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Concanavalin A–Polysaccharides binding affinity analysis using a quartz crystal microbalance

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ABSTRACT

There is no comparative data available on the binding constants of Concanavalin A (Con A) and glycogen and Con A–mannan using quartz crystal microbalance (QCM), cost and time efficient system for biosensor analysis. It is hypothesized that a QCM can be used in its flow injection mode to monitor the binding affinity of polysaccharides to an immobilized lectin, Con A. The biosensor is prepared by immobilizing Con A on a 5 MHz gold crystal by carbodiimide crosslinking chemistry. The attachment efficiency is monitored by Fourier Transform Infrared Spectroscopy. Equilibrium association and dissociation constants describing Con A–polysaccharides interaction are determined in a saturation binding experiment, where increasing concentrations of polysaccharides are run on a Con A-immobilized gold crystal surface, and the frequency shifts recorded on the frequency counter. The molecular weights (MW) of glycogen from Oyster and mannan from *Saccharomyces cerevisiae* are determined by size exclusion chromatography. The MW for glycogen and mannan are 604 ± 0.002 kDa and 54 ± 0.002 kDa, respectively. The equilibrium association and dissociation constants for Con A–glycogen and Con A–mannan interactions are $K_A = 3.93 \pm 0.7 \times 10^6\ M^{-1}/K_D = 0.25 \pm 0.06\ \mu M$ and $K_A = 3.46 \pm 0.22 \times 10^5\ M^{-1}/K_D = 2.89 \pm 0.20\ \mu M$ ($n=3$), respectively. Their respective frequency and motional resistance shifts relationship ($\Delta F/\Delta R$) are 37.29 ± 1.55 and 34.86 ± 0.85 Hz/Ω ($n=3$), which support the validity of Sauerbrey's rigidity approximation. This work suggests that Con A–mannan complex could be potentially utilized for insulin delivery and the targeting of glucose-rich substances and glycoproteins when fast drug release is desired.

1. Introduction

The design of core–shell novel drug delivery systems relies mostly on the interaction between a coating or cross-linking agent and a triggering element. In the case of using Con A, such delivery systems rely primarily on a competitive binding event between a free carbohydrate and a polysaccharide cross-linker. This then makes the binding constants a fundamental parameter for a rational design of lectin based drug delivery systems, especially when a control release is expected. QCM comparative study of Con A–polysaccharides binding affinity using different structurally based polysaccharides namely glycogen and mannan, especially in the same condition has not been published previously. It is crucial, though, to challenge the ligands against the lectin using the same method, as the binding affinity might differ from one technique to another. Therefore for an accurate data interpretation in such studies, a single comparative study is required. Besides the availability of a wide number of binding affinity studies, they rarely go beyond one ligand interaction. This might perhaps be due to certain challenges (price, stability of reagents over the course of the experiment, complexity related to the experiments, calibration, etc.) involved in the respective techniques employed. However, quartz crystal microbalance (QCM) is cost effective, easy to set up, does not need any calibration, fast, and has similar sensitivity and detection limits compared to other methods such as surface plasmon resonance (SPR). These unique characteristics of QCM allow easy, cost and time efficient comparative studies.

Introduced by Sauerbrey (1959), the basic theory of the QCM correlates the mass change per unit area at the QCM electrode surface to the observed change in oscillation frequency of the crystal. QCMs have been proven to be reliable in the field of electrochemistry (Paulo Tde et al., 2013; Sun et al., 2013a, 2013b), in petroleum characterization (Pejcic et al., 2011; Ueyama et al., 2002), in environmental chemistry (Nicolini et al., 2012), material science (Pei et al., 2010) and more interestingly in the development of biosensors (Bouchet-Spinelli et al., 2013; Eom et al., 2013; Guntupalli et al., 2013; Wangchareansak et al., 2013) which are valuable for disease

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diagnosis. QCMs also found a great application in the monitoring of the affinity constants defining a given substrate–ligand interaction (Lebed et al., 2006; Li et al., 2013; Mori et al., 2009; Tan et al., 2013). Besides the fact that it does not require any calibration, one of the most exciting features of the QCM is its ability to quantify a mass change at the nano-gram level. This makes QCM an easy to set up and robust technique for bioaffinity analysis. Data obtained from QCMs are shown to be in accordance with other well-established techniques such as SPR (Cho et al., 2004; Laricchia-Robbio and Revoltella, 2004), fluorescence spectroscopy (Ha et al., 2004; Hisao, 2000), atomic force microscopy (Caruso et al., 1997; Coffey and Krim, 2001) and photoelectron microscopy (Kristensen et al., 2003).

Polysaccharides and glycoconjugates are known to play a crucial role in biological processes such as cell activation, differentiation and apoptosis (Lebed et al., 2006) but also in cellular recognition, transmission of information, immunity and tumorigenesis (Compain and Martin, 2001; Koeller and Wong, 2000; Mori et al., 2009; Pei et al., 2005; Varki, 1993). Glycogen, known as "animal starch" for its resemblance with starch found in plants, is the principal storage form of glucose (energy) in animal cells (Flatt, 1995). It is a highly branched polymer in which small chain of glucose molecules are linked by α-D-(1→4) linkage and α-D-(1→6) branching points (Zhang et al., 2001). In human, glycogen is mostly found in the liver but can also be found in muscles and brain glial cells where its proportion is significantly lower (Alberti et al., 1980; Hochachka and Storey, 1975; Kong et al., 2002).

Lectins are proteins representing a class of carbohydrate binding agents. They are primarily extracted from plants and show binding specificity to certain sugar moieties. Originally extracted from the jack-bean, Con A is a member of the legume lectin family and binds to sugars, glycoproteins, and glycolipids, containing internal and non-reducing terminal α-D-mannosyl and α-D-glucosyl groups (Brewer, 1985; Gray and Glew, 1973; Goldstein et al., 1965a, 1965b). Like most lectins, Con A is a homotetramer with each subunit composed of 235 amino acids and having a molecular weight of 26.5 kDa. At pH below 6, Con A exists as a dimer and is in its tetrameric form at pH above 6. To depict its binding activity, the protein requires metal ions. Agrawal and Goldstein have shown that each subunit has one Ca^{2+} and Mn^{2+} binding sites (Agrawal and Goldstein, 1968; Brewer, 1985).

The binding of Con A to mannose and glucose containing oligosaccharides has been intensively studied and to date, several carbohydrate binding constants to Con A are known (Dam et al., 2000; Duverger et al., 2003; Gray and Glew, 1973; Lebed et al., 2007). Yet, a thorough investigation of the binding constants of polysaccharides to Con A remains to be investigated. Particularly, it is crucial to understand the interaction of Con A and glycogen considering the emerging interest in drug delivery systems using such interaction as a design strategy (Chinnayelka and McShane, 2004; Sato et al., 2005, 2012; Zhu et al., 2011; Yuri et al., 1995). In addition, this study intends to provide binding affinity constants, based on the determined molecular weights by size exclusion chromatography (SEC), for a thorough characterization of Con A–glycogen and Con A–mannan interactions. Furthermore, the present study provides a model for a better understanding of protein-polysaccharides in-vitro interaction.

In this study, it is hypothesized that a QCM biosensor can be used in a flow injection setting to monitor the binding affinities of polysaccharides (glycogen and mannan) to an immobilized lectin, Con A. The binding constants of Con A to these polysaccharides could then be utilized rationally in the design of stimuli-sensitive drug delivery systems to target high mannose or glucose containing glycoproteins or surfaces.

To study the binding kinetics of the polysaccharides to Con A, the lectin is first immobilized onto a gold crystal by EDC chemistry and the immobilization is validated by Fourier Transform InfraRed (FTIR) spectroscopy.

2. Material and methods

2.1. Material

2.1.1. Quartz crystal microbalance

A Stanford Research System (SRS) QCM200 apparatus (Sunnyvale, CA) is used in this experiment. The QCM200 System is a stand-alone instrument with a built-in frequency counter and resistance meter. It includes controller, crystal oscillator electronics, crystal holder, and quartz crystals. In this study, 1 in. diameter, 5 MHz AT-cut, plano–plano Chromium/gold (Cr/Au) quartz crystals from SRS are used for each measurement in a flow injection mode. Two single injection syringe pumps are used to deliver the ligands (polysaccharides) and the free buffer (Tris Buffer Saline). Series resonance frequency and resistance are measured and displayed directly on the front panel. Therefore, SRS-QCM200 software is installed on a computer for real-time display, analysis, and storage.

2.1.2. Chemicals

Concanavalin A (Con A) from *Canavalia ensiformis* (Jack bean) Type VI, glycogen from Oyster, mannan from *Saccharomyces cerevisiae*, *N*-hydroxysuccinimide (NHS), 11-mercaptoundecanoic acid (MUA, 95%), 4-morpholineethanesulfonic acid sodium salt (MES), sulfuric acid (H_2SO_4, puriss. p.a., 95–97% (T)), hydrogen peroxide solution (H_2O_2, 30 wt% in H_2O, ACS reagent), 200 proof absolute ethanol, hydrochloric acid (ACS reagent, 37%), calcium chloride dehydrate ($CaCl_2 \cdot 2H_2O$, ACS reagent, ≥99%) and manganese(II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$, Reagent Plus®, ≥99%), phosphate buffered saline (PBS) pH 7.4 and Tris Buffer Saline (TBS) pH 8.0 are purchased from Sigma-Aldrich (Milwaukee, WI). 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) is purchased from Thermo Scientific (Rockford, IL). The PBS and TBS are prepared according to the manufacturer protocol and the pH is checked before any experiment. The TBS is supplemented with Mn^{2+} and Ca^{2+} and used as running buffer without any further treatments.

2.2. Methods

2.2.1. Fourier transform infrared (FTIR) spectroscopy

The Agilent Cary 630 FTIR instrument (Newark, DE) equipped with a diamond crystal having a single reflection and a nominal angle of 45° is used in its Attenuated Total Reflectance (ATR) mode for the acquisition of all IR spectra. A resolution of 4 cm^{-1} and a sample scan of 32/s is set as parameters and the effective pathlength is 1.1 μm. Prior to any IR analysis, the QCM crystals are dried under a nitrogen gas stream and allowed to make an intimate contact with the ATR diamond crystal surface by pressing them with a pressure clamp. FTIR spectra are collected and analyzed with MicroLab PC and MicroLab Lite softwares (versions 4.0, Newark, DE), respectively.

2.2.2. Size exclusion chromatography (SEC)

Glycogen and mannan molecular weight are determined by size exclusion chromatography. Briefly, 7 dextran standards covering a molecular weight range from 5.2 to 668 kDa are run on SEC (Waters, Milford, MA) equipped with a differential refractometer detector to draw a calibration curve. The equation of the calibration curve is $Y = -0.3367X + 10.351$ with a coefficient of determination of $R^2 = 0.9877$ indicating a strong correlation between the retention time (X) and the base 10 logarithm of the molecular weights (Y). Samples are dissolved in 0.1 M of sodium nitrate ($NaNO_3$) at pH 7 and each experiment is conducted at a flow rate of 1 ml/min on a 7.8 × 300 mm WAT011525 Ultrahydrogel column (Waters, Milford, MA). Data are acquired and analyzed on Millennium^{32} software version 3.20 (Waters, Milford, MA) and glycogen and mannan molecular weight

are derived from their respective retention time. Each experiment is performed in triplicate.

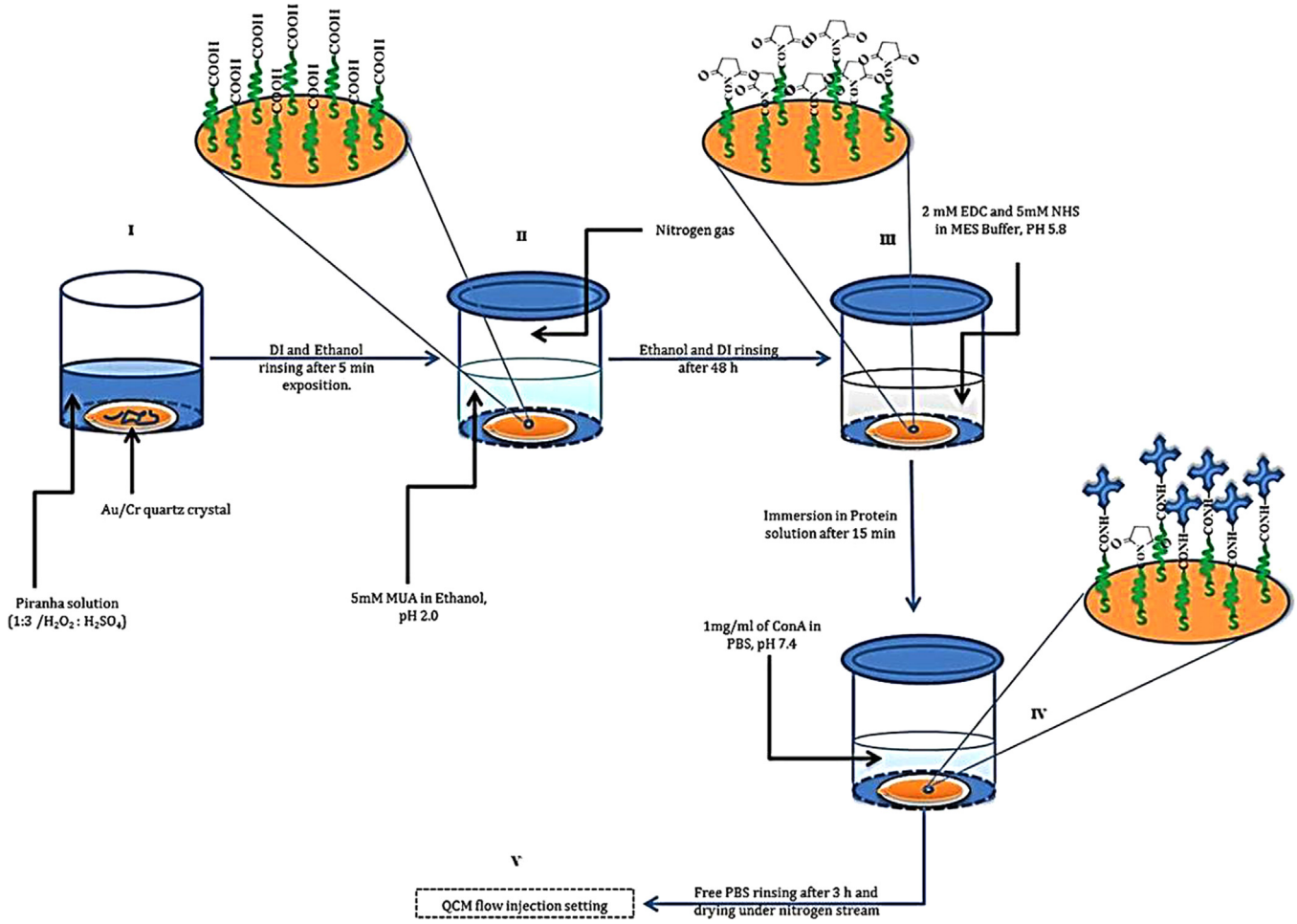
2.2.3. QCM measurement

2.2.3.1. Preparation of the gold crystal surface. Prior to any experiment, a Cr/Au QCM crystal is cleaned by exposing it to a piranha solution prepared as described below. Basically, the cleaning solution is prepared in an appropriate container by carefully adding 1 part per volume of hydrogen peroxide to 3 parts per volume of sulfuric acid to reach a ratio of 1:3 (Caruso et al., 1997). *Caution: Piranha solution should be handled with extreme care and only small volume should be prepared at any time*. For safety purposes, the piranha solution should be prepared under a well-functioning hood and crystals should be handled with appropriate tweezers. After 5 min in the piranha solution, the crystals are removed and cleaned twice with Millipore Q water (DI water) and ethanol before drying under a nitrogen stream.

2.2.3.2. Con A immobilization. As shown earlier by Lebed et al. (2006), Con A can readily adsorb onto a clean gold crystal surface. Even though the mechanism involved in such physisorption is not fully explained, it appears that the lectin binds loosely and can completely wipe out the crystal surface when the free buffer is then run on it. To avoid such an undesirable effect, a covalent attachment of the lectin is performed through zero linker (EDC/NHS) chemistry by coupling the N-terminal of the protein with the carboxylic head group of the MUA.

To immobilize Con A on the quartz crystal, the method of Caruso et al. (1997) is used with minor adaptations. Briefly, in order to provide the gold surface with the carboxylic acid function, a clean gold crystal is incubated in an ethanolic solution of 5 mM of MUA at pH 2.0 for 48 h. To avoid thiol oxidation and ensure the success of the self-assembling, the container is tightly sealed and backfilled with dry nitrogen prior to storage. The success of the MUA attachment resides in the intrinsic property of the alkanethiols moieties to chemisorb onto gold surfaces (Pensa et al., 2012). It has been proven (Love et al., 2005) that the sulfur atom of the alkanethiol forms a coordinate (dative covalent) bond with the gold metal surface. After 48 h of incubation, the crystal is then removed and thoroughly rinsed with ethanol and DI water before immersion in a 0.1 M MES buffer containing 0.5 M NaCl at a pH 5.8. EDC and NHS are subsequently added to the buffer to reach a concentration of 2 mM and 5 mM, respectively. The mixture is allowed to react for at least 15 min. The crystal is then retrieved and redisposed in 1 mg/ml of Con A in PBS for 3 h, after which the surface is cleaned again with a free PBS buffer and placed in the flow cell chamber for QCM frequency acquisition. Scheme 1 shows an illustration of the steps involved in the lectin immobilization.

2.2.3.3. QCM frequency acquisition. A QCM200 is used in a flow injection mode to monitor the lectin and polysaccharides interaction. Briefly, two injection pumps, one for the free running buffer and the second for the polysaccharide solutions, are connected to a dual flow



Scheme 1. Step by Step illustration of Con A immobilization on the QCM gold surface. Special care should be taken in handling Piranha solution under a fume hood. Once the crystal is cleaned and dry under nitrogen, it is disposed in 5mM of Mercaptoundecanoic acid for 48h for carboxylic acid functionalization. Con A is then immobilized by EDC chemistry using 2mM EDC and 5mM NHS in MES buffer (pH5.8). The reagent mixture is gently shaken to insure even distribution of reagents during EDC coupling.

connector. The flow rates are set to 3 ml/h in each measurement. The free buffer is first run to rule out the buffer effect and the flow is switched to the polysaccharide solution after a stable baseline is reached. Five different concentrations covering a range from 0.1 to 1 mg/ml are prepared in the running buffer for both glycogen and mannan, and the relative frequency shifts and mass increases following the binding are recorded on a computer through the QCM200 software. Each experiment is performed in triplicate. The Sauerbrey Eq. (1) is used to fit the frequency shift to the mass change on the crystal surface.

$$\Delta F = -C_f \times \Delta m \tag{1}$$

where ΔF, C_f and Δm are respectively the frequency shift (resonance frequency change), the crystal sensitivity factor, and the relative mass change. C_f is a fundamental property of the quartz crystal and is expressed by Eq. (2).

$$C_f = \frac{2nf_0^2}{(\rho_q \mu_q)^{0.5}} \tag{2}$$

In Eq. (2), n is the number of harmonic at which the crystal is driven, f_0 the resonant frequency of the fundamental mode of the crystal, ρ_q the density of quartz and μ_q the shear modulus of quartz. In this experiment, these parameters are $n=1$, $f_0=5.0$ MHz, $\rho_q = 2.648\ g/cm^3$ and $\mu_q = 2.947 \times 10^{11}\ g/cm/s^2$, respectively. The sensitivity factor C_f is 56.6 Hz/μg/cm^2.

3. Results and discussion

Fig. 1A summarizes the FTIR spectra obtained at each step of the lectin immobilization process. The spectrum A-a is obtained from the clean and unmodified QCM gold crystal. The FTIR spectrum acquired after the MUA attachment (Fig. 1A-b) on the gold crystal shows distinctive peaks between 2980 and 2850 cm^{-1}, which are due to the symmetric and asymmetric stretch mode of CH_2 in the MUA backbone (Corn, 1996; Plant, 1998). Furthermore, features at around 1440 and 1700 cm^{-1} are related to the COOH groups in the MUA (Ojamae et al., 2006). The spectrum obtained after the EDC/NHS chemistry (Fig. 1A-c)

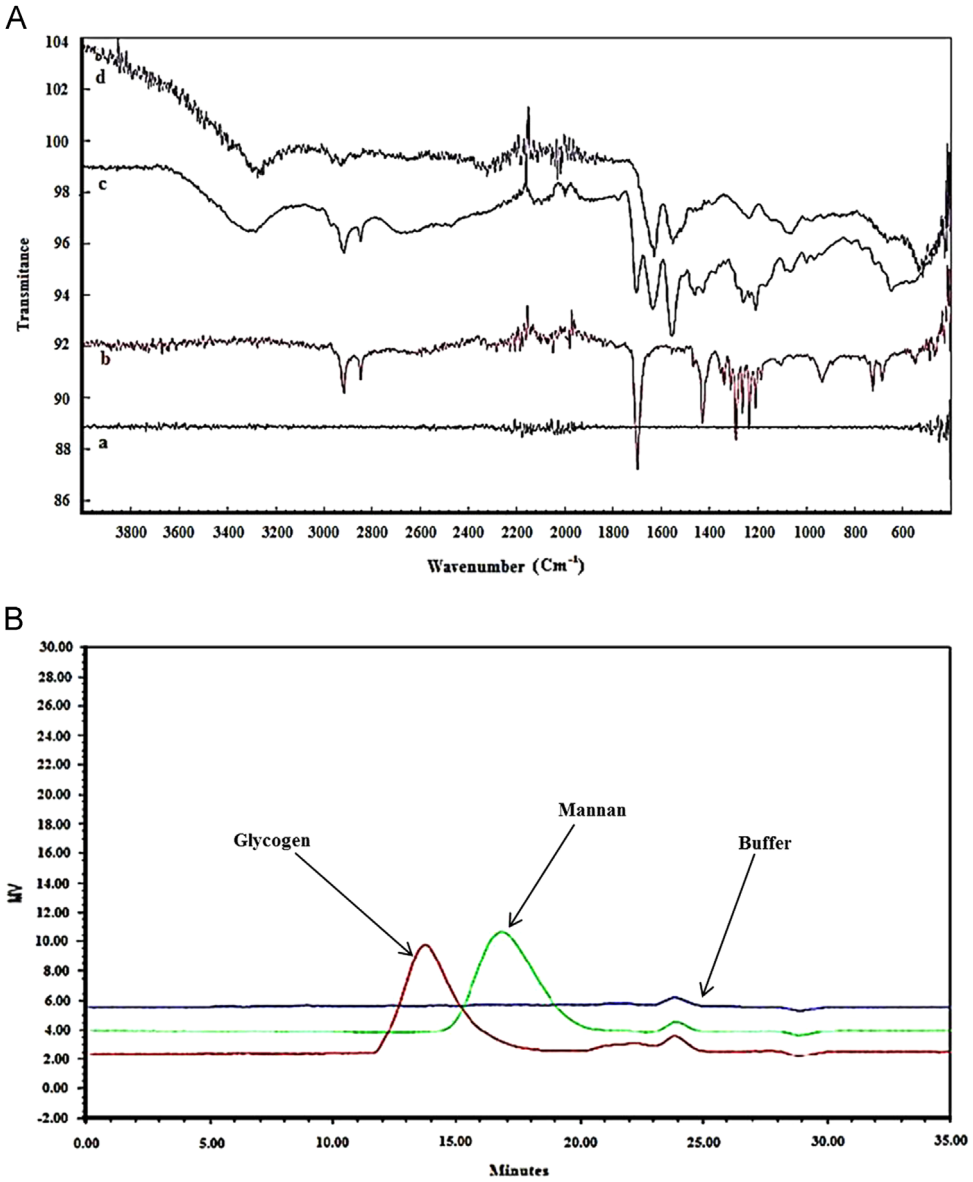


Fig. 1. (A) FTIR/ATR spectra of the clean QCM gold crystal (a) followed by 11-mercaptoundecanoic acid (MUA) attachment (b), before EDC/NHS chemistry (c) and finally Con A immobilization (d) onto the QCM crystal. (B) Size exclusion chromatograms of sodium nitrate (N_aNO_3) (buffer), glycogen and yeast mannan.

shows two characteristic bands at around 1715 and 1650 cm^{-1}, which are assigned to the carbonyl stretch mode of the COO-NHS ester moiety (Corn, 1996; Plant, 1998). After the Con A attachment, amide I and amide II bands at 1540 and 1640 cm^{-1} can be distinguished and are indicative of a successful Con A covalent attachment on the gold surface (Fig. 1A-d) (Sam et al., 2010; Yi et al., 2013).

Glycogen and mannan molecular weights, determined by SEC/GPC, are found to be 604 ± 0.002 kDa and 54 ± 0.002 kDa ($n=3$), respectively. The typical chromatograms from these analyses are shown in Fig. 1B.

Once Con A is immobilized on the gold surface, different ligand concentrations are flowed on the Con A-immobilized QCM crystal. In this work, each experiment is run on a separate crystal and the polysaccharide solutions are injected when a stable baseline is reached as shown in Fig. 2a. The resonance frequency is gradually decreasing while increasing the glycogen and mannan concentration as displayed in Fig. 2b and c and are found to be following a Langmuir-type adsorption isotherm (Eq. (3)) as shown in Fig. 3(a and b).

$$\Delta F = \Delta F_{max} \frac{K_A[\text{ligand}]}{1 + K_A[\text{ligand}]} \tag{3}$$

where ΔF represents the frequency shift observed on the QCM frequency counter, ΔF_{max} the maximum frequency shift at saturation, and K_A represents the equilibrium association constant and [ligand] the concentration of polysaccharide. At saturation, the binding and dissociation rates of ligands to the available binding sites of the lectin are identical and reach a steady state (Hulme and Trevethick, 2010).

The reciprocal of Eq. (3) gives the equation of a straight line (Eq. 4) from which one can derive the ΔF_{max} from the y-intercept and the equilibrium association constant (K_A) from the slope as shown in Fig. 3 (a′ and b′). The equilibrium dissociation constant which is the reciprocal of K_A, is obtained from Eq. (5).

$$\frac{1}{\Delta F} = \frac{1}{K_A \times \Delta F_{max} \times [\text{ligand}]} + \frac{1}{\Delta F_{max}} \tag{4}$$

$$K_D = \frac{1}{K_A} \tag{5}$$

The expression of Eq. (4) for glycogen is $Y = 4 \times 10^{-10} X + 1.57 \times 10^{-3}$ with a coefficient of determination of $R^2 = 0.9940$ indicating a very good correlation for this polysaccharide. The calculated ΔF_{max} is 637 Hz (95% CI: 584–700 Hz) and the equilibrium association and dissociation constants value are $K_A = 3.93 \pm 0.7 \times 10^6\ M^{-1}$ and $K_D = 0.25 \pm 0.06\ \mu M$, respectively. These values are found to be consistent with others (Tan et al., 2007) obtained using an electrochemical piezoelectric quartz crystal impedance (EPQCI) method ($K_A = 1.48 \times 10^6\ M^{-1}$ and $K_D = 0.4\ \mu M$).

For mannan, the fitted Eq. (4) is $Y = 4.15 \times 10^{-9} + 1.43 \times 10^{-3}$ with a coefficient of determination of $R^2 = 0.9772$, suggesting a good correlation. The ΔF_{max} is found to be 697 Hz (95% CI: 583–867 Hz) and the equilibrium association and dissociation constants are $K_A = 3.46 \pm 0.22 \times 10^5\ M^{-1}$ and $K_D = 2.89 \pm 0.20\ \mu M$, respectively. The K_D values reported so far for the interaction of the yeast mannan with the Con A measured on QCM are in the range of 0.1–1.1 μM (Horisberger, 1980; Mislovicova et al., 2002). Binding affinity (K_D) obtained from the binding between Con A and the immobilized yeast mannan is reported to be 0.4 μM (Pei et al., 2005). Compared to our study, in which the lectin is immobilized, Pei et al. opted instead for a method in which yeast mannan is immobilized on the gold crystal, which might explained the slight difference in affinity values. In fact, it has been proven that different affinity values can be obtained in SPR experiments for the same interaction considering whether the lectin or the ligand

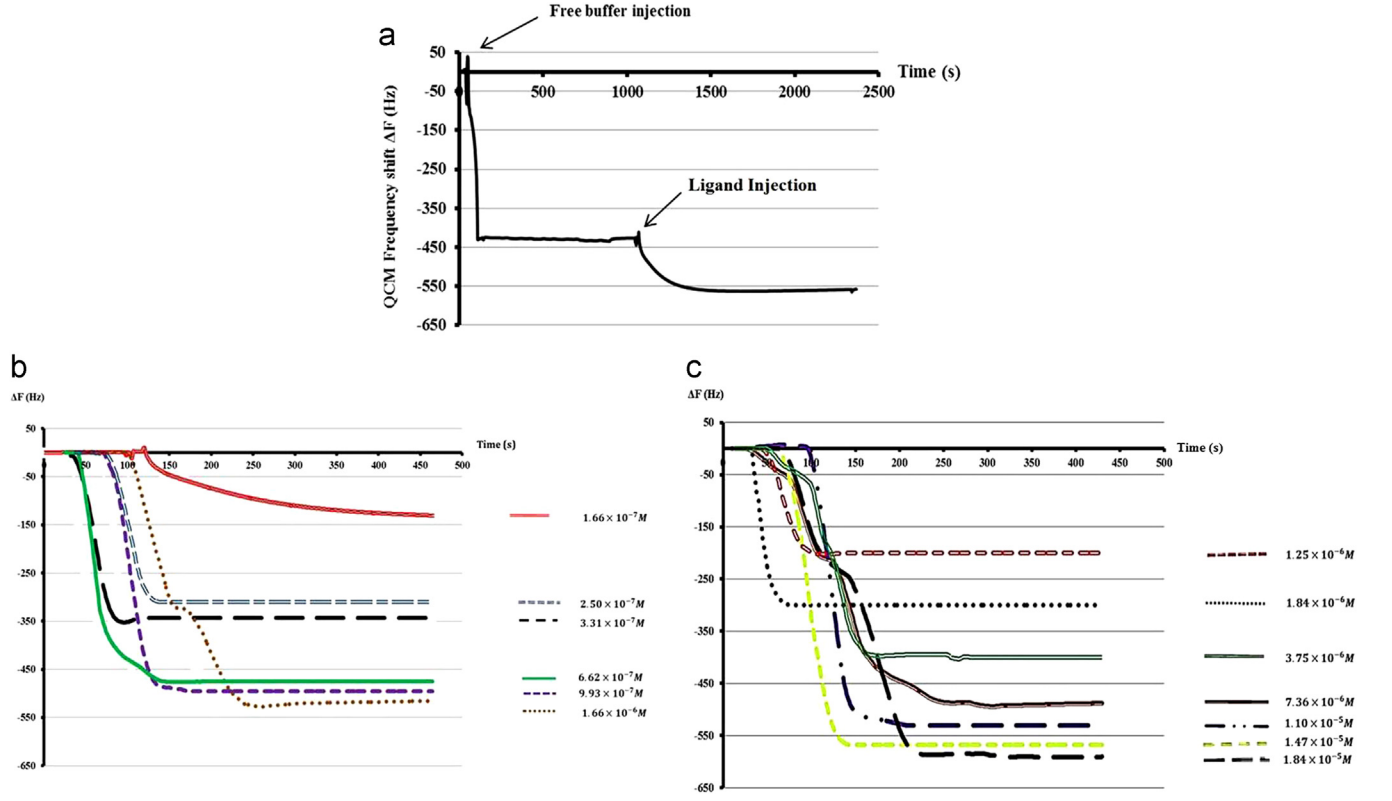


Fig. 2. (a) Frequency shift observed with polysaccharide injection after a baseline is reached with free buffer (0.05 M Tris-buffer saline, 1 mM Ca^{2+}, 1 mM Mn^{2+}, pH 8.0). Time dependent QCM resonance frequency shifts for glycogen (b) and mannan (c) at different concentrations.

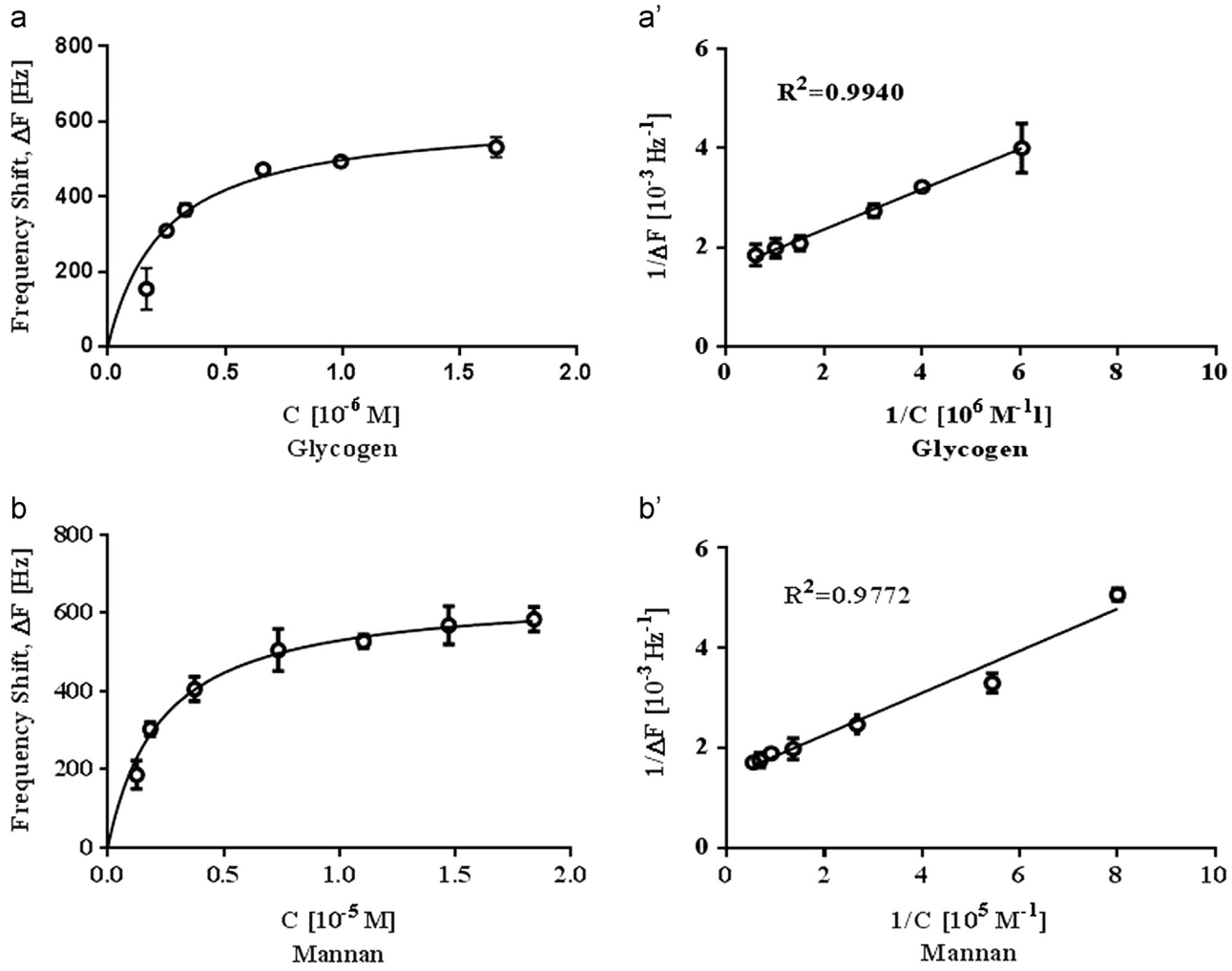


Fig. 3. Saturation binding curves for glycogen (a) mannan (b) and their respective reciprocal curves (a') and (b') obtained from Equation (4).

is immobilized on the crystal. Lectin based assays, however, are known to yield accurate binding affinity measurements than ligand immobilized assay (Duverger et al., 2003).

Con A is known to have higher affinities to mannose containing oligosaccharides compared to glucose containing moieties (Mori et al., 2009; Ohyama et al., 1985). However, in this study, a greater affinity is found for Con A to glycogen compared to yeast mannan. Initially observed by Tan et al. (2007) using an EPQCI method, this pattern is explained to be partly due to the greater compaction of the association between Con A and glycogen compared to mannan and Con A. The steric hindrance caused by the glycogen macromolecule is also thought to have an influence on the combination between Con A and glycogen. In addition to the steric effect, the huge difference in the polysaccharides molecular weights could explain the result obtained in this study. Indeed, it is reported (Mueller et al., 2000) that the binding affinity of glucans polymers to (1→3)-β-D-glucan receptors in a human monocyte-like cell line is, in part, dependent on their molecular weights. In fact, this group observed that higher glucan molecular weights resulted in higher affinity values. Similar results are reported for the antitumor activity of Schizophyllan (Kemasa Kojima et al., 1985).

Furthermore, the higher affinity to glycogen could be due to the branching pattern of both glycogen and yeast mannan (Fig. 4). Indeed, in the past decades, it has been extensively proven that sugar residues must obey a set of criteria in order to interact with Con A. Thus, it is generally accepted that hydroxyl groups on the C-3, C-4 and C-6 positions are critical in Con A and a sugar residue interaction with a higher requirement at the C-6 position as shown by Goldstein and others (Brewer, 1985; Goldstein et al., 1974). Moreover, glycogen main chains glucose monomers are connected by 1→4 glycosidic bonds with only few 1→6 branches linkages at every 12 glucose residues (Roach et al., 2012). This branching arrangement allows more glucose residues to be available for interaction with Con A. Unlike for glycogen, yeast mannan main chains monomers are linked by 1→6 glycosidic bonds with several 1→3 glycosidic linkages found throughout the branches of the macromolecule (Cawley and Ballou, 1972; Jones and Ballou, 1969; Nakajima and Ballou, 1974). The presence of 1→4 glycosidic bonds in yeast mannan is also reported (Kwiatkowski and Kwiatkowski, 2012). This arrangement in contrast does not allow a wide range of mannose residues to successfully interact with Con A because of a higher unavailability of the C-6, C-4 and C-3 hydroxyl groups compared to glycogen.

Sugar responsive Con A–glycogen layers and insulin delivery systems prepared by the Layer-By-Layer strategy have been proven to disrupt and release their content often within an hour. (Zhu et al., 2011; Sato et al., 2005; Yuri et al., 1995)). In this strategy, insulin is released upon a competitive binding of the triggering saccharide (glucose, mannose, fructose, etc.,) to Con A. Even though such systems are considered to be promising as such, their release profile could be speed up with the use of lower affinity

polysaccharides such as mannan from *S. cerevisiae*. Such a fast-acting delivery systems will represent an alternative to the actual urgent need for innovative insulin delivery methods (Al-Tabakha and Arida, 2008; Timko et al., 2014).

Along with the frequency shift, the motional resistance shift following the binding of the polysaccharides to Con A is recorded in each experiment. The motional resistance expresses the loss in mechanical energy dissipated to the medium and the quartz interior and is induced by viscoelasticity changes occurring on the quartz surface and changes of solution viscosity and density (Lebed et al., 2006). Using a Butterworth-Van Dyke equivalent circuit model, Martin et al. have established the linear relationship (Eq. (6)) between the change in series resonance resistance (ΔR) of the quartz oscillator and the liquid density and viscosity during a liquid loading (Stephen et al., 1991).

$$\Delta R = \frac{n \times \omega_s \times L_u}{\pi} \times \sqrt{\frac{2\omega_s \times \rho_L \times \eta_L}{\rho_q \times \mu_q}} \quad (6)$$

where ΔR represents the change in series resonance resistance in Ω, n the number of sides in contact with the liquid, ω_s the angular frequency at series resonance ($2\pi f_s$) and L_u the induction for the unperturbed (dry) resonator. ρ_L and η_L are the density and shear viscosity of the liquid, respectively. ρ_q and μ_q are the density and shear modulus of quartz. In our study, the resistance is recorded from the frequency counter as the experiment progresses.

Fig. 5 shows the relationship between the motional resistance change and the resonant frequency shift (ΔR vs ΔF) corresponding to the polysaccharides binding to Con A. The fitting equations are $y = 0.027 \times x + 0.303$ for glycogen and $y = 0.029 \times x - 0.086$ for mannan and the coefficients of determination are $R^2 = 0.9908$ and $R^2 = 0.9963$, respectively. From the fitting curves, it is found that the reciprocal of the slopes ($\Delta F/\Delta R$) are 37.29 ± 1.55 Hz/Ω (95% CI: 41.95–33.56) for glycogen and 34.86 ± 0.85 Hz/Ω (95% CI: 37.11–32.86) for mannan.

Lebed et al. (2006) found a $\Delta F/\Delta R$ value of 29.41 Hz/Ω for the interaction of carboxypeptidase *Y* to an immobilized Con A on a 5 MHz QCM gold electrode in Tris-buffered saline. Characterizing the interaction between Con A and glycogen, Tan et al. found $\Delta F/\Delta R$ values ranging from 41.6 to 43.2 Hz/Ω for the adsorption of glycogen, BSA and Con A onto a 9 MHz quartz crystal in PBS (Tan et al., 2007). Low $\Delta F/\Delta R$ values are generally associated with viscoelastic or damping effects (Lebed et al., 2006). However, the values obtained in this study, as compared to others, are typical of a bulk liquid effect instead of the process related to the association of the polysaccharides to Con A (Lebed et al., 2006; Tan et al., 2007).

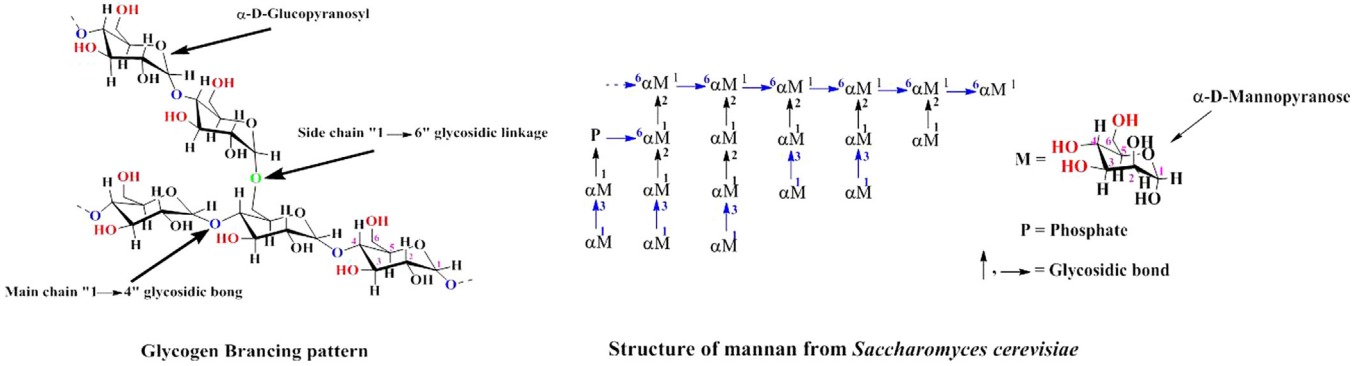


Fig. 4. Molecular branching pattern in glycogen from Oyster and mannan from *Saccharomyces cerevisiae*. Mannan main chain monomers are linked by 1→6 glycosidic bonds with several 1→3 linkages throughout the branches. Those linkages are shown in blue color in mannan's structure. Glycogen main chain monomers are 1→4 linked with only few 1→6 glycosidic bonds occurring each 12 glucose residues. The 1→4 linkages are shown in blue in glycogen's structure and the 1→6 linkage in green. Hydroxyl groups at position 3, 4 and 6, shown in red are critical for Con A interaction with a greater requirement at position 6. Unavailability of the critical hydroxyl groups prevents binding to Con A.

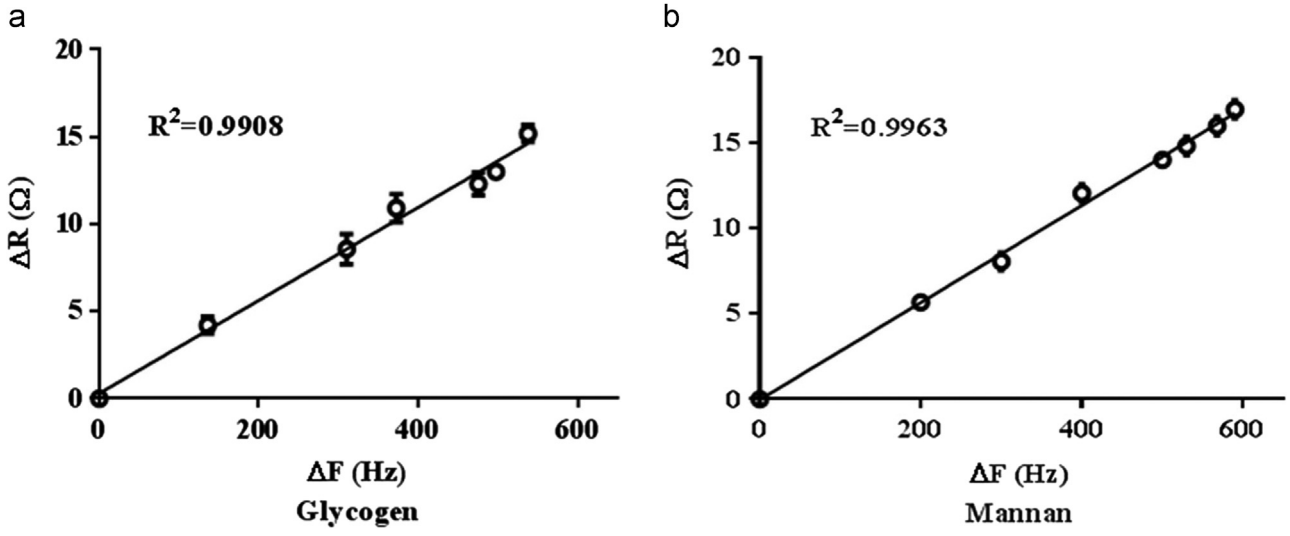


Fig. 5. Relationships (ΔR vs ΔF) between the motional resistance (ΔR) and the resonant frequency (ΔF) changes corresponding to specific glycogen (a) and mannan (b) adsorption on a Con A layer on QCM crystal surface.

That is, the frequency shift observed is primarily due to the mass effect, and Sauerbrey's rigidity approximation is valid (Fu et al., 2008).

4. Conclusion

Using a quartz crystal microbalance in its flow injection mode, we have been able to obtain affinity binding constants describing the interaction between two polysaccharides (glycogen and mannan) and Con A. The equilibrium dissociation constant for the interaction Con A–glycogen is $K_D = 0.25 \pm 0.06\ \mu M$ which is about 12 fold lower than the equilibrium dissociation constant describing the interaction Con A–mannan ($K_D = 2.89 \pm 0.20\ \mu M$). That is, Con A, a mannose specific lectin, is found to have a higher affinity to glycogen from Oyster, a glucose base polysaccharide, than to mannan from *S. cerevisiae*, a mannose based polysaccharide. This observation is mainly due to a steric effect, the molecular weight and the branching pattern of both polysaccharides. Con A being a homotetramer with a pH dependency conformational change, it will be useful to investigate how critical the pH can impact polysaccharides binding affinity to the lectin. Based on the underlying results, it is hypothesized that the Con A-–mannan interaction can be used as a design strategy in targeting glycogen containing substances and derivatives as a drug delivery system. Future studies will also show how this work might find a valuable application as a fast acting drug delivery system for insulin delivery in the treatment of diabetes and the targeting of glycoproteins for other disease therapies.

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References

Agrawal, B.B., Goldstein, I.J., 1968. Arch. Biochem. Biophys. 124 (1), 218–229.
Alberti, K.G., Batstone, G.F., Foster, K.J., Johnston, D.G., 1980. J. Parenter. Enteral Nutr. 4 (2), 141–146.
Al-Tabakha, M.M., Arida, A.I., 2008. Indian J. Pharm. Sci. 70 (3), 278–286.
Bouchet-Spinelli, A., Reuillard, B., Coche-Guerente, L., Armand, S., Labbe, P., Fort, S., 2013. Biosens. Bioelectron. 49, 290–296.
Brewer, F., 1985. Einstein Q. J. Biol. Med. 3 (1), 55–60.
Caruso, F., Rodda, E., Furlong, D.N., Niikura, K., Okahata, Y., 1997. Anal. Chem. 69 (11), 2043–2049.
Cawley, T.N., Ballou, C.E., 1972. J. Bacteriol. 111 (3), 690–695.
Chinnayelka, S., McShane, M.J., 2004. J. Fluorescence 14 (5), 585–595.
Cho, Y.K., Kim, S., Kim, Y.A., Lim, H.K., Lee, K., Yoon, D., Lim, G., Pak, Y.E., Ha, T.H., Kim, K., 2004. J. Colloid Interface Sci. 278 (1), 44–52.
Compain, P., Martin, O.R., 2001. Bioorg. Med. Chem. 9 (12), 3077–3092.
Corn, B.L.F.a.R.M., 1996. Anal. Chem. 68 (18), 3187–3193.
Dam, T.K., Roy, R., Das, S.K., Oscarson, S., Brewer, C.F., 2000. J. Biol. Chem. 275 (19), 14223–14230.
Duverger, E., Frison, N., Roche, A.C., Monsigny, M., 2003. Biochimie 85 (1-2), 167–179.
Eom, S., Yu, E., Choi, S.J., 2013. Anal. Biochem. 443 (1), 78–80.
Flatt, J.P., 1995. Am. J. Clin. Nutr. 61 (4), 952S–959S.
Fu, Y., Chen, C., Xie, Q., Xu, X., Zou, C., Zhou, Q., Tan, L., Tang, H., Zhang, Y., Yao, S., 2008. Anal. Chem. 80 (15), 5829–5838.
Gray, R.D., Glew, R.H., 1973. J. Biol. Chem. 248 (21), 7547–7551.
Goldstein, I.J., Hollerman, C.E., Merrick, J.M., 1965a. Biochim. Biophys. Acta 97, 68–76.
Goldstein, I.J., Hollerman, C.E., Smith, E.E., 1965b. Biochemistry 4, 876–883.
Goldstein, I.J., Reichert, C.M., Misaki, A., 1974. Ann. N.Y. Acad. Sci. 234 (0), 283–296.
Guntupalli, R., Sorokulova, I., Olsen, E., Globa, L., Pustovyy, O., Vodyanoy, V., 2013. J. Visual. Exp. (75))
Ha, T.H., Kim, S., Lim, G., Kim, K., 2004. Biosens. Bioelectron. 20 (2), 378–389.
Hisao, O.Y.N.K.F.H.M., 2000. Anal. Sci. 16 (11), 1113–1119.
Hochachka, P.W., Storey, K.B., 1975. Science 187 (4177), 613–621.
Horisberger, M., 1980. Biochim. Biophys. Acta 632 (2), 298–309.
Hulme, E.C., Trevethick, M.A., 2010. Br. J. Pharmacol. 161 (6), 1219–1237.
Jones, G.H., Ballou, C.E., 1969. J. Biol. Chem. 244 (3), 1052–1059.
Lebed, K., A.J.K., Forro', L., Lekka, M., 2007. Acta Phys. Pol. A 111, 2.
Kemasa Kojima, K.T., W.I., Yanaki, T., 1985. Agric. Biol. Chem. 1 (50), 231–232.
Koeller, K.M., Wong, C.H., 2000. Nat. Biotechnol. 18 (8), 835–841.
Kong, J., Shepel, P.N., Holden, C.P., Mackiewicz, M., Pack, A.I., Geiger, J.D., 2002. J. Neurosci. 22 (13), 5581–5587.
Kristensen, E.M., Rensmo, H., Larsson, R., Siegbahn, H., 2003. Biomaterials 24 (23), 4153–4159.
Kwiatkowski, S., Kwiatkowski, S.E., 2012. The Complex World of Polysaccharides. InTech, pp. 47–70
Laricchia-Robbio, L., Revoltella, R.P., 2004. Biosens. Bioelectron. 19 (12), 1753–1758.
Lebed, K., Kulik, A.J., Forro, L., Lekka, M., 2006. J. Colloid Interface Sci. 299 (1), 41–48.
Li, X., Pei, Y., Zhang, R., Shuai, Q., Wang, F., Aastrup, T., Pei, Z., 2013. Chem. Commun. 49 (85), 9908–9910.
Love, J.C., Estroff, L.A., Kriebel, J.K., Nuzzo, R.G., Whitesides, G.M., 2005. Chem. Rev. 105 (4), 1103–1169.
Mislovicova, D., Masarova, J., Svitel, J., Mendichi, R., Soltes, L., Gemeiner, P., Danielsson, B., 2002. Bioconjugate Chem. 13 (1), 136–142.
Mori, T., Toyoda, M., Ohtsuka, T., Okahata, Y., 2009. Anal. Biochem. 395 (2), 211–216.
Mueller, A., Raptis, J., Rice, P.J., Kalbfleisch, J.H., Stout, R.D., Ensley, H.E., Browder, W., Williams, D.L., 2000. Glycobiology 10 (4), 339–346.
Nakajima, T., Ballou, C.E., 1974. J. Biol. Chem. 249 (23), 7679–7684.
Nicolini, C., Adami, M., Sartore, M., Bragazzi, N.L., Bavastrello, V., Spera, R., Pechkova, E., 2012. Sensors (Basel) 12 (12), 17112–17127.
Ohyama, Y., Kasai, K., Nomoto, H., Inoue, Y., 1985. J. Biol. Chem. 260 (11), 6882–6887.
Ojamae, L., Aulin, C., Pedersen, H., Kall, P.O., 2006. J. Colloid Interface Sci. 296 (1), 71–78.
Paulo Tde, F., de Sousa, T.P., de Abreu, D.S., Felicio, N.H., Bernhardt, P.V., Lopes, L.G., Sousa, E.H., Diogenes, I.C., 2013. J. Phys. Chem. B 117 (29), 8673–8680.
Pei, Z., Anderson, H., Aastrup, T., Ramstrom, O., 2005. Biosens. Bioelectron. 21 (1), 60–66.
Pei, Z., Ma, X., Ding, P., Zhang, W., Luo, Z., Li, G., 2010. Sensors 10 (9), 8275–8290.
Pejcic, B., Myers, M., Ranwala, N., Boyd, L., Baker, M., Ross, A., 2011. Talanta 85 (3), 1648–1657.
Pensa, E., Cortes, E., Corthey, G., Carro, P., Vericat, C., Fonticelli, M.H., Benitez, G., Rubert, A.A., Salvarezza, R.C., 2012. Acc. Chem. Res. 45 (8), 1183–1192.
Plant, D.J.V.C.W.M.V.I.S.A.L., 1998. Organic Anal. Chem. 14 (24), 6916–6923.
Roach, P.J., Depaoli-Roach, A.A., Hurley, T.D., Tagliabracci, V.S., 2012. Biochem. J. 441 (3), 763–787.
Sam, S., Touahir, L., Salvador Andresa, J., Allongue, P., Chazalviel, J.N., Gouget-Laemmel, A.C., Henry de Villeneuve, C., Moraillon, A., Ozanam, F., Gabouze, N., Djebbar, S., 2010. Langmuir 26 (2), 809–814.
Sato, K., Kodama, D., Anzai, J., 2005. Anal. Sci. 21 (11), 1375–1378.
Sato, K., Takahashi, S., Anzai, J., 2012. Anal. Sci. 28 (10), 929–938.
Sauerbrey, G., 1959. Z. Phys. 155, 206–222.
Stephen, J. Martin, V.E.G., Gregory C., Frye, 1991. Anal. Chem. 63 (20), 2272–2281.
Sun, Y.X., Ren, K.F., Wang, J.L., Chang, G.X., Ji, J., 2013a. Am. Chem. Soc.: Appl. Mater. Interfaces 5 (11), 4597–4602.
Sun, Y.X., Ren, K.F., Zhao, Y.X., Liu, X.S., Chang, G.X., Ji, J., 2013b. Langmuir 29 (35), 11163–11168.
Coffey, T., M.A., Krim, J., 2001. Condens. Matter 13 (21), 4991–4999.
Tan, L., Lin, P., Chisti, M.M., Rehman, A., Zeng, X., 2013. Anal. Chem. 85 (18), 8543–8551.
Tan, L., Xie, Q., Yao, S., 2007. Bioelectrochemistry 70 (2), 348–355.
Timko, B.P., Arruebo, M., Shankarappa, S.A., McAlvin, J.B., Okonkwo, O.S., Mizrahi, B., Stefanescu, C.F., Gomez, L., Zhu, J., Zhu, A., Santamaria, J., Langer, R., Kohane, D. S., 2014. Proc. Nat. Acad. Sci. U.S.A. 111 (4), 1349–1354.
Ueyama, S., Hijikata, K., Hirotsuji, J., 2002. Water Sci. Technol. 45 (4-5), 175–180.
Varki, A., 1993. Glycobiology 3 (2), 97–130.
Wangchareansak, T., Sangma, C., Ngernmeesri, P., Thitithanyanont, A., Lieberzeit, P. A., 2013. Anal. Bioanal. Chem. 405 (20), 6471–6478.
Yi, C., Sun, Y., Song, B., Tian, W., Qi, Q., Zheng, Y., Dai, Y., Jiang, W., 2013. Nanotechnology 24 (43), 435704.
Zhu, Yi, Weijun, T., Changyou, G., 2011. Soft Mater. 7 (12), 5805–5815.
Yuri, L., Katsuhiko, A., Izumi, I., Toyoki, K., 1995. J. Chem. Soc., Chem. Commun. 22, 2313–2314.
Zhang, S.Z., Zhao, F.L., Li, K.A., Tong, S.Y., 2001. Talanta 54 (2), 333–342.