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Preparation of avidin-containing polyelectrolyte microcapsules and their uptake and release properties

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Abstract Avidin-containing polyelectrolyte microcapsules were prepared by a layer-by-layer deposition technique and uptake and release of biotin-labelled fluorescein (b-FITC) was studied. The polyelectrolyte microcapsules were prepared by coating the surface of calcium carbonate ($CaCO_3$) microparticles containing avidin-poly(styrene sulfonate) (PSS) conjugate, followed by dissolution of $CaCO_3$ core in an ethylenediaminetetraacetic acid solution. Release of avidin from the microcapsules was markedly suppressed due to formation of a high molecular weight of avidin-PSS conjugate in the microcapsules. The uptake of b-FITC into the microcapsules was highly enhanced through a strong binding of b-FITC to avidin, as compared to the uptake into avidin-free microcapsules. Release of b-FITC from the microcapsules was accelerated upon addition of biotin, 2-iminobiotin, or lipoic acid in the solution due to the competitive binding of the additives to the binding site of avidin.

Keywords Polyelectrolyte microcapsule · Avidin · Biotin · Uptake and release · Fluorescein

Introduction

Layer-by-layer (LbL) thin films have extensively been studied for developing functional films and coatings. LbL thin films can be prepared by an alternate deposition of two kinds of polymers through the electrostatic force of attraction, hydrogen bonding and biological affinity [1–6]. The materials employed for this purpose include synthetic polymers [3], proteins [4, 5] and polysaccharides [6]. LbL

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films have been applied to separation and purification [7], biosensors [8, 9] and controlled release [10–12].

Recently, polyelectrolyte microcapsules have been constructed by coating the surface of a microparticle core with LbL film followed by dissolution of the core material [13–18]. The polyelectrolyte microcapsules thus prepared have found applications to sensors and delivery devices. In this context, we have developed microcapsules by coating the surface of concanavalin A (Con A)-loaded calcium carbonate ($CaCO_3$) microparticles with LbL film composed of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) followed by dissolution of the $CaCO_3$ core in ethylenediaminetetraacetic acid (EDTA) solution [17]. The microcapsules thus prepared could be used for the fluorometric detection of sugars through sugar-Con A interactions. Insulin-containing microcapsules have also been developed by using Con A for controlled release of insulin in response to glucose [18].

The present study reports the preparation of polyelectrolyte microcapsules containing avidin and their uptake and release properties for biotin-labelled fluorescein (b-FITC). Avidin is a protein found in egg white and is known to strongly bind biotin and analogues [19]. In fact, we have found that b-FITC can be successfully encapsulated in the microcapsules through avidin–biotin interactions and is released effectively in the presence of biotin and analogues.

Experimental section

Materials

Poly(sodium styrenesufonate) (PSS; average molecular weight (MW), ~500,000) was obtained from Scientific Polymer Products, Inc. (NY, USA). An aqueous solution (20%) of poly(allylamine hydrochloride) (PAH; MW, ~150,000) was purchased from Nittobo Co. (Tokyo, Japan). Avidin, tetramethylrhodamine-labelled avidin (TRITC-avidin), and b-FITC were commercially available products. All other reagents used were of the highest grade and used without further purification. Chemical structures of PSS, PAH and b-FITC were illustrated in Fig. 1.

Apparatus

Optical density and fluorescence spectra were measured with UV–visible spectrophotometer (UV-3100PC, Shimadzu Co., Japan) and fluorescence spectrophotometer (FL-5300PC, Shimadzu Co., Japan), respectively. Optical and fluorescence microscope images were recorded on CBMT-15 (Carton Co.) and IX-70 (Olympus Co.).

Preparation of avidin-loaded microcapsules

Calcium carbonate ($CaCO_3$) microparticles as core material for constructing microcapsules were prepared according to the reported procedure with a slight

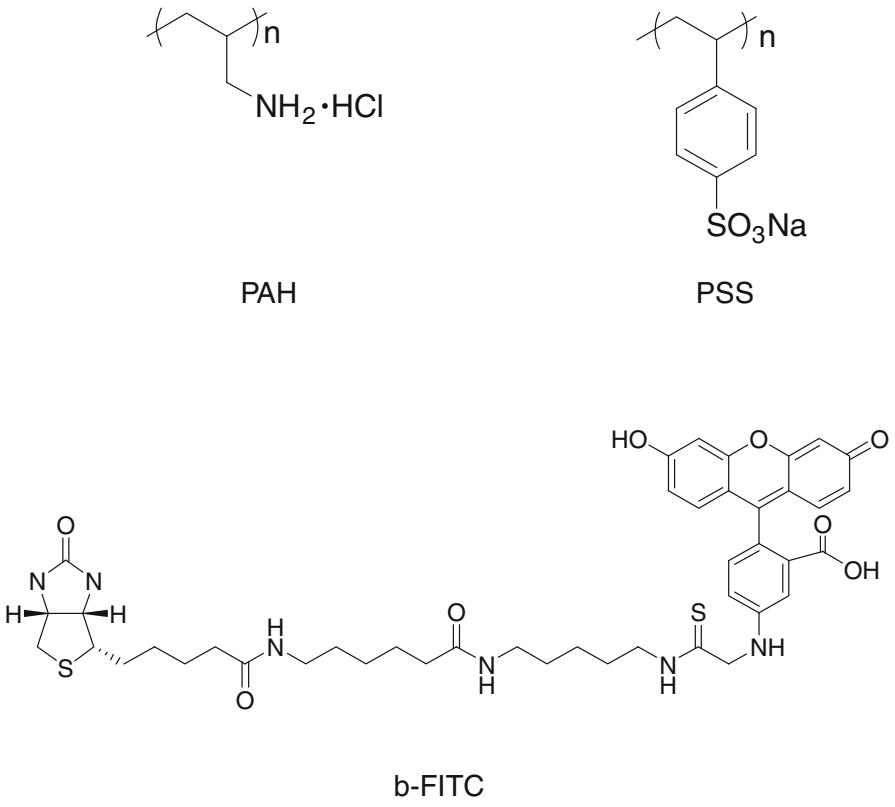

Fig. 1 Chemical structures of PAH, PSS and b-FITC

modification [17, 18]. Briefly, an avidin-PSS conjugate was prepared by mixing 5 mL of solutions of avidin and PSS (0.1 mg/mL, 0.1 M Tris–HCl buffer at pH 7.4) and the solution was left standing for 30 min, and then $CaCl_2$ (111 mg) was added. PSS was used for electrostatically encapsulating positively charged avidin in $CaCO_3$ particles. The resulting mixture was added into 5 mL of aqueous solution containing $(NH_4)_2CO_3$ (96 mg) and PSS (20 mg). The mixture was stirred for 30 min and resulting microparticles were collected by centrifugation and washed with water. The size of $CaCO_3$ particles thus prepared was 5–10 μm. The whole amounts of $CaCO_3$ particles collected were dispersed in a PAH solution (1 mg/mL, 0.1 M Tris–HCl buffer at pH 7.4) under gentle stirring for 15 min to deposit a first PAH layer on the surface of the $CaCO_3$ particles. The PAH-coated $CaCO_3$ particles were collected and washed in the working buffer. Then, the $CaCO_3$ particles were dispersed in a PSS solution (1 mg/mL, 0.1 M Tris–HCl buffer at pH 7.4) in a similar manner to deposit a second PSS layer on the surface. The alternate deposition of PAH and PSS was repeated 5 times to prepare 5-bilayer $(PAH\text{-}PSS)_5$ film on the $CaCO_3$ particles. The ζ-potential of the LbL film-coated $CaCO_3$ particles was measured after each deposition of the film for 10 particles in 10 mM NaCl solution (pH 7.0), using

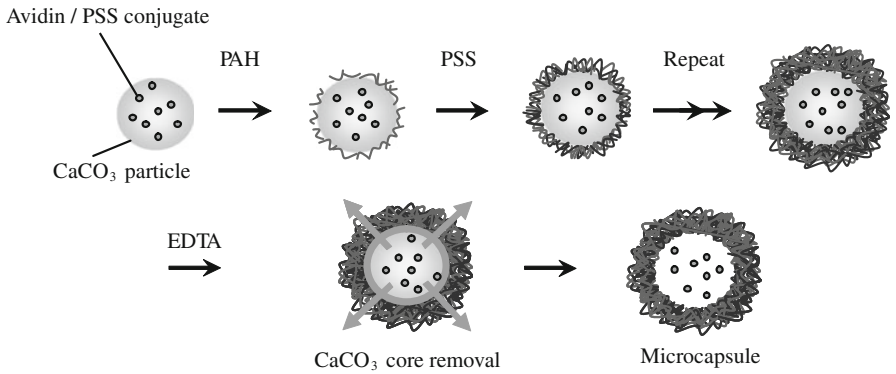

Fig. 2 A procedure for the preparation of polyelectrolyte microcapsules containing avidin-PSS conjugate

a ζ-potential analyzer (ZEECOM/ZC-2000, Microtec Co., Funabashi, Japan). The $(PAH\text{-}PVS)_5$ film-coated $CaCO_3$ particles were then dispersed in 0.2 M ethylenediaminetetraacetic acid (EDTA) solution for 5 min to dissolve $CaCO_3$ core. The EDTA treatment was repeated 5 times. The hollow microcapsules thus prepared were kept in 0.1 M Tris–HCl buffers before use. Figure 2 illustrates the procedure for the preparation of microcapsules.

Release of avidin from the microcapsules

Release of avidin from the microcapsules was evaluated by fluorescence method using TRITC-avidin. Microcapsules containing avidin-PSS conjugate were dispersed in 0.1 M buffer (pH 4.0, 7.0 and 9.0) in an optical cell (10 × 10 mm) for fluorescence measurements and gently stirred. The concentration of the microcapsules was adjusted so that the optical density is 0.9 at 600 nm. The solution was centrifuged for every 15 min and fluorescence intensity of the supernatant was measured at 580 nm to estimate the concentration of TRITC-avidin released. It was separately ascertained that the microcapsules are not decomposed under stirring during the measurements.

Uptake of b-FITC into microcapsule and its release

Microcapsules containing avidin-PSS conjugate were dispersed in 0.1 M Tris–HCl buffer (pH 7.0) containing 1 μM b-FITC in an optical cell (10 × 10 mm) for fluorescence measurements (OD at 600 nm, 0.9). The solution was gently stirred and centrifuged for every 15 min to record the fluorescence intensity of the supernatant at 520 nm. For evaluating release of b-FITC from the microcapsule, b-FITC-bound microcapsules were dispersed in 0.1 M Tris–HCl buffer (pH 7.0) in the optical cell (10 × 10 mm) (OD at 600 nm, 0.9). The release of b-FITC was monitored by recording fluorescence intensity in the supernatant in a similar manner. All measurements were carried out at ambient temperature (ca. 20 °C).

Results and discussion

Preparation of microcapsules containing avidin-PSS conjugate

Prior to the preparation of microcapsules, we have evaluated the amount of avidin encapsulated in the microparticles using TRITC-avidin in place of avidin. The $CaCO_3$ microparticles prepared were dissolved in diluted HCl solution to evaluate the amount of TRITC-avidin and PSS. Figure 3 shows the efficiency in the encapsulation of TRITC-avidin in the $CaCO_3$ microparticles as a function of the concentration of TRITC-avidin in the feeding solution used for $CaCO_3$ preparation. About 30, 18 and 9% of total amount of TRITC-avidin used were included in the $CaCO_3$ microparticles when the TRITC-avidin concentration was 0.1, 0.5 and 1.0 mg/mL. Judging from the results, we used 0.1 mg/mL TRITC-avidin solution for preparing $CaCO_3$ microparticles throughout this study. In fact, 3 mg of TRITC-avidin and 110 mg of PSS were encapsulated per 1 g of $CaCO_3$ microparticles.

Figure 4 shows an optical microscope image of TRITC-avidin-containing $CaCO_3$ particles and fluorescence microscope images of TRITC-avidin-containing $CaCO_3$ particles and TRITC-avidin-containing $(PAH/PSS)_5$ micorcapsules. The microscope image exhibits fluorescence emission originating from the labelled TRITC, confirming successful encapsulation of TRITC-avidin in the microcapsules. The size of microcapsules prepared was 5–10 μm, as determined with microscope. The loading of TRITC-avidin in the microcapsules was evaluated by dissolving the TRITC-avidin-containing $(PAH/PSS)_5$ microcapsules in 0.1 M NaOH solution. Fluorometric measurements of the solution revealed that 1 g of dried hollow microcapsules contains ca. 20 mg of TRITC-avidin. In a separate experiment, we have confirmed a successful deposition of PAH and PSS on the $CaCO_3$ particles by recording ζ-potential of the coated $CaCO_3$ particles after each deposition (Fig. 5).

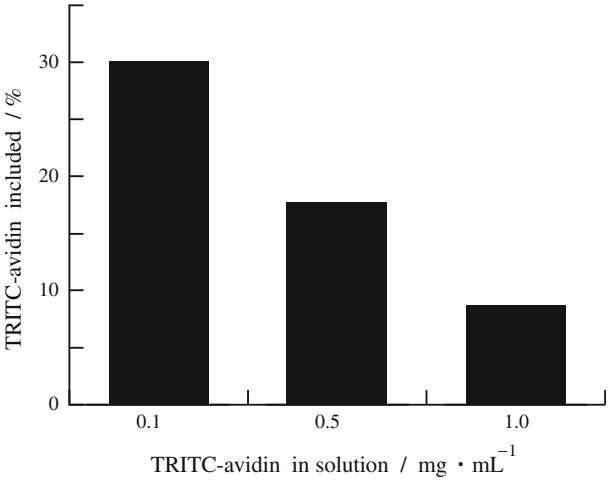

Fig. 3 The loading of TRITC-avidin in the $CaCO_3$ microparticles as a function of TRITC-avidin concentration in the solutions used

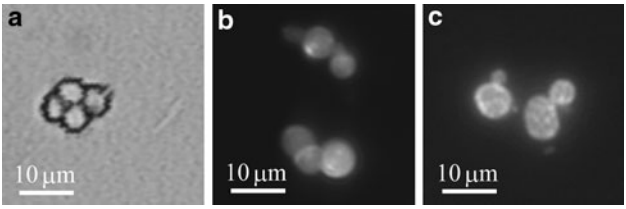

Fig. 4 An optical microscope image of TRITC-avidin-PSS conjugate containing $CaCO_3$ particles (**a**) and fluorescence microscope images of TRITC-avidin-PSS conjugate containing $CaCO_3$ particles (**b**) and TRITC-avidin-PSS conjugate containing $(PAH/PSS)_5$ micorcapsules (**c**)

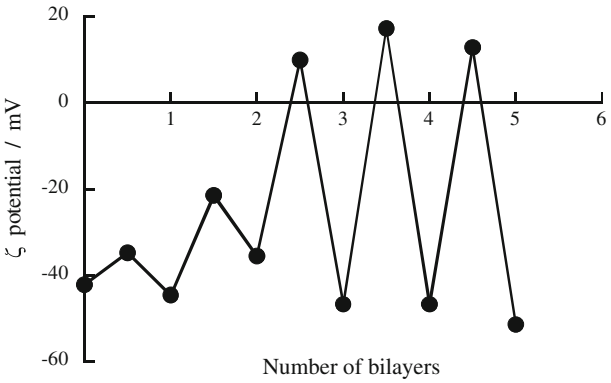

Fig. 5 Zeta potential of $CaCO_3$ microparticles coated with $(PAH/PSS)_n$ films in 10 mM NaCl solution (pH7.0)

The ζ-potential exhibited positive and negative values after deposition of PAH and PSS, respectively, though the charge reversal was not clear in the first few layers probably due to a low surface coverage.

Release of avidin from microcapsules

Polyelectrolyte shell of microcapsules is often permeable for ions and molecules. It has been reported that, in some cases, macromolecules such as proteins are also released from polyelectrolyte microcapsules depending on pH and ionic strength of medium [20]. Therefore, we have evaluated the leakage of encapsulated avidin from the microcapsules. Figure 6 plots released % of TRITC-avidin from $(PAH/PSS)_4$, $(PAH/PSS)_5$ and $(PAH/PSS)_6$ microcapsules. The $(PAH/PSS)_4$ microcapsules exhibited ca. 20% release of TRITC-avidin while the release from the microcapsules with thicker shell was ca. 10% at pH 7.0. The effects of pH and ionic strength in medium on the release were also studied and found that the effects of these variables in medium were rather small. For all cases, the release of TRITC-avidin was 10–20% under the experimental conditions tested. It is noted that the release was observed at the initial stage within 15 min and thereafter no further release was detected. These results suggest that the release of TRITC-avidin may arise from

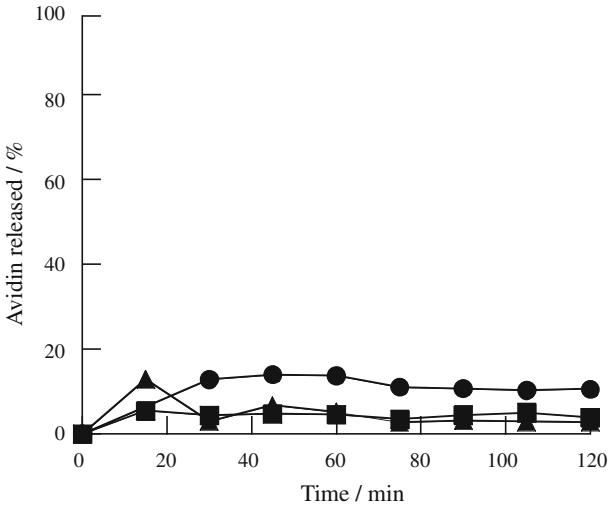

Fig. 6 Release of TRITC-avidin from the $(PAH/PSS)_4$ (*filled circle*), $(PAH/PSS)_5$ (*filled triangle*) and $(PAH/PSS)_6$ microcapsules (*filled square*) at pH 7.0

only those located at the microcapsule shell and that TRITC-avidin encapsulated inside the microcapsule did not release. The suppressed release of TRITC-avidin may be ascribed to the formation of large size of TRITC-avidin-PSS conjugates in the microcapsules. It is likely that the PAH/PSS polyelectrolyte shell of the microcapsule is less permeable for the large-sized TRITC-avidin-PSS conjugates. Thus, the present results show that release of proteins from polyelectrolyte microcapsules can be effectively suppressed by forming conjugates with an oppositely charged polyelectrolyte. From a similar point of view, Nayak and McShane have employed an in-site polymerization method to suppress the release of peroxidase (HRP) from polyelectrolyte microcapsule [21].

Uptake of b-FITC by microcapsule and its release

Avidin is a tetramer protein found in egg white and is known to strongly bind biotin and analogues (the binding constant to biotin, ca. 10^{15} M^{-1}) [19]. It is thus reasonable to expect that the avidin-containing microcapsules effectively encapsulate biotin and derivatives. Figure 7 shows the time-course of uptake of b-FITC into $(PAH/PSS)_5$ microcapsules. We have separately obtained the binding constant of avidin to b-FITC to be 2.4×10^9 M^{-1} based on a Scatchard plot [22]. The avidin-containing $(PAH/PSS)_5$ microcapsule effectively bound b-FITC, the loading being 0.6×10^{-9} mol after 15 min and 1.1×10^{-9} mol after 180 min. In contrast, the loading of b-FITC in the avidin-free microcapsule was 0.3×10^{-9} mol after 180 min. The uptake of b-FITC by the avidin-containing microcapsule was highly enhanced as compared to the uptake by the microcapsule without avidin. This is due to the binding of b-FITC to avidin in the microcapsule. This view is further

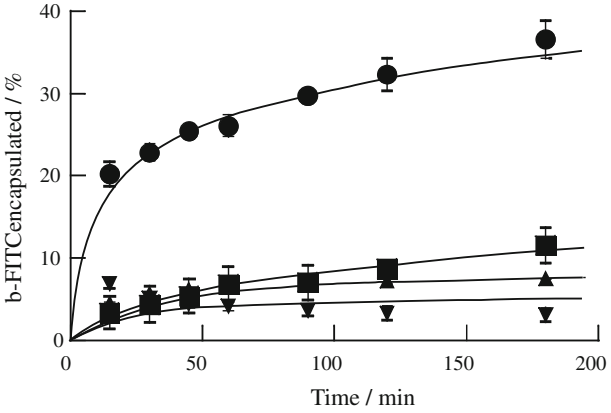

Fig. 7 Uptake of b-FITC into $(PAH/PSS)_5$ microcapsules with (*filled circle*) and without (*filled square*) avidin-PSS conjugate. The plot shown by (*filled inverted triangle*) is uptake of b-FITC into the microcapsule containing avidin-PSS conjugate whose binding sites had been masked with biotin. The uptake of biotin-free FITC was also shown by (*filled triangle*). The experiments were carried out in 1 mM solutions of b-FITC and biotin-free FITC at pH 7.0

supported by the fact that the uptake of biotin-free fluorescein was not enhanced. In addition, the enhanced uptake of b-FITC was not observed when the binding sites of avidin in the microcapsule had been masked in advance by biotin. A minor fraction of encapsulated b-FITC may be bound to the $(PAH/PSS)_5$ shell of microcapsules in view of the fact that loaded amounts of b-FITC or biotin-free fluorescein in the latter three cases are nearly comparable to each others.

Avidin is known to bind biotin as well as analogues such as lipoic acid and 2-iminobiotin less strongly than to biotin [19]. It is thus reasonable to assume that the encapsulated b-FITC may be released from the microcapsule in the presence of an appropriate amount of biotin and analogues. In this context, we have previously reported that the release of 2-(4′-hydroxyphenylazo)benzoic acid from avidin-containing LbL film is triggered upon addition of biotin [23]. Figure 8 shows the release of b-FITC from the $(PAH/PSS)_5$ microcapsule in the presence 1 mM of biotin and analogues. b-FITC released from the microcapsule slowly even in the absence of biotin and analogues. In contrast, the release rate was markedly enhanced in the presence of biotin in the solution, confirming that b-FITC was replaced by added biotin to diffuse out of the microcapsule. Lipoic acid and 2-iminobiotin also accelerated the release of b-FITC to some extent. This is reasonable because the binding constants of lipoic acid and 2-iminobiotin are reported to be ca. 10^6 and 10^3 M^{-1} in neutral solution [24]. These results clearly show that the release of encapsulated biotin-derivatives can be regulated by adding biotin or analogues.

Figure 9 shows the effects of pH on the release of b-FITC from the microcapsules in the presence of 0.1 mM biotin. The rate of release was higher at pH 9.0 than at pH 7.0 and 4.0, which is in line with the higher permeability of PAH-PSS microcapsule in basic media [20].

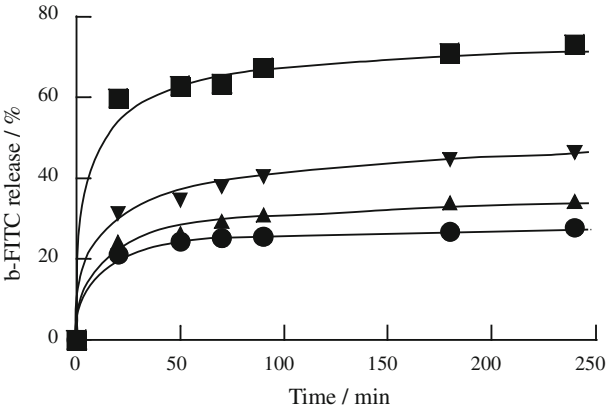

Fig. 8 Release of b-FITC from the $(PAH/PSS)_5$ microcapsules in the presence of 1 mM biotin (*filled square*), 1 mM lipoic acid (*filled inverted triangle*) and 1 mM 2-iminobiotin (*filled triangle*). The release profile of b-FITC in the absence of any additive is also shown (*filled circle*). The experiment was carried out at pH 7.0

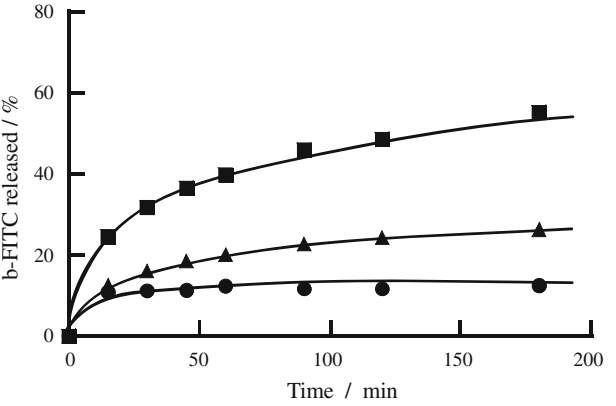

Fig. 9 Release of b-FITC from the $(PAH/PSS)_5$ microcapsules in the presence of 0.1 mM biotin at pH 4.0 (*filled circle*), pH 7.0 (*filled triangle*) and pH 9.0 (*filled square*)

Conclusion

The present study demonstrated that biotin-labelled low-molecular-weight compound (i.e., b-FITC) can be successfully encapsulated in polyelectrolyte microcapsules through avidin–biotin binding. The release rate of b-FITC from the microcapsule was accelerated by adding biotin or analogues. The present microcapsules may find future applications in drug delivery systems.

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