Novel Formulations of Vitamins and Insulin by Nanoengineering of Polyelectrolyte Multilayers around Microcrystals

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Abstract: Microcapsules loaded with vitamin K_3 (VK_3), biotin, or insulin were prepared by using a novel coating technology based on the layer-by-layer (LbL) deposition of oppositely charged polyelectrolytes onto microcrystal templates. This produced multilayered, polymeric shells of varying thickness around the crystalline cores. Dissolution of the core material (VK_3 with ethanol, biotin with basic solution, and insulin with acidic solution), resulted in its release through the shells. Microelectrophoresis was employed to monitor the microcrystal coating process; confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) were used to verify multilayer coating and the formation of hollow polymer shells following removal of the microcrystal templates. The release rates of both VK_3 and insulin decreased as the wall thickness (the number of polyelectrolyte layers deposited onto the microcrystal cores), increased. The release time could be varied by a factor of more than ten, depending on the number of polyelectrolyte layers applied. Following the addition of 70 mass % ethanol, the solubility of VK_3 increased by as much as 170-fold, resulting in an increased rate of VK_3 release. By selecting appropriate polymer materials for the shells, and by controlling the number of polyelectrolyte layers applied, shells of various thickness, stiffness, aqueous solubility, dispersibility, biocompatibility, and permeability can be constructed.

Keywords: adsorption · controlled release · drug delivery · microcapsules · UV/Vis spectroscopy · vitamins

Introduction

Microencapsulation as a technique for the formulation of drugs offers many advantages, such as tailored release, site-specific drug delivery, minimizing deleterious side effects, prolonging time of activity, protecting sensitive drugs from enzymatic or acidic degradation in the GI tract, and taste masking.[1–11] Because of the various requirements of drug development, several conventional encapsulating systems, such as liposomes, microparticles, and microemulsions, are used for the fabrication of micro- and nanosized shells.[12–23] Unfortunately, many of these techniques do not readily permit the manipulation of permeability, mechanical and chemical stability, surface charge, and biocompatibility. Moreover, all of these techniques are associated with the use of organic solvents,[24] incomplete release, and the initial burst effect.[25]

A well-controlled and economical system for drug delivery can be achieved by taking advantage of the nature of crystalline structures, which protect the integrity (biological, physical, and/or chemical) of the therapeutic agent within the lattice structure during processing, upon storage, and after drug delivery.[26] Low crystal solubility in the intestinal fluid is also essential for sustained and effective release.[27]

The human body is dependent on a total of 13 vitamins, many of which are unstable or can be destroyed during modern food-processing practices.[28] To prevent vitamin deficiencies and to maintain adequate vitamin stores, many people take daily vitamin supplements in the form of a tablet or capsule. In view of this and the use of various vitamins in the pharmaceutical and food industries, it is vital to study the stability and dispersion of vitamins within complex matrices, such as drugs and foodstuffs.

Hydrophobic VK_3 is absorbed by humans through the small and large intestines.[29,30] This process is initiated by the high solubility of VK_3 in fats. Due to the current trend

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of reducing dietary fat intake, the occurrence of fat-soluble-vitamin deficiencies is likely to increase.[31] Vitamin supplementation may reduce the risk of deficiency, but only if the vitamins are solubilized and absorbed through the digestive tract. Thus, there has been an intense interest in strategies to deliver hydrophobic VK_3 microcrystals in a stable and aqueous form.[32,33]

Formulating therapeutic insulin for delivery at both controlled rates and for prolonged periods presents many challenges.[34] The preservation of insulin's native conformation is a prerequesite for the maintenance of its biological activity.[34,35] However, the greatly enhanced stability of the crystalline state observed for small molecules may not apply to a crystalline protein. It has been found that crystalline insulin is less stable than the corresponding amorphous form.[36,37] Crystals may rapidly "melt" or dissolve when exposed to an environment different from their supersaturated crystallization medium.[37] Moreover, crystals may loose their integrity upon mechanical processing, such as stirring or shaking, or simply during transfer by, for example, pipetting.[38] Therefore, neither crystallization nor traditional encapsulation alone may be sufficient to formulate insulin with both desirable release characteristics and sufficient physical robustness to allow it to be handled as a solid dosage form.[39]

One strategy to overcome these difficulties is the use of the layer-by-layer (LbL) technique, based on the assembly of alternating layers of oppositely charged species onto the surface of drug microcrystals.[40] The thickness of the resulting capsule wall is adjustable on the nanometer scale and, together with wall composition, affects wall permeability.[42] The availability of a large variety of polyelectrolyte species for coating means that appropriately paired layers can be selected to reach the desired stability and compatibility.[41]

In this study, the colloidal templating LbL technique was used to encapsulate VK_3 and insulin microcrystals with biodegradable and environmentally benign polyelectrolytes, at a moderate temperature without the use of organic solvent. The release rate of the drugs was controlled by varying the number of polyelectrolyte layers and the ratio of ethanol to water. This approach was also extended to the encapsulation of another vitamin, biotin. The purpose of this study was to achieve a better understanding of the properties of permeation of vitamins and insulin through multilayer capsules, and to evaluate the prospects of polyelectrolyte microencapsulation for use in controlled drug release.

Results and Discussion

The sequential deposition of the oppositely charged polyelectrolyte layers on the microcrystal templates was confirmed by enhanced dispersability and microelectrophoresis measurements. Figure 1 shows the ζ-potential as a function of the number of polymer coating layers for VK_3 microcrystals. VK_3 crystals exhibited a value of around -50 mV when exposed to poly(sodium 4-styrenesulfonate) (PSS), indicating that the VK_3 crystal surface is covered with negatively charged PSS. On the other hand, when the positively charged poly(allylamine hydrochloride) (PAH) formed the outermost layer, a ζ-potential of around $+50$ mV was measured. This data confirms the recharging of the microcrystal surface due to adsorption of the polyelectrolytes onto each layer, explaining the dispersability of the microcrystals in aqueous solution. The adsorbed polyelectrolyte multilayers coat the microcrystals, thereby protecting them from aggregation by ionic and/or steric interactions of the thin coating already associated with each microcrystal particle. VK_3 microcrystals could not be readily dispersed when the polyelectrolyte PAH was used; however, they could be dispersed by exposure to PSS, which can be explained by the presence of hydrophobic interactions between PSS and the microcrystals. It has been suggested that the hydrophobic aromatic groups of PSS are associated with the hydrophobic surface of the VK_3 microcrystal, and that the ionic groups of PSS enable the successful adsorption and consequent charging of the crystal surface.[40a]

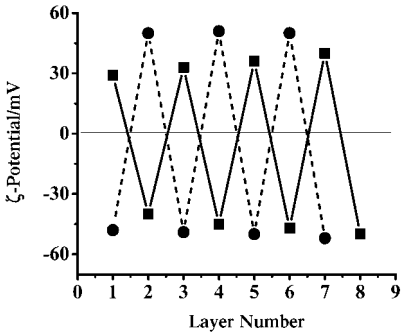

Figure 1. ζ-Potential of PSS/PAH multilayers on VK_3 microcrystals (●) and PLL/AAS multilayers on insulin microcrystals (■) as a function of the number of layers. Surface charge reversal is due to adsorption of each subsequent polyelectrolyte layer. Negative values; PSS or AAS deposition, positive values; PAH or PLL adsorption.

Insulin, a 51-amino-acid polypeptide of MW 5800, exhibits an apparent polydispersity index (pI) of 5.3. The use of positively charged poly-L-lysine hydrobromide (PLL) or PAH as a first layer to coat insulin crystals results in polycation deposition on the surface of the negatively charged insulin. This may be accounted for by electrostatic interaction. The insulin microcrystals coated with alternate layers of PLL and alginic acid sodium salt (AAS) yielded alternating ζ-potentials of approximately $+30$ to $+40$ mV for those having an outermost PLL layer, and approximately -40 to -50 mV for particles with outermost AAS layers (Figure 1). For both PSS/PAH and AAS/PLL systems, the sign of the values obtained is in accordance with the charge on the species deposited. This data clearly demonstrates a reversal of the surface charge, which is characteristic of polyelectrolyte multilayer growth on colloidal templates.[40a,41a]

Confocal laser scanning microscopy (CLSM) was employed to investigate the morphology of the microcrystals and to verify their coating with polymer multilayers. Because insulin is nonfluorescent, a fluorescently labeled polyelectrolyte, PAH labeled with rhodamine B isothiocyanate (PAH–RITC), was used as the outermost layer. Figure 2a and Figure 3a show how VK_3 and insulin microcrystals, re-

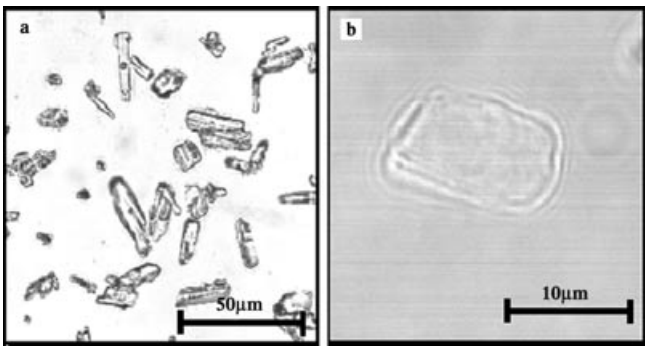

Figure 2. CLSM transmission images: a) VK_3 crystals coated with $(PSS/PAH)_4$ multilayers, and b) the resulting hollow polymer capsule following dissolution of the crystal core in ethanol.

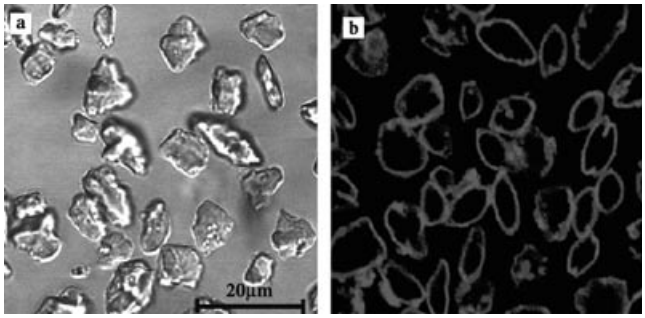

Figure 3. a) CLSM transmission image of insulin microcrystals coated with $(PLL/AAS)_4$/PAH–RITC multilayers. b) CLSM fluorescence image of the hollow $(PLL/AAS)_4$/PAH–RITC capsules following dissolution of the crystal cores in 0.01 N HCl.

spectively, have been well separated from each other as a result of the colloidal stabilization of the polyelectrolyte multilayer (PEM) coating. No noticeable changes in the size or shape of the drug crystals within the PEM capsule were observed upon coating by using the stepwise, regular assembly of oppositely charged polyelectrolyte layers.

Exposure of the coated VK_3 crystals to ethanol, or of the enveloped insulin crystals to 0.01 N HCl, resulted in the successful formation of hollow polymer capsules (Figure 2b and Figure 3b). The pores in the polymer microcapsules are large enough to permit the bulk solution to diffuse into the capsules, where it dissolves the drug crystals, and also for the dissolved VK_3 or insulin molecules to diffuse out through the capsule walls rather than by breaking open the capsules. Close inspection of the capsules reveals a slight swelling of the shells caused by the increased osmotic pressure during crystal dissolution. Following complete dissolution, the capsules shrink as a consequence of the equilibrium established between the osmotic pressure inside and outside the capsules. The remaining polyelectrolyte shells of the microcrystals are now clearly visible.

Important advantages of this VK_3 crystal templating procedure are its versatility and its general applicability. This process was extended to encapsulate biotin. The exposure of the coated biotin crystals to 2 M NH_4OH resulted in the shrinking of the crystal cores, demonstrating the dissolution of biotin inside the shells within a few seconds (Figure 4a). These results are consistent with the observation that the turbidity of the polymer-coated microcrystal suspensions decreased upon addition of the corresponding solvent. The re-

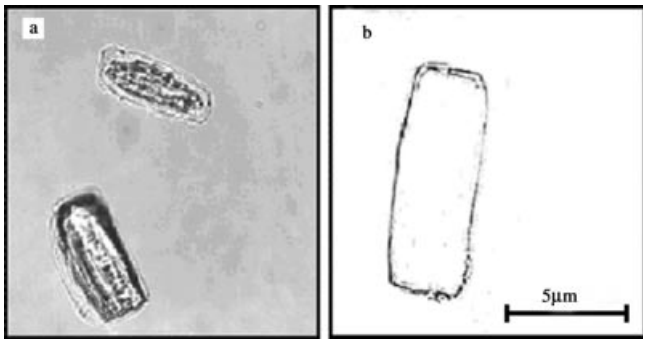

Figure 4. CLSM transmission images: a) Biotin crystals coated with $(PSS/PAH)_4$ multilayers in dissolution, and b) the resulting hollow capsule following dissolution of the crystal core in 2 M NH_4OH.

sulting hollow capsules preserve their integrity and the shapes of the original crystals, as shown in Figure 4b. This indicates that, during removal of the core, only a low osmotic pressure is transiently established, which can be resisted by the polymer. This finding is consistent with earlier reports that polymer multilayers are permeable to low molecular weight species.[40]

The representative atomic force microscopy (AFM) image of a hollow microcapsule of air-dried polymer $(PLL/AAS)_4$ templated onto insulin microcrystals is shown in

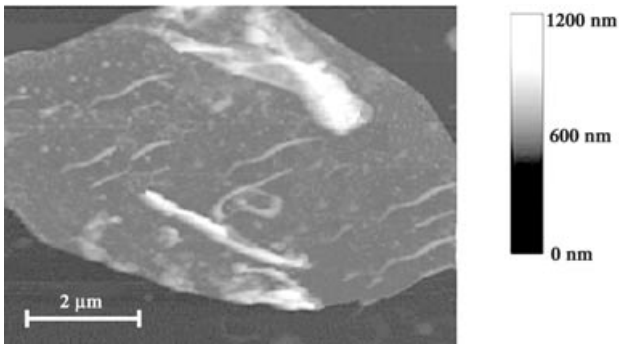

Figure 5. AFM image of an air-dried hollow polymer capsule, produced by coating insulin microcrystals with four bilayers of PLL/AAS, followed by acid dissolution of the core.

Figure 5. Despite the creases and folds caused by capsule collapse, the capsule surface appears quite smooth. By measurement of the narrowest part of the capsule (32 ± 5 nm), assumed to be twice the thickness of the polymer capsule wall, the average thickness per polyelectrolyte layer was estimated to be approximately 2.0 ± 0.3 nm, similar to the value in previous reports.[40,41a] On the basis of this data, the thickness of the polyelectrolyte multilayers is estimated to range from around 2 to 40 nm, that is, thin-walled microcapsules composed of 1–20 polyelectrolyte layers. Accord-

ingly, the release rate of encapsulated substances would be expected to decrease as the shell thickness increases. The thickness of the polyelectrolyte multilayer shell can be controlled with nanometer precision simply by varying the number of polyelectrolyte layers deposited.

Due to its speed, sensitivity, and feasibility,[40d,43] the spectrophotometric method was selected to monitor the release of insulin and VK_3 molecules through the polymer capsule wall following dissolution of the core templates. Figure 6

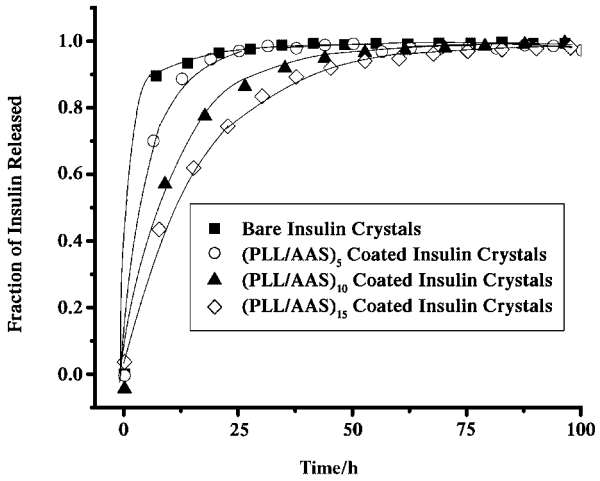

Figure 6. Release-time profiles of insulin microcrystals, coated with 0, 5, 10, and 15 bilayers of PLL/AAS, in pH 7.4.

shows the release-time profiles for insulin microcapsules, coated with a different number (0, 5, 10, 15) of (PLL/AAS) bilayers, in a PBS buffer solution of pH 7.4. The release time increases as the number of polyelectrolyte multilayers deposited onto the microcrystals increases. For example, the time required for the release of 80% of the insulin microcrystals coated with 15 (PLL/AAS) bilayers is more than ten times that required for the uncoated crystals.

Figure 7 shows the release-time profiles for VK_3 microcrystals, coated with a different number (0, 5, 10, 15) of (AAS/PLL) bilayers, in 70% ethanol. The bare VK_3 microcrystals dissolve faster than the encapsulated crystals. As expected, the release rate of the VK_3 crystals decreases as the wall thickness increases. The release of 95% of the crystals enveloped with 0, 5, 10, and 15 bilayers of AAS/PLL takes approximately 80 ± 10, 125 ± 10, 240 ± 10, and 335 ± 10 s, respectively. Clearly, the first five layers have a smaller effect on the delay of release than the subsequent layers, indicating that these initial layer pairs have a greater permeability. This is especially true for the release of the final 20% of VK_3 crystals, which diffuse into the bulk solution at a much lower rate than the initial 80%. There are clearly two different release mechanisms at work here. Due to the high solubility of VK_3 in 70% ethanol, a large osmotic pressure gradient between the capsule interior and the bulk solution is established. This may result in a pressure-driven convective flow, perhaps through transient pores created by the widening of the mesh within the polyelectrolyte multilayer. However, at an advanced state of release, diffusion of the drug

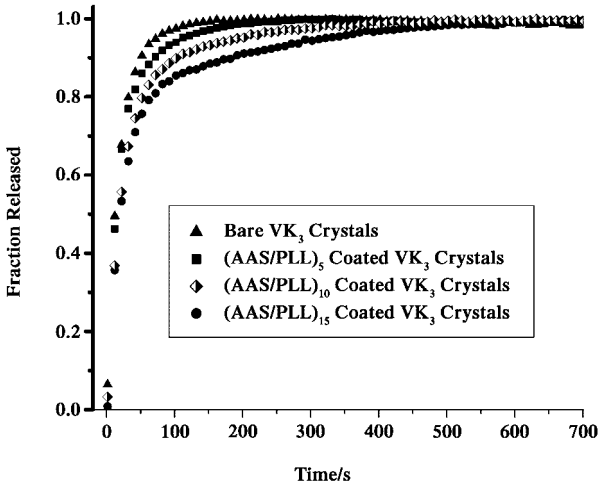

Figure 7. Release-time profiles of VK_3 microcrystals, coated with 0, 5, 10, and 15 bilayers of AAS/PLL, in 70% ethanol.

molecules becomes the prevalent mechanism, leading to a naturally much slower rate of release.

Further experiments were conducted to clarify the influence of solubility on release. The release of VK_3 from polyelectrolyte-coated microcrystals was studied as a function of the ratio of ethanol to water in the dissolution medium. Figure 8 shows the VK_3 release-time profiles for capsules coated with 15 polyelectrolyte bilayers of AAS/PLL in sol-

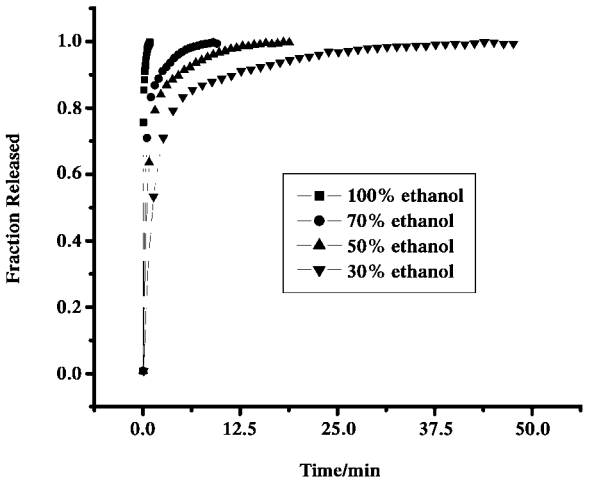

Figure 8. Release-time profiles of VK_3 microcrystals, coated with 15 bilayers of AAS/PLL, in 30, 50, 70, and 100% ethanol.

vents with different ethanol/water ratios. The release rate is much faster in pure ethanol than in pure water. As the ethanol/water ratio increases, the rate of release increases. In pure water, the release of 95% of the drug takes approximately 5 h (data not shown), compared with less than 1 min in pure ethanol. The dramatic difference in release rates can be attributed mainly to differences in solubility: In pure water, VK_3 has a very low solubility of 151 $mg\,L^{-1}$; however, in 10% ethanol, the solubility increases approximately 2-fold, and by addition of 70% ethanol, solubility is enhanced

by a factor of about 170.[33] Another contributing factor may be swelling of the coating in ethanol.

The dramatic increase in release times with decreasing solubility of the drug supports suggestions of a two-stage release mechanism. Moreover, the curves in Figure 8 are inconsistent with monoexponential behavior. It is expected that at the early stages of release, the drug concentration within the capsules is very high, presumably close to its saturation solubility in bulk solution.[40] This results in a large concentration gradient across the capsule wall and a consequently high osmotic pressure within the capsule. The drug molecules, together with the solvent, are then forced through the capsule wall as a result of the quasi-stationary hydrostatic pressure difference. This convective flow may occur through some pores that had been created by the induced lateral wall tension. Notably, during the first stage, the solvent crosses the wall in both directions; coming in by diffusion and leaving mostly by convective flow. At a later stage, the pressure gradient drops, existing pores may reseal, and a comparatively slow diffusion mechanism finally takes over, which is responsible for the release of the remaining drug molecules.

Therefore, drug release is governed simultaneously by the saturation solubility of the drug, the permeability of the PEM capsule, and, possibly, its resistance to the opening of pores. This explains why the release rate increases as the saturation solubility of the encapsulated drug increases, but decreases as the thickness of the capsule wall increases. The dependence on shell thickness may be explained by either the existence of pores that are successively closed by the deposition of further layers, or by thickness-dependent diffusion. Accordingly, the release rate of encapsulated substances is expected to decrease as shell thickness increases.

From the mechanism of release discussed above, it follows that, to effectively slow down the release of highly soluble compounds, it is necessary to first of all reduce the permeability of the capsule wall to the solvent. By reducing this, a greater overall effect on the release of highly soluble drugs is expected than by simply reducing the diffusion of the drug itself through the wall. This study demonstrates the limitations of novel capsule technology, but also suggests possibilities of how to develop capsules with desirable release features.

Conclusion

The microencapsulation of drugs by the LbL method avoids harsh preparative procedures and the use of organic solvent or high temperatures, thus minimizing the potential loss of drug activity. The drugs incorporated into the microcapsules during this procedure suffered no alteration with respect to the pure, standard drug. Many problems of drug formulation, such as release, delivery, control of concentration in the organism, and duration of effect, may be solved by the customized formation of microcrystal shells. The drug release rate decreases as the thickness of the wall increases, but increases as the solubility of the drug in the dispersion solution increases. Although the capsule walls are very thin (on the nanometer scale), comprising only about 1% by weight of the capsule, they are tough and homogeneous. Therefore, no initial burst of the encapsulated drug is observed for PEM microencapsulation systems. The release of VK_3 from the PEM is heavily dependent on solvent, exhibiting minimum release in water and maximum release in ethanol. The encapsulated VK_3 crystals display high dispersability in aqueous solutions and high stability during storage. This technique may also be an elegant method for the preparation of injectable forms of substances that are insoluble in water.

As the organic templates are in the solid crystalline state and, therefore, at their maximum density, the loading capacity of layer-by-layer encapsulations is very high. The high density of material within the capsule, combined with a nanometer-thin capsule wall, results in an extremely high ratio of encapsulated material mass to wall mass. In addition, the ratio of bioactivity to capsule volume surpasses that of all other techniques. Reduced interference to the host organism is expected due to the small amount of capsule material needed, which is of particular interest for in vitro applications. Furthermore, the wall materials are nontoxic and nonaggressive. In summary, the layer-by-layer engineering of PEM coating on vitamins and insulin crystal templates represents a new route for the development of special formulations to optimize drug delivery and availability.

Experimental Section

Materials: Bovine insulin (MW 5734), biotin, vitamin K_3, poly-L-lysine hydrobromide (PLL, MW 30000–70000), and alginic acid sodium salt (AAS) were all obtained from Sigma. Poly(sodium 4-styrenesulfonate) (PSS, MW 70000) and poly(allylamine hydrochloride) (PAH, MW 8000–11000) were purchased from Aldrich. PAH labeled with rhodamine B isothiocyanate (PAH–RITC) was synthesized according to a method reported previously.[40a] The sterilized phosphate buffer was purchased from Cellgro. The aqueous solutions were prepared by using ultrapure water with a resistivity greater than 18.2 MΩ, obtained by means of a USF Deutschland GmbH Purelab Plus purification system.

Encapsulation of microcrystals: Crystals (200 mg) were finely ground by using an agate mortar and a small amount of dispersant (2 mg mL^{-1} alginate for VK_3, 2 mg mL^{-1} poly-L-lysine for insulin or biotin). The ground crystals were then dispersed in deionized (100 mL) water. The dispersion was ultrasonicated for 3 min and allowed to stand for a suitable time, during which time the larger crystals sedimented. The resulting products were washed twice with water. The precharged microcrystals were then coated by the consecutive adsorption of poly-L-lysine and alginate, with slight modifications to the procedure reported previously.[40] A poly-L-lysine solution (1.5 mL of 2 mg mL^{-1}, containing 0.5 M NaCl) was added to a 2-mL centrifuge tube containing microcrystals (50 mg), to which alginate had been added as the first layer. Similarly, an alginate solution (1.5 mL of 2 mg mL^{-1}, containing 0.5 M NaCl) was added to microcrystals, to which poly-L-lysine had been adsorbed as the first layer. After an adsorption time of 10 min, the dispersion was centrifuged at 3000 g for 5 min. The supernatant was removed by careful decantation and the coated microcrystals were washed twice in water to remove any unbound polyelectrolyte. Alternating polyelectrolyte layers were deposited until the desired number of multilayers was reached.

Release of VK_3: The release of the polyelectrolyte-multilayer-coated crystals was conducted in a 1-cm quartz cuvette under constant magnetic stirring at room temperature (ca. 20 °C). The kinetics of the process were monitored by recording the change in absorbance at 335 nm. The total drug concentration was kept below the saturation solubility (151 mg L^{-1}

in pure water), so that the solution would not reach saturation during the release process.

Release of insulin: The insides of the test tubes and cuvettes used were initially coated with Sigmacote (Sigma) to prevent adsorption of the released peptides. Polyelectrolyte-multilayer-coated insulin crystals (50 mg) were then suspended in a phosphate buffer solution (10 mL of 0.01 M PBS, pH 7.4) in glass test tubes and maintained at room temperature under constant stirring. Supernatants were collected at appropriate time intervals by centrifugation at 3000 g for 5 min. Fresh buffer (10 mL) was added to the pellets and incubation continued. The insulin content of the supernatants was determined by using a UV/Vis spectrophotometer at 276.4 nm.

The microelectrophoretic mobilities were measured in pure water by using a Malvern Zetasizer 3000 HS. The mobilities u were converted into the ζ-potentials by using the Smoluchowski relation $\zeta = u\eta/\varepsilon_0\varepsilon$, in which η and $\varepsilon_0\varepsilon$ are the viscosity and permittivity of the solution, respectively.

Confocal micrographs were taken by using a confocal laser scanning system TCS NT "Aristoplan" from Leica, equipped with a 100×1.4 oil immersion objective.

The AFM images were obtained by means of a Digital Instruments Nanoscope IIIa in tapping mode. Samples were prepared by applying a drop of the capsule solution onto a freshly cleaved mica substrate. After the capsules were allowed to settle, the substrate was extensively rinsed in pure water and then dried under a gentle stream of nitrogen.

All drug release measurements were carried out on a Cary model 4E UV/Vis spectrophotometer (Varian).

Acknowledgment

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[1] K. G. Das, *Controlled Release Technique*, Wiley Interscience, New York, **1983**.
[2] Y. Haga, S. Inoue, T. Sato, R. Yosomiya, *Angew. Makromol. Chem.* **1986**, *139*, 49–61.
[3] E. Bourgeat-Lami, J. Lang, *J. Colloid Interface Sci.* **1998**, *197*, 293–308.
[4] K. E. Uhrich, S. M. Cannizzaro, R. S. Langer, K. M. Shakesheff, *Chem. Rev.* **1999**, *99*, 3181–3198.
[5] P. R. Hari, T. Chandy, C. P. Sharma, *J. Microencapsulation* **1996**, *13*, 319–329.
[6] K. Park, *Controlled Drug Delivery: Challenges and Strategies*, American Chemical Society, Washington, DC, **1997**.
[7] R. Langer, *Nature* **1998**, *392*, 5–10.
[8] I. McCullosch, S. W. Shalaby, *Tailored Polymeric Materials for Controlled Delivery Systems*, American Chemical Society, Washington, DC, **1998**.
[9] T. L. Whateley, *Macroencapsulation of Drugs*, Harwood Academic, Amsterdam, **1992**.
[10] R. Arshady, *J. Controlled Release* **1991**, *17*, 1–21.
[11] J. Kreuter, *Colloidal Drug Delivery Systems*, Marcel Dekker, New York, **1994**.
[12] J. L. Richardson, N. F. Farraj, L. Illum, *Int. J. Pharm.* **1992**, *88*, 319–325.
[13] P. Edman, E. Bjork, L. Ryden, *J. Controlled Release* **1992**, *21*, 165–172.
[14] A. K. Kwong, S. Chou, A. M. Sun, M. V. Sefton, M. F. A. Goosen, *J. Controlled Release* **1986**, *4*, 47–62.
[15] K. B. Choudhari, V. Labhaserwar, A. K. Dorle, *J. Microencapsulation* **1994**, *11*, 319–325.
[16] M. Mesiha, F. Plakogiannis, S. Vejosoth, *Int. J. Pharm.* **1994**, *111*, 213–216.
[17] K. Okumura, S. Iwakawa, T. Yoshida, T. Seki, F. Komada, *Int. J. Pharm.* **1992**, *88*, 63–73.
[18] I. Morishita, M. Morishita, K. Takayama, I. Machida, T. Nagai, *Int. J. Pharm.* **1993**, *91*, 29–37.
[19] F. Lim, A. M. Sun, *Science* **1980**, *210*, 908–910.
[20] S. J. Shuttleworth, S. M. Allin, P. K. Sharma, *Synthesis* **1997**, 1217–1239.
[21] I. Cohen, H. Li, J. L. Hougland, M. Mrksich, S. R. Nagel, *Science* **2001**, *292*, 265–267.
[22] J. P. McGee, S. S. Davis, D. T. O'Hagan, *J. Controlled Release* **1995**, *34*, 77–86.
[23] I. Lacik, M. Brissova, A. V. Anilkumar, A. C. Powers, T. Wang, *J. Biomed. Mater. Res.* **1998**, *39*, 52–60.
[24] N. Elvassore, A. Bertucco, P. Caliceti, *Ind. Eng. Chem. Res.* **2001**, *40*, 795–800.
[25] a) L. Vayssieres, C. Chaneac, E. Tronc, J. P. Jolivet, *J. Colloid Interface Sci.* **1998**, *205*, 205–212; b) C. D. Herzfeldt, R. Kummel, *Drug Dev. Ind. Pharm.* **1983**, *9*, 767–793.
[26] a) C. M. Yip, M. R. DeFelippis, B. H. Frank, M. L. Brader, M. D. Ward, *Biophys. J.* **1998**, *75*, 1172–1179; b) C. M. Yip, M. L. Brader, B. H. Frank, M. R. DeFelippis, M. D. Ward, *Biophys. J.* **2000**, *78*, 466–473.
[27] S. D. Putney, P. A. Burke, *Nat. Biotechnol.* **1998**, *16*, 153–157.
[28] A. T. Diplock in *Fat-Soluble Vitamins: Their Biochemistry and Applications, Vitamin E* (Ed.: A. T. Diplock), Technomic Publishing Co. Inc., Lancaster, **1985**, pp. 171–186.
[29] H. E. Gallo-Torres in *Vitamin E: A Comprehensive Treatise, Absorption* (Ed.: J. M. Lawrence), Marcel Dekker, New York, **1980**, pp. 170–193.
[30] F. Weber in *Digestion and Absorption of Nutrients, Absorption of Fat-Soluble Vitamins* (Eds.: H. Bickel, Y. Schutz), Hans Huber, Vienna, **1983**, pp. 55–65.
[31] J. W. Suttie in *Fat-Soluble Vitamins: Their Biochemistry and Applications, Vitamin K* (Ed.: A. T. Diplock), Technomic Publishing Co. Inc., Lancaster, **1985**, pp. 239–249.
[32] E. Browning, *The Vitamins, Vol. 18,* Bailliere, Tindall, & Cox, Baltimore, MD, **1931**.
[33] M. D. Dubbs, R. B. Gupta, *J. Chem. Eng. Data* **1998**, *43*, 590–591.
[34] a) F. G. Banting, C. H. Best, *J. Lab. Clin. Med.* **1922**, *7*, 464–472; b) J. Brange, *Galenics of Insulin: The Physico-Chemical and Pharmaceutical Aspects of Insulin and Insulin Preparations*, Springer, Berlin, **1987**.
[35] a) J. Brange, *Stability of Insulin*, Kluwer Academic, Boston, **1994**; b) U. Grau, C. D. Saudek, *Diabetes* **1987**, *36*, 1453–1459.
[36] J. S. Liu, D. R. Rigsbee, C. Stotz, M. J. Pikal, *J. Pharm. Sci.* **2002**, *91*, 1853–1862.
[37] B. Shenoy, Y. Wang, W. Shan, A. L. Margolin, *Biotechnol. Bioeng.* **2001**, *73*, 358–369.
[38] M. J. Pikal, D. R. Rigsbee, *Pharm. Res.* **1997**, *14*, 1379–1387.
[39] P. Hildebrandt, A. Berger, A. Volund, C. Kuhl, *Diabetic Med.* **1985**, *2*, 355–359.
[40] a) Z. F. Dai, H. Zastrow, S. Leporatti, E. Donath, H. Möhwald, B. X. Peng, *J. Dispersion Sci. Technol.* **2002**, *23*, 555–562; b) Z. F. Dai, A. Voigt, E. Donath, H. Möhwald, *Macromol. Rapid Commun.* **2001**, *22*, 756–762; c) X. Y. Shi, F. Caruso, *Langmuir* **2001**, *17*, 2036–2042; d) X. P. Qiu, S. Leporatti, E. Donath, H. Möhwald, *Langmuir* **2001**, *17*, 5375–5380.
[41] a) Z. F. Dai, H. Möhwald, *Chem. Eur. J.* **2002**, *8*, 4751–4755; b) Z. F. Dai, H. Möhwald, B. Tiersch, L. Dähne, *Langmuir* **2002**, *18*, 9533–9538; c) Z. F. Dai, L. Dähne, H. Möhwald, B. Tiersch, *Angew. Chem.* **2002**, *114*, 4191–4194; *Angew. Chem. Int. Ed.* **2002**, *41*, 4019–4022.
[42] a) Z. F. Dai, A. Voigt, S. Leporatti, E. Donath, L. Dähne, H. Möhwald, *Adv. Mater.* **2001**, *13*, 1339–1342; b) Z. F. Dai, L. Dähne, E. Donath, H. Möhwald, *Langmuir* **2002**, *18*, 4553–4555; c) Z. F. Dai, L. Dähne, E. Donath, H. Möhwald, *J. Phys. Chem. B* **2002**, *106*, 11501–11508.
[43] F. Bugamelli, M. A. Raggi, I. Orienti, V. Zecchi, *Arch. Pharm.* **1998**, *331*, 133–138.

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