pH-stable hyperbranched poly(ethyleneimine)-maltose films for the interaction with phosphate containing drugs†

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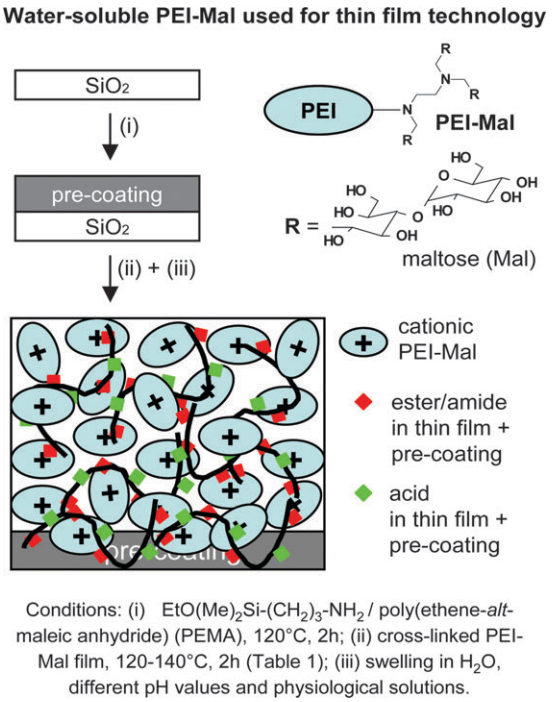
Maltose-decorated hyperbranched poly(ethyleneimine) is a promising candidate as drug carrier attributed by electrostatic interactions. Here, we report the fabrication of stable hyperbranched poly(ethyleneimine)-maltose (PEI-Mal) films capable of swelling and their features to load and release phosphate containing drugs as being observed by ellipsometric study.

During the last decade, advances in nanotechnology have led to increased interest in organic and polymeric smart thin films.[1–6] Thus, switchable, controllable and responsive surfaces and films have been developed for various applications spreading from data storage over microfluidic devices to bio-applications. Moreover, the possibility to adapt for example the protein adsorption/desorption and cell adhesion at the liquid/solid interface is a challenging task in the field of bio-applications (drug delivery, biofouling, glycomics, cell culture, *etc.*). Recent studies, using linear, hyperbranched and dendritic glycopolymers in thin film technology,[7–13] addressed *e.g.*, (a) the interactions between (oligo-)saccharide surfaces and biomacromolecules and biological entities,[7] (b) the controlled degradation of films to release biomolecules,[8] (c) the selective binding/release of fibroblast growth factor,[10] and (d) a significant enantiospecific interaction of D-ascorbic acid over L-ascorbic acid at chiral polyelectrolyte multilayers.[12] Inspired by that and additional work[14] as well as recent breakthroughs in facile formation of precoatings[15] we developed thin hydrogel films based on hyperbranched PEI-Mal[16] being highly responsive towards phosphate containing drugs. For the first time, water-soluble hyperbranched glycopolymers are used in the formation of media-stable hydrogel films which are capable of reversible swelling.

Hyperbranched polymers, meaning highly branched, narrowly disperse and globular macromolecules,[17] are promising materials in the field of nanocarriers and polymeric therapeutics and diagnostics.[18] Decoration of hyperbranched PEI and other dendritic scaffolds with PEG or (oligo-)saccharide units is carried out primarily to enhance the biocompatibility. This also leads to multivalent materials possessing potential binding affinities to biomacromolecules.[19] One successful approach was the synthesis of hyperbranched PEI with different oligosaccharide architectures using the reductive amination method in basic solution. These materials showed high potential as carrier molecules for enhanced ATP uptake in cells.[16] Thus, hyperbranched PEI-Mal with dense maltose shell (Scheme 1; details of PEI-Mal as structure A in ref. 16) was selected as the main component for the formation of thin hydrogel films. These were used to evaluate the binding features of cross-linked PEI-Mal towards phosphate containing drugs such as ATP, AMP, *etc.*



Scheme 1 Preparation of PEI-Mal films capable of swelling which were used for the interaction study with phosphate containing drugs.

A two-step approach [(i) + (ii) in Scheme 1] was carried out to prepare thin films. First, adopting a method from Pompe *et al.*,[15] an acid/anhydride-functionalized pre-coating with film thickness between 4–8 nm was achieved by the conversion of poly(ethene-*alt*-maleic anhydride) (PEMA) with (3-aminopropyl)-dimethylethoxysilane film, attached on Si wafer, after annealing and hydrolysis steps. Then, a spin-coating process followed to deposit PEI-Mal with PEMA as cross-linker. Finally the film preparation was finished by annealing and washing/drying steps. Thus, reproducible thin films [Scheme 1: dry film after (ii)] with defined film thickness (*d* determined by ellipsometric measurements) (Table 1) were realized. The PEI-Mal films

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Table 1 Film thickness (d) and refractive index (n), considering data from different experiments, of dry and swollen films after a two-step approach [Scheme 1 with (i) and (ii)] and swelling degree (SD, determination in ESI†) in water; using 7.5 wt% PEMA as cross-linker refers to the total amount of PEI-Mal in aqueous solution

	Dry film		Swollen film		
PEI-Mal (wt%)	d/nm	n	d/nm	n	SD
4	63	1.559	78	1.448	0.24
6	96	1.543	273	1.376	1.8
8	158	1.529	395	1.407	1.5

show basically d (60–160 nm) which depends on preparation parameters used in this study (Tables 1 and S1, ESI†). As a consequence of the acid/anhydride groups in the precoating[20] and in the cross-linker, esterification reactions as the main conversion step took place to attach PEI-Mal on the precoating surface and to crosslink the PEI-Mal macromolecules within thin films.

In this context the chemical cross-linking of cellulose with PEMA[20] was also established using similar cross-linking conditions as mentioned in this paper to provide ester bonds as cross-linking points for exhibiting water-stable cellulose films. Moreover, model reaction of maltose with PEMA in thicker films was carried out to preferably show the formation of ester bonds and the presence of free acid groups (ESI†). Further, minor amidation reaction is also possible where more the tails or loops of PEMA can penetrate through the dense maltose shell of PEI-Mal to find residual secondary amino groups[16] in PEI-Mal for initiating chemical conversion in thin films. Both functional groups, ester and amide groups, as cross-linking points in the PEI-Mal films were not detectable by IR, but first indication for ester groups by XPS study (ESI†) to establish water-stable and swellable PEI-Mal hydrogel films.

Further, the addition of defined amounts of the polymeric cross-linker PEMA (7.5 wt% towards the total amount of PEI-Mal; Table 1) in aqueous solution guaranteed the formation of thicker ($\leq$200 nm) and stable films which showed no significant reduction of d after washing and drying steps (ESI†).

Moreover, a swelling study of the PEI-Mal films in water (Table 1) by ellipsometry showed that films with cross-linker PEMA have reasonable swelling degrees (SD) of 0.24 to 1.8. Additionally, electrokinetic measurements outlined that the cross-linked films possess an isoelectronic point at about pH 7.8 indicating that the films have a cationic excess charge at lower pH values (ESI†). A final study on the film morphology revealed generally closed and smooth films in dry and swollen states (ESI†; AFM study).

However, considering all parameters (fixation and cross-linking conditions) to prepare water-stable PEI-Mal films, swelling and stability (mentioned below) behavior at different pH values and results[12,20] from literature, one can preferentially exclude the main formation of electrostatically cross-linked PEI-Mal macromolecules with PEMA where the cross-linker PEMA is used as a minor component in the cross-linking process. In opposite to our films, water-stable polyelectrolyte multilayers based on PEI-Mal with structure A were established by using layer-by-layer technique without annealing step.[12]

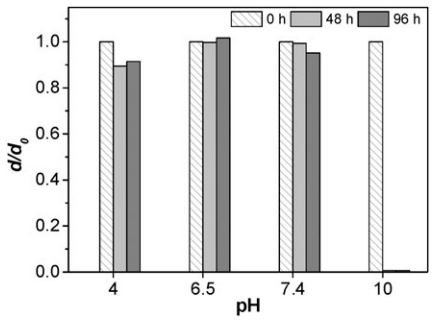

Fig. 1 Stability of PEI-Mal films, cross-linked with PEMA, at different pH values (degradation at pH 10); ratio of d after storage in pH (and drying step) *versus* original dry film thickness d_0 (after annealing and extraction in water) is given.

For potential bio-application the stability of the PEI-Mal films (Fig. 1 and ESI†) was tested at different pH values. Cross-linked PEI-Mal films possessed a high stability at pH 4, 6.5 and 7.4 up to 4 days, but showed rapid degradation at pH 10. Further, the SD of PEI-Mal films cross-linked with PEMA were compared using different physiological solutions and pH values (ESI†). Similar SD ($\sim$1.5) was found in all cases, except for 1 M NaCl solution where a SD of $\sim$2.0 was detected. At all ellipsometric measurements used to determine d after swelling a fast response of the films cross-linked with PEMA was observed down to $\leq$30 s. This fast swelling behavior stimulated us to test the response of the cross-linked PEI-Mal hydrogel films with the highest SD towards phosphate containing drugs.

For the interaction study by *in situ* ellipsometric measurements we selected the following nucleic acid solutions to be added to PEI-Mal films, pre-swollen in water: ATP, AMP, CTP, CMP.‡ The nucleic acids are used as model drugs to evaluate generally the depot properties of PEI-Mal films with regard to short-term or stepwise release of the model drugs. Especially, nucleoside analogs which act as cytotoxic metabolites are of biological relevance in cancer chemotherapy.[14] Fig. 2 presents the total interaction process with ATP and AMP. Here, each change of solution is immediately accompanied by an alteration of the raw data of Δ and Ψ as a qualitative measure for the interaction of the agent with the swollen film. From the full spectroscopic data set of Δ and Ψ d and refractive index n can be determined as quantitative results using an appropriate optical model for fitting (Table 2 and ESI†).[21]

One surprising result is that d of the swollen films is reduced in the presence of the triphosphate containing drugs ATP and CTP (Table 2 and ESI†). It can be assumed that the reduction of d after the addition of ATP and CTP resulted from strong intra-/intermolecular interactions of both negatively charged drugs with the cationic PEI-Mal macromolecules[16] in the thin hydrogel films. XPS results confirmed the presence of remaining ATP in the dried films after two rinsing steps (Fig. 2 and ESI†). In contrast, a typical osmotic behavior of the films after the addition of AMP and CMP was observed resulting in a slightly increased d of the films. Thus, AMP and CMP behave similar as phosphate anions in PBS buffer resulting in an increase of d. One additional indication will be given for the different interaction properties of mono- and triphosphate containing

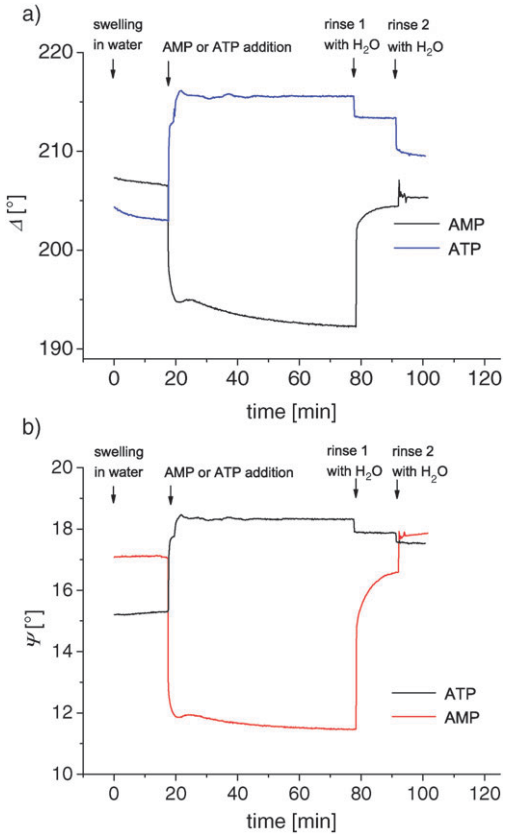

Fig. 2 Dynamic scan of the experimental ellipsometric parameters Δ (a) and Ψ (b) (*e.g.* for λ = 535.5 nm) during the swelling in water, addition of AMP or ATP (both c = 0.5 mg/ml) and rinsing process with water of PEI-Mal films cross-linked with PEMA.

drugs towards PEI-Mal hydrogel films that the PEI macromolecule decorated with a dense maltose shell showed the lowest cationic charge density over a broad pH range in comparison to other PEI-Mal macromolecules described in ref. 16. Thus, the combination of cationic charge density from PEI-Mal and free acid groups (ESI†; electrokinetic measurement) of the cross-linker PEMA in the hydrogel film also tailors the interaction properties of the nucleic acid derivatives as mentioned above.

Also, three different release behaviors are observed during the rinsing process: whereas a stepwise and clearly incomplete release is found for ATP (*e.g.* see Δ scan in Fig. 2), but also nearly no release for ATP (Table 2) and CTP. However, a fast and nearly total release of AMP (*e.g.* see Δ scan in Fig. 2) and CMP is observed. The long-term encapsulation in such films as observed for ATP or CTP could be applied for other drugs

Table 2 Film thickness after the addition of ATP using PEMA as cross-linker for PEI-Mal; thickness d (nm) and refractive index n at λ = 667.9 nm from ellipsometry

	Film 1		Film 2		Film 3	
Condition	d	n	d	n	d	n
Dry state	87	1.541	86	1.537	98	1.544
Swelling in water	243	1.401	216	1.403	283	1.371
ATP addition	209	1.413	195	1.420	256	1.392
Rinse 1 in water	212	1.408	197	1.410	259	1.389
Rinse 2 in water	210	1.406	196	1.409	264	1.387

(*e.g.* FATP)[14] with intrigue to biological experiments like cell growth on such PEI-Mal hydrogel films where it is possible to tailor the release of the pre-encapsulated drug by immediate uptake of the cell.

Our study has shown that collecting of the ellipsometric "raw data" Δ and Ψ by *in situ* measurements can directly be used as a qualitative sensor to monitor the interactions of small drugs in swollen thin hydrogel films in real-time. Especially for the monophosphate containing drugs (ESI†), just evaluation of the final d and n values using an optical model limits the information on the interaction of PEI-Mal films with phosphate containing drugs which can be, however, enhanced by looking *in situ* at the experimental $\Delta - \Psi$ data set.

For future work the developed stable and responsive thin hydrogel films of PEI-Mal are adaptable to the interaction/separation of (anionic) bio-active compounds (*e.g.* small phosphate containing drugs). Especially, the stepwise and tuneable release of (anionic) drugs and bioactive components from those films exhibits a high potential in bio-applications like controlled cell growth on those film surfaces but also monitoring of various biological processes. Further work is needed to evaluate the content of drugs after the rinsing steps, *e.g.* combining Vis-ellipsometric technique with other structure-sensitive methods (*e.g.* IR-ellipsometry[22]) and to down-size and pattern those films for application in bio-sensorics.

Experimental

Materials

Phosphate buffered saline (PBS) tablets were purchased from Aldrich, one tablet was dissolved in 200 ml MilliQ water to get a buffer solution with pH 7.4. Sodium chloride and the phosphate containing drugs [cytidine 5′-monophosphate disodium salt, cytidine 5′-triphosphate disodium salt, adenosine 5′-monophosphate disodium salt solution and adenosine 5′-triphosphate disodium salt solution] were purchased from Sigma-Aldrich (Munich, Germany). The buffer solution with a pH 4 and 10 was purchased from Merck (Darmstadt, Germany). PEI-Mal is an abbreviation for hyperbranched poly(ethyleneimine)-maltose. Maltose monohydrate was used as purchased from Fluka. Poly(ethylenimine) (PEI as general abbreviation; Lupasol G100 with M_w = 5000 g mol^{-1}) were received from BASF SE (Ludwigshafen, Germany).

Preparation of the pre-coating

The method was adopted from Pompe *et al.*[15] Further details are presented in ESI.†

Preparation of PEI-Mal film

PEI was modified with maltose using reductive amination according to the method reported in ref. 16 in the paper. The addition of cross-linker to aqueous PEI-Mal solution was necessary using poly(ethene-*alt*-maleic anhydride) (PEMA). PEMA was hydrolyzed prior to use as an acid containing cross-linker.[15] Different concentrations, meaning PEI-Mal and cross-linker, in MilliQ water after filtration were used for the preparation of thin films in a spin coating process (6000 rpm/30 s); further details in Tables S1 and S2 (ESI†).

Stable PEI-Mal films were achieved with 7.5 wt% PEMA relating to the total PEI-Mal concentration (3 wt%–10 wt%) in aqueous solution (Fig. S2, ESI†). Here, attachment on pre-coating and PEMA cross-linked PEI-Mal films can be attained by putting the films, deposited on Si wafers, in an oven for 2 h at 120 °C. After cooling down and rinsing with water stable PEI-Mal films were received (Tables 1, S1 and S2, ESI†).

Determination of film thickness by spectroscopic ellipsometry

The determination of film thickness (d) and refractive index (n) of the PEI-Mal films, the *in situ* swelling and the interaction experiments with phosphate containing drugs were performed by using a multiwavelength ellipsometer in the spectral range of 380–900 nm and an incidence angle of 70° [Rotating Compensator Ellipsometer Alpha-SE, J. A. Wollam Co., USA]. n and d were calculated from the obtained ellipsometric data Δ and Ψ using the model (Si/SiO_2/polymer/ambient) and the Cauchy relation to describe the wavelength dependence of n of the polymer. Further, details are explained in Reference: Y. Mikhailova *et al.*, *Colloids Surf., A: Physicochemical and Engineering Aspects*, 2006, **279**, 20–27. The equilibrium out of plane swelling degree (SD) of thin polymer films was calculated according to the following formula: $SD = (d - d_0)/d_0$, where d is the thickness of the swollen film and d_0 the film thickness of the dry or pre-swollen film depending on experiments carried out. For swelling experiments a quartz cell with an incidence angle of 70° was used.

Swelling experiment in different solutions

The swelling measurements were run in MilliQ water (pH = 6.5; also for Table 1) and in other aqueous solutions [PBS-buffer with pH 7.4, sodium chloride solution (c = 0.1 and 1 M) and another buffer solution with pH 4]. The PEI-Mal films, attached on Si wafers, were put into the quartz cell and fixed with a sample holder. For each experiment 3500 µl of the swelling solution were injected to the cell and the ellipsometric swelling measurements were directly started. The experiment was finished when the equilibrium of Δ and Ψ was achieved.

Acknowledgements

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Notes and references

‡ Cytidine 5′-monophosphate disodium salt (CMP), cytidine 5′-triphosphate disodium salt (CTP), adenosine 5′-monophosphate disodium salt solution (AMP), adenosine 5′-triphosphate disodium salt solution (ATP) and 5-fluoroadenine arabinoside (FATP).

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