International Journal of Pharmaceutics 468 (2014) 121–132

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Quantifying the release of lactose from polymer matrix tablets with an amperometric biosensor utilizing cellobiose dehydrogenase

Patrik Knöös [a,1,*], Christopher Schulz [b,1,**], Lennart Piculell [a], Roland Ludwig [c], Lo Gorton [b], Marie Wahlgren [d,***]

[a] Division of Physical Chemistry, Lund University, Box 124, Lund SE-22100, Sweden
[b] Department of Biochemistry and Structural Biology, Lund University, Box 124, Lund SE-22100, Sweden
[c] Food Biotechnology Laboratory, Department of Food Sciences and Technology, BOKU University of Natural Resources and Life Sciences, Vienna, Austria
[d] Division of Food Technology, Lund University, Box 124, Lund SE-22100, Sweden

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 28 March 2014
Accepted 30 March 2014
Available online 12 April 2014

Keywords:
Controlled release
Polymer matrix
Biosensors
Cellobiose dehydrogenease
Lactose release
Release mechanisms

ABSTRACT

The release of lactose (hydrophilic) from polymer tablets made with hydrophobically modified poly (acrylic acid) (HMPAA) have been studied and compared to the release of ibuprofen, a hydrophobic active substance. Lactose is one of the most used excipients for tablets, but lactose release has not been widely studied. One reason could be a lack of good analytical tools. A novel biosensor with cellobiose dehydrogenase (CDH) was used to detect the lactose release, which has a polydiallyldimethylammonium chloride (PDADMAC) layer that increases the response. A sample treatment using polyethylenimine (PEI) was developed to eliminate possible denaturants. The developed methodology provided a good approach to detect and quantify the released lactose. The release was studied with or without the presence of a model amphiphilic substance, sodium dodecyl sulphate (SDS), in the release medium. Ibuprofen showed very different release rates in the different media, which was attributed to hydrophobic interactions between the drug, the HMPAA and the SDS in the release medium. The release of hydrophilic lactose, which did not associate to any of the other components, was rapid and showed only minor differences. The new methodology provides a useful tool to further evaluate tablet formulations by a relatively simple set of experiments.

1. Introduction

During pharmaceutical development of tablet formulations, emphasis is put on the release of the active substance. The information obtained by this strategy will in many cases not be sufficient to reveal the underlying mechanisms of the release under varying release conditions. If the release of other compounds in the formulation is investigated, additional information on the release mechanism can be obtained. In this work we present the investigation of a model polymer matrix tablet containing Pemulen™ TR2, lactose and ibuprofen. Pemulen™ TR2 is a commercially available cross-linked and hydrophobically modified poly(acrylic acid), henceforth referred to as HMPAA. We have previously studied the potential of HMPAA in tablet formulations, (Knöös et al., 2013a; Knöös et al., 2013b), where it has shown promising abilities to control the release of hydrophobic compounds. However the mechanism of release from these tablets is not yet fully understood.

Lactose is one of the most used excipients in the pharmaceutical industry (Aulton and Cooper, 1988). From a release point of view lactose can be regarded as a model hydrophilic substance and can thus be studied simultaneously with the active substance to give complementary information on the behavior of the formulation. In our case it provides an opportunity to compare the releases of a hydrophobic and a hydrophilic substance. To the authors' knowledge there are only few previous studies on the release of lactose in tablet formulations (Eadala et al., 2009; Gao et al., 1996). One reason could be that there is currently a lack of good analytical tools to study lactose release from pharmaceutical agents.

Various analytical methods are used to directly analyze lactose in dairy products, such as polarimetry, infrared absorption, gravimetry (Watson, 1994), HPLC, (Kwak and Jeon, 1988; West

* Corresponding author. Tel.: +46 46 222 81 63; fax: +46 46 222 44 13.
** Corresponding author. Tel.: +46 46 222 01 03; fax: +46 46 222 41 16.
*** Corresponding author. Tel.: +46 46 222 83 06; fax: +46 46 222 46 22.
E-mail addresses: patrik.knoos@fkem1.lu.se (P. Knöös), Christopher.Schulz@biochemistry.lu.se (C. Schulz), marie.wahlgren@food.lth.se (M. Wahlgren).
[1] These authors contributed equally and are listed alphabetically.

http://dx.doi.org/10.1016/j.ijpharm.2014.03.060
0378-5173/

and Llorente, 1981) or enzymatic assays (Essig and Kleyn, 1983; Kleyn, 1985; Watson, 1994). Those methods are inconvenient for the purposes in the present investigation due to the requirement of mercury (for polarimetry and infrared absorption), large sample volumes (10 or 25 g of milk sample for chromatography or gravimetry respectively) or expensive reagents (enzymatic assays utilizing NADH). Enzymes have also been used for the construction of biosensors allowing the direct or indirect conductometric (Marrakchi et al., 2008) or amperometric (Adányi et al., 1999; Eshkenazi et al., 2000; Ferreira et al., 2003) detection of lactose, however, these are not commercially available and not standardized yet.

Cellobiose dehydrogenase (CDH) has been shown to be a potential candidate for the construction of amperometric lactose biosensors with the advantages of only minimal sample preparation, higher specificity compared to non-enzymatic methods and the requirement of low sample volumes (Glithero et al., 2013; Safina et al., 2010; Stoica et al., 2006; Tasca et al., 2013; Yakovleva et al., 2012). CDH is an extracellular, two-domain, monomeric, enzyme expressed by saprophytic and by some pathogenic fungi (Zamocky et al., 2006). The enzyme consists of a catalytic dehydrogenase domain, DH_{CDH}, containing FAD as cofactor and a cytochrome domain, CYT_{CDH}, containing heme *b* as cofactor and a linker region connecting the two domains. CDH is able to oxidize various sugars including the natural substrate cellobiose, but also lactose, and depending on the phylum also glucose. During substrate oxidation at the DH_{CDH} the cofactor FAD is fully reduced. The electrons are then sequentially transferred to the CYT_{CDH} domain via an internal electron transfer step (IET) (Henriksson et al., 2000; Ludwig et al., 2010, 2013). In vivo the CYT_{CDH} is reoxidized possibly by a copper dependent lytic polysaccharide monooxygenase (CaZy auxilliary family AA9 (Cantarel et al., 2009)) (Beeson et al., 2011; Langston et al., 2011; Phillips et al., 2011). For the construction of a biosensor utilizing CDH the electrode can either reoxidize directly the reduced CYT_{CDH} domain in what is denoted a direct electron transfer (DET) step, as in the presented study or an additionally introduced redox mediator is used to shuttle electrons from the DH_{CDH} and/or CYT_{CDH} domain to the electrode (Ludwig et al., 2013).

In a previous investigation the polycation polyethylenimine (PEI) was found to enhance the analytical signal of a lactose biosensor constituted of a graphite electrode, the PEI promotor layer and the enzyme CDH from *Myriococcum thermophilum* (*Mt*CDH) (unpublished results). The PEI layer was suggested to increase the enzyme load onto the biosensor surface by electrostatic interactions between the polycation and the negatively charged enzyme. Also a possible increase in the electron transfer, either the IET and/or the DET was suggested due to the positive charges present in PEI based on earlier finding on the beneficial effect of Ca^{2+} on the activity of CDH (Schulz et al., 2012). In the present study, CDH from *Corynascus thermophilus* (*Ct*CDH) is used instead of *Mt*CDH due to its higher activity in the human physiological pH present in the samples to be measured. Furthermore, the applicability of the polycation polydiallyldimethylammonium chloride (PDADMAC) as a promotor layer for the construction of an amperometric lactose biosensor using CDH is investigated. In contrast to PEI, the quaternary amine containing PDADMAC is positively charged independent on the solution pH. This assures a tight electrostatic binding between *Ct*CDH (pI = 3.8 (Harreither et al., 2011)) and the promotor layer, even at basic or alkaline pH where PEI starts to be deprotonated. The combination of *Ct*CDH with PDADMAC has the potential to be used as a fast and sensitive lactose biosensor enabling the detection of lactose release from drug containing tablets.

The drug in a polymer matrix tablet can primarily be released via two mechanisms, either by diffusion through the swollen tablet or by erosion together with the polymer (Colombo et al., 2000; Maderuelo et al., 2011; Skoug et al., 1993; Tahara et al., 1995). The gelatinous semi-dilute polymer solution formed outside the dry tablet core, conventionally referred to as the "gel layer", is considered to determine the release and dissolution of the polymer tablets (Colombo et al., 2000). The properties of the gel layer are important for the performance of the tablet for controlled release and an increased strength (i.e., viscosity) of the gel layer leads to an increased thickness (Maderuelo et al., 2011). This results in a slower dissolution of the tablet and a longer way for the drug to diffuse in order to be released, thus a slower drug release (Colombo et al., 2000; Efentakis et al., 2007).

It has been shown that the diffusion of hydrophobic or amphiphilic substances can be affected by the addition of surfactant in a polymer matrix, especially for hydrophobically modified polymers (HM-polymers) (Bramer et al., 2009; Paulsson and Edsman, 2002). Moreover, owing to hydrophobic interactions, surfactants generally affect the viscosity of HM-polymer solutions (Dualeh and Steiner, 1990; Magny et al., 1992), and can also solubilize such HM-polymers that, owing to extensive hydrophobic association, are not soluble in water (Dualeh and Steiner, 1990; Piculell et al., 2001). Surfactant sensitivity could potentially affect the performance of tablets of HM-polymers in vivo, due to the varying composition of amphiphilic compounds in the intestine (Abrahamsson et al., 1999; Dressman et al., 1998).

Previous investigations have shown that the release of hydrophobic substances from HMPAA tablets can be manipulated by adding surfactants and/or changing the ionic strength in the surrounding medium (Knöös et al., 2013a). The behavior was further studied by NMR Chemical Shift Imaging, revealing clear differences in the concentration profiles of the polymer during dissolution that could be correlated to the polymer solubility and phase behavior (Knöös et al., 2013b). In pure water, the tablets showed only minor swelling and a rapid disintegration and release of non-soluble but swollen tablet/polymer particles were seen, which increased the release rate. Addition of buffer to the medium and/or surfactant at concentrations above the CMC increased the solubility of the HM-polymer and the tablets swelled indefinitely. A thicker gel layer was formed and the release of a model hydrophobic substance was slowed down. A hypothesis in these initial experiments with HMPAA was that not only the polymer solubility, but also interactions between a hydrophobic active substance and the hydrophobic substituents (hydrophobes) on the polymer played a major role for the drug release. Lactose is a hydrophilic substance and should not associate with either the hydrophobes or the surfactant. It is thus interesting to see how this molecule differs in its release behavior compared to a hydrophobic or amphiphilic drug.

This work evaluates the potential of a new analytical technique for lactose analysis that could be used within the pharmaceutical industry to quickly and easily quantify the release of lactose from pharmaceutical formulations. The technique is used to gain a better understanding of the dissolution of controlled release tablets based on HMPAA via studying the release of lactose and ibuprofen. Traditionally the sodium salt of ibuprofen is not considered to be poorly soluble as it has solubility above 100 mg/ml (Bustamante et al., 2000); in fact, it is often formulated as an immediate release tablet. However, the acid form has a much lower solubility, 55.4 μM (Yazdanian et al., 2004). Furthermore, ibuprofen has an amphiphilic character and will form mixed micelles with both cationic and anionic surfactants (Bramer et al., 2006; Stephenson et al., 2006). Thus, ibuprofen was chosen, as it will likely interact with the hydrophobic polymer matrix as compared to lactose that will not show such interactions. SDS is used to examine the effects of surface-active compounds. SDS is a well-studied anionic surfactant that is also approved for oral

formulation. It has been established that SDS has a similar effect as bile salts (50/50 mixture of sodium taurocholate and sodium deoxycholate) on the release from Pemulen™ TR2 tablets (Knöös et al., 2013a).

2. Materials and methods

2.1. Materials

Pemulen™ TR2 NF (lots no. CC11MCU893 and 0100834373) was kindly provided by Lubrizol Advanced Materials (Brussels, Belgium). According to the supplier, the polymer consists of poly (acrylic acid), cross-linked with allylpentaerytritol, and contains 52–62 wt% of COOH groups. Pemulen is also hydrophobically modified with grafted C10–C30 alkyl-chains via ester bonds.

Ibuprofen sodium salt (lots no. 038K0755 and 87H0764) was purchased from Sigma–Aldrich Chemicals (Steinheim, Germany) and used as delivered. Sodium dodecyl sulphate (SDS) was acquired from VWR International (Poole, England) and used as delivered. The critical micelle concentration (CMC) for SDS is 8.3 mM in pure water (Holmberg, 2003) and was determined to be 1.8 mM at 37 °C in 0.1 M phosphate buffered solution, pH 7.2 using a tensiometer and a de Noüy ring.

The following chemicals were used during the tablet production: lactose, talc and magnesium stearate, which were of analytical grade. A (70:30) mixture of ethanol (99.5%) and 0.001 M aqueous HCl was used as granulation fluid.

For the development and application of the lactose biosensor the following chemicals were used: branched polyethylenimine (M_w = 25,000 g/mol, M_n = 10,000 g/mol), polydiallyldimethylammonium chloride (20 wt% in H_2O, M_w = 100,000–200,000 g/mol), β-lactose, hydrochloric acid (37%) were purchased from Sigma–Aldrich Chemicals. Sodium 4-*n*-octylbenzenesulphonate (SOBS) was purchased from TCI Europe N.V. (Zwijndrecht, Belgium). Cellobiose dehydrogenase from *Corynascus thermophilus* (*Ct*CDH) was obtained following a published procedure (Harreither et al., 2012). The enzyme was stored in a 50 mM sodium acetate buffer at pH 5.5 at a concentration of 9.4 mg/ml (determined spectroscopically at 280 nm as in Ref. (Harreither et al., 2012)).

2.2. Dissolution experiments

2.2.1. Tablet preparation

Tablets with compositions as specified in Table 1 were prepared as described previously (Knöös et al., 2013a). First, the dry ingredients lactose, polymer and ibuprofen were mixed in an intensive mixer (Kitchen Aid, Model K5SS), for 5 min. The granulation process started with gently spraying granulation fluid over the powder, with interruptions to allow the fluid to absorb. The procedure was continued until the desired properties of the granulation were achieved. The granules were then sieved through a 2 mm sieve, and if the particle size was too large, a food-processer (Philips, Cucina) was used to reduce it. The granules were then dried in an oven at 50 °C over night and were sieved again through a 2 mm sieve. The dried granules were mixed with lubricants (talc and magnesium stearate) in a cone blender for 2 min after each addition.

The tablets were manufactured with a single-punch tableting machine (Diaf, Type TM 20, Denmark) to a weight of around 400 ± 20 mg (n = 10). Appropriate settings were applied to ensure a good hardness and weight of the tablets. Hardness and friability were measured according US Pharmacopeia methods. All tablets achieved a hardness above 5 kp and a friability less than 1%.

Table 1
Compositions of tablets used in this study. The amount of ibuprofen was chosen from an experimental perspective in order to the match detection levels of the analyses.

Ingredient	Amount (wt%)
Polymer	30
Ibuprofen	10
Lactose	58
Talc	1
Magnesium stearate	1

2.2.2. Release studies

Dissolution experiments were carried out at 37 °C in a USP dissolution apparatus II (Prolabo Intelligent dissolution tester, Novakemilab, Enskede, Sweden) with a paddle speed of 100 rpm. For experiments in buffered solution the fluid was continuously withdrawn and transferred by means of a pump to a spectrophotometer (Cary 50 Bio UV–visible, Varian, Belrose, Australia), which measured the ibuprofen concentration in the vessels at the absorption of λ = 222 nm. The spectrophotometer measured according to the following cycles; 1) every minute for 10 min 2) every 10 min until one hour 3) every 20 min until six hours 4) every hour until completed experiment. For all remaining experiments, aliquots (V_a = 1 ml) were manually withdrawn from the vessels. The release of lactose was measured for all cases in the aliquots and analyzed as described below. The released amount (%release), for lactose and ibuprofen was calculated according to

$$\%\text{release} = \frac{[c_s \times (V_o - V_a(n_a - 1))] + \sum_{n=0}^{n_a-1}(V_a \times c_{s,n})]}{m_s} \quad (1)$$

where the concentration of ibuprofen or lactose from the analyzed aliquots is given by c_s and the initial volume of the vessel is V_0 (=800 ml). V_a and $c_{s,n}$ denote the volume and the concentration in the aliquots and the number of aliquots is denoted n_a. The amount of ibuprofen or lactose in the tablets is given by m_s.

The media used were 0.1 M phosphate buffered solution, pH 7.2, or water deionized in a Milli-Q water apparatus. The pH was not monitored during dissolution in pure water. SDS was added in varying amounts, yielding concentrations between 0 and 10 mM (in phosphate buffered solution) and 0–20 mM (in water).

2.2.3. Gravimetric erosion and swelling studies

Experiments to determine the erosion and swelling of the polymer tablets were performed, where the tablets were removed from the vessels and weighed. The experiments were carried out under the same conditions as the release studies above and with the same apparatus. Triplicates of tablets were studied during specified time intervals and were at designated times picked up with a spoon and placed on a pre-weighed mesh. Care was taken to avoid excess fluid on the mesh and to ensure that the whole tablet was extracted. The diameter of the swollen tablet was measured at the same time, both the total diameter and the core diameter. The tablets were then dried at 55 °C until no further weight loss was seen. Furthermore, aliquots were withdrawn from the USP vessels, at the time of tablet removal, to determine the amount of released lactose and ibuprofen (according to Eq. (1)). Using the measured dried weights (W_d), the released amount of polymer from the tablets could be calculated from the following mass-balance

$$W_d = m_{pol} + m_{ibu} + m_{lactose} \quad (2)$$

Here the mass of ibuprofen and lactose in the tablets (m_{ibu} and $m_{lactose}$) are obtained as

$$m_{ibu} = 0.1 \times W_i - c_{ibu} \times V_0 \quad (3a)$$

$$m_{lactose} = 0.58 \times W_i - C_{lactose} \times V_0 \quad (3b)$$

and W_i is the initial weight, $c_{lactose}$ and c_{ibu} the concentrations of lactose and ibuprofen, respectively, in the vessels with a volume of V_0. Here, we assume that the added lubricants (talc and magnesium stearate), which were added in low amounts did not contribute significantly to the total tablet weight. The amount of polymer in the dried tablet can thus be written as

$$m_{pol} = W_d - (m_{ibu} + m_{lactose}) \quad (4)$$

and the released fraction of polymer (release (pol)) can be calculated from

$$\text{release(pol)} = \left[\frac{0.3 \times (W_t - (m_{ibu} + m_{lactose}))}{0.3 \times W_i}\right] \quad (5)$$

The data from the gravimetric experiments were further used to calculate the relative swelling (RS) and the release of total dry mass (RM), which were defined as

$$RS = \frac{W_w - W_d}{W_d} \quad (6)$$

$$RM = \left(\frac{W_i - W_d}{W_i}\right) \times 100 \quad (7)$$

2.3. Lactose analysis

2.3.1. Solutions

For the precipitation of SDS, a solution of 2.5 mM PEI in 0.1 M PBS was prepared. At this high concentration the activity of the hydronium ions does not equal to its concentration, so the pH was stepwise adjusted with HCl so that a final 50 times diluted PEI solution had a pH of 7.2.

2.3.2. Preparation of the lactose biosensor

Spectrographic graphite electrodes (d = 3.05 mm, Alfa Aesar GmbH & Co., KG, Karlsruhe, Germany) were polished on wet emery paper (P1200 from Norton, Saint-Gobain Abrasives AB, Sollentuna, Sweden) and cleaned with Milli-Q water and allowed to dry. If not differently stated, the electrodes were immersed for 10 min in a 4 wt% aqueous solution of PDADMAC for the immobilization of the polycation PDADMAC. The PDADMAC modified electrodes were rinsed with Milli-Q water and allowed to dry. Enzyme modification was done by adding a drop of 4 μl of a solution of 9.4 mg/ml *Ct*CDH. All prior steps were performed at room temperature. The electrodes were kept in the fridge at 4 °C between 0.5 and 3 h until use.

2.3.3. Sample preparation

The samples taken during tablet dissolution contained SDS of concentrations between 0 and 20 mM present in either water or 0.1 M PBS, pH 7.2. SDS is known to be a denaturant for proteins able to also irreversibly inactive CDH used for the construction of the lactose biosensor (Fang et al., 1998) and had to be removed or inactivated. Branched PEI was shown to be able to form hydrophobic, coagulating complexes with SDS (Mészáros et al., 2003). Depending on the pH the polycation-surfactant complex formation is driven by electrostatic and hydrophobic interactions

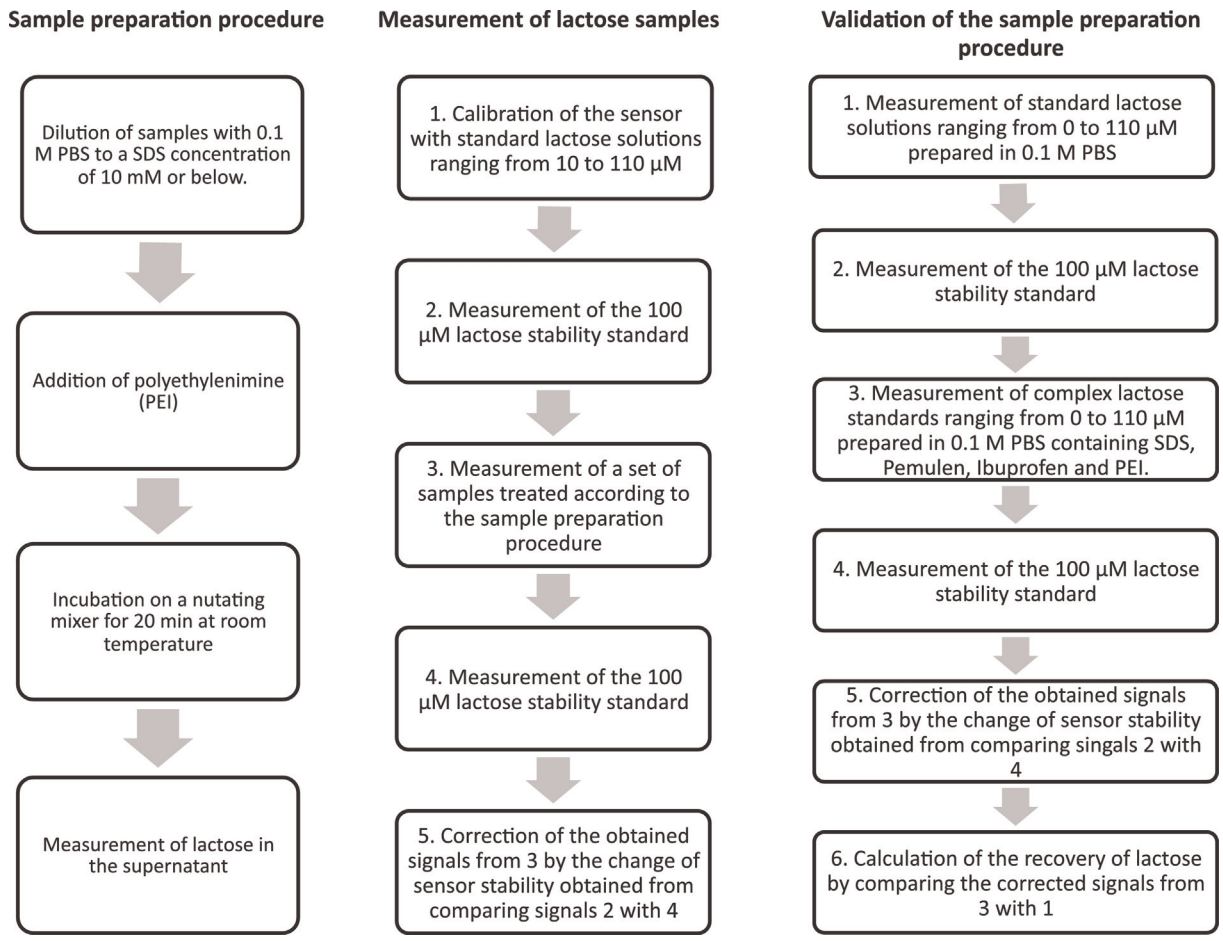

Fig. 1. Analytical flow chart describing the sample preparation procedure (left), the measurement of lactose samples (middle) and the validation of the sample preparation procedure (right).

(Li et al., 2000; Wang et al., 2006) partly accompanied by an uptake of protons (Bystryak et al., 1999; Mészáros et al., 2003). The ability of the PEI-SDS complex to form a hydrophobic coagulate is used for the establishment of a sample preparation procedure to diminish SDS present in the samples.

The SDS- and lactose-containing samples taken during tablet dissolution were diluted with 0.1 M PBS, pH 7.2 to an SDS concentration of ≤10 mM SDS and stored in a 1.5 ml polypropylene microtube. Twenty microliter of the 2.5 mM PEI solution was added to each 1 ml sample containing lactose and 10 mM SDS. If SDS concentrations were below 10 mM the volumes of the PEI solution added were adjusted accordingly. The samples containing PEI, SDS and lactose were incubated for 20 min on a nutating mixer (Gyromini, LabNet, Woodbridge, New Jersey, USA) at room temperature. A precipitate formed and adsorbed to the wall of the microtube. The supernatant was transferred into a new microtube and the lactose concentration was measured. Lactose samples in a 0.1 M PBS, pH 7.2 matrix, which did not contain SDS, were measured directly without a PEI treatment. Lactose samples in a H_2O matrix were diluted 1:5 with carrying buffer independently on their SDS content to adjust the matrix to the pH and the ionic strength of the carrying buffer (0.1 M PBS, pH 7.2). A flow chart of the sample preparation procedure is given in Fig. 1.

2.3.4. Measurement of lactose with flow injection analysis with amperometric detection

The lactose biosensors were rinsed with Milli-Q water prior to use to remove loosely adsorbed enzyme molecules. The biosensors were mounted into a flow-through, electrochemical wall-jet cell housing also an Ag|AgCl reference (0.1 M KCl) and a platinum wire counter electrode (Appelqvist et al., 1985). The electrodes were connected to an analogue potentiostat (Zäta Elektronik, Höör, Sweden) to control the applied potential and measure the currents obtained from the enzyme catalyzed lactose oxidation. The applied potential was 0 mV versus the Ag|AgCl (0.1 M KCl) reference electrode. The current signals were plotted on a strip chart recorder (Kipp & Zonen, Delft, The Netherlands). The flow cell was also connected to a six-valve injector (former Rheodyne, now IDEX Health & Science, Oak Harbor, USA) to control the injection of the samples. The samples were loaded into an injection loop with a volume of 20 μl. The injector was connected to a peristaltic pump (Gilson, Villier-le-Bel, France) pumping 0.1 M PBS, pH 7.2 carrying buffer at a speed of 0.5 ml/min to transport the samples from the injector to the flow cell containing the biosensor. Each sample or standard solution was injected at a rate of approximately one injection/min and at least three times and the average of the obtained signals was evaluated. Each newly mounted biosensor was calibrated with lactose standards with concentrations of 10, 30, 50, 70, 90 and 110 μM covering the linear range of the biosensor. It followed the injection of a lactose stability standard with a concentration of 100 μM to monitor and correct for changes in the biosensor response with time. Subsequently the supernatant of the samples, which have gone through the sample preparation procedure, were analyzed. In case the signals from the samples lay outside the linear range, they were further diluted with carrying buffer and measured again. The lactose stability standard was injected after each set of samples and the signals from the set of samples measured before were corrected by the possible deviation in sensor stability. A flow chart of the measurement of lactose samples is given in Fig. 1.

2.3.5. Validation of the sample preparation procedure

The lactose biosensor was calibrated using standard lactose solutions with concentrations of 10, 30, 50, 70 110, 500 and 5000 μM prepared in 0.1 M PBS, pH 7.2 according to the procedure described above. Complex standard lactose solutions containing equal concentrations of lactose (as above) prepared in 0.1 M PBS, pH 7.2 additionally containing 50 mM SDS and the tablet contents of 0.15 mg/ml Pemulen and 0.05 mg/ml Ibuprofen were treated according to the sample preparation procedure. Their analytical signals were measured and the recovery of lactose was calculated using the linear equation valid for the linear range of the biosensor. This was repeated independently from each other for 5 electrodes. A flow chart of the validation of the sample preparation procedure is given in Fig. 1.

3. Results

3.1. Lactose analysis

To detect lactose concentrations in samples taken during dissolution experiments of polymer matrix tablets also partly containing SDS, a sample preparation procedure to remove SDS and a lactose biosensor was developed to enable a fast, selective and sensitive detection of lactose in small sample volumes with minimal sample preparation. Therefore an amperometric measurement set-up was chosen utilizing graphite electrodes modified with the lactose oxidizing enzyme *Ct*CDH to gain selectivity and a PDADMAC promotor layer to the enhance sensitivity for the detection of lactose.

3.1.1. Signal enhancement of the lactose biosensor by a PDADMAC promoter layer

Based on previous findings on the beneficial effect of PEI on the response of *Mt*CDH modified biosensors (see Section 1), the applicability of the polycation PDADMAC as a promotor layer for the construction of *Ct*CDH modified biosensors was investigated. In Table 2 the apparent kinetic parameters for *Ct*CDH immobilized either on bare graphite or on PDADMAC modified graphite electrodes are summarized. The maximal catalytic current density J_{max} gained from lactose oxidation could be enhanced 120 times to 40.9 μA/cm^2 when PDADMAC was used as a promoter layer. Consequently for all lactose biosensors PDADMAC was used as a promotor layer. This increase in the maximal current density J_{max} can be attributed to a higher loading of enzyme onto the electrode surface, as suggested also for PEI/*Mt*CDH modified graphite electrodes (unpublished results). At the investigated pH of pH 7.2 *Ct*CDH is negatively charged (pI = 3.8 (Harreither et al., 2011)) allowing it to be bound electrostatically to the polycation PDADMAC. Also the IET within the enzyme or the final electron transfer from the enzyme to the electrode might be increased by PDADMAC due to a screening of repulsive, negative charges present on either the interface of the two domains of the enzyme or on the graphite electrode allowing a better electrical communication, as previously suggested for PEI (unpublished results) and $CaCl_2$ (Schulz et al., 2012).

The apparent K_M (K_M^{app}) was not changed significantly in the presence of PDADMAC indicating no mass transfer limitations of lactose to the immobilized enzyme through the PDADMAC layer.

Table 2
Calculated maximal current densities J_{max} and K_M^{app} obtained from the fitted response curves of SPG/*Ct*CDH lactose biosensors for lactose standard solutions without promotor layer (−PDADMAC), with promotor layer (+PDADMAC) and for complex lactose standard solutions treated with the sample preparation procedure (+SDS, +PEI) measured in a 0.1 M PBS, pH 7.2 buffer at an applied potential of 0 mV versus Ag|AgCl (0.1 M KCl). For each condition the response curves of three electrodes have been fitted individually and the averages and standard deviations of the obtained parameters have been calculated and are given in the table.

	−PDADMAC	+PDADMAC	+PDADMAC, +SDS, +PEI
J_{max}/nA cm^{-2}	340 ± 200	40,890 ± 8530	46,400 ± 7990
K_M^{app}/μM	760 ± 90	560 ± 100	600 ± 70

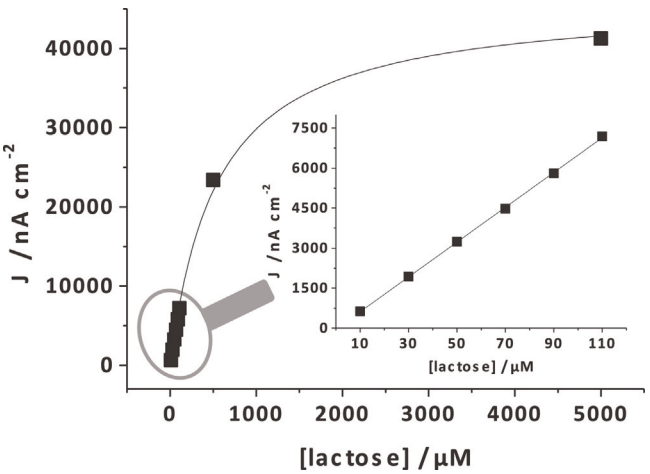

Fig. 2. Amperometric response of a SPG/PDADMAC/*Ct*CDH lactose biosensor to lactose standard solutions and linear range of the biosensor (inset) measured in a 0.1 M PBS, pH 7.2 buffer at an applied potential of 0 mV versus Ag|AgCl (0.1 M KCl).

The variations in the response between equally modified electrodes are rather high (see standard deviation in Table 2), so for the latter measurement of lactose in the samples, each used biosensor was calibrated using six standard lactose concentrations prior to use.

In Fig. 2 a typical response curve of a graphite/PDADMAC/*Ct*CDH lactose biosensor is shown. Since it is an enzymatic biosensor the signal gained from high substrate concentrations (here above 110 μM lactose) levels off as described by the Michaelis–Menten equation (Michaelis and Menten, 1913). The linear range of the biosensor response was between 1 and 110 μM using standard lactose solutions but only the range between 10 and 110 μM was used for the measurements of lactose in the samples, as discussed later (see inset Fig. 2).

3.2. Evaluation of removal of SDS by PEI

SDS present in the lactose containing samples, at concentrations between 0 and 20 mM, had to be removed or reasonably decreased to avoid the denaturation of *Ct*CDH used for the construction of the lactose biosensor. The measurement of untreated, 1 mM SDS, 100 μM lactose containing standards lead to irreproducible and rapid, irreversible decreases in the sensor response. Various methods were investigated to remove SDS from the lactose containing samples based on either ionic interactions (anion exchange chromatography) or hydrophobic interaction (incubation with commercially available polystyrene beads). Ion exchange chromatography was found to change the ionic strength/composition of the sample leading to additional, interfering electrochemical signals not related to the oxidation of lactose. The addition of the polystyrene beads to the SDS/lactose containing samples lead to a significant, apparent decrease in the lactose concentration in the samples. Possibly capillary liquid entrapped between the pre-soaked beads diluted the samples in an irreproducible manner. Disadvantageous was also the long incubation time necessary for SDS to bind to the polystyrene beads.

A method to precipitate the negatively charged SDS by the addition of branched PEI was developed. The precipitation of the PEI/SDS complex is due the formation of an uncharged complex between SDS and branched PEI (Mészáros et al., 2003; Wang et al., 2006). The amount and concentration of PEI necessary to be added to complex lactose standards (containing 10 mM SDS) were optimized with respect to a sufficient precipitation of SDS, high biosensor viability, minimal sample dilution and minimization of the sensor response to excess PEI. This led to the sample preparation procedure as described in Section 2.

To evaluate how much SDS could be removed by the sample preparation procedure the UV active anionic surfactant SOBS was used instead of SDS. It has a similar molecular weight, also a sulfate head group but shows UV absorption with an absorption maximum at 261 nm (experimentally determined). Applying the developed sample preparation procedure to a 10 mM SOBS solution 96 ± 1% ($n = 3$) of SOBS could be removed from the sample as determined photometrically. Assuming a comparable clearance by PEI for SDS as for SOBS, this means a residual leftover of SDS in PEI treated samples in the μM range. Furthermore, it was evident that the viability of the lactose biosensor was not decreased by the residual SDS. In fact the residual SDS seemed to affect the stability of the lactose biosensor positively, as indicated by an average 1.06 times higher response to the lactose stability standard after the measurement of PEI treated complex lactose standards (determined experimentally, see Section 2 or Fig. 1 Validation of the sample preparation procedure). A similar trend was observed for the measurement of PEI treated samples taken from the dissolution experiments. After an average of 80 measurements of PEI treated SDS/lactose samples the sensors responded in average 1.12 ± 0.08 ($n = 4$) higher to the lactose stability standard of 100 μM (determined experimentally, see Section 2 or Fig. 1). These sensor drifts were corrected as described in Section 2. There is possibly residual SDS binding to PDADMAC (Mukherjee et al., 2011) and/or CtCDH. How this might have altered the biosensor response was not further investigated. The dilution of the sample by the addition of PEI was only 2% and was neglected.

The injection of blank samples treated with the sample preparation procedure gave signals as high as a standard lactose solution with a concentration of average 6 ± 1.4 μM ($n = 5$). Excess PEI possibly changes the ionic strength of the sample, which creates a pseudo-response due to the recharge of the electrochemical double layer of the biosensor. Consequently only analytical signals higher or equal to the one of a 10 μM lactose standard solution were evaluated.

In Fig. 3 the average absolute recovery of lactose in complex lactose standard solutions treated with the sample preparation procedure is plotted versus the designated lactose concentration. The lactose concentrations of the complex, PEI treated standards are slightly overrated in their designated concentrations by

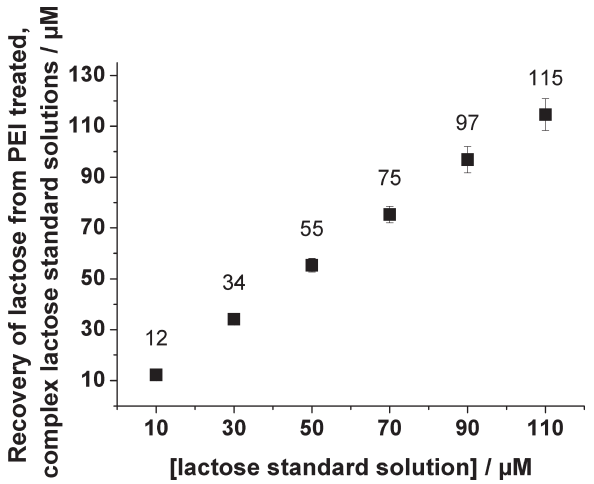

Fig. 3. Recalculated average recovery of lactose (see label) from complex lactose standard solutions containing lactose, 10 mM SDS, 0.03 mg/ml Pemulen and 0.01 mg/ml ibuprofen in 0.1 M PBS, pH 7.2 after treatment with the sample preparation procedure using PEI to precipitate SDS. The error bars indicate the standard deviation of five individually performed experiments.

maximum average +7 ± 5 μM for 90 μM lactose (n = 5). Thus, even if the sensor response was corrected by the response to the lactose stability standard the lactose concentrations in the complex lactose standards (containing 10 mM SDS) were still overrated, possibly caused by residual SDS and/or PEI with a standard deviation of ±5 μM (random error). However, the sensor responses were not corrected for the apparent systematic overrating of the real lactose concentrations, since the lactose samples were present in different matrices (PBS and H_2O), contained various concentrations of SDS (0 to 20 mM) and PEI and had to be diluted differently depending on their individual lactose concentration to end up in the linear measuring range of the biosensor. Consequently, the systematic error of maximum +7 μM was treated as a random error and all measured lactose concentrations in the samples are seen to have an overall random error of maximum ±13% ((±7 μM ± 5 μM)/90 μM).

3.3. Dissolution and release of HMPAA tablets

The release of ibuprofen and lactose for tablets dissolving in water and in buffered solutions are shown in Fig. 4, with and without SDS added to the surrounding media. Based on the behavior observed in previous work (Knöös et al., 2013a), the SDS concentration was chosen to be well above the CMC values of the surfactant (see Section 2) in water and buffer, respectively. The behavior of the tablets in pure water might seem irrelevant for pharmaceutical development of controlled release formulations. However, water was used for comparison with previous performed experiments in water, where the polymer showed very low swelling and differed in release characteristics compared to buffered solutions (Knöös et al., 2013a).

Fig. 4 reveals strong variations in the release patterns depending on the presence or absence of both buffer and surfactant. A rapid and, within error, simultaneous release of lactose and ibuprofen was seen in pure water. Here, it was also observed that the tablet had completely disintegrated after 24 h. In all other experiments, the dissolution of the tablets was more or less slow, and the release profiles differed between ibuprofen and lactose. For ibuprofen, the addition of surfactant to either water or buffer slowed down the release with a retained linear profile. In some cases the tablets appeared to release more than 100% when fully dissolved (see Fig. 4c). This is most likely due to accumulation of tablet particles in the flow cells of the UV/vis apparatus (see Section 2). Accumulated particles increase the background absorbance, affecting the calculated concentration of ibuprofen. As in our previous study (Knöös et al., 2013a), the tablets in this study show very long release times for ibuprofen, due to a high content of polymer. This tablet design was chosen in order to avoid possible experimental variations due to tablet inhomogeneities. However, the aim of the present study is to evaluate the release of lactose and possible effects of amphiphiles, not to optimize the release time. Preliminary results indicate that the dissolution times can be decreased by decreasing the polymer content.

The release of lactose from the tablets was more rapid than the ibuprofen release, except in pure water, and most of the lactose had been released already after 24 h in all media. In contrast to the results for ibuprofen, only minor effects on the lactose release were seen if buffer and/or SDS was added to the release medium. Tablets

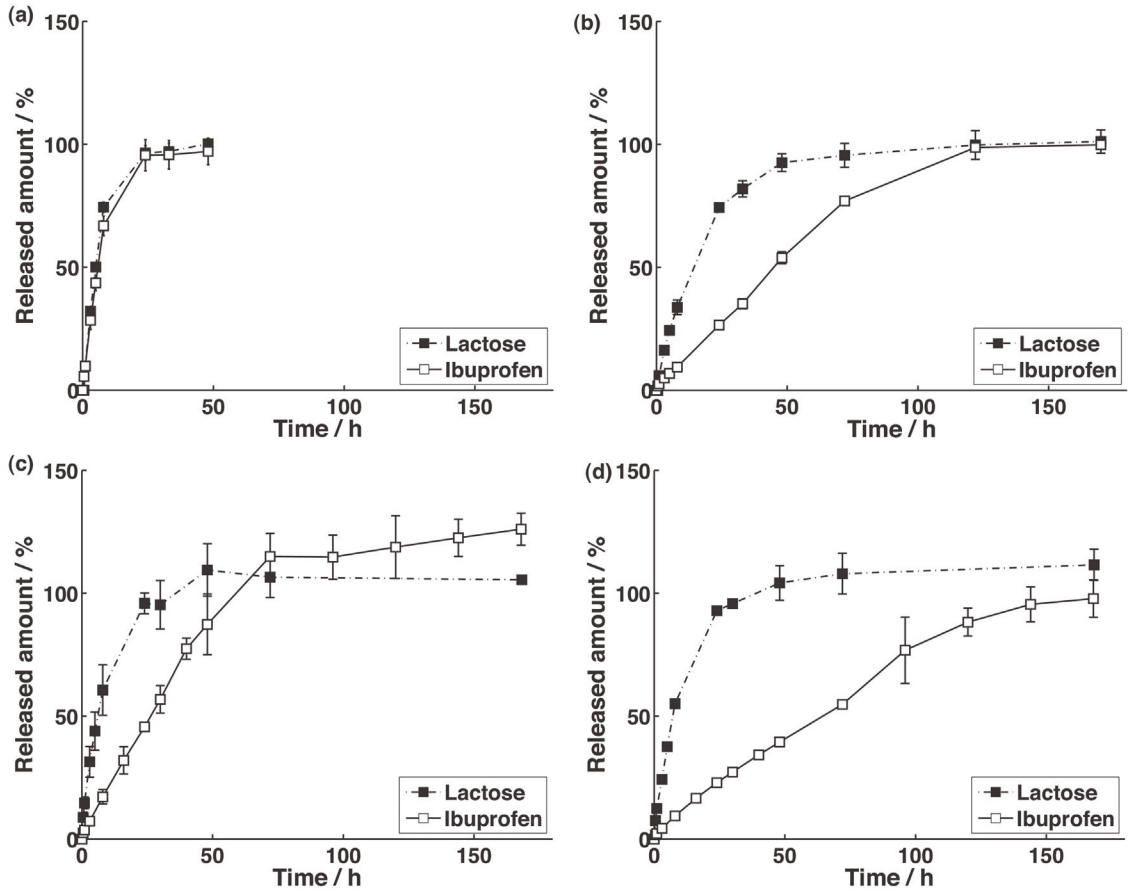

Fig. 4. The release of lactose (filled squares) and ibuprofen (open squares) from HMPAA tablets in water without (a) or with (b) 20 mM SDS, and in buffer solutions without (c) or with (d) 10 mM SDS. The released amount is calculated according to Eq. (1) and error bars indicate the standard deviation (n = 3).

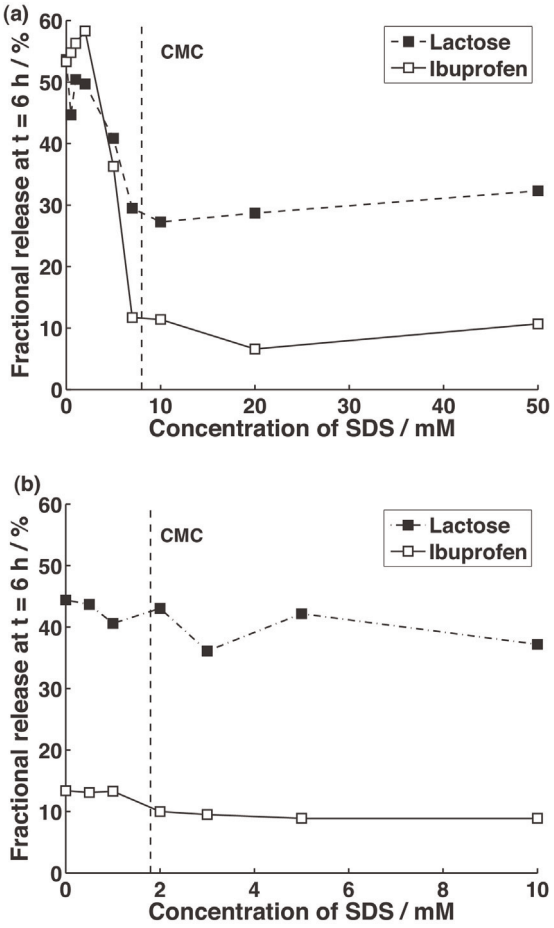

Fig. 5. Released fractions at t = 6 h in water (a) and buffer (b) solutions with varying concentration of SDS. The release for lactose is given by filled squares and ibuprofen by open squares. The values are an average of two tablets. The dashed vertical lines indicate the CMC values for SDS in the two solutions, water and buffer, respectively.

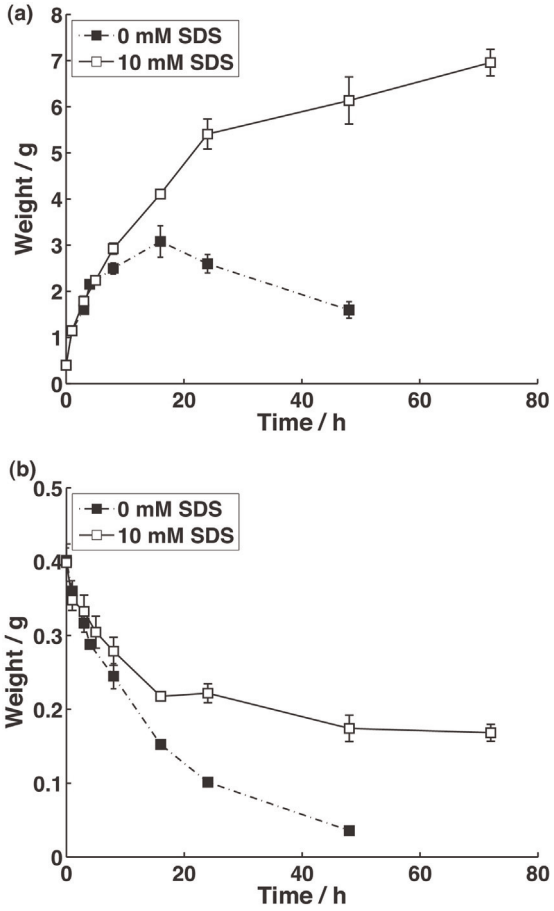

Fig. 6. Measured wet (a) and dry (b) weights of tablets dissolving in buffered solutions with (open symbols) and without (filled symbols) addition of 10 mM SDS. Error bars indicate the standard deviation ($n = 3$), except at zero hour which is the average value of all tablets where n is equal to 21 or 24 for pure buffer and buffer with surfactant, respectively.

dissolving in buffered solution showed almost no difference in lactose release rates with or without SDS in the bath, whereas the release in water was slowed down somewhat upon addition of 20 mM SDS.

To further compare the effects of the dissolution medium and surfactant concentration on the release of lactose and ibuprofen, the released fractions of the two compounds were evaluated at $t = 6$ h (Fig. 5) for a series of different surfactant concentrations (0–20 mM in water and 0–10 mM in buffer). The trends observed for ibuprofen have already been described in (Knöös et al., 2013a), but here we can compare with the behavior of lactose. For tablets dissolving in water the two model substances showed a qualitatively similar response to surfactant added to the medium (Fig. 5a). Here, a decrease in released amount with increasing surfactant concentration was observed below the CMC, followed by a leveling off at higher concentrations. The decrease in release rate below the CMC was quite steep for ibuprofen, but less pronounced for lactose. In buffered solution (Fig. 5b), the released amounts of the two substances differed at all surfactant concentrations. The release of ibuprofen was slightly, but significantly, affected by the addition of surfactant, again with a leveling off above the CMC. We note that the released amount of ibuprofen was similar in both media above the CMC. For lactose, the scatter in the data does not allow us to draw any conclusions regarding effects of surfactant addition. We note that the trends of Fig. 5 confirm the trends observed in the results of Fig. 4.

3.4. Water uptake, erosion and swelling

In order to further study the dissolution and release behavior of HMPAA tablets, gravimetric studies were performed, where tablets were, at designated times, picked up from the baths and the wet and dry weights were noted. For this purpose we chose to study the behavior only in buffered solution, which is more relevant for in vivo behavior. It was also possible to calculate the released amount of the polymer from the measured wet and dry weights.

The weights of the wet and dried tablets from the gravimetric studies are shown in Fig. 6 for tablets dissolving in buffered solution with and without 10 mM SDS added to the release media. In surfactant-free buffer the tablets showed a rapid increase in wet weight and a maximum was reached at 16 h. At later times the tablets started to decrease in size and at 48 h only small amounts of the tablets were left. For tablets dissolving in the presence of surfactant, the wet weight continued to increase throughout the duration of the experiment (72 h). In surfactant solution the tablets showed an initial rapid decrease of the dry weight, which leveled off at later times, and a large fraction of the dry tablet was still left at 72 h of dissolution. In the presence of surfactant the tablet erosion became slower and the initial decrease was most likely due to the rapid lactose release, as seen above.

The values for the relative swelling and release of total dry mass, calculated from Eqs. (6) and (7), respectively, are shown in

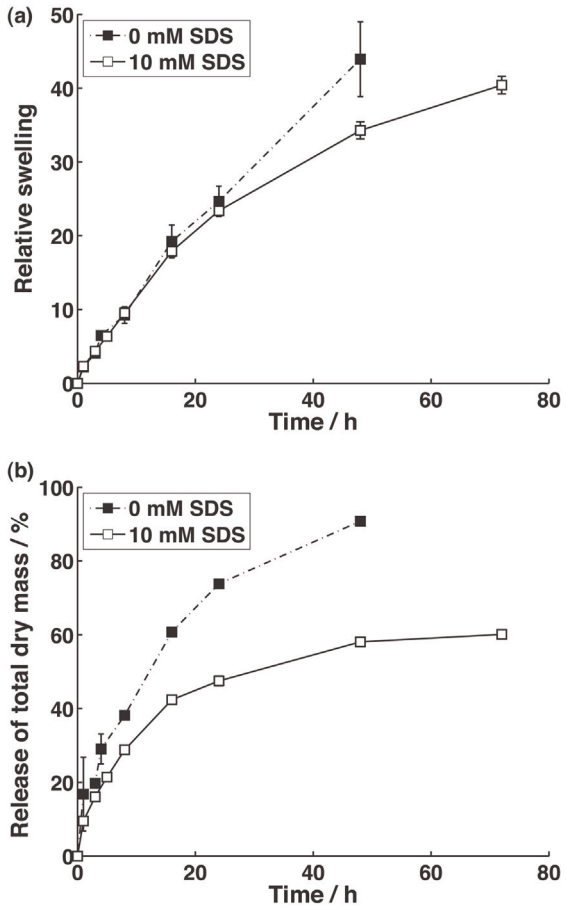

Fig. 7. The relative swelling (a) and release of total dry mass tablets (b) dissolving in pure buffer (filled symbols) and in 10 mM SDS buffer solution (open symbols). Error bars indicate the standard deviation ($n = 3$).

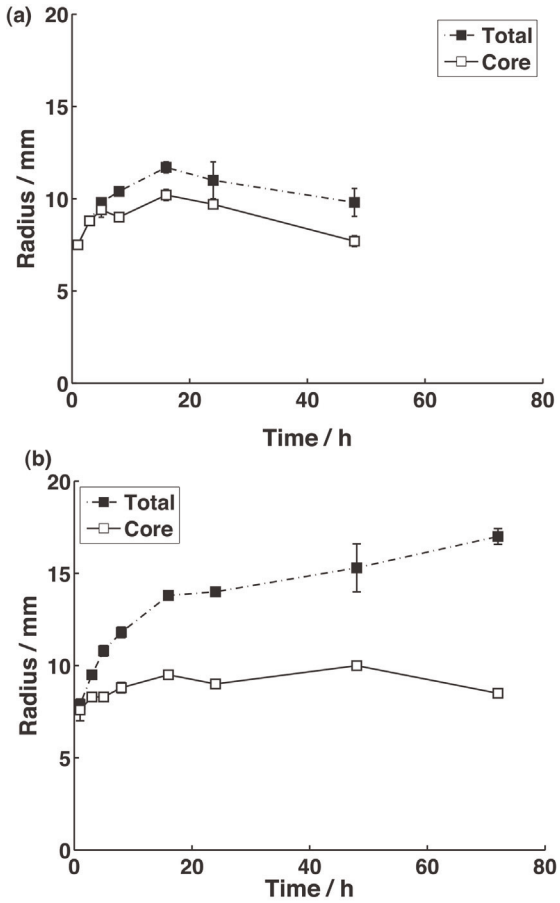

Fig. 8. Measured total (filled) and core (unfilled) radii of the swollen tablets at indicated times, dissolving in pure buffer (a) and in 10 mM SDS buffer solution (b). Error bars indicate the standard deviation ($n = 3$).

Fig. 7. The systems with and without SDS showed a similar swelling during the first hours of dissolution (Fig. 7a), however, after 24 h the data started to differ and the matrices in surfactant-free buffer were more swollen. It seems likely that the rapid initial solvent penetration was mainly driven by the osmotic pressure caused by the large fraction of lactose in the tablets. As the lactose content decreased due to the rapid lactose release, the differences caused by the presence or absence of surfactant in the medium became more obvious. At later stages the relative swelling slowed down in the surfactant solution, whereas the rate of relative swelling was similar throughout the dissolution period in surfactant-free buffer. The value for the relative swelling should at long times approach a value corresponding to the concentration of polymer present at the erosion front (Körner et al., 2005). However, even at the termination of the experiment this limiting case was presumably not reached, since the tablets were still far from complete dissolution – especially the tablets in surfactant solution.

The release of total dry mass (Fig. 7b) for the two systems differed more than the relative swelling. In surfactant-free buffer the tablets continued to erode throughout the dissolution time, but for tablets in buffer with surfactant the erosion seemed to slow down and at later times the rate of erosion was very slow. The results confirm the conclusions put forward above and show that the erosion of the tablets slowed down upon surfactant addition. Moreover, the results show that the initial decrease tablet mass is mostly due to the release of lactose. Once the lactose is released the tablet weight does not seem to decrease instead we are left with a swollen polymer matrix.

The swelling behavior of the tablets could be further established visually and via measuring the sizes of the extracted tablets (Fig. 8). All dissolving tablets were surrounded by transparent gel layers and in the middle of the tablet a white "core" could be seen, which at closer examination showed a sponge-like texture. Comparing the gel layer thickness (i.e., total radius – core radius) between the two media showed that the gel layers increased in thickness with added SDS. In surfactant-free buffer the tablets started to decrease in size after 16 h of dissolution, whereas the tablets in buffer with surfactant continued to grow throughout the experimental period. Moreover the gel layer increased in thickness throughout the dissolution time for both media and the tablet core started to decrease in size at later times. The results are quite consistent with the results above from the gravimetric studies. In the presence of surfactant, the tablets erode very slowly and seem to swell continuously. Furthermore, the results follow the previous observations that the largest effects on addition of surfactant was seen for the gel layer thickness (Knöös et al., 2013a; Knöös et al., 2013b).

The releases of all three major components (polymer, ibuprofen and lactose) of the tablets from the gravimetric studies are shown in Fig. 9. The releases of lactose and ibuprofen followed the patterns already seen in Fig. 4. Nonetheless, with some small differences, presumably due to the fact that all data points in Fig. 9 correspond to different tablets; each experiment was obviously terminated when the tablet was removed from the bath for gravimetric analysis. The release of the polymer with time in surfactant-free buffer was similar to that of ibuprofen, indicating

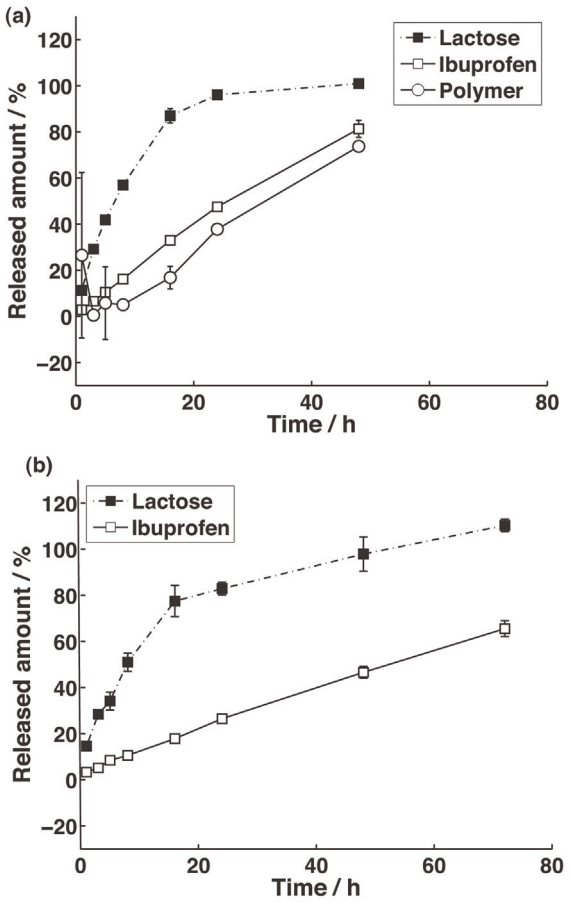

Fig. 9. Release of ibuprofen (filled squares), lactose (open squares) and polymer (open circles) from tablets dissolving in pure buffer (a) and in 10 mM SDS buffer solution (b). The polymer release is not shown for tablets dissolving in surfactant solution, for description of the release the reader is referred to the text. Error bars indicate the standard deviation ($n = 3$).

that the two releases were correlated (Skoug et al., 1993), as discussed above. The diffusional release of ibuprofen from the tablet was sufficiently slow so that the tablet erosion significantly contributed to the total release. When adding surfactant to the system the released amount of polymer was too small to be determined by our gravimetric approach. In fact the dry weight of the extracted tablets was higher than the initial polymer content, possibly due at least in part to the absorption of buffer and SDS from the medium (these contributions to the dry mass are neglected in Eq. (4) above). This indicates two things, a very slow release of the polymer and binding of SDS to the polymer matrix.

3.5. Drug release from polymer tablets

From the release and gravimetric studies, three distinct release scenarios for tablets containing the two compounds ibuprofen and lactose in different release media can be identified. In part, the differences are due to the interactions of the HMPAA polymer with both buffer and surfactant. In addition, lactose and ibuprofen interact very differently with the other components in the studied systems.

In the first scenario, for the release into water, both lactose and ibuprofen show a similar release patterns, indicating similar release mechanisms. In pure water, the tablets showed only a limited swelling and formed thin gel layers, as observed previously and confirmed here (Knöös et al., 2013a). Furthermore, eroded hydrated tablet particles were present in the bath that disintegrated from the tablet matrix. During our previous studies we have established that the polymer has poor water solubility and has a maximum swelling equal to ca. 25 wt% polymer content (Knöös et al., 2013b). This means that the polymer layer can only swell to this polymer concentration and the convection will shear off hydrated insoluble polymer/tablet particles (Knöös et al., 2013a; Knöös et al., 2013b). This leads to a rapid disintegration of the tablet that determines the release of both substances.

In the second scenario, for the release into phosphate buffer, the releases of lactose and ibuprofen differ. Lactose is released comparatively rapidly, whereas the releases of polymer and ibuprofen almost coincide, which indicates that ibuprofen is released mainly due to erosion of the tablet. In buffer, the carboxylic acid groups of HMPAA become partly charged (Laguecir et al., 2006; Mylonas et al., 1999). The tablet then swells more than in water and a thicker gel layer is formed that leads to slower erosion, thus a slower release of ibuprofen (compared to water). The release of lactose is marginally slower in surfactant-free buffer (compared to pure water), due to the decreased erosion of the tablets in buffer. The different release profiles for the polymer and lactose indicates that lactose is primarily released by a rapid diffusional transport, which is faster than the tablet erosion.

In the third scenario, for release into surfactant solutions, the releases of all three components differ. In the presence of surfactant (above CMC) the polymer is solubilized and the tablets can swell indefinitely (Knöös et al., 2013a). The thickness of the gel layer is then determined by the balance of the cohesive forces between the polymer molecules and the applied force from the convection (Borgquist et al., 2006; Körner et al., 2005). Furthermore, the surfactant most likely strengthens the polymer matrix by increasing the lifetime of the temporary cross-links formed by the hydrophobes (Magny et al., 1992). The tablets formed thick gel layers and showed a very slow dissolution/erosion. Lactose has about the same release rate as in surfactant-free media indicating that it is released through a rapid diffusion that is only slightly slowed down by the increased thickness of the swollen and stagnant polymer matrix. The effect of added surfactant on the lactose release is marginal in buffer, but significant in pure water (Figs. 4 and 5), where added surfactant also has the strongest effect on the swelling and disintegration of the polymer matrix and thus could effect the diffusion path of lactose. The release of ibuprofen is very slow but, nevertheless, faster than the polymer release.

It remains to discuss the interactions experienced by lactose and ibuprofen in the studied systems. Lactose is an uncharged hydrophilic substance that dissolves in water without associating with either the hydrophobes on the polymer or the surfactant. Ibuprofen, on the other hand, should have a quite complex behavior in the swollen HMPAA tablets. Although added in the salt form, ibuprofen can be partially protonated by proton exchange with the abundant acrylic acid groups' chain of the polymer. The studied system will thus be composed of a mixture of sodium salt and the acid form of ibuprofen, at varying water contents, depending on the degree of water penetration. Moreover, in the outermost part of the tablet, the ratios of the two forms will be affected by the penetrating phosphate buffer. (In the interior of the tablet, the carboxylate/carboxylic acid system will clearly be the dominating buffer system.) On top of this, ibuprofen will interact with the added surfactant and/or the hydrophobes and form mixed aggregates. A detailed modeling is clearly beyond the scope of this paper. However, hydrophobic interactions with the other components must decrease the concentration of molecularly dissolved ibuprofen in water. As stated above ibuprofen is known to form mixed micelles with SDS (Stephenson et al., 2006). We also know that the penetrating surfactant will form mixed micelles with the HMPAA hydrophobes (Piculell et al., 2001). It is therefore likely that a majority of the ibuprofen molecules will be associated with

the mixed aggregates formed by the hydrophobes and/or SDS. In media containing sufficient concentrations of SDS, the release of ibuprofen should thus depend on the diffusion through the gel layer of a small fraction of ibuprofen molecules molecularly dissolved in water; hence, it will be very slow (Bramer et al., 2009).

4. Conclusions

In the present study the release of lactose (hydrophilic) and ibuprofen (hydrophobic) from dissolving drug tablets containing lactose, ibuprofen and HMPAA were investigated in the presence or absence of the surfactant SDS in the dissolution bath. The lactose release was analyzed quantitatively by a novel lactose biosensor utilizing the sugar-oxidizing enzyme *Ct*CDH immobilized on a PDADMAC modified graphite electrode. The promotor layer PDADMAC increased the analytical signals by on average 120 times improving the sensitivity of the sensor possibly due to an increased loading of sensing *Ct*CDH molecules onto the biosensor and an increased interdomain or heterogeneous electron transfer from the enzyme to the electrode. The linear measuring range of lactose was between 10 and 110 μM lactose with a standard deviation of 13%. A fast and easy sample preparation procedure was developed to precipitate denaturating SDS from lactose containing samples by the addition of PEI forming a precipitate due to hydrophobic and electrostatic interactions. The addition of PEI diluted the samples by less than 2% and eliminated around 96% of the SDS within only 20 min enabling a fast and easy measurement of lactose present in samples taken during the dissolution of the tablets.

The release of lactose and ibuprofen differed vastly and the release of ibuprofen from tablets with HMPAA was greatly affected by the presence of surfactant and/or buffer, while the lactose release showed only minor differences. The release behavior of ibuprofen and lactose could be correlated to their different interactions with the polymer and/or the surfactant. A comparison of the release between these two tablet components, combined with studies on the swelling and disintegration of the polymer matrix, allowed us to identify and distinguish between diffusion and erosion controlled release mechanisms from the same tablet formulation. In principle three different scenarios for release could be deduced. For the first scenario, both substances showed similar release profiles and the releases were determined by the rapid tablet erosion. In the second scenario the substances differed, where lactose was mostly dependent on the transport and diffusion through the swollen tablets. Here, ibuprofen was primarily released with the polymer as the latter eroded. In the final scenario, the presence of surfactant slowed down the erosion of the tablets, and none of the three major components showed similar releases. The release of lactose was about the same as in surfactant-free buffer indicating that the rapid diffusion is only slightly slowed down. The release of ibuprofen was instead very slow, nevertheless faster than the polymer release, and the release became dependent on the slow diffusional transport of ibuprofen through the swollen tablets. The strategy and analyses used in this paper are able to further validate previous hypotheses (Knöös et al., 2013a; Wahlgren et al., 2009) and clearly show that the hydrophobic interactions between ibuprofen and the hydrophobically modified polymer are important for the release. By relatively simple dissolution tests and analyses, a deeper understanding of the system could be achieved.

Acknowledgements

The Research School in Pharmaceutical Sciences (FLÄK) is gratefully recognized for financial support (PK) as well as the European Commission ("Chebana" FP7-PEOPLE-2010-ITN-264772, CS, LG).

References

Abrahamsson, B., Roos, K., Sjogren, J., 1999. Investigation of prandial effects on hydrophilic matrix tablets. Drug Development and Industrial Pharmacy 25, 765–771.
Adányi, N., Szabó, E.E., Váradi, M., 1999. Multi-enzyme biosensors with amperometric detection for determination of lactose in milk and dairy products. European Food Research and Technology 209, 220–226.
Appelqvist, R., Marko-Varga, G., Gorton, L., Torstensson, A., Johansson, G., 1985. Enzymatic determination of glucose in a flow system by catalytic oxidation of the nicotinamide coenzyme at a modified electrode. Analytica Chimica Acta 169, 237–247.
Aulton, M.E., Cooper, J.W., 1988. Pharmaceutics: The Science of Dosage Form Design, first ed. Churchill Livingstone, Edinburgh, New York.
Beeson, W.T., Phillips, C.M., Cate, J.H.D., Marletta, M.A., 2011. Oxidative cleavage of cellulose by fungal copper-dependent polysaccharide monooxygenases. Journal of the American Chemical Society 134, 890–892.
Borgquist, P., Körner, A., Piculell, L., Larsson, A., Axelsson, A., 2006. A model for the drug release from a polymer matrix tablet – effects of swelling and dissolution. Journal of Controlled Release 113, 216–225.
Bramer, T., Dew, N., Edsman, K., 2006. Catanionic mixtures involving a drug: a rather general concept that can be utilized for prolonged drug release from gels. Journal of Pharmaceutical Sciences 95, 769–780.
Bramer, T., Frenning, G., Grasjo, J., Edsman, K., Hansson, P., 2009. Implications of regular solution theory on the release mechanism of catanionic mixtures from gels. Colloids and Surfaces B: Biointerfaces 71, 214–225.
Bustamante, P., Pena, M.A., Barra, J., 2000. The modified extended Hansen method to determine partial solubility parameters of drugs containing a single hydrogen bonding group and their sodium derivatives: benzoic acid/Na and ibuprofen/Na. International Journal of Pharmaceutics 194, 117–124.
Bystryak, S.M., Winnik, M.A., Siddiqui, J., 1999. Unusual conductivity changes for sodium dodecyl sulfate solutions in the presence of polyethyleneimine and polyvinylamine. Langmuir 15, 3748–3751.
Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2009. The carbohydrate-active enzymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Research 37, D233–D238.
Colombo, P., Bettini, R., Santi, P., Peppas, N.A., 2000. Swellable matrices for controlled drug delivery: gel-layer behaviour, mechanisms and optimal performance. Pharmaceutical Science & Technology Today 3, 198–204.
Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharmaceutical Research 15, 11–22.
Dualeh, A.J., Steiner, C.A., 1990. Hydrophobic microphase formation in surfactant solutions containing an amphiphilic graft copolymer. Macromolecules 23, 251–255.
Eadala, P., Waud, J.P., Matthews, S.B., Green, J.T., Campbell, A.K., 2009. Quantifying the 'hidden' lactose in drugs used for the treatment of gastrointestinal conditions. Alimentary Pharmacology & Therapeutics 29, 677–687.
Efentakis, M., Pagoni, I., Vlachou, M., Avgoustakis, K., 2007. Dimensional changes, gel layer evolution and drug release studies in hydrophilic matrices loaded with drugs of different solubility. International Journal of Pharmaceutics 339, 66–75.
Eshkenazi, I., Maltz, E., Zion, B., Rishpon, J., 2000. A three-cascaded-enzymes biosensor to determine lactose concentration in raw milk. Journal of Dairy Science 83, 1939–1945.
Essig, A.M., Kleyn, D.H., 1983. Determination of lactose in milk: comparison of methods. Journal: Association of Official Analytical Chemists 66, 1514–1516.
Fang, J., Liu, W., Gao, P.J., 1998. Cellobiose dehydrogenase from schizophyllum commune: purification and study of some catalytic, inactivation, and cellulose-binding properties. Archives of Biochemistry and Biophysics 353, 37–46.
Ferreira, L.S., Souza Jr, M.B., Trierweiler, J.O., Hitzmann, B., Folly, R.O.M., 2003. Analysis of experimental biosensor/FIA lactose measurements. Brazilian Journal of Chemical Engineering 20, 07–13.
Gao, P., Skoug, J.W., Nixon, P.R., Ju, T.R., Stemm, N.L., Sung, K.C., 1996. Swelling of hydroxypropyl methylcellulose matrix tablets. 2. Mechanistic study of the influence of formulation variables on matrix performance and drug release. Journal of Pharmaceutical Sciences 85, 732–740.
Glithero, N., Clark, C., Gorton, L., Schuhmann, W., Pasco, N., 2013. At-line measurement of lactose in dairy-processing plants. Analytical and Bioanalytical Chemistry 405, 3791–3799.
Harreither, W., Felice, A.K.G., Paukner, R., Gorton, L., Ludwig, R., Sygmund, C., 2012. Recombinantly produced cellobiose dehydrogenase from *Corynascus thermophilus* for glucose biosensors and biofuel cells. Biotechnology Journal 7, 1359–1366.
Harreither, W., Sygmund, C., Augustin, M., Narciso, M., Rabinovich, M.L., Gorton, L., Haltrich, D., Ludwig, R., 2011. Catalytic properties and classification of cellobiose dehydrogenases from ascomycetes. Applied and Environmental Microbiology 77, 1804–1815.
Henriksson, G., Johansson, G., Pettersson, G., 2000. A critical review of cellobiose dehydrogenases. Journal of Biotechnology 78, 93–113.
Holmberg, B.J., Kronberg, B., Lindman, B., 2003. Surfactants and Polymers Aqueous Solutions, second ed. John Wiley & Sons Ltd., Chichester.

Kleyn, D.H., 1985. Determination of lactose by an enzymatic method. Journal of Dairy Science 68, 2791–2798.
Kwak, H.S., Jeon, I.J., 1988. Comparison of high performance liquid chromatography and enzymatic method for the measurement of lactose in milk. Journal of Food Science 53, 975–976.
Körner, A., Larsson, A., Piculell, L., Wittgren, B., 2005. Molecular information on the dissolution of polydisperse polymers: mixtures of long and short poly(ethylene oxide). The Journal of Physical Chemistry B 109, 11530–11537.
Knöös, P., Onder, S., Pedersen, L., Piculell, L., Ulvenlund, S., Wahlgren, M., 2013a. Surfactants modify the release from tablets made of hydrophobically modified poly(acrylic acid). Results in Pharma Sciences 3, 7–14.
Knöös, P., Topgaard, D., Wahlgren, M., Ulvenlund, S., Piculell, L., 2013b. Using NMR chemical shift imaging to monitor swelling and molecular transport in drug-loaded tablets of hydrophobically modified poly(acrylic acid) – methodology and effects of polymer (in) solubility. Langmuir 29, 13898–13908.
Laguecir, A., Ulrich, S., Labille, J., Fatin-Rouge, N., Stoll, S., Buffle, J., 2006. Size and pH effect on electrical and conformational behavior of poly(acrylic acid): simulation and experiment. European Polymer Journal 42, 1135–1144.
Langston, J.A., Shaghasi, T., Abbate, E., Xu, F., Vlasenko, E., Sweeney, M.D., 2011. Oxidoreductive cellulose depolymerization by the enzymes cellobiose dehydrogenase and glycoside hydrolase 61. Applied and Environmental Microbiology 77, 7007–7015.
Li, Y., Ghoreishi, S.M., Warr, J., Bloor, D.M., Holzwarth, J.F., Wyn-Jones, E., 2000. Binding of sodium dodecyl sulfate to some polyethyleneimines and their ethoxylated derivatives at different pH values: electromotive force and microcalorimetry studies. Langmuir 16, 3093–3100.
Ludwig, R., Harreither, W., Tasca, F., Gorton, L., 2010. Cellobiose dehydrogenase: a versatile catalyst for electrochemical applications. ChemPhysChem 11, 2674–2697.
Ludwig, R., Ortiz, R., Schulz, C., Harreither, W., Sygmund, C., Gorton, L., 2013. Cellobiose dehydrogenase modified electrodes: advances by materials science and biochemical engineering. Analytical and Bioanalytical Chemistry 405, 3637–3658.
Magny, B., Iliopoulos, I., Audebert, R., Piculell, L., Lindman, B., 1992. Interactions between hydrophobically modified polymers and surfactants. Progress in Colloid and Polymer Science-Springer 89, 118–121.
Mylonas, Y., Staikos, G., Ullner, M., 1999. Chain conformation and intermolecular interaction of partially neutralized poly(acrylic acid) in dilute aqueous solutions. Polymer 40, 6841–6847.
Mészáros, R., Thompson, L., Bos, M., Varga, I., Gilányi, T., 2003. Interaction of sodium dodecyl sulfate with polyethyleneimine: surfactant-induced polymer solution colloid dispersion transition. Langmuir 19, 609–615.
Marrakchi, M., Dzyadevych, S.V., Lagarde, F., Martelet, C., Jaffrezic-Renault, N., 2008. Conductometric biosensor based on glucose oxidase and beta-galactosidase for specific lactose determination in milk. Materials Science and Engineering: C 28, 872–875.
Maderuelo, C., Zarzuelo, A., Lanao, J.M., 2011. Critical factors in the release of drugs from sustained release hydrophilic matrices. Journal of Controlled Release 154, 2–19.
Mukherjee, S., Dan, A., Bhattacharya, S.C., Panda, A.K., Moulik, S.P., 2011. Physicochemistry of interaction between the cationic polymer poly(diallyldimethylammonium chloride) and the anionic surfactants sodium dodecyl sulfate, sodium dodecylbenzenesulfonate, and sodium *N*-dodecanoylsarcosinate in water and isopropyl alcohol–water media. Langmuir 27, 5222–5233.
Michaelis, L., Menten l, M., 1913. Die kinetik der invertinwirkung. Biochemische Zeitschrift 49, 333–369.
Paulsson, M., Edsman, K., 2002. Controlled drug release from gels using lipophilic interactions of charged substances with surfactants and polymers. Journal of Colloid and Interface Science 248, 194–200.
Phillips, C.M., Beeson, W.T., Cate, J.H., Marletta, M.A., 2011. Cellobiose dehydrogenase and a copper-dependent polysaccharide monooxygenase potentiate cellulose degradation by neurospora crassa. ACS Chemical Biology .
Piculell, I., Thuresson, K., Lindman, B., 2001. Mixed solutions of surfactant and hydrophobically modified polymer. Polymers for Advanced Technologies 12, 44–69.
Safina, G., Ludwig, R., Gorton, L., 2010. A simple and sensitive method for lactose detection based on direct electron transfer between immobilised cellobiose dehydrogenase and screen-printed carbon electrodes. Electrochimica Acta 55, 7690–7695.
Schulz, C., Ludwig, R., Micheelsen, P.O., Silow, M., Toscano, M.D., Gorton, L., 2012. Enhancement of enzymatic activity and catalytic current of cellobiose dehydrogenase by calcium ions. Electrochemistry Communications 17, 71–74.
Skoug, J.W., Mikelsons, M.V., Vigneron, C.N., Stemm, N.L., 1993. Qualitative evaluation of the mechanism of release of matrix sustained-release dosage forms by measurement of polymer release. Journal of Controlled Release 27, 227–245.
Stephenson, B.C., Rangel-Yagui, C.O., Pessoa, A., Tavares, L.C., Beers, K., Blankschtein, D., 2006. Experimental and theoretical investigation of the micellar-assisted solubilization of ibuprofen in aqueous media. Langmuir 22, 1514–1525.
Stoica, L., Ludwig, R., Haltrich, D., Gorton, L., 2006. Third-generation biosensor for lactose based on newly discovered cellobiose dehydrogenase. Analytical Chemistry 78, 393–398.
Tahara, K., Yamamoto, K., Nishihata, T., 1995. Overall mechanism behind matrix sustained-release (sr) tablets prepared with hydroxypropyl methylcellulose-2910. Journal of Controlled Release 35, 59–66.
Tasca, F., Ludwig, R., Gorton, L., Antiochia, R., 2013. Determination of lactose by a novel third generation biosensor based on a cellobiose dehydrogenase and aryl diazonium modified single wall carbon nanotubes electrode. Sensors and Actuators B: Chemical 177, 64–69.
Watson, C.A., 1994. Official and Standardized Methods of Analysis, third ed. Royal Society of Chemistry, Cambridge.
West, L.G., Llorente, M.A., 1981. High performance liquid chromatographic determination of lactose in milk. Journal of the Association of Official Analytical Chemistry 64, 805–807.
Wang, H., Wang, Y., Yan, H., Zhang, J., Thomas, R.K., 2006. Binding of sodium dodecyl sulfate with linear and branched polyethyleneimines in aqueous solution at different pH values. Langmuir 22, 1526–1533.
Wahlgren, M., Christensen, K.L., Jorgensen, E.V., Svensson, A., Ulvenlund, S., 2009. Oral-based controlled release formulations using poly(acrylic acid) microgels. Drug Development and Industrial Pharmacy 35, 922–929.
Yakovleva, M., Buzas, O., Matsumura, H., Samejima, M., Igarashi, K., Larsson, P.-O., Gorton, L., Danielsson, B., 2012. A novel combined thermometric and amperometric biosensor for lactose determination based on immobilised cellobiose dehydrogenase. Biosensors and Bioelectronics 31, 251–256.
Zamocky, M., Ludwig, R., Peterbauer, C., Hallberg, B.M., Divne, C., Nicholls, P., Haltrich, D., 2006. Cellobiose dehydrogenase – a flavocytochrome from wood-degrading, phytopathogenic and saprotropic fungi. Current Protein Peptide Science 7, 255–280.