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A pH-responsive, low crosslinked, molecularly imprinted insulin delivery system

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ABSTRACT

A new type of insulin delivery system capable of better self-regulating the release of insulin was reported in this study. This insulin delivery system was made of a low crosslinked insulin-imprinted hydrogel that exhibited pH-dependent interpolymer interactions between poly(methacrylic acid) (PMAA) and poly(ethylene glycol) (PEG). At acidic conditions (such as pH 3.5), this delivery system resembled a highly crosslinked imprinted hydrogel and demonstrated a relatively slow release due to the formation of the PMAA-PEG complexes, which significantly increased physical crosslinking within the hydrogel interior and largely fixed the imprinted networks. On the contrary, at neutral or basic conditions (such as pH 7.4), this delivery system was comparable to a non-imprinted hydrogel and caused a rapid release resulting from the dissociation of the PMAA-PEG complexes. Unlike previously reported non-imprinted hydrogels and highly crosslinked imprinted polymers, which lack either molecular recognition ability or switchable imprinted networks, this unique insulin delivery system was composed of tunable and low crosslinked imprinted networks, which thereby enabled better self-regulation of insulin delivery.

Keywords: Molecular imprinting; hydrogels; controlled release; insulin

Songjun Li, one of editors-in-chief of Advanced Materials Letters, is a professor of Chemistry. He did PhD from the Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, China in 2005. Subsequently, he was appointed by Central China Normal University (CCNU) as an associate professor with research field in Physical Chemistry. He doubles also as an invited professor in the University of Jinan, China with interest in Analytic Chemistry. From 2005 to 2008, he has worked as the deputy head in the Chemical Experimental Center of CCNU. He had had also one year's working experience at the University of Wisconsin, USA as a post-doctoral fellow from Sep. 2008 to Aug. 2009. Now, he is working in the Cranfield University, UK as the Marie Curie Incoming Fellow. During the past 10 years, he, as the PI and first author excluding non-PI and co-authors, has published about 40 papers in international peer-reviewed journals. He has also edited four books in prestigious publishers typically as in Wiley-VCH, Germany, Bentham Science, USA and Nova Science, USA, etc. He has been the editorial member for American Journal of Environmental Sciences, International Journal of Physical Sciences, Journal of Public Health and Epidemiology, the Open Electrochemistry Journal and Journal of Computational Biology and Bioinformatics Research. He has been invited beyond 100 times to be the reviewer for international journals. His recent interest is focused on designing and developing novel, highly substrate-selective catalysts with molecular imprinting.

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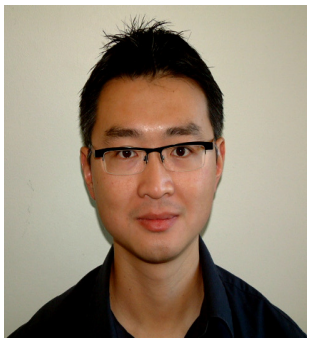

Yi Ge, Associate editor of Advanced Materials Letters, obtained his bachelor's in Biopharmaceutics and went on to an MPhil in Pharmaceutical Chemistry at the Aston University, UK. In 2000, he moved to the University of Sheffield for a PhD in Chemistry. He was employed as a research scientist in a UK pharmaceutical company and was then a postdoctoral research associate at the Imperial College London, UK before joining Cranfield Health at Cranfield University, UK as a member of academic staff in 2006. One of his expertise areas is the design, synthesis and characterization of polymeric materials, particularly those with novel structural and functional features, such as molecularly imprinted polymers, hyperbranched polymers, and bio-conjugated polymers, and their practical applications across Chemistry and Biology.

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Introduction

There is much interest in the controlled release of insulin because of its importance in treating diabetes [**1,2**]. Approximately 20 million people have diabetes in the United States alone [**3**]. In usual cases, insulin is administered to the patients with a twice-daily injection treatment. This is a relatively painful process that encourages patient non-compliance. Hence, there is a need to find new ways to administer insulin with less pain. Prominent among the ongoing effort is to develop oral hydrogel delivery systems [**4**]. Compared with the conventional injection treatment, significant advantages in the hydrogels-based delivery are the slow release of drugs because of the interaction between the encapsulated drugs and hydrogel networks. Thus, the release profile of drugs is usually lagged behind compared with that of the injection treatment. For the release of insulin, such a lagging effect will allow more insulin molecules to be delivered to favorable regions within the gastrointestinal tract [**5**]. As such, the efficacy of dosage is improved. However, the main drawback in such hydrogel systems is that the significant leakage of insulin in stomach occurs during the delivery process. The concomitant inactivation of insulin is usually caused by the digestive enzymes in stomach [**6**]. To overcome this drawback, Peppas et al. [**7**] and Kumar et al. [**8**] have suggested to use poly(methacrylic acid-*g*-ethylene glycol) (P(MAA-*g*-EG)) as the 'smart' carrier for the controlled release of insulin. In the acidic environments of stomach, the P(MAA-*g*-EG) hydrogels were unswollen, preventing the encapsulated insulin from proteolytic degradation. On the contrary, in the neutral and/or basic environments of intestine, the P(MAA-*g*-EG) hydrogels were swollen, causing the rapid release of insulin. As such, the inactivation of insulin by the digestive enzymes in stomach was largely inhibited. However, two important problems are still remained behind the P(MAA-*g* -EG)-based systems: (1) such delivery systems essentially lack molecular recognition ability for insulin. Thus, it is difficult to efficiently self-regulate the release of insulin [**9**]; and (2) the reloading of insulin in such systems is usually a result of the nonspecific adsorption of insulin, which is not necessarily helpful for the efficacy of dosage. Thus, the development of new types of insulin delivery systems with more sophisticated designs is greatly needed.

Known as a 'from-key-to-lock' technology, molecular imprinting provides a promising option for the development of novel delivery systems [**10,11**]. This technology makes molecularly imprinted polymers (MIPs) capable of highly recognizing their imprint species (i.e., their template molecules). To fabricate the MIP, template and functional monomers are first allowed to form a self-organized architecture via molecular self-assembly. Polymerization with a high content of crosslinker (up to ~70 mol.%) is then performed to fix the self-organized architecture. The imprinted template is subsequently removed from the highly crosslinked matrix, leaving behind the template's memory [**12**]. Thus, using this principle, one may expect that a new type of insulin delivery system capable of better self-regulating the release of insulin can be developed. Nevertheless, as the delivery carriers capable of tunable networks, hydrogel systems usually contain a crosslinking degree as low as 5-10 mol.% [**13,14**]. For the preparation of MIPs, such a low crosslinking degree will significantly decrease the molecular recognition ability. Thus, additional physical or post-polymerized crosslinking methods have to be used to prop up and fix the imprinted networks [**15,16**]. The fixation of the imprinted networks makes the prepared hydrogel capable of specific recognition for the template

drugs. On the contrary, a deformation and distortion of the imprinted networks causes loss of molecular recognition ability and accordingly accelerate the release of the encapsulated drugs. In this way, molecularly imprinted hydrogels are capable of better self-regulating the release of the encapsulated drugs. As such, the self-regulation of access in this new insulin delivery system is significantly improved as compared with that in conventional hydrogel systems. Furthermore, using molecular imprinting, the reloading of insulin is essentially a result of the specific adsorption of insulin, which thereby leads to the increased efficacy of dosage.

To the best of our knowledge, for the first time, a smart molecularly imprinted hydrogel system (namely "MIHG-R") for insulin delivery was developed here (cf. **Scheme 1**). This delivery system was composed of a low crosslinked (8.0 mol.%) insulin-imprinted hydrogel made of poly(methacrylic acid) (PMAA) and poly(ethylene glycol) (PEG). At acidic conditions, the formation of the PMAA-PEG complexes significantly increased physical crosslinking within the hydrogel networks, fixing the imprinted networks and largely inhibiting the release of insulin. On the contrary, at neutral and basic conditions, the dissociation of the PMAA-PEG complexes caused a deformation and distortion of the imprinted networks, which accelerated the release of insulin. In this way, this novel insulin delivery system was capable of more pH-sensitively self-regulating the release of insulin.

For comparison, two control hydrogels (namely "MIHG" and "NIHG-R") were also prepared under comparable conditions (cf. **Table 1**). The MIHG is a highly crosslinked insulin-imprinted hydrogel and does not have significant interpolymer interactions present within its networks. The NIHG-R is the conventional pH-sensitive non-imprinted hydrogel. During the release process, both MIHG and NIHG-R in principle may provide border conditions for MIHG-R, which thereby significantly promoted this study. The objective of this study is to demonstrate that the new generation of insulin delivery system capable of better self-regulation can be fabricated using this novel design.

Experimental

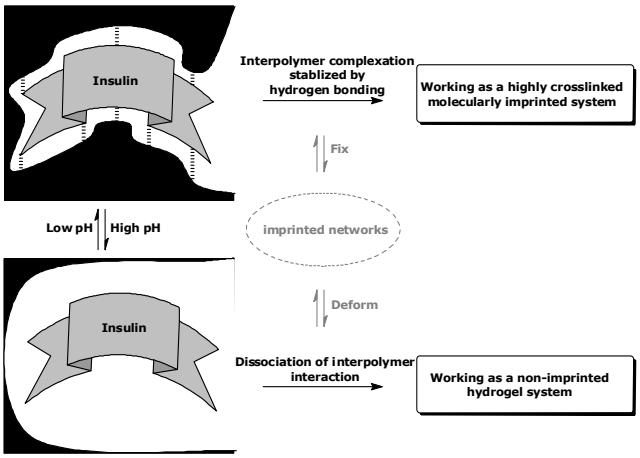
Preparation of hydrogels

The chemicals available from Sigma-Aldrich were of analytic grade and used as received, except methacrylic acid (MAA), which was freshly distilled prior to use. In triplicate (cf. **Scheme 2**), the template (crystalline porcine insulin), monomers (MAA and PEG(200) monomethacrylate, in a 1:1 ratio of MAA and EG-unit), crosslinker (N,N'-methylenebisacrylamide (MBA)), initiator (ammonium persulfate (APS)) and accelerator (N,N,N',N'-tetramethylethylene diamine (TMEDA)) were dissolved in diluted HCl solution (1μmol L^{-1}; 20 mL) to form a homogeneous system. After being deoxygenated with sonication under nitrogen, the mixture was irradiated with ultraviolet light (365 nm) overnight. The resulting hydrogel was sliced up and extensively washed with water to remove the imprinted insulin. The prepared hydrogel sample (viz., MIHG-R) was subsequently dried in a vacuum vessel at room temperature.

The two controls, i.e., MIHG and NIHG-R, were also prepared under comparable conditions (cf. **Table 1**). The MIHG was prepared as the MIHG-R except that MAA was replaced with the same amount of MBA, in order to achieve the highly crosslinked networks. The NIHG-R was the pH-sensitive non-imprinted hydrogel and therefore no template was used during the preparation process.

FTIR spectra

The FTIR spectra of the prepared hydrogel samples were studied using a FTIR apparatus (Nicollet MX-1E, USA). The scanned number was 100 cycles and the scanned range was from 550 to 4000 cm^{-1}.



Scheme 1. Proposed mechanism for the modulated release by MIHG-R.

Swelling behavior

The swelling of the prepared hydrogel samples was studied at room temperature using a batch format. In triplicate, dried samples of these hydrogels were immersed into various pH solutions (phosphate-citrate buffer solution (PCBS)) for 8 h. After blotting up the water adhered on the surface, these wet samples were weighed (W_t) and then dried in a vacuum vessel up to a constant weight (W_d). The swelling ratio was calculated using the equation as follows and finally the average of the triplicate runs was reported:

$$S = \frac{W_t - W_d}{W_d} \times 100\%$$

Dynamic desorbing cyclic voltammetry

The potential to reduce/oxidize a bound molecule depends on the binding constant. A high binding constant requires more energy to overcome the binding, thereby causing a larger redox potential. Thus, dynamic desorbing cyclic voltammetry (DCV) can provide valuable information on the binding behavior between the prepared polymers and insulin [**17,18**]. Using an electrochemical workstation equipped with a three-electrode configuration (Pt-working and counter electrodes; Ag/Ag^{+}-ref.) (CHI-

600, USA), the hydrogel samples (20 mg) that pre-adsorbed with about 60μg insulin were placed into a cuvette encircled by a diffusion-eliminated sonication apparatus (supporting electrolyte: 0.01 mmol mL^{-1} KNO_3; 10 mL of PCBS solutions). The transiently desorbed insulin were rapidly scanned by the workstation up to 20 cycles until the stable DCV diagram was reached (scanning range, -50 ~ -1000 m*V*; scanning rate, 25 m*V* · s^{-1}).

Table 1. Synthesis composition of hydrogels.

Composition	MIHG-R	MIHG	NIHG-R
Insulin	10 mg	10 mg	0 mg
MAA	1.29 g (15 mmol)	0 g	1.29 g (15 mmol)
PEG(200) monomethacrylate	1.55 g (15 mmol EG-unit)	1.55 g (15 mmol EG-unit)	1.55 g (15 mmol EG-unit)
MBA	0.24 g (1.56 mmol)	1.53(i.e., 0.24+1.29)g (9.92 mmol)	0.24 g (1.56 mmol)
APS	0.4 g	0.4 g	0.4 g
TMEDA	0.1 g	0.1 g	0.1 g

Release behavior

The release behavior of the prepared hydrogels was studied at room temperature. To avoid the effect of unspecific sorption, the amount of insulin uploaded into these hydrogels was precisely controlled based on the proportion of insulin used during the preparation of the hydrogels as shown in **Table 1** (except that monomers were replaced with the same amount of hydrogels). Subsequently, in triplicate, each hydrogel system (0.06 g, dried state) was placed into the PCBS solution (1 mL). The amount of insulin released over time was determined with a HPLC. The average of the triplicate runs was reported.

Results and discussion

FTIR and morphology analysis

In order to confirm the imprinting behavior, **Fig. 1** presents the FTIR spectra of the prepared hydrogel samples. Four main absorption bands (3100-3400, 2800-3000, ~1750, and 950-1100 cm^{-1}) appeared in these spectra. In basic conditions [**19**], these absorption bands may be attributed to the stretching of O-H/N-H, C-H, C=O, and C-C, respectively. For comparison, the spectra of insulin and the MIHG-R precursor (i.e., the MIHG-R system where the imprinted insulin had not yet been removed from the polymeric matrix) were also included in **Fig. 1**. The MIHG-R precursor displayed the absorption band of insulin at 1400-1600 cm^{-1}, which was probably attributed to the C-N and C=N containing in insulin [**20**]. After washing, the spectrum of the resulting hydrogel (i.e., MIHG-R) became comparable to that of NIHG-R. These results suggest that the imprinting of insulin did occur during the preparation process, as expected.

In order to further confirm the imprinting behavior, **Fig. 2** presents the digital images of these prepared hydrogels. The template insulin was the white powder (**a**). The MIHG-R (**c**), MIHG (**d**), and NIHG-R (**e**) were yellow and gel-like in morphology. Although the MIHG-R precursor (**b**) displayed also gel-like in morphology, it was greater white largely due to the presence of the white insulin molecules in the yellow MIHG-R networks. Associated with the preparation of these hydrogels (cf. **Scheme 2**), this result reflects again a consequence of insulin imprinting.

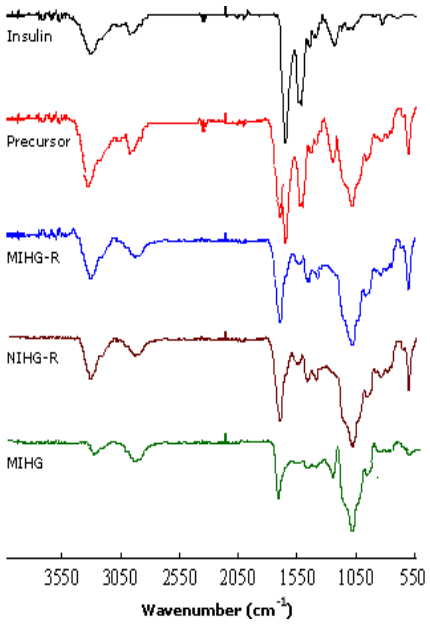


Fig. 1. FTIR spectra of the prepared hydrogel samples.

Swelling behavior

Fig. 3 presents the swelling curves of the prepared hydrogels. The MIHG demonstrated a relatively low and pH-independent swelling ratio, probably due to the highly crosslinked polymer networks and its non-pH-sensitive composition. By contrast, the MIHG-R and NIHG-R demonstrated a much larger dependence on the pH condition. The significant increased swelling in both MIHG-R and NIHG-R occurred at ~ pH 5.5, which was consistent with the pK_a of PMAA [**21**]. Below pH 5.5, both MIHG-R and NIHG-R showed a relatively low swelling ratio. Conversely, above pH 5.5, both MIHG-R and NIHG-R showed a much higher swelling ratio. This result, as previously explained, can be attributed to the inter polymer interaction between PMAA and PEG. The lower swelling ratio observed below pH 5.5 may be due to the formation of the PAAM-PEG complexes, which largely inhibited the access of water to the hydrogel networks. Above pH 5.5, the dissociation of carboxylic protons from PMAA essentially relaxed the inter polymer interaction, thereby allowing access to the hydrogel interior. As a result, both MIHG-R and NIHG-R showed a much higher swelling ratio at higher pH values.

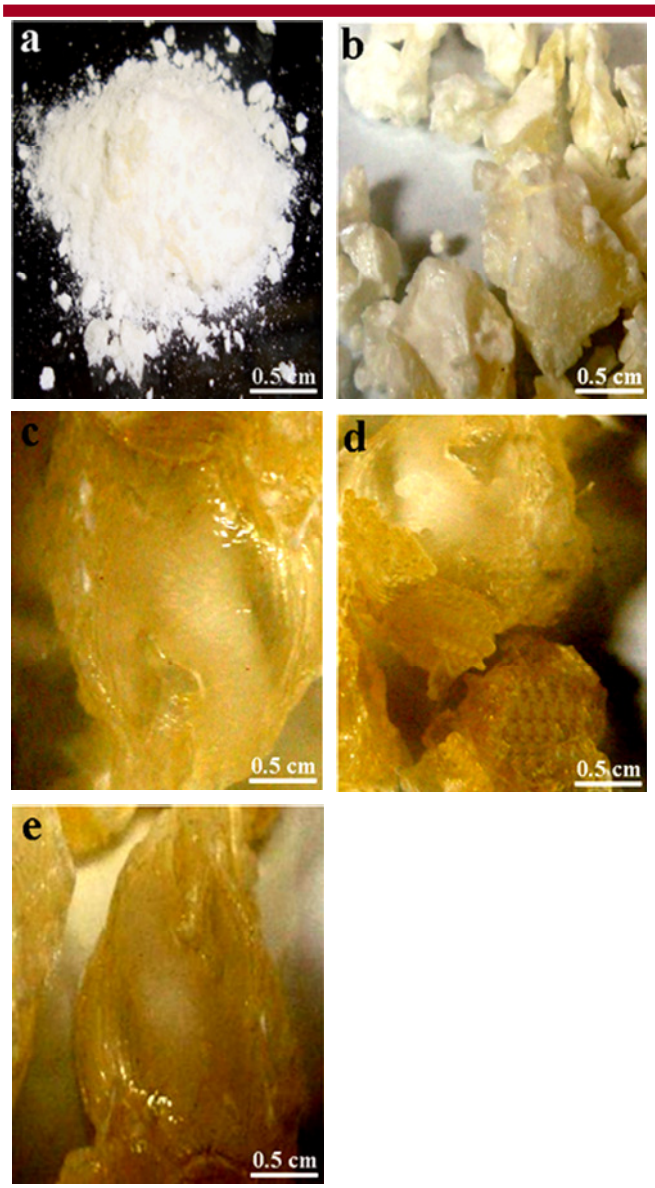
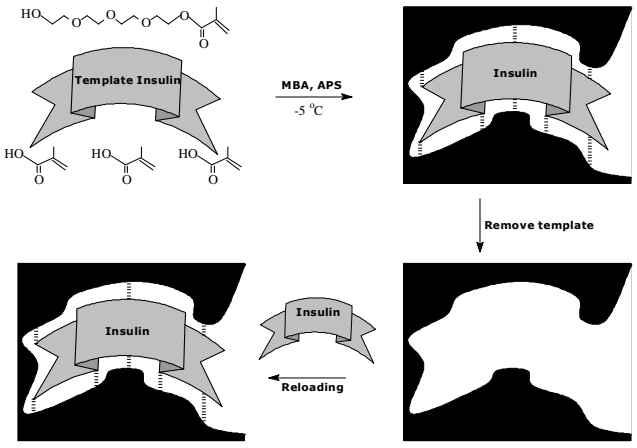


Fig. 2. Digital images of the prepared hydrogel samples. (a: insulin; b: MIHG-R precursor; c: MIHG-R; d: MIHG; e: NIHG-R).

Interaction between hydrogels and insulin

In order to further study the inter polymer interaction, **Fig. 4** presents the DCV diagrams of free insulin and the insulin bound by MIHG-R. For comparison, two typical pH values, i.e., 3.5 and 7.4 (either lower or higher than the observed transitional value of MIHG-R (i.e., pH 5.5), were selected. At pH 3.5, the free insulin demonstrated an oxidation peak at 648.7 m*V*, while the insulin bound by MIHG-R exhibited an oxidation peak at 709.8 m*V*. Thus, the binding interaction between MIHG-R and insulin at pH 3.5 increased the oxidation potential by 61.1 m*V*. Under comparable conditions, the binding interaction between MIHG-R and insulin at pH 7.4 only increased the oxidation potential by 5.1 m*V*. The binding interaction between MIHG-R and insulin appeared to be stronger at pH 3.5 than at pH 7.4.

The oxidation potentials of the insulin bound by both controls along with that by MIHG-R were listed in **Table 2**, in order to ascertain the binding interaction between MIHG-R and insulin. At pH 3.5, the oxidation potential of the insulin bound by MIHG-R was nearly comparable to that bound by MIHG (709.8 vs. 714.9 m*V*). On the contrary, at pH 7.4, the oxidation potential of the insulin bound by MIHG-R became almost as low as that by NIHG-R (614.4 vs. 611.1 m*V*). This result strongly suggested that the binding interaction between MIHG-R and insulin was highly specific and pH-tunable.



Scheme 2. Technical outline for the preparation of MIHG-R.

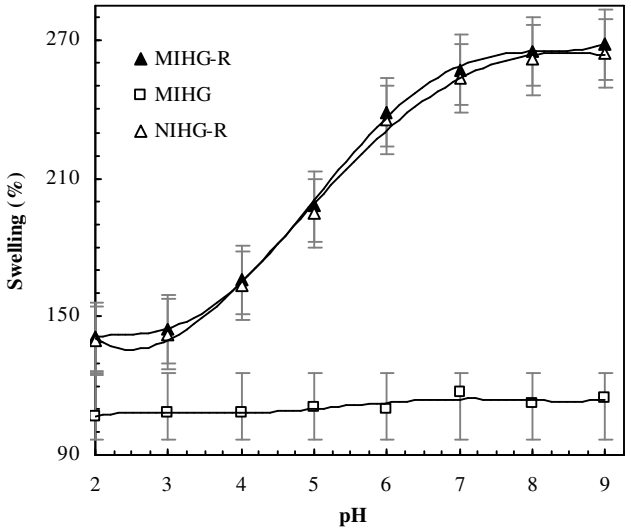


Fig. 3. Swelling curves of the prepared hydrogels.

As further noted from pH 3.5 to pH 7.4 (cf. **Table 2**), the increase of pH values led to the decrease of oxidation potential by 39.4, 95.4, 40.3, and 66.2 mV, respectively. Thus, the binding interaction between these prepared hydrogels and insulin actually decreased by 56, 1.9 and 26.8 mV. The binding interaction between MIHG-R and insulin demonstrated the strongest dependence on the change of pH values. By contrast, the highly crosslinked MIHG system was largely independent from the change of pH values. This result further confirmed that the specific and pH-tunable interaction between MIHG-R and insulin was essentially a result of the pH-sensitive imprinted

networks. As previously explained, at pH 3.5 the specific interaction between MIHG-R and insulin may be attributed to the formation of the PMAA-PEG complexes, which significantly increased physical crosslinking within the hydrogel interior and largely fixed the imprinted networks. On the contrary, at pH 7.4, the dramatically decreased interaction between MIHG-R and insulin can be due to the dissociation of the PMAA-PEG complexes, which distorted the imprinted networks and thereby caused loss of molecular recognition ability.

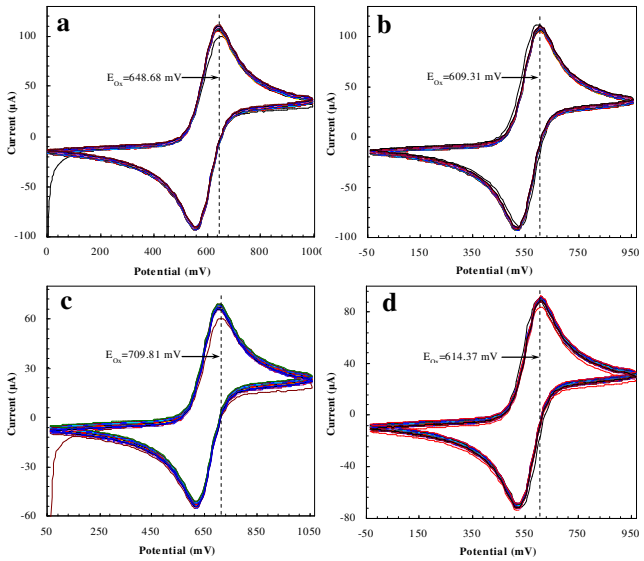


Fig. 4. DCV diagrams of the free insulin and that binding to MIHG-R. (*a*: free insulin at pH 3.5; *b*: free insulin at pH 7.4; *c*: binding insulin at pH 3.5; *d*: binding insulin at pH 7.4)

Table 2. Oxidation potentials (mV) of insulin binding to the prepared hydrogels.

System	pH 3.5	pH 7.4	Effect of pH
Free insulin	648.7	609.3	39.4
Insulin-MIHG-R	709.8	614.4	95.4
Insulin-MIHG	714.9	673.6	41.3
Insulin-NIHG-R	677.3	611.1	66.2

Release behavior

Fig. 5a and **b** present the release of insulin from these prepared hydrogels. In order to monitor the pH-regulated release, two typical pH values i.e., 3.5 and 7.4, were selected again for a contrastive study. The MIHG system demonstrated the similar release at both pH 3.5 and pH 7.4. By contrast, the MIHG-R and NIHG-R systems demonstrated a lower release of insulin at pH 3.5 than at pH 7.4. Compared with NIHG-R, the MIHG-R system demonstrated a stronger pH-dependent release. At pH 3.5, the MIHG-R resembled the MIHG and demonstrated a low release of insulin. On the contrary, at pH 7.4, the MIHG-R became approximately comparable to the NIHG-R and demonstrated a rapid release of insulin. This result suggested that the MIHG-R did demonstrate pH-regulated release. As specially noted in **Fig. 3**, the MIHG-R and NIHG-R did not demonstrate significant differences in the swelling ratio, probably due to their same chemical composition (cf. **Table 1**). Thus, the pH-regulated release by MIHG-R should be attributed to the tunable imprinted networks, as expected. Again, this result indicated that the tunable imprinted networks did play the exact role on regulating the release behavior of insulin.

Insulin reloading

With a low crosslinking degree, the removal and reloading of drugs from molecularly imprinted hydrogels may distort and deform the imprinted networks [**22,23**]. Thus, drug removal and reloading may affect the release behavior. **Fig. 6a** and **6b** present the effect of insulin removal and reloading on the release profile, in order to further study the pH-regulated mechanism. At pH 3.5, the MIHG-R system subject to insulin removal and reloading demonstrated a faster release as compared with its precursor system (i.e., the MIHG-R system where the imprinted insulin had not yet been removed). Conversely, at pH 7.4, the MIHG-R system subject to insulin removal and reloading demonstrated the release behavior as in its precursor system. Associated with the nature of MIHG-R, it appeared that molecular recognition by the MIHG-R was propped up by the weak interactions that can be modulated by the pH condition. This result was fully consistent with the interpolymer interaction between PMAA and PEG. The association and dissociation between PMAA and PEG may induce significant changes in the physical crosslinking with the hydrogel interior, which thereby fixed/distorted the imprinted networks. Again, this result suggests that better self-regulated release by the MIHG-R is essentially a result of the tunable imprinted networks.

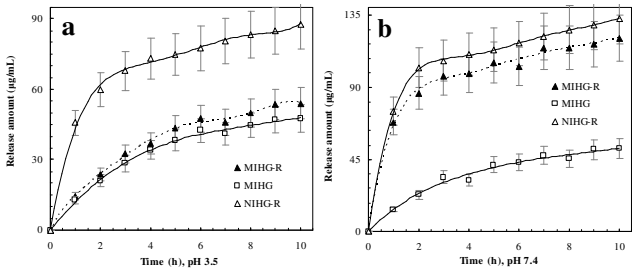


Fig. 5. Release profiles of insulin from the prepared hydrogels (a: pH 3.5; b: pH 7.4).

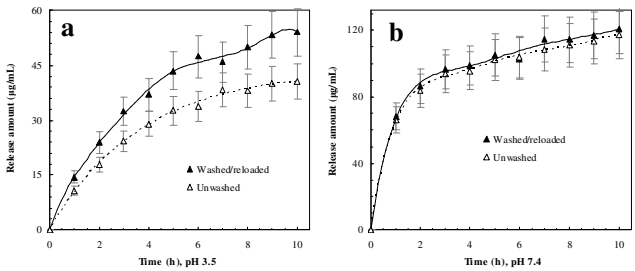


Fig. 6. Effect of insulin removal and reloading on the release behavior (a: pH 3.5; b: pH 7.4).

Conclusion

A new type of insulin delivery system capable of better self-regulating the release of insulin was developed. This delivery system was composed of a low crosslinked insulin-imprinted hydrogel made of PMAA and PEG. Because of the unique interpolymer interaction between PMAA and PEG, this delivery system can resemble either a highly crosslinked imprinted hydrogel or a non-imprinted delivery system. The use of the low chemical crosslinking allowed for tunable imprinted networks. This design indicates that the novel insulin delivery system capable of better self-regulating the release of insulin can be fabricated using the low-crosslinked molecular imprinting strategy.

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