Author's Accepted Manuscript

www.elsevier.com/locate/talanta

Design of an aptamer-based magnetic adsorbent and biosensor systems for selective and sensitive separation and detection of thrombin

Gulay Bayramoglu, Cengiz Ozalp, Merve Oztekin, Ulku Guler, Bekir Salih, M. Yakup Arica

PII: S0039-9140(18)30858-0
DOI: https://doi.org/10.1016/j.talanta.2018.08.048
Reference: TAL18969

To appear in: *Talanta*

Received date: 10 June 2018
Revised date: 11 August 2018
Accepted date: 17 August 2018

Cite this article as: Gulay Bayramoglu, Cengiz Ozalp, Merve Oztekin, Ulku Guler, Bekir Salih and M. Yakup Arica, Design of an aptamer-based magnetic adsorbent and biosensor systems for selective and sensitive separation and detection of thrombin, *Talanta,* https://doi.org/10.1016/j.talanta.2018.08.048

Design of an aptamer-based magnetic adsorbent and biosensor systems for selective and sensitive separation and detection of thrombin

Gulay Bayramoglu[1,2]*, Cengiz Ozalp[3,4], Merve Oztekin[1], Ulku Guler[5], Bekir Salih[5], and M. Yakup Arica[1]

[1]Biochemical Processing and Biomaterial Research Laboratory, Gazi University, 06500 Teknikokullar, Ankara, Turkey

[2] Department of Chemistry, Faculty of Sciences, Gazi University, 06500 Teknikokullar, Ankara, Turkey

[3] Department of Bioengineering, Konya Food & Agriculture University, 42080 Konya, Turkey

[4] Research and Development Center for Diagnostic Kits (KIT-ARGEM), Konya Food and Agriculture University, 42080 Konya, Turkey

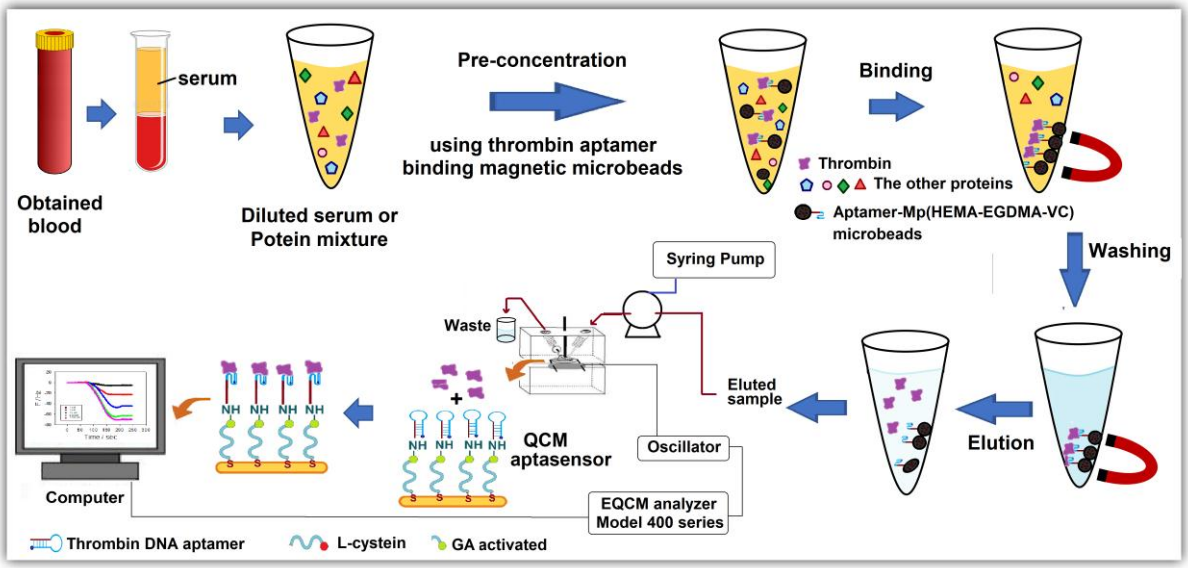
[5] Hacettepe University, Department of Chemistry, 06800 Beytepe, Ankara, Turkey

Abstract

An aptasensor was designed for sensitive detection of thrombin using in biological fluids by integrating a magnetic aptamer-microbeads. To achieve this goal, the surface of gold plated QCM crystals was coated with L-cysteine and a thrombin binding DNA aptamer was immobilized on the L-cysteine coated QCM crystals surface via glutaraldehyde coupling. The binding interactions of thrombin to QCM crystals were characterized. Magnetic poly(2-

hydroxyethyl methacrylate-ethylene glycol dimethacrylate-vinylene carbonate), Mp(HEMA-EGDMA-VC) microbeads were synthesized and thrombin binding aptamer (TBA) was immobilized. The Mp(HEMA-EGDMA-VC)-TBA microbeads were effectively adsorbed thrombin from serum in a relatively short contact time (ca. 5.0 min), and the eluted protein from Mp(HEMA-EGDMA-VC)-TBA was transferred to the QCM aptasensor that showed a specific detection of thrombin from serum. The detection limit of thrombin using aptasensor was 1.00 nmol L^{-1}. The calculation dissociation constant of the aptasensor was 68.5 nmol L^{-1}. The selectivity of the aptasensor system was tested with three different proteins (i.e., elastin, immunoglobulin G (IgG) and human serum albumin (HSA)) and showed high specificity to thrombin. The aptasensor was regenerated by washing with NaOH solution, and repeatedly used until 20 cycles without a change in the performance.

Graphical abstract



Keywords: Thrombin, Aptamers, Serum, Aptasensor, Magnetic microbeads

1. Introduction

Thrombin is a specific serine protease which is the main component of the blood coagulation cascade. The important function of thrombin is cleaving of fibrinogen to fibrin. [1-3]. Meanwhile, thrombin is also an important biomarker for diseases associated with coagulation abnormalities, cardiovascular diseases and especially in the detection of pulmonary metastatic tumors [4-6]. Thus, the amount of thrombin in blood indicates the physiological function of human body. In the resent years, due to the medical importance of thrombin several quantitative detection methods have been developed such as aptamer based-sensor systems, [7,8] spectrophotometric, fluorimetric and electrochemical etc. [9,10].

Aptamers are small single stranded DNA or RNA oligonucleotides that show high selectivity to their matching target molecules, such as; inorganic or organic small molecules, peptides, proteins and microbial cells. Aptamers are generally selected via Systematic Evolution of Ligands by Exponential Enrichment (SELEX), which was first developed by Tuerk and Gold [11]. Like proteins, aptamers can be able to fold with a specific binding properties [12-14]. Thus, aptamers gain a molecular recognition ability that can bind specifically to the corresponding target. Moreover, selected aptamers sequence can be multiplied in a test tube and during these aptamer production processes different functional groups can be incorporated on the aptamer structure at desired positions. For that reason, the functionalized aptamer molecules gain ability for fixation to a solid matrix under given experimental conditions. On the other hands, proteins can be fixed to solid matrices in a arbitrary position due to the presence of several reactive sites (i.e., -NH_2, -SH, -COOH and –OH) which may hinder the target binding site, therefore, decrease the binding potential of the fixed protein to its target molecule [15-18]. So, aptamer interacts with its target with high specific affinity, and it can be used as an alternative ligand in specific molecular recognition

for different biosensor applications. The aptamer-based biosensors have been reported for several types of applications such as metal ions, small organic complex molecules, proteins, or whole cells [19-22]. Additionally, aptamer-immobilized magnetic solid supports have been used for purification of several biological molecules as well as a precursor for enrichment of target molecule from complex sample mixtures. Thus, interference of the other possible interactive molecules with biosensors has been prevented. Aptamers have several advantages over protein based ligands such as; they are prepared biochemical method, and selected in vitro without use of animal. They are considerably economic to produce large quantity and controllable yields and stable over long periods of storage time under mild conditions. Thus, aptamers are highly important novel ligand molecules to be used for diagnostic and therapeutic purposes. Many aptamer ligands such as thrombin aptamer, anti-human IgE aptamers, and whole cell aptamers (e.g., E. coli and Salmonella) have been used for construction of biosensor [23-25].

QCM based biosensor can be used for detection from small molecules to cells [30,31]. The detection principle of the QCM biosensor system is based on the reduction in frequency change which is linearly proportional to the added mass on the crystal, which is known as Sauerbrey relation [29-33]. In this system, specific affinity ligand is fixed on the biosensor chip for interaction with its target molecule. Thus, a facile and specific interaction can be generated for the fast determination of target molecule in the complex samples.

Magnetic matrices have been used for separation of proteins, pre-concentration of various small and macromolecules from biological fluids to eliminate interference substance in the samples [34]. The main necessitating property of the magnetic materials is that after laden adsorbent with its target molecule can be removed from medium by using an external magnetic force [24]. The acrylic based materials have used for preparation of magnetic support materials and can be prepared in required size and geometry. In addition, they can be

synthesized with required pore size and large surface area, and functionalized with the desired groups. In this work, we presented a direct method for detection of thrombin in human serum by conjunction of a pre-concentration system with QCM-aptasensor. To the best of our knowledge, the work is the first report of a new aptamer-QCM system to detect thrombin in serum samples. Purposely, thrombin specific DNA aptamer was used as specific binding ligand for isolation and detection of thrombin from solution and human serum. In the first step, the thrombin binding aptamer was immobilized on the magnetic poly(2-hydroxyethyl methacrylate-ethylene glycol dimethacrylate- vinylene carbonate), Mp(HEMA-EGDMA-VC), microbeads via ring opening reaction and used for enrichment of thrombin from diluted human serum. The aptamer-immobilized microbeads were characterized by FTIR and SEM. The sequestered thrombin was eluted and fed into a QCM-aptasensor for quantitative detection of thrombin in the solutions and diluted serum samples.

2. Materials and Methods

2.1. Materials

2-hydroxyethyl methacrylate (HEMA) and 4-Vinyl-1,3-dioxolan-2-one (vinyl ethylene carbonate) (VC) monomers were supplied from Sigma-Aldrich Chemical Co. (Germany). Ethylene glycol dimethacrylate (EGDMA) (Sigma-Aldrich) was used as a cross-linker. Thrombin aptamer GGTTGGTGTGGTTGGTTTTTTTTTTTT [AmC6T], (5'–NH_2 modified, (MW=8558) was obtained from Sigma-Aldrich. α-Thrombin, human serum albumin (HSA), human immunoglobulin (IgG), and elastin were purchased from Sigma-Aldrich. **A**zobisisobutyronitrile (AIBN), glutaraldehyde, polyvinyl alcohol (PVA) and L-cysteine were also supplied from Sigma-Aldrich. Distilled water from a Millipore filter (Merck, Darmstadt, Germany) was used in all experiments.

2.2. Preparation of the magnetic nanoparticles and Mp(HEMA-EGDMA-VC) microbeads

The magnetic nanoparticles were prepared via thermal co-precipitation method by mixing Fe^{+3} and Fe^{+2} chlorine and sulfate salts, respectively, at 2:1 molar ratio as described previously [24,34]. The magnetic poly(2-hydroxyethyl methacrylate- ethylene glycol dimethacrylate-vinylene carbonate), Mp(HEMA-EGDMA-VC), microbeads were prepared via suspension polymerization. A three necked reaction vessel was furnished with mechanical mixer, reflux condenser and N_2 gas inlet. The organic phase containing HEMA (7.0 mL), VC (7.0 g), EGDMA (6.0 mL), 1.0 g of Fe_3O_4 particles, and PVA (5.0%, 15 mL, as stabilizer) was mixed together with 0.2 g of AIBN as initiator in 20 mL of toluene. The mixture was added into reaction vessel and placed in an ultrasonic water-bath and sonicated at 200 W for 10 min (Bransonic 2200, England) for the mixing of the polymerization ingredient. Then, the reactor was operated as described previously [35].

2.3. Characterization of the Mp(HEMA-EGDMA-VC) microbeads

The specific surface area of the magnetic microbeads was determined by the surface area apparatus and calculated using the BET (Brunauer, Emmett, and Teller) method [36]. The surface properties of the Mp(HEMA-EGDMA-VC) microbeads was studied using scanning electron microscopy (SEM; ZEIZZ, Evo 50). ATR-FTIR measurements of the magnetic "Fe_3O_4" nanoparticles, Mp(HEMA-EGDMA-VC) and Mp(HEMA-EGDMA-VC)-aptamer microbeads were realized using a spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA) equipped with an Universal ATR accessory. The magnetization of Fe_3O_4 particles and Mp(HEMA-EDGMA-VC) microbeads versus the applied magnetic field was detected with a vibrating-sample magnetometer (VSM; Model 155, Digital Measurement System Inc., Westwood, MA, USA). The phase formation and crystallographic state of the pure-Fe_3O_4

nanoparticles and Mp(HEMA-EDGMA-VC) microbeads were analyzed by X-ray diffraction (XRD) technique as described previously [24]. The amount of available cyclic carbonate on the microbeads was determined gravimetrically described in the literature [37]. The water content of the Mp(HEMA-EGDMA-VC) microbeads was determined as described previously [38].

2.4. Immobilization of thrombin aptamer on Mp(HEMA-EGDMA-VC) microbeads via cyclic reactive carbonate group

The Mp(HEMA-EGDMA-VC) microbeads containing cyclic carbonate groups were directly coupled with $–NH_2$ modified thrombin specific aptamer. To achieve this, the Mp(HEMA-EGDMA-VC) microbeads (about 250 mg) were incubated in Tris-HCl buffer (20 mmol L^{-1}, pH 8.5) for 1.0 h, and added to the same fresh buffer containing thrombin binding aptamers with pendant —NH_2 groups (2.0 mL, 2.0 μmol mL aptamer). The coupling reaction was realized at room temperature for 3.0 h. The chemistry of reaction is presented in **Figure 1**. The amount of immobilized aptamer was determined spectrophotometrically at 265 nm (using an UV–vis Nanodrop 2000; Nanodrop products, Wilmington, USA). The amount of immobilized aptamer and the immobilization efficiency were calculated by subtraction of the aptamer amount in the final and the initial solutions.

2.5. Adsorption and pre-concentration studies of thrombin using Mp(HEMA-EGDMA-VC)-aptamer microbeads

Thrombin specific DNA aptamer immobilized Mp(HEMA-EGDMA-VC) microbeads were used for pre-concentration of thrombin from samples. Adsorption of thrombin on the Mp(HEMA-EGDMA-VC)-aptamer microbeads was studied at pH 7.4 (Tris HCl buffer, 20 mmol L^{-1}). The batch experiments were accompanied using a certain amounts of Mp(HEMA-

EGDMA-VC)-aptamer microbeads (5 mg) at pH 7.4 and at 25 °C for 10 min. For each set experiment, the aptamer magnetic-beads were washed two times with 1.0 mL of Tris HCl buffer solution (pH 7.4, 20 mmol L^{-1}, containing 100 mmol L^{-1} NaCl) to remove nonspecifically adsorbed thrombin. Then, the adsorbed thrombin was eluted using 20 mM NaOH solution. All experiments were studied in triplicate with 5.0 mg of Mp(HEMA-EGDMA-VC)-TBA microbeads at 25 °C, and at a rotating rate of 150 rpm for 5 min. Then, the Mp(MMA-VC-EGDMA)-TBA microbeads were separated with an external magnet from the medium and the amount of adsorbed thrombin was determined as described above.

2.6. Magnetic enrichment of thrombin using Mp(HEMA-EGDMA-VC) microbeads

The Mp(HEMA-EGDMA-VC)-TBA microbeads were used to isolate thrombin in aqueous medium and the diluted human serum containing different amount of spiked thrombin (0-100 nM). The adsorption conditions were similar as described above. The Mp(HEMA-EGDMA-VC)-TBA microbeads of 5.0 mg were transferred into 250 μL of sample solution containing different amount of thrombin. The eluted thrombin was used in the thrombin activity assay or QCM studies.

2.7. Preparation of QCM sensor crystal for determination of α-thrombin

The QCM sensor chip was cleaned sodium hydroxide solution (1.0 mol L^{-1}) for 20 min and hydrochloride acid solution (1.0 mol L^{-1}) for 15 min to obtain a clean gold crystal surface. The crystal was washed with ethanol and water, then dried under nitrogen stream and placed in the QCM flow cell holder as described previously [26]. Then, the crystal was continuously contacted with L-cysteine solution (5 mg mL^{-1}, pH 8.5) for 8.0 h, at dead end operation mode by means of an injection pump. After the reaction, self-assembled monolayers with pendant amine and carboxyl groups via gold-thiolate bonds were generated on the crystal surface. The

non-specifically adsorbed L-cysteine was removed by washing with ethanol and purified water. The conjugated L-cysteine on the crystal was reacted with glutaraldehyde (0.5%) for 4 h to convert the amine group to an amine-reactive glutaraldehyde related enol groups. After modification of gold surface for thrombin specific aptamer immobilization, the QCM chip was placed again follow cells and modified face of the chip was exposed to the amine labeled thrombin binding aptamer (2 µmol aptamer mL in Tris-HCl buffer pH 8.5). Immobilization reaction of aptamer was carried out at room temperature for 6.0 h. According to manufacturer's instruction guide (CH Instruments), a net change of 1.0 Hz matches to 1.34 ng of mass change on the chip surface of 0.196 cm^2 area.

2.8. Determination of thrombin amount using QCM aptasensor

Thrombin binding aptamer immobilized QCM-aptasensor was used for the detection of thrombin from solution and diluted serum or fibrinogen-precipitated plasma samples. The fibrinogen was precipitated out as described by Bini *et al.* (2007) [40]. The quartz crystal microbalance system (model CHI 400A, CH Instruments, Texas, USA) with a continuous flow cell, oscillator and an analyzer was used to construct QCM sensor for the thrombin determination. The sensor chip with immobilized aptamer was positioned into the continuous flow cell. A syringe injecting pump was used for injecting samples and the system was operated as described earlier. The amount of mass on the QCM chip was calculated using Sauerbrey equation [32]. Although Sauerbrey equation is for gas phase and ignores associated water molecules, we used it as an approximation for mass calculations.

$$\Delta F = -2F_0^2 \Delta m / A\,[\mu_q \rho_q]^{0.5} \quad \Delta F = -2F_0^2 \Delta m / A\,[\mu_q \rho_q]^{0.5} \qquad (1)$$

where F_0 is the resonant frequency of the fundamental mode of the crystal, Δm is the surface mass loading, A is the area of the gold disk coated onto the crystal, ρ_q is the density of the

crystal (2.684 x 10^9 ng cm^{-3}), and μ_q is the shear modulus of quartz (2.947 10^{20} ng cm^{-1} s^2). $\Delta m/A$ is the mass-increase per unit of area (ng cm^{-2}).

Thrombin stock solution (1000 nmol L^{-1}) was used to prepare standard solutions with thirteen different concentrations (0.5, 0.75, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0, 75.0, 100.0, 150.0, 250.0 and 500 nM) in Tris–HCl buffer (20 mmol L^{-1}, pH 7.4), and the sample cell suspension was injected through the QCM flow cell at a flow rate of 1.0 mL min^{-1}, and the frequency shift of each sample was recorded. Regeneration of the biosensor chip was carried out by contacting with 5.0 mL of 20 mmol L^{-1} NaOH solution with 1.0 mL min^{-1} flow-rate. The real-time data was presented after applying a negative exponential smoothing by SigmaPlot 11 (Systat software Inc.).

2.9. Activity assay of thrombin preparations

The initial activity of the free and aptamer captured thrombin was assayed by using an artificial peptide substrate (i.e., β-Ala-Gly-Arg-*p*-nitroanilide**;** Sigma catalog: T3068). Additionally, the activities of the thrombin in serum and solution were also determined spectrophotometrically at 405 nm. The increase in the color of enzymatic release of p-nitroanilide from artificial peptide substrate of thrombin was followed for 15 min after addition of thrombin as described in Sigma catalog T3068.

Thrombin reaction with artificial peptide substrate is:

β-Ala-Gly-Arg-*p*-nitroanilide → *β*-Ala-Gly-Arg + *p*-nitroanilide (2)

In this reaction thrombin cleaved the artificial substrate at arginine site and p-nitroanilide released. The reaction was carried out in Tris-HCl buffer (20 mmol L^{-1} pH 7.4 containing 100 mmol L^{-1} of NaCl, 10.0 mmol L^{-1} of KCl, 1.0 mmol L^{-1} of $MgCl_2$ and 1.0 mmol L^{-1} of $CaCl_2$. The thrombin concentration was varied between 10 and 100 nmol L^{-1}. The thrombin laden

aptamer-magnetic beads on the beads were separated from solutions using an external magnet and then used in the thrombin activity assay studies.

3. Results and discussion

3.1. Characterization Mp(HEMA-EGDMA-VC) microbeads

The Mp(HEMA-EGDMA-VC) microbeads were synthesized via suspension polymerization, and a size distribution of 50-150 μm and with a specific surface area of 28.7 m^2 g^{-1} were used for isolation thrombin from solutions and diluted human serum. The amount of available cyclic carbonate groups of the Mp(HEMA-EGDMA-VC) microbeads was 2.27 mmol per gram of the microbeads. The water content and density of the microbeads were 67% and 1.27 g cm^{-3}, respectively.

The entrapment of Fe_3O_4 nano-particles in the polymeric microbeads and immobilization of thrombin binding aptamer on the Mp(HEMA-EGDMA-VC) microbeads were confirmed by ATR-FTIR studies (**Figure S1**). The ATR-FTIR spectra of the Fe_3O_4, Mp(HEMA-EGDMA-VC) and Mp(HEMA-EGDMA-VC)-aptamer were obtained in the range 400-4000 cm^{-1}. As can be seen from **Figure S1a**, the Fe_3O_4 particles show a broad band at around 3300 cm^{-1}, indicative of the presence of -OH groups in the structure of Fe_3O_4 nano-particles. The peak at around 590 cm^{-1} for the pristine Fe_3O_4 nano-particles is due to the out-of-plane bending vibration of Fe-O on Fe_3O_4. For Mp(HEMA-EGDMA-VC) microbeads, the carbonyl peak with the characteristic ester group was observed at 1735 cm^{-1}. The -OH group vibration and aliphatic stretching vibration peaks of HEMA are seen at 3475 cm^{-1} and 2995 cm^{-1}, respectively (**Figure S1b**). Another important band at 1815 cm^{-1} for carbonyl stretching vibration of Mp(HEMA-EGDMA-VC) as a cyclic carbonate function is also observed. Following the aptamer ligand attachment, a new peak at 1458 cm^{-1} is observed for the stretching vibrations of C-N groups of the aptamer ligand. Additionally, characteristic bands

for the stretching peaks of C-H aromatic stretch are also seen at around 2865 and 2947 cm^{-1}, and the peaks at 1524 cm^{-1} can be due to the stretching vibrations of C=C groups, representative of the presence of aromatic moieties of the nucleotide in the aptamer structure (**Figure S1c**). Above all, clear disparities of the FTIR spectrum between pristine Mp(HEMA-EGDMA-VC) and Mp(HEMA-EGDMA-VC)-TBA microbeads were observed. Thus, the formation of the covalent bound between thrombin binding aptamer and cyclic carbonate groups on the microbeads was proved.

The SEM analysis gives information about the surface properties of the Mp(HEMA-EGDMA-VC) microbeads. The SEM image of the Mp(HEMA-EGDMA-VC) microbeads clearly shows that the microbeads are spherical and nearly uniform in size (**Figure S2**). Additionally, the Mp(HEMA-EGDMA-VC) microbeads have also a porous surface structure.

The XRD patterns of pure Fe_3O_4 particles and Mp(HEMA-EGDMA-VC) microbeads are presented in **Figure 2**. Here, there are eight apparent diffraction peaks in the 2θ range of 10°–80° at 30.42°, 36.05°, 43.79°, 52.98°, 56.73°, 63.78°, 71.24° and 74.06° which could readily be attributed to (220), (311), (400), (422), (511), (440), (620) and (533), respectively. These reflection peaks agree well with the X-ray diffraction data cards (ICDD card No. 88-0315). As observed from **Figure 2a and b**, Fe_3O_4 particles and Fe_3O_4 particles entrapped polymer network have the same diffraction peaks corresponding to spinel configuration of Fe_3O_4, respectively. Thus, the XRD pattern of Fe_3O_4 in the Mp(HEMA-EGDMA-VC) microbeads (**Figure 2b**) was very similar with the pure Fe_3O_4 nanoparticles, that demonstrating protection of the magnetic structure and not notably altered after entrapment in polymer network [33].

The magnetic properties of Fe_3O_4 nanoparticles and Mp(HEMA-EGDMA-VC) microbeads were characterized using a vibrating sample magnetometer (VSM), which is generally utilized for the quantitation of saturation magnetization values of the magnetic

materials. The representative magnetization curves of Fe_3O_4 particles and Mp(HEMA-EGDMA-VC) microbeads are presented in **Figure S3**. The saturation magnetization values were 48.7 and 26.2 emu g^{-1} at room temperature, respectively. These results showed that the magnetic property of the Fe_3O_4 particles was conserved in the polymer network. Whereas the saturation magnetization value of Mp(HEMA-EGDMA-VC) microbeads was significantly decreased compared to the bare Fe_3O_4 particles. This may be due to reduction in the amount of the magnetic moments per unit weight because of the diamagnetic contribution of the polymer network.

3.2. Isolation of thrombin from diluted serum with aptamer immobilized magnetic microbeads

A functional cyclic carbonate group carrying monomer was used for covalent immobilization of thrombin binding aptamer on the microbeads. The cyclic carbonate groups in the polymer structure allow convenient immobilization of various molecules (such as proteins, nucleic acids, lipids and sugars) through specifically amine groups via cyclic ring opening coupling reaction. The Mp(HEMA-EGDMA-VC) microbeads with reactive cyclic carbonate groups were readily reacted under mild experimental conditions with thrombin binding aptamer containing primary amine group ($-NH_2$). The microbeads were used for isolation of thrombin from aqueous medium and diluted serum samples spiked with external thrombin. Additionally, thrombin can be selectively isolated from samples with a single step purification method using Mp(HEMA-EGDMA-VC)-TBA microbeads, and thrombin laden microbeads can also be separated from complex serum samples using an external magnet without needing any other equipment.

The performance of the Mp(HEMA-EGDMA-VC)-TBA microbeads was tested using 10-fold diluted human serum sample which spiked with 50 nmol L^{-1} thrombin. The adsorption

efficiency of the spiked thrombin at 50 nmol L^{-1} was 93.7% with a standard deviation of 2.8% in the diluted serum. From this result, the Mp(HEMA-EGDMA-VC)-TBA microbeads was successfully isolated thrombin from 10-fold diluted serum samples, thus, the Mp(HEMA-EGDMA-VC)-TBA system can be used for isolation of target molecules from complex samples.

In general, thrombin molecules were captured from aqueous medium and diluted serum samples by using 5.0 mg of Mp(HEMA-EGDMA-VC)-TBA microbeads. Then it was eluted into 250 µL NaOH (or Tris HCl buffer Bölüm 2.6) (0.1 mol L^{-1}), neutralized with acetic acid and directly fed onto QCM sensor to obtain corresponding frequency changes. Batch-wise thrombin isolation experiments were performed at room temperature for 10 min contact time. Then, the Mp(HEMA-EGDMA-VC)-TBA microbeads were separated from the samples using an external magnet. The use of aptamer sequence both for capturing and subsequent detection ensures high specificity, similar to a sandwich assay. In fact, we reported a similar strategy (i.e., nanoparticle-based purification) for highly specific determination of pathogenic bacteria from the milk or blood samples by coupling QCM-aptasensor or aptamer-gated silica nanoparticles, respectively [14,21]. The specificity of Mp(HEMA-EGDMA-VC)-TBA microbeads was also studied with different four proteins such as elastase, IgG, HSA and thrombin . These tested proteins did not cause any hindrance for the isolation of thrombin from Tris-HCl buffer and diluted serum samples. The amounts of binding of these proteins onto Mp(HEMA-EGDMA-VC)-TBA microbeads were too small to be assigned, therefore, data was not shown. The high specificity of the thrombin binding aptamer to thrombin can be due to the specific association and selective interaction.

3.3. Operation of thrombin binding QCM-aptasensor

QCM biosensor is facile method for label less real-time analysis of biomolecules compared to other techniques such as chromatography, electrophoresis, mass spectrometry, and ultracentrifugation [14,26]. Due to this advantage, QCM biosensors have increasingly been the preferred method for monitoring interactions between small molecules and proteins [14,26]. Therefore, as described above, we employed magnetic isolation method before QCM-aptasensor detection for a highly sensitive and specific thrombin detection system in a two-step procedure. Similarly, in this report, thrombin was purified by Mp(HEMA-EGDMA-VC)-TBA microbeads and subsequent detection was obtained by QCM-aptasensor developed by using the same aptamers. First, L-cysteine amino acid was used for the creation of a self-assembled monolayer on the gold surface of the QCM chip. Then, it was functionalized with bifunctional agent (i.e., glutaraldehyde). A reactive aldehyde group was generated on the chip surface for covalent fixation of the amine labeled thrombin binding aptamer. As a backfilling molecule 6-mercapto 1-hexanol was used to minimize non-specific binding. A typical immobilization experiment of thrombin binding aptamer resulted in 14.37 Hz decrease in frequency after injection of 10 µM aptamer solution through a continuous flow cell for 150 seconds (**Figure 3A**). QCM biosensor is a bulk acoustic wave measurement technique; changes in piezoelectric quartz crystal are measured after electrical excitation, which are linearly proportional to the mass on the sensor chip surface, as described by Sauerbrey equation [33]. A surface coverage of 98.2 ng cm^{-2} thrombin binding aptamer was obtained according to this equation. An addition, the amount of interacted mass on the QCM chip surface is measured in real-time, allowing the researcher to closely follow mass changes. Thus, it is possible to follow how particularly small masses that are being added or removed to the QCM biosensor chip.

3.4. QCM-aptasensor coupled to Mp(HEMA-EGDMA-VC) microbeads and detection of thrombin in samples

To study of the applicability of this QCM-aptasensor in biological samples, the determination of thrombin from diluted human serum samples was realized. Serum is a biological fluid and obtained after coagulation of whole blood, therefore, does not contain any coagulation protein. As described above, any interfering substances present in human serum sample were effectively removed by subsequent magnetic isolation process as evidenced by QCM aptasensor records. The magnetically separation of the thrombin laden Mp(HEMA-EGDMA-VC)-TBA microbeads was confirmed as an appropriate tactic for an successful quantification of thrombin in serum samples. The human serum samples were spiked with thrombin at various concentrations, magnetic isolation procedure was applied and the eluent was subjected to QCM analysis or to biochemical analysis of thrombin amount with artificial substrate T3068 as described above.

For QCM analysis, the eluted thrombin from Mp(HEMA-EGDMA-VC)-TBA microbeads was fed into QCM-aptasensor for real-time quantification of thrombin in solution and serum samples. The change in QCM-aptasensor response is directly proportional with the increase of the spiked thrombin concentration in serum samples (**Figure 3B**) and the absolute frequency changes increased along with thrombin concentrations, such that frequency change exhibited a one-to-one Langmuir binding relationship. The affinity constant (K_d) was calculated to be 68.5 nmol L^{-1}, which is in agreement with literature. The range of thrombin concentration in a range of 0-100 nmol L^{-1} followed a linear relationship with the frequency change **(Figure 4)**. Whereas serum sample lacking any addition of thrombin produced only small frequency changes was observed about 3.00 ± 1.23 Hz. As the frequency change depends on thrombin specific-binding induced mass change on the surface of the aptamer-chip compared to unspecific adsorption, signal change should be relatively insensitive to

control proteins present in human serum. To evaluate the binding specificity of the QCM-aptasensor to thrombin, control experiments with non-target molecules (elastin IgG and HSA) were performed at 100 nM concentrations. The changes in signal intensities for elastin, IgG and HSA were extremely feeble, almost the same as the blank (i.e., Tris-HCl buffer). Even at high concentrations of interfering proteins, QCM frequency changes were not significantly different. Additionally, the frequency intensity of thrombin in existence of the tested protein was nearly the same with that in Tris–HCl buffer solution **(Figure 5)**. This result showed that other proteins were not interacted with the thrombin binding aptamer on the QCM sensor chip. The presence of these substances did not cause any hindrance for the detection of thrombin. In fact, Bini *et al* (2007) previously reported QCM aptasensor for detection in diluted serum and plasma samples [40]. However, diluting samples resulted in sensitivity levels which are not sufficient for direct thrombin detection in real samples. The excellent selectivity and sensitivity suggest a promising detection system for thrombin in complex serum. The resultant aptamer-based QCM sensor exhibited high selectivity and reproducibility toward thrombin. It could be used to detect thrombin in dilutes serum samples, suggesting potential application in clinical settings.

3.5. Equilibrium binding studies of thrombin on the QCM biosensor

The thrombin binding aptamer functionalized QCM chip was placed in the continuous flow cell holder. Then, the direct binding performance of thrombin to the QCM biosensor chip surface was studied in order to determine the limit of detection for thrombin (**Figure 4**). Thrombin solutions in Tris-HCl buffer (20 mmol L^{-1}, pH 7.4) at various concentrations were injected continuously to flow cell. The non-specifically interacted thrombin was disinterested by washing with Tris HCl buffer solution (pH 7.4, 20 mmol L^{-1}, containing 200 mmol L^{-1} NaCl). Contacting of thrombin solution with aptamer-chip resulted in a reduction in frequency

values. The frequency change values were plotted against the thrombin concentrations to obtain a binding calibration curve (**Figure 4**). The aptamers-chip of the biosensor was also utilized for the determination of thrombin amounts in 10-fold diluted serum sample spiked with thrombin. Serum samples were spiked with thrombin at various concentrations, magnetic isolation procedure was applied and the eluent were injected on thrombin binding aptamer immobilized sensor chips. QCM biosensor data with the thrombin-aptamer chip was analyzed for the evaluation of equilibrium isotherm parameters using Scatchard, Langmuir and Freundlich models.

The Scatchard: $\Delta F/[C] = 1/K_d\,[(\Delta F_{max} - \Delta F)]$ (3)

The Langmuir: $\Delta F = (\Delta F_{max}\,[C])/(K_d + [C])$ (4)

The Freundlich: $\Delta F = K_F\,[C]^{1/n}$ (5)

Where ΔF_{max} is the maximal frequency shift for fully saturated surface (Hz), ΔF is the frequency shift (Hz) at a given concentration of thrombin, C is the amount of thrombin (nmol L^{-1}), K_d (nmol L^{-1}) is the equilibrium dissociation constant and it is equal reverse of the equilibrium association constant ($1/K_a$) and 1/n and K_F are Freundlich exponent. K_a (nmol L^{-1}) and K_d (nmol L^{-1}) are forward and reverse equilibrium constants; subscript "max" donate maximum. For binding of thrombin on the aptamer-chip of the biosensor, the Langmuir model was better fitted the experimental data than those of the Scatchard and Freundlich model (Table 1).

Scatchard analysis of the amount of thrombin bound at the surface as a function of the concentration of thrombin in the buffer gave the value for the equilibrium dissociation constant (K_d, 62.5 nmol L^{-1}in Table 1). These results correlated well with values reported in literature [37]. The calculated value for the regression coefficient (R^2) at equilibrium adsorption conditions obtained in this work by QCM of the binding system is 0.789. The linearity of the Langmuir model is well-suited compared to the Scatchard model. The

maximal frequency shift for fully saturated surface for thrombin (ΔF_{max}) was 73.95 (Hz), and the calculated ΔF_{max} value was very close to the experimental value (64.25 Hz) (Table 1). The affinity constant (K_d) and regression coefficient (R^2) were calculated to be 68.5 nmol L^{-1} and 0.992, respectively, which is in agreement with literature [37,38]. As shown in **Figure 4** the absolute frequency changes, increases along with thrombin concentrations, such that frequency change exhibited a one-to-one Langmuir binding relationship. Freundlich model is well fitted to heterogeneous surfaces. The regression coefficient was 0.989. For the Freundlich model, the magnitude of n values was found to be high (1.46) adequate for binding of thrombin from aqueous medium (Table 1).

The designed QCM aptasensor was used with thrombin solutions and diluted serum samples, which spiked with known concentrations (0-100 nmol L^{-1}) of thrombin for construction of a calibration curve. The limit of detection and the limit of quantification for thrombin were found to be 1.0 and 100 nmol L^{-1}, respectively. It should be reported that, the presented Mp(HEMA-EGDMA-VC)-TBA system and QCM-aptasensor have a good efficiency in the detection of thrombin in solutions and diluted serum samples.

3.6. Sensitivity and stability of QCM aptasensor

The sensitivity and stability are important factors for the convenient application of biosensors. Therefore, these factors were studied using newly designed QCM-aptasensor. A 15 samples of 10-fold diluted human serum spiked with three different thrombin concentrations (i.e., 10, 50 and 100 nM) were analyzed using QCM-aptasensor. Each value of thrombin concentration analyzed in this test corresponds to the average obtained from 5 repeated studies. The QCM-aptasensor showed average deviation between 2.4 and 3.7%. These results showed that the signal response for the same concentration of thrombin can be recovered with an insignificant

error. The response of the QCM-aptasensor was highly sensitive for serum spiked with thrombin.

These QCM-aptasensor data were correlated by using thrombin assay in 10-fold diluted serum, The serum was spiked with three different concentration of thrombin (i.e., 10, 50 and 100 nM) and assayed using T3068 thrombin artificial substrate, (i.e., β-Ala-Gly-Arg-*p*-nitroanilide diacetate) by incubating 2.0 h at 37 ◦C. The amount of human thrombin in the 10-diluted serum was determined at A_{405} nm. A calibration curve was obtained by the measurement of thrombin at various concentrations. The human serum is obtained after coagulation of blood and the serum does not include any coagulation proteins. Thus, the external addition of thrombin does not influence the thrombin spiked serum [40]. The result obtained by thrombin assay method has a lower SD value than that of 3.9%. The detection of thrombin using artificial T3068 substrate was in the range of 102.4-103.6%, indicating that the accuracy and precision of the presented QCM-aptasensor method was sensible. The amount of detected thrombin was same using thrombin assay method as in QCM-aptasensor. The detection limit of some thrombin binding aptamer biosensors was compared for detection of thrombin with their linear range (Table 2). Noticeably, the QCM-aptasensor can be utilized for detection of thrombin in the biological fluids with conjunction magnetic pre-concentration system. The QCM-aptasensor has a wide linear range and sensitive, and that is a significant advantage of the presented work.

The stability of the QCM-aptasensor was studied at room temperature at 30-day intervals. The Δm data is obtained for the 20 cycles (data not shown). The response of the QCM-aptasensor for each run was similar. It should be noted that the QCM aptasensor response Δm is linearly associated at with same concentration of thrombin (i.e., 100 nM spiked thrombin in 10-fold diluted). During the repeated use period, there was no significant

change in the aptasensor responses. Thus, the QCM-aptasensor offered good shelf-life stability for 60 days at room temperature.

4. Conclusions

The methods available for thrombin detection in serum are very complicated, and time-consuming. Hence, a novel QCM-aptasensors were constructed for label-free detection of thrombin in solution and diluted serum samples. In addition, an aptamer-magnetic isolation system was designed and integrated to the QCM aptamer-sensor. Thrombin binding aptamers were effectively incorporated on the surface of the L-cysteine modified QCM chip. These results showed that Mp(HEMA-EGDMA-VC)-TBA microbeads have high reusability during isolation and operation of QCM-aptasensor. The constructed QCM-aptasensor for thrombin detection has resulted a good analytical performance in terms of lower detection limit (1.0 nmol L^{-1}), a wider linear range (1.0–100.0 nmol L^{-1}) and QCM-aptasensor does not lost its initial capture capacity for thrombin when stored at room temperature for 60 days. These results showed that the cyclic carbonate groups of Mp(HEMA-EGDMA-VC) microbeads can be functionalized with different ligands and can be used as a potential magnetic support in separation and pre-concentration of mark out proteins from biological fluids.

References

[1] Y. Liu, H. Wang, S. Li, H. Gong, C. Chen, X. Chen, C. Cai, Ultrasensitive detection of thrombin based on MoS2-aptamer biosensors by resonance light scattering technique, Sens. Actuators B 258 (2018) 402–407.

[2] R. Yang, S. Liu, Z. Wu, Y. Tan, S. Sun, Core-shell assay based aptasensor for sensitive and selective thrombin detection using dark-field microscopy. Talanta 182 (2018) 348–353.

[3] Z. Chen, M. Sun, F. Luo, K. Xu, Z. Lin, L. Zhang, Stimulus-response click chemistry based aptamer-functionalized mesoporous silica nanoparticles for fluorescence detection of thrombin, Talanta 178 (2018) 563–568.

[4] N. Jo, B. Kim, S.-M. Lee, J. Oh, I. H. Park, K. J. Lim, J.-S. Shin, K.-H. Yoo, Aptamer-functionalized capacitance sensors for real-time monitoring of bacterial growth and antibiotic susceptibility, Biosens. Bioelectron. 102 (2018) 164–170.

[5] M. Liu, J. Li, B. Li, A colorimetric aptamer biosensor based on cationic polythiophene derivative as peroxidase mimetics for the ultrasensitive detection of thrombin, Talanta 175 (2017) 224–228.

[6] L. Kuang, S.-P. Cao, L. Zhang, Q.-H. Li, Z.-C. Liu, R.-P. Liang, J.-D. Qiu, A novel nanosensor composed of aptamer bio-dots and gold nanoparticles for determination of thrombin with multiple signals, Biosens. Bioelectron. 85 (2016) 798–806.

[7] S. Centi, G. Messina, S. Tombelli, I. Palchetti, M. Mascini, Different approaches for the detection of thrombin by an electrochemical aptamer-based assay coupled to magnetic beads. Biosens. Bioelectron. 23 (2008) 1602–1609.

[8] T. Hianik, V. Ostatna, M. Sonlajtnerova, I. Grman, Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin, Bio electrochemistry 70 (2007) 127–133.

[9] Y. Wang, X. He, K. Wang, X. Ni, J. Su, Z. Chen, Electrochemical detection of thrombin based on aptamer and ferrocenylhexanethiol loaded silica nanocapsules, Biosens. Bioelectron. 26 (2011) 3536–3541.

[10] Y. Tao, Y. Jiang, W. Li, B. Cai, Rapid magnetic solid-phase extraction combined with ultra-high performance liquid chromatography and quadrupole-time-of-flight mass spectrometry for analysis of thrombin binders from a crude extract and injection of Erigeron breviscapus, RSC Adv., 2016, 6, 34782.

[11] C Tuerk, L Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505-510.

[12] C.M. Collins, S. Yui, C.E.S. Roberts, I.Kojic, Thrombin detection using a piezoelectric aptamer-linked immune-sorbent assay, Anal. Biochem. 443 (2013) 97–103.

[13] T., Mairal, V.C. Ozalp, P. L. Sanchez, M. Mir, I. Katakis, C.K. O'Sullivan, Aptamers: molecular tools for analytical applications, Anal. Bioanal. Chem. 390 (2008) 989–1007.

[14] V.C. Ozalp, G. Bayramoglu, Z. Erdem, M.Y. Arica, Pathogen detection in complex samples by quartz crystal microbalance sensor coupled to aptamer functionalized core-shell type magnetic separation. Analytica Chimica Acta. 853 (2015) 533-540.

[15] Z. Wang, J.-C. Zhao, H.-Z. Lian, Hong-Y, Chen Aptamer-based organic-silica hybrid affinity monolith prepared via “thiol-ene” click reaction for extraction of thrombin Talanta, 138 (2015) 52–58.

[16] X. Yu, F. Chen, R. Wang, Y. Li, Whole-bacterium SELEX of DNA aptamers for rapid detection of *E.coli* O157:H7 using a QCM sensor. J. Biotechnol. 266 (2018) 39–49.

[17] B. Chen, Q. Ye, K. Zhou, Y. Wang Adsorption and separation of HCV particles by novel affinityaptamer-functionalized adsorbents, J. Chromatogr. B 1017–1018 (2016) 174–181.

[18] B. Jin, S. Wang, M. Lin, Y. Jin, S. Zhang, X. Cuib, Y. Gong, A. Li, F. Xu , T. J. Lu, Upconversion nanoparticles based FRET aptasensor for rapid and ultrasenstive bacteria detection, Biosens. Bioelectron. 90 (2017) 525–533.

[19] F. Ahour, M.K. Ahsani, An electrochemical label-free and sensitive thrombin aptasensor based on graphene oxide modified pencil graphite electrode, Biosens. Bioelectron. 86 (2016) 764–769.

[20] B.A. Borsa, B.G. Tuna, F.J. Hernandez, L.I. Hernandez, G. Bayramoglu , M.Y. Arica, V.C. Ozalp, *Staphylococcus aureus* detection in blood samples by silica nanoparticle-oligonucleotides conjugates. Biosens. Bioelectron. 86 (2016) 27–32.

[21] M. Kavruk, O. Celikbicak, V. C. Ozalp, B.A. Borsa, F.J. Hernandez, G. Bayramoglu, B. Salih, M.Y. Arica, Antibiotic loaded nanocapsules functionalized with aptamer gates for targeted destruction of pathogens. Chem. Commun. 51 (2015) 8492.

[22] Q. Luan, X. Xiong, N. Gan, Y. Cao, T. Li, D. Wu, Y. Dong, F. Hu. A multiple signal amplified colorimetric aptasensor for antibiotics measurement using DNAzyme labeled Fe-MIL-88-Pt as novel peroxidase mimic tags and CSDP target-triggered cycles, Talanta 187 (2018) 27–34.

[23] C. Forier, E. Boschetti, M. Ouhammouch, A. Cibie, F. Duconge, M. Nogre, M. Tellier, D. Bataille, N. Bihoreau, P. Santambien, S. Chtouroua, G. Perret, DNA aptamer affinity ligands for highly selective purification of human plasma-related proteins from multiple sources. J. Chromatogr. A, 1489 (2017) 39–50.

[24] G. Bayramoglu, V.C. Ozalp, U. Dincbal, M.Y. Arica, Fast and sensitive detection of *Salmonella* in milk samples using aptamer-functionalized magnetic silica solid phase and MCM-41-aptamer gate system, ACS Biomater. Sci. Eng. 4 (2018) 1437−1444.

[25] Y. Liu, X. Tang, F. Liu, K. Li. Selection of ligands for affinity chromatography using quartz crystal biosensor, Anal. Chem. 77 **(**2005) 4248-4256.

[26] G. Bayramoglu, M.Y. Arica, Development of a sensitive method for selection of affinity ligand for trypsin using quartz crystal microbalance sensor. Bioprocess Biosyst. Eng. 35 (2012.) 423–431.

[27] T. Ramon-Marquez, A.L. Medina-Castillo, A. Fernandez-Gutierrez, J.F. Fernandez-Sanchez, Evaluation of two sterically directed attachments of biomolecules on a coaxial nanofibre membrane to improve the development of optical biosensors, Talanta 187 (2018) 83–90.

[28] T. Ramon-Marquez, A.L. Medina-Castillo, N. Nagiah, A. Fernandez-Gutierrez, J. F. Fernandez-Sanchez, A multifunctional material based on co-electrospinning for developing biosensors with optical oxygen transduction, Anal. Chim. Acta 1015 (2018) 66-73.

[29] T.-Y. Lin, C.-H. Hu, T.-C. Chou, Determination of albumin concentration by MIP-QCM sensor, Biosens. Bioelectron. 20 (2004) 75-81.

[30] G. Bayramoglu, V. C. Ozalp, M. Yilmaz, U. Guler, B. Salih , M.Y. Arica, Lysozyme specific aptamer immobilized MCM-41 silicate for single-step purification and quartz crystal microbalance (QCM)-based determination of lysozyme from chicken egg white, Micropor. Mesopor. Mater. 207 (2015) 95-104.

[31] T. Nezu, M. Taira, S. Saitoh, K. Sasaki, Y. Araki, Viscoelastic adlayers of collagen and lysozyme studied using quartz crystal microbalance with dissipation monitoring, Int. J. Biol. Macromol. 46 (2010) 396-403.

[32] G.Z. Sauerbrey, The use of quartz oscillators for weighing thin layers and for micro-weighing, Z. Phys. 155 (1959) 206–222.

[33] X. Yu, F. Chend, R. Wang, Y. Li, Whole-bacterium SELEX of DNA aptamers for rapid detection of *E.coli* O157:H7 using a QCM sensor. J. Biotechnol. 266 (2018) 39–49.

[33] G. Bayramoglu, A. Akbulut, M.Y. Arica, Removal of metal complexed azo dyes fromaqueous solution using tris(2-aminoethyl) amine ligand modified magnetic p(GMA-EGDMA) cationic resin: Adsorption, isotherm and kinetic studies. Chem. Eng. Res. Des. 124 (2017) 85–97.

[34] G. Bayramoglu, M.Y. Arica, Enzymatic removal of phenol and p-chlorophenol in enzyme reactor: horseradish peroxidase immobilized on magnetic beads, J. Hazard. Mater. 156 (2008) 148-155.

[35] S. Brunrunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers. J. Amer. Chem. Soc. 60 (1938) 309-315.

[36] G. Bayramoglu, B. Karagoz, M.Y. Arica, Cyclic-carbonate functionalized polymer brushes on polymeric microspheres: Immobilized laccase for degradation of endocrine disturbing compounds, J. Ind. Eng. Chem. 60 (2018) 407–417.

[37] G. Bayramoglu, M.Y. Arica, O. Celikbicak, B. Salih, Removal of bisphenol A from aqueous medium using molecularly surface imprinted microbeads. Chemosphere 150 (2016) 275-284.

[38] G. Bayramoglu, E. Yavuz, B. F. Senkal, M. Y. Arica, Glycidyl methacrylate grafted on p(VBC) beads by SI-ATRP technique: Modified with hydrazine as a salt resistance ligand for adsorption of invertase, Colloid. Surf. A: Physicochem. Eng. Aspects. 2009, 345, 127–134.

[39] Y.-S. Chen, C.-S. Chang, S.-Y. Suen, Protein adsorption separation using glass fiber membranes modified with short-chain organosilicon derivatives, J. Memb. Sci. 2007, 305, 125–135.

[40] A. Bini, M. Minunni, S. Tombelli, S. Centi, M. Mascini, Analytical performances of aptamer-based sensing for thrombin detection, Anal. Chem. 2007, 79, 3016-3019.

[41] H. Chen, F. Yuan, S. Wang, J. Xu, Y. Y. Zhang, L. Wang Aptamer-based sensing for thrombin in red region via fluorescence resonant energy transfer between NaYF4:Yb,Er up conversion nanoparticles and gold nanorods, Biosens. Bioelectron. 2013, 48, 19–25.

[42] F. Chen, Y. Liu, R. Liao, H. Gong, C. Chen, X. Chen, C. Cai, 4- Reduced graphene oxide as a resonance light-scattering probe for thrombin detection using dual-aptamer-based dsDNA Anal. Chim. Acta 2017, 985, 141-147.

[43] Y. Wen, C. Peng, D. Li, L. Zhuo, S. He, L. Wang, Q. Huang, Q.-H. Xu, C. Fan, Metal ion-modulated graphene-DNAzyme interactions: design of a nanoprobe for fluorescent detection of lead(II) ions with high sensitivity, selectivity and tunable dynamic range, Chem. Commun., 2011, 47, 6278–6280.

Figure 1. Preparation of thrombin binding aptamer-immobilized Mp(HEMA-EGDMA-VC) microbeads. The microbeads were used for specific capture of thrombin from diluted serum samples.

Figure 2. The XRD patterns (A) γFe_3O_4 and (B) Mp(HEMA-EGDMA-VC) microbeads

Figure 3. (A) Frequency change upon immobilization of thrombin binding aptamer on functionalized QCM chip. (B) Frequency change at different Aptamer-thrombin concentrations.

Figure 4. Thrombin at different concentrations was injected on the QCM-aptasensor chip to obtain the linear part of the thrombin-aptamer interaction.

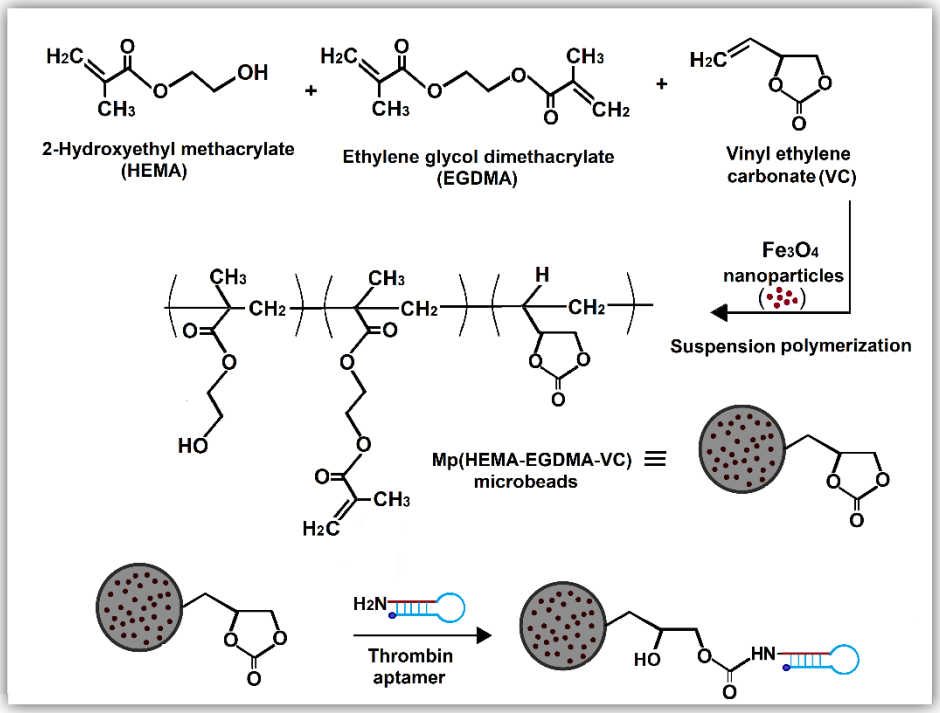
Figure 5. Specificity (n=3) of thrombin binding aptamer for thrombin. The concentrations of HSA, elastin and IgG were 100 nmol L^{-1}. The possible interferences were tested in 10-fold diluted serum or fibrinogen-precipitated plasma samples with QCM-aptasensor for detecting thrombin, elastin, IgG and HSA. Samples were first spiked in diluted serum or fibrinogen-precipitated plasma and magnetically isolated from sample mixture and the eluted isolates were directly fed into QCM sensor chip.

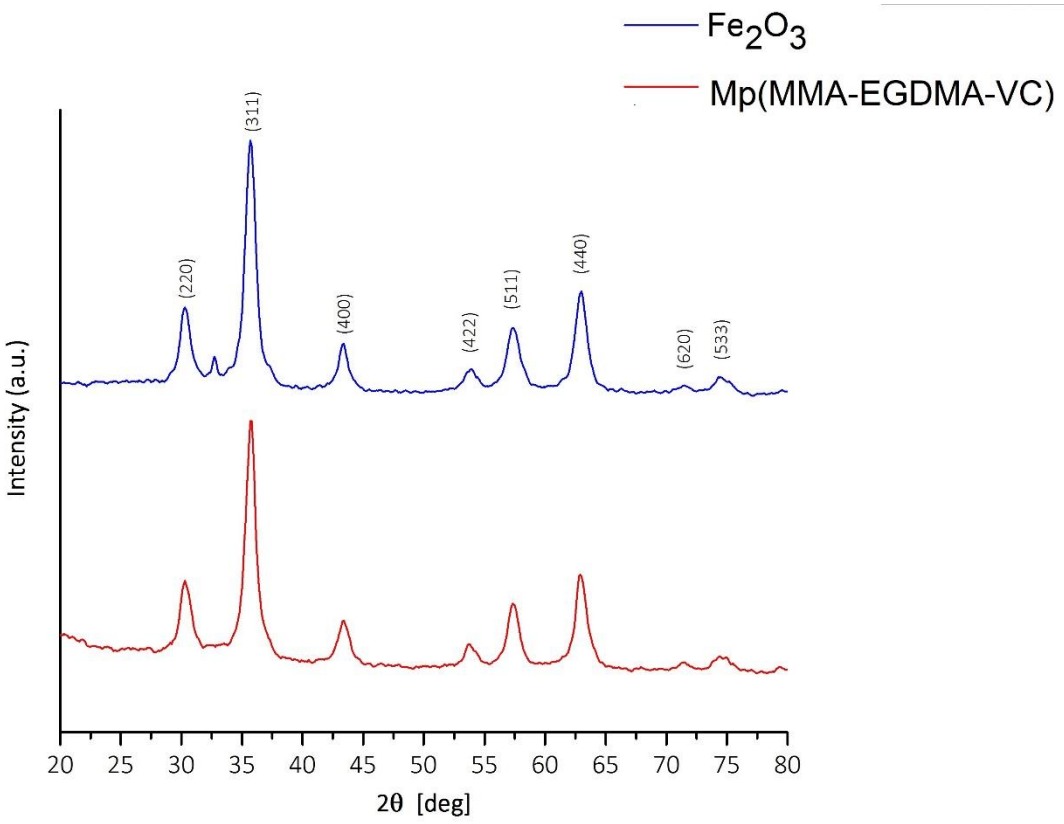
Table 1. Isotherm models constants and correlation coefficients for thrombin from aqueous solutions on the QCM-aptasensor chips.

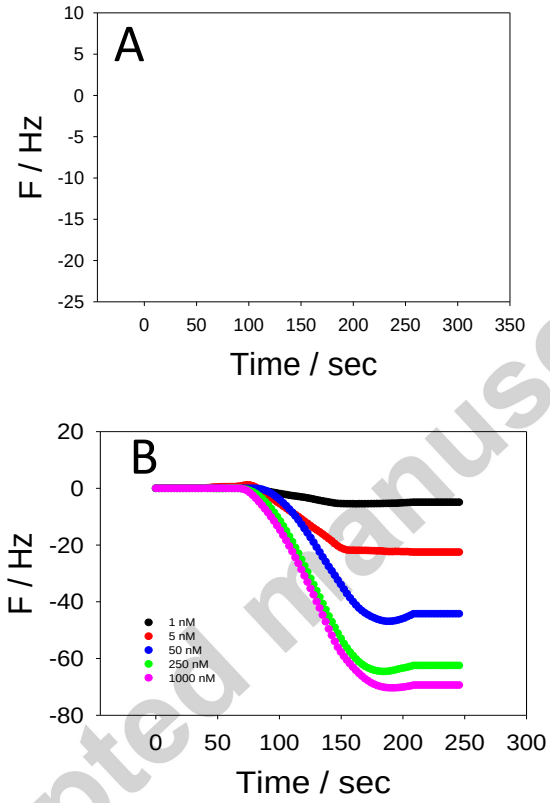
Exp	Scatchard plot			Langmuir constant			Freundlich constant		
ΔF_m (Hz)	ΔF_{max} (Hz)	K_d (nmol L^{-1})	R^2	ΔF_{max} (Hz)	K_d (nmol L^{-1})	R^2	n	K_F	R^2
64.3	73.8	62.5	0.789	74.0	68.5	0.992	1.46	1.52	0.989

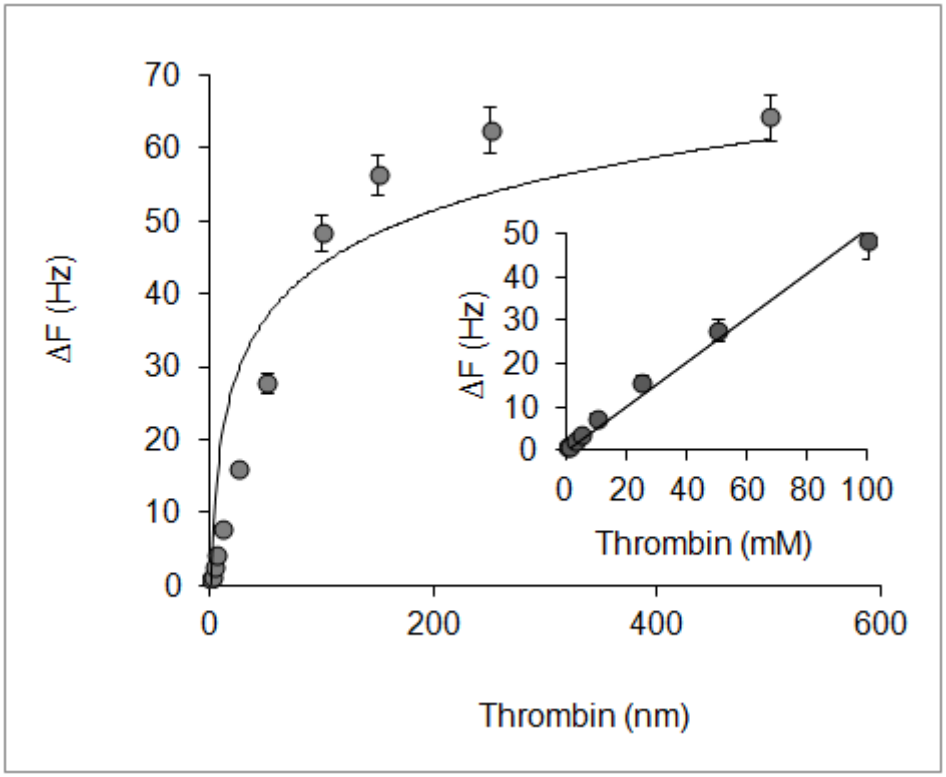
Table 2. Comparison of aptamer utilized biosensors reported for thrombin detection.

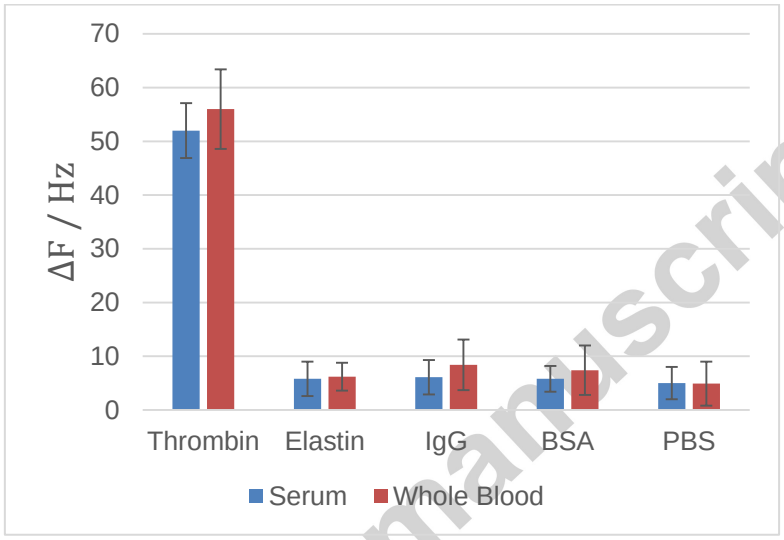
Sensors	Linear range	Detection limit	Refs.
Fluorescence	2.5–90 nmol L^{-1}	1.5 nmol L^{-1}	41
Resonance light scattering	10-500 pmol L^{-1} and 1–60 nmol L^{-1}	0.71 pmol L^{-1}	1
Chehemiluminescence	2.5×10^{-10} to 1×10^{-9} mol L^{-1}	8.3×10^{-11} mol·L^{-1}	29
Resonance light-scattering	0.5-75 nmol L^{-1}	8.60 nmol L^{-1}	42
Electrochemistry	3.0-80.0 nmol L^{-1}	3.0 nmol L^{-1}	43
Resonance light-scattering	0–35 nmol L^{-1}	1.05 nmol L^{-1}	6
QCM-aptasensor	50-200 nmol L^{-1}	50 nmol L^{-1}	40
QCM-aptasensor	1.0-100 nmol L^{-1}	1.0 nmol L^{-1}	In this work




Fe2O3
Mp(MMA-EGDMA-VC)
(220)
(311)
(400)
(422)
(511)
(440)
(620)
(533)
Intensity (a.u.)
20 25 30 35 40 45 50 55 60 65 70 75 80
2θ [deg]




70
60
50
40
30
20
10
0
ΔF (Hz)
0
200
400
600
Thrombin (nm)
50
40
30
20
10
0
ΔF (Hz)
0
20
40
60
80
100
Thrombin (mM)



Highlights

- A QCM-aptasensor is constructed for the determination of thrombin from serum.
- The aptamers on magnetic beads and QCM crystal chips has high specificity to thrombin.
- Thrombin detection limit of the QCM-aptasensor was 1.0 nM.
- The combination of aptasensor with pre-concentration offered a sensitive detection of thrombin.
- The binding of thrombin to QCM-aptasensor was well described Langmuir model.