gold surface of quartz crystal with different injection sequences

With the injection way of “microgel-after-protein”, a FIB layer was firstly formed on the gold surface of quartz crystal. The frequency shifts ( $\Delta f_3/3$ ) of about  $-74$  Hz and  $-84$  Hz at  $12$  °C and  $37$  °C meant that the thicknesses of adsorbed FIB layers were about  $12.8$  nm and  $14.5$  nm at  $12$  °C and  $37$  °C, respectively (Fig. 4), which were almost twice of the dimension of FIB. More FIB proteins were adsorbed and multilayer of FIB was formed on the gold surface at  $37$  °C. In other words, the adsorbed FIB completely covered the gold surface of quartz crystal at  $12$  °C and  $37$  °C. With the subsequence injection of microgels, the adsorption of microgels on FIB layers was indeed studied (cf. Scheme 1). Figure 4 indicates that poly(DEGMMA-*co*-MEA) microgels did not adsorb onto the FIB layers. The slight increase of frequency during the injection of microgels could be attributed to desorption of loose adsorbed FIB. The results of Fig. 4 suggested that there was not noticeable interaction between FIB and poly(DEGMMA-*co*-MEA) microgels. It was understandable because FIB has a pI value of  $5.5$  and was negative charged in Tris-HCl buffer solution (pH =  $7.4$ ). Poly(DEGMMA-*co*-MEA) microgels were also negative charged in Tris-HCl buffer solution due to the use of initiator KPS and anionic surfactant SDS. The repulsive interaction between FIB and poly(DEGMMA-*co*-MEA) microgels resisted the adsorption of poly(DEGMMA-*co*-MEA) microgels on FIB layers.

The injection sequence of “microgel-after-protein” was then used to study the interaction of poly(DEGMMA-*co*-MEA) microgels and the model proteins, BSA and LYS. Figure 5 shows the frequency shift ( $\Delta f_3/3$ ) and half-band-half shift ( $\Delta F_3/3$ ) of quartz crystal for the interaction of poly(DEGMMA-*co*-MEA) microgels and bovine serum albumin (BSA) at (a)  $12$  °C and (b)  $37$  °C. The adsorption amounts of BSA on gold surface were less than those of FIB. From the frequency shifts ( $\Delta f_3/3$ ) of  $-18$  Hz at  $12$  °C and  $-12$  Hz at  $37$  °C, the thicknesses of adsorbed BSA layers were estimated to be about  $2$ – $3$  nm, which were smaller than the size of BSA. Albumin has a dimension of  $8$  nm  $\times$   $8.7$  nm  $\times$   $6$  nm<sup>[32]</sup> and a hydrodynamic radius of  $3.4$  nm in buffer solution<sup>[44]</sup>. These results indicated that the adsorbed BSA did not form a complete protein layer on gold surface. With the subsequence injection of poly(DEGMMA-*co*-MEA) microgels, the frequency of quartz crystal did decrease. The decrease of frequency was larger for the collapsed microgels at  $37$  °C (Fig. 5b). The frequency shifts ( $\Delta f_3/3$ ) were about  $-7$  Hz for M20 microgels and  $-10$  Hz for M0 microgels adsorbed on BSA surface at  $37$  °C, corresponding to the estimated thickness of  $1$  nm for the microgels with Eq. (1), which indicated that only a few microgels were adsorbed onto the surface. In swollen state at  $12$  °C, the adsorption of microgels onto the surface was even less. The existence of MEA had not observable effect on the interaction of poly(DEGMMA-*co*-MEA) microgels and BSA. Note that BSA has a pI value of  $4.7$  and was also negatively charged in Tris-HCl buffer solution (pH =  $7.4$ ). There were also repulsive interaction between negatively charged poly(DEGMMA-*co*-MEA) microgels and BSA. Therefore, the frequency decrease observed in Fig. 5 might be likely attributed to the adsorption of a few microgels on the uncovered gold surface.

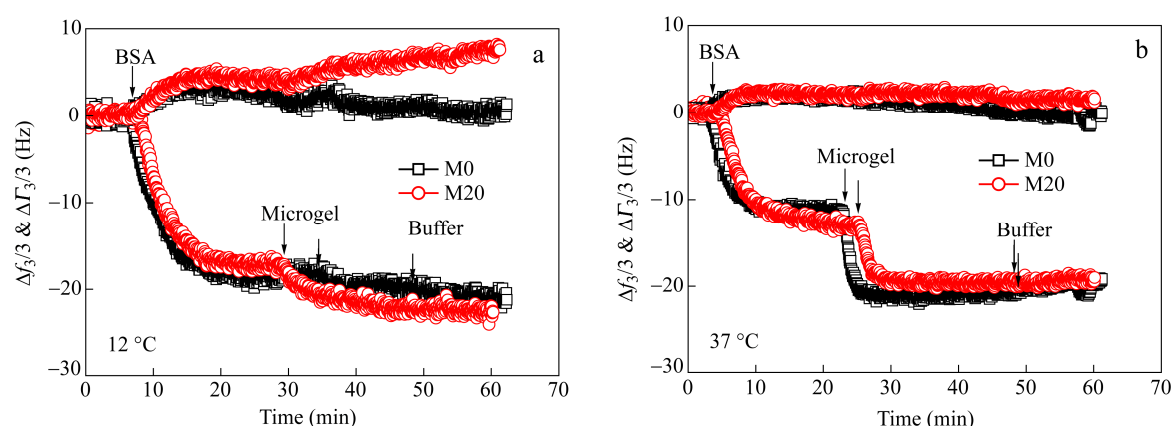


**Fig. 5** Frequency shift ( $\Delta f_3/3$ , solid symbol) and half-band-half shift ( $\Delta \Gamma_3/3$ , open symbol) of quartz crystal at 3<sup>rd</sup> overtone for the interaction of poly(DEGMMA-co-MEA) microgels and BSA as a function of time at (a) 12 °C and (b) 37 °C

The injection sequence was “microgel-after-protein”.

Figure 6 shows the frequency shift ( $\Delta f_3/3$ ) and half-band-half shift ( $\Delta \Gamma_3/3$ ) of quartz crystal for the interaction of poly(DEGMMA-co-MEA) microgels and lysozyme (LYS) as a function of time at (a) 12 °C and (b) 37 °C. Figure 6(a) indicates that LYS was strongly adsorbed on gold surface of quartz crystal, leading to the frequency shifts ( $\Delta f_3/3$ ) of about -34 Hz or -37 Hz at 12 °C and -24 Hz at 37 °C. By using Eq. (1), the thicknesses of adsorbed LYS layers were calculated to be about 6 nm at 12 °C and 4 nm at 37 °C, respectively, which were larger than the size of LYS. Note that lysozyme has a dimension of 3 nm × 3 nm × 4.5 nm<sup>[33]</sup>. The adsorbed LYS formed a dense protein layer on gold surface with complete coverage. With the subsequent injection of poly(DEGMMA-co-MEA) microgels, the frequency of quartz crystal strongly decreased, indicating the strong adsorption of the microgels on the LYS layer. Again, the decrease of frequency was larger for the collapsed microgels at 37 °C (Fig. 6b). In swollen state at 12 °C, with the injection of poly(DEGMMA-co-MEA) microgels, a quick decrease of frequency was observed and the frequency reached its minimum value and then increased again to an equilibrium value.  $\Delta f_3/3$  of -43 Hz was obtained for M0 microgels adsorbed on LYS surface at the equilibrium state. However, 0 Hz frequency shift was obtained for M20 microgels adsorbed on LYS surface at 12 °C, which indicated that there was not adsorption for the swollen M20 microgels on LYS surface at equilibrium state. At 37 °C, a two-step adsorption processes was clearly identified for the adsorption of poly(DEGMMA-co-MEA) microgels (both M0 and M20 microgels) on LYS surface (Fig. 6b). In the first step, the frequency shift linearly decreased with the adsorption time. In the second step, a sharp and quick frequency decrease was clearly observed and the adsorption process then reached the equilibrium state. The frequency shifts ( $\Delta f_3/3$ ) were about -119 Hz for M20 microgels and -151 Hz for M0 microgels adsorbed on LYS layer at 37 °C, which were corresponding to the estimated thicknesses of about 21 nm and 26 nm, respectively. The results of Fig. 6 suggested that there was strong interaction between poly(DEGMMA-co-MEA) microgels and LYS. It is reasonable because LYS has a pI value of 11.1 and is positively charged in Tris-HCl buffer solution (pH = 7.4). However, the poly(DEGMMA-co-MEA) microgels was negatively charged in Tris-HCl buffer solution (pH = 7.4). The strong adsorption of poly(DEGMMA-co-MEA) microgels on LYS surface could be mainly attributed to the electrostatic interaction between the negatively charged microgels and positively charged LYS layers. The collapsed microgels had smaller surface area and hence higher negative surface charge density, which increased the electrostatic interaction between the collapsed microgels and LYS surface, leading to the larger adsorption of microgels at 37 °C. Furthermore, the incorporation of MEA into the microgels weakened the interaction of poly(DEGMMA-co-MEA) microgels and LYS, leading to the less adsorption of M20 microgels on LYS surface as compared to that of M0 microgels.

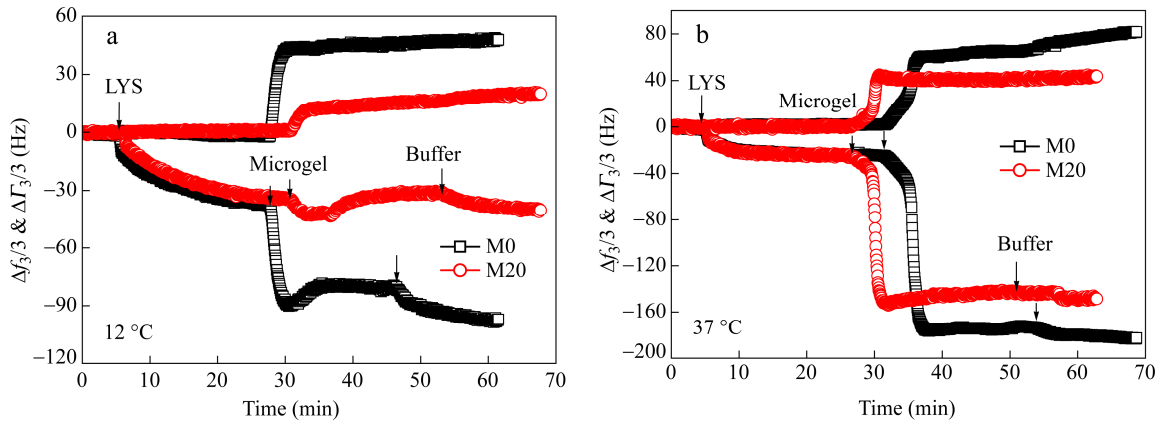


**Fig. 6** Frequency shift ( $\Delta f_3/3$ , solid symbol) and half-band-half shift ( $\Delta \Gamma_3/3$ , open symbol) of quartz crystal at 3<sup>rd</sup> overtone for the interaction of poly(DEGMMA-co-MEA) microgels and LYS as a function of time at (a) 12 °C and (b) 37 °C

The injection sequence was "microgel-after-protein".

The structure changes of poly(DEGMMA-co-MEA) microgels due to the interaction with the adsorbed LYS could be referred to the plot of  $\Delta \Gamma_3/3$  versus  $-\Delta f_3/3$  during the adsorption process of microgels on the adsorbed LYS layers. Figure 7 shows the plot of  $\Delta \Gamma_3/3$  versus  $-\Delta f_3/3$  for the adsorption of M0 and M20 microgels on the adsorbed LYS layers at 37 °C. Note that the resonance frequency and half-band-half width of quartz crystal with adsorbed LYS layers were taken as the new reference states. For M0 microgels, the  $\Delta \Gamma_3/3$  first increased linearly with  $-\Delta f_3/3$  with a slope ( $\Delta \Gamma_3/3$  vs  $-\Delta f_3/3$ ) of about 1.06 at the beginning of adsorption. The evolution of  $\Delta \Gamma_3/3$  was then slowly bent to a smaller slope of 0.29 and increased linearly with  $-\Delta f_3/3$  again. With the continuous adsorption of M0 microgels,  $\Delta \Gamma_3/3$  was bent to an even smaller slope of 0.17 and increased linearly with  $-\Delta f_3/3$  before the rinsing step. For M20 microgels,  $\Delta \Gamma_3/3$  also increased linearly with  $-\Delta f_3/3$  with a slope of  $\sim 1.06$  at the early stage of adsorption. Similarly, the evolution of  $\Delta \Gamma_3/3$  was slowly bent to a smaller slope of 0.3 and again increased linearly with  $-\Delta f_3/3$  before the rinsing step. These results indicated that the viscoelastic properties of the adsorbed poly(DEGMMA-co-MEA) microgels were changed due to the interaction with LYS. The decrease of slope ( $\Delta \Gamma_3/3$  vs  $-\Delta f_3/3$ ) suggested that the adsorbed poly(DEGMMA-co-MEA) microgels increased their rigidity during the adsorption process. It was understandable because the strong electrostatic interactions between the negatively charged microgels and the positively charged LYS layers might cause the adsorbed microgels to be denser in the contact with LYS layers with increasing contact times, leading to the increase of rigidity and the decrease of  $\Delta \Gamma_3/3$  value.

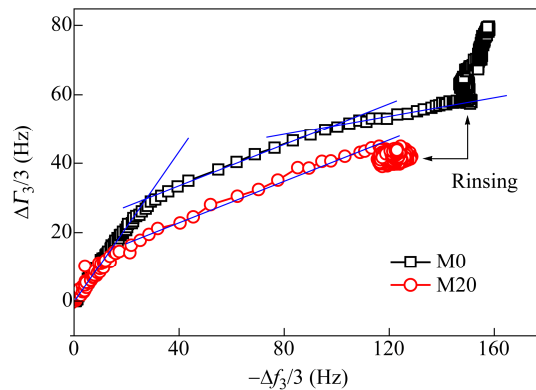


**Fig. 7** Plots of half-band-half width shift ( $\Delta \Gamma_3/3$ ) versus negative frequency shift ( $-\Delta f_3/3$ ) for the adsorption of M0 and M20 poly(DEGMMA-co-MEA) microgels on the adsorbed LYS layers at 37 °C (The solid lines were the guides to the eyes.)

## CONCLUSIONS

The poly(DEGMMA-co-MEA) microgels were thermo-sensitive and exhibited a volume phase transitive temperature (VPTT) of 14 °C–22 °C. The incorporation of hydrophobic comonomer MEA shifted the VPTT of poly(DEGMMA-co-MEA) microgels to lower temperatures. QCM results indicated that the interfacial interaction of poly(DEGMMA-co-MEA) microgels and the adsorbed proteins was mainly dominated by the electrostatic interaction between the microgels and the proteins. Due to the negative charge of poly(DEGMMA-co-MEA) microgels in Tris-HCl buffer solution (pH = 7.4), the microgels did not adsorb on the negatively charged FIB and BSA layers but strongly adsorbed on the positively charged LYS layers. The incorporation of MEA weakened the interaction between poly(DEGMMA-co-MEA) microgels and LYS. The electrostatic interaction between poly(DEGMMA-co-MEA) microgels and LYS led to the increase of rigidity for the adsorbed microgels during the adsorption process.

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