Biosensors and Bioelectronics 141 (2019) 111467

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Designing and fabrication of new VIP biosensor for the rapid and selective detection of foot-and-mouth disease virus (FMDV)

Heba A. Hussein[a,c,1], Rabeay Y.A. Hassan[a,b,1], Rasha Mohamed El Nashar[d,**], Samy A. Khalil[e], Sayed A. Salem[c], Ibrahim M. El-Sherbiny[a,*]

[a] Nanomaterials Laboratory, Center for Materials Science, Zewail City of Science and Technology, 6th October City, 12578, Giza, Egypt
[b] Applied Organic Chemistry Department, National Research Centre (NRC), Dokki, 12622, Giza, Egypt
[c] Virology Department, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Egypt
[d] Chemistry Department, Faculty of Science, Cairo University, Giza, 12613, Egypt
[e] Microbiology Department, Faculty of Veterinary Medicine, Alexandria University, Egypt

ARTICLE INFO

Keywords:
Foot and mouth disease virus (FMDV)
Virus imprinted polymers (VIPs)
Screen printed electrode (SPE)
Biomimetic virus biosensors

ABSTRACT

Foot and mouth disease virus (FMDV), is a highly contagious virus due to its ease of transmission. FMDV has seven genetically distinguished serotypes with many subtypes within each serotype. The traditional diagnostic methods of FMDV have demonstrated many drawbacks related to sensitivity, specificity, and cross-reactivity. In the current study, a new viral imprinted polymer (VIP)-based biosensor was designed and fabricated for the rapid and selective detection of the FMDV. The bio-recognition components were formed via electrochemical polymerization of the oxidized O-aminophenol (*O*-AP) film imprinted with FMDV serotype O on a gold screen-printed electrode (SPE). The overall changes in the design template have been investigated using cyclic voltammetry (CV), atomic force microscopy (AFM), Field emission-scanning electron microscopy (FE-SEM), and Fourier-transform infrared spectroscopy (FT-IR). Optimal conditions were achieved through investigating the capturing efficiency, binding stability, selectivity and life-time of the developed biosensor. The results depicted a high selectivity of the biosensor to the serotype O over all other genus serotypes A, SAT2 and Lumpy skin disease virus (LSDV), as well as, the inactivated serotype O. The limits of detection (LOD) and quantification (LOQ) were around 2 ng/mL and 6 ng/mL, respectively, in addition to the tested repeatability and reproducibility values with a variance coefficient of 1.0% and 3.6%, respectively. In comparison with the reference methods (ELISA and PCR), the analysis of saliva real samples using the developed affordable biosensor offered 50 folds lower LOD with the possibility of an on-line monitoring in the field with no prior sample treatment.

1. Introduction

Foot and mouth disease virus (FMDV), is a major biological whip that threatens the livestock in many countries all over the world. FMDV has seven genetically distinct serotypes, known as serotype O, A, C, Asia1 and Southern Africa Territories (SAT1, SAT2 and SAT3) with a wide antigenic diversity within each serotype (Longjam et al., 2011). Serotype O is the most prevalent one and spreads quickly worldwide, especially in Egypt, where it has been accused in many outbreaks of diseases since 1960s (Aidaros, 2002; Reid et al., 2000). Thus, the early detection of the virus, especially serotype O, in clinical samples is urgently needed due to its fast-infectious rate leading to high mortality rates in infected animals. Traditional diagnosis of FMDV is based on the virus isolation on tissue culture system, especially on baby hamster kidney-21 (BHK-21) cell lines, followed by the viral antigen identification using enzyme linked immuno-sorbent assay (ELISA) (Amaral-Doel et al., 1993; Reid et al., 2002). Molecular identification is also performed using thermal amplification by reverse transcriptase-polymerase chain reaction (RT-PCR) (Callens and De Clercq, 1997; King et al., 2006b; Vangrysperre and De Clercq, 1996) and Real-time PCR (Chen et al., 2011; Moniwa et al., 2007; Rasmussen et al., 2003; Reid et al., 2000), which have been utilized as confirmatory tools for accurate diagnosis of FMDV infection. However, these currently used techniques require complicated laboratory facilities, besides being time consuming as they require time that ranges from 4 h for ELISA to 24 h upon using PCR and reaching 72 h in the case of performing tissue

* Corresponding author. Nanomaterials & Nanomedicine Zewail City for Science and Technology, 6th of October City, Giza, Egypt.
** Corresponding author.
E-mail addresses: rashaelnashar@gmail.com (R.M. El Nashar), ielsherbiny@zewailcity.edu.eg (I.M. El-Sherbiny).
[1] Both authors contributed equally.

https://doi.org/10.1016/j.bios.2019.111467
Received 8 April 2019; Received in revised form 28 May 2019; Accepted 21 June 2019
Available online 25 June 2019
0956-5663/

culture. Therefore, the early, rapid and accurate diagnosis of the disease is a corner stone for control of the virus.

Virus biosensors represent an alternative method for rapid response, highly sensitive and selective quantitative diagnosis of viruses (Xu et al., 2012). These biosensors have biological sensing elements either for the whole virus particles or for specific epitopes and viral proteins in addition to a signal transducer and amplification component (Gramsbergen et al., 2003; Vestergaard et al., 2007). The bio-recognition components mainly have a bio-affinity role which should confer a sensitive and selective binding to the target analytes (Xu et al., 2012).

Molecularly imprinted polymers (MIPs) were found to be very promising candidates in various biosensing applications. This was attributed to their capability to enhance the accuracy, sensitivity and selectivity towards different biological analytes (Blanco-López et al., 2004; Mayes and Whitcombe, 2005; Moczko et al., 2013). MIPs involve the creation of artificial recognition binding sites in crosslinked polymeric matrices via either covalent or non-covalent interactions between the functional monomers and the active sites of the template (Mayes and Whitcombe, 2005). These specific binding sites have a mimicry property of the “key and lock” of the template included in the shape and size after its removal. MIPs were incorporated in different immunoassay applications such as enzyme-linked immuno-sorbent assay represented by synthesis of a nano-MIP of vancomycin, and the prepared MIPs were then deposited onto the microtitre plate for detection of vancomycin in porcine plasma (Chianella et al., 2013).

Viral imprinted polymers (VIPs) involve the preparation of a synthetic polymeric matrix entrapping the virus to produce specific binding sites for the template (virus) (Bolisay et al., 2006; Sangma et al., 2017; Yang et al. 2013, 2017). Recently, VIPs were used for the electrochemical detection of Zika virus using on-surface imprinted polymers and graphene oxide composites (Tancharoen et al., 2019) (Tancharoen et al., 2019). VIPs were also used for detection of human adenovirus (AdV) selected as a model virus facilitating the development and application of a rapid virus quantification (Gast et al., 2018) (Gast et al., 2018), as well as, other viruses such as human influenza, Dengue virus, Japanese encephalitis virus and human immuno-deficiency virus to facilitate their detection (Malik et al., 2017).

Many materials were reported in literature for the electrochemical deposition, including conductive and nonconductive polymers such as pyrrole, anilines, aminophenols and thiophenols. *O*-aminophenol (*O*-AP), for instance, has been widely utilized in many biosensing applications, where it can be polymerized electrochemically in various media; acidic, neutral and alkaline. For example, *O*-AP was electrochemically polymerized on gold electrodes for sensing of nicotine as a template and the resulting layer was arranged smoothly and simply (Wu et al., 2006). In another study, the electropolymerization of *O*-AP was carried out in the presence of acetaminophen as a template, subsequently after deposition of gold nanoparticles on the surface of a gold electrode (Menon et al., 2018).

This work aims at presenting new bio-recognition components formed by the electrochemical polymerization of the oxidized *O*-AP to form polymeric film (POAP) imprinted with FMDV serotype O on gold screen-printed electrodes for the construction of the VIP biosensors. The overall changes on the electrodes have been investigated using cyclic voltammetry (CV) and linear sweep voltammetry (LSV) with setting of the analytical sensitivity or limits of detection (LOD) and quantification (LOQ).

2. Material and methods

2.1. Reagents, chemicals and biological materials

O-aminophenol (*O*-AP), polyethylene glycol-6000 (PEG-6000), tetrahydrofuran (THF, ACS grade, 99%), triton and PBS tablets were purchased from Sigma-Aldrich (USA). Anhydrous citric acid was provided by ADWIC Co., Egypt. Dialysis sacks (average flat width 35 mm, MWCO 12.000 Da) were obtained from Sigma-Aldrich (USA). ELISA commercial kit (IZSLER, Pirbright, UK), Patho-Gene spin for RNA extraction (INTRON Biotechnology, Korea), Master Mix, dtec RT-qPCR (Genetic PCR solutions, Spain) were used. Baby hamster kidney cell line (BHK_{21}) was obtained from Pirbright (Italy), and modified minimum essential medium with Earle's salts was purchased from Sigma-Aldrich (USA). FMDV serotype O was kindly provided by Foot and Mouth disease department, Vaccine and Serum Research Institute (VSRI), Egypt, while FMDV serotype A, SAT-2 and Lumpy skin disease virus (LSDV) were obtained from virology department, Animal Health Research Institute (AHRI), Egypt.

2.2. Equipment

All electrochemical measurements were performed at room temperature using a computer-controlled Gamry Potentiostat/Galvanostat/ZRA G750. The electrochemical experiments were carried out in a home-made electrochemical cell with a total volume of 4 mL. DropSens three electrode type screen printed cable was used for connecting the 3-electrode systems consisting of Au as a working and counter electrode with Ag/AgCl as a reference electrode. Atomic force microscopy (AFM) with autoprobe cp-research head (Thermomicroscope, Sunnyvale, California, USA) was used for characterization, and was operated in a non-contact mode using antimony (n) doped Si probe (Bruker, FESP, USA) with the aid of proscan 1.8 software for controlling the scan parameters along with IP 2.1 software for image analysis. Field emission-scanning electron Microscope (FE-SEM) (Quanta FEG250, Czech). Fourier-transform infrared spectroscopy (FT-IR) (Thermo-Fisher Scientific, Nicolet is10, USA). Ultra-cooling centrifuge (Centurion Scientific, K3 Series, UK), ultra-low temperature freezer of −86 °C (Operon Co., Korea), applied biosystems Verti thermal cycler, and step one thermo cycler (Thermo Fisher Scientific, Germany) were used.

2.3. Virus propagation

FMDV serotype O was propagated in BHK-21 cell line with modified minimum essential medium with Earle's salts which was supplied by 1–2% fetal calf serum at 37 °C in 5% CO_2 incubator for 18–24 h. The produced virus was purified and concentrated by centrifugation at 8000 rpm in a cooling centrifuge for 15 min, then concentrated via dialysis and PEG-6000 for 18 h at 4 °C. The virus stock was stored at −80 °C and the titer of the virus was expressed in $\log_{10}$ $TCID_{50}$ (Ramakrishnan, 2016).

2.4. Fabrication of the VIP biosensor

The gold electrodes were washed with THF for 5 min, followed by soaking in 5 mL of a triton solution (1%), rinsed by distilled water and PBS, and then left to dry until use in electrochemical polymerization.

Prior to the imprinting of FMDV serotype O on the gold chip (with the active surface area of 3 mm), 75 µL of FMDV particles suspension (187.5 ng/mL) were mixed with 75 µL of *O*-AP solution (5 mg/mL) and completed to 15 mL using PBS. Then, electropolymerization process (40 cycles) was carried out at a potential ranging from −0.4 V to 1.0 V with a scan rate of 50 mV/s. As a control, non-imprinted polymer (NIP) was prepared using similar procedures in absence of the FMDV. The surfaces of the prepared NIPs and VIPs were examined using CV, atomic force microscopy (AFM), Field emission-scanning electron microscopy (FE-SEM), and Fourier-transform infrared spectroscopy (FT-IR).

2.5. Removal of the virus particles from the imprinted matrix

For creating the template imprinted cavities, FMDV particles were removed from the imprinted matrix via soaking the VIP biosensor in a solution of 0.2% citric acid or an equal volume of citric acid and triton at room temperature. Washing in sonicating water bath was also,

tested. Different time intervals (5–25 min) were tried to determine the most effective soaking period in the washing solution.

For testing the efficiency of the created VIP cavities for selective capturing of FMDV particles, VIP vs NIP were incubated for 5 min in different virus concentrations (3.74, 18.64, 37.30, 55.90 and 74.60 ng/mL, respectively). Then, the VIP or NIP was rinsed by Milli-Q water and PBS to flush away any non-captured virus particles avoiding the non-specific binding. Afterwards, the changes in Faraday current of VIP and NIP were considered for measuring the voltammetric signal of each sensor before and after the incubation with the virus particles.

2.6. Virus biosensor selectivity testing

FMDV serotype A (39.19 ng/mL), SAT-2 (33.6 ng/mL), LSDV (113.7 ng/mL) and inactivated FMDV serotype O (inactivation was attained by incubation in 0.2% citric acid for 30 min) were propagated and treated in the virology department, Animal Health Research Institute, Egypt. The washed VIPs were incubated in each of the above-mentioned virus particles (6 µL: 4.94 mL 0.1M PBS) for 5–15 min at room temperature for testing the effect of their interference with the target virus (live FMDV serotype O as a positive control (44.79 ng/mL)).

2.7. Bench application of the fabricated virus biosensor with real samples

The field samples were obtained from real FMDV-infected and suspected animals with clinically appeared lesions from (i) epithelial tongue tissue or ruptured vesicles from the mouth, and (ii) heart tissues as post-mortem lesions. Then, the samples were prepared, under sterile conditions, by grinding the tissue (tongue and heart) with sterile sand and centrifuged with maintaining the medium (with Earle's salts). A combination of antibiotic solutions (100 iu penicillin and 100 µL kanamycin/mL) were added to the collected supernatant for at least 3 h incubation to avoid bacterial contamination of the virus suspension followed by storing the samples at −20 °C till measured. The samples were analyzed without any further treatments at the same previously mentioned sterilization conditions. On the other hand, a known concentration (56 ng/mL) of the virus particles and negatively tested samples were considered as positive and negative controls, respectively.

2.8. Confirmation using ELISA and Real-Time PCR

The samples were tested with ELISA procedures, which were applied for antigen detection and serotyping of FMDV serotypes O, A, C, Asia1 and SAT1-2. The test procedures and the preparation of reagents were performed according to the guiding instructions in the kit. On the other hand, another confirmation was attained using Real-Time PCR where the virus RNA was extracted by using the commercial RNA extraction kits. Thermo-cycling of the extracted RNA was done in a master mix solution for RT-qPCR reaction, and the reaction was run for 40 cycles.

3. Results and discussion

3.1. FMDV imprinting on the screen-printed electrodes (SPE)

Imprinting of FMDV particles on the gold surface of the SPE was performed using the electropolymerization technique. To ensure the formation of a compact and uniform imprinted layer that covers the whole surface, different number of voltammetric cycles were tested. The number of voltammetric cycles varied from 10 to 80 at a constant scan rate (50 mV/s) as given in Fig. 1A.

The capturing efficiency of each prepared electrode was used as the base for the electrode evaluation. A significant enhancement of FMDV capturing was obtained at 40 cycles of electropolymerization of the MIP, as shown in Fig. 1B. Therefore, 40 cycles was selected as the optimum for further investigations.

3.2. FMDV removal from the MIP matrix

The removal of FMDV particles from the developed MIP matrix, for creating specific recognition sites, was performed using either 0.2% citric acid or a combination of citric acid and triton X-100 (1%). The results depicted the powerful effect of citric acid solely to efficiently remove the captured FMDV from the VIP surface as given in Fig. 2A. The efficiency of citric acid alone for the FMDV removal is attributed to its lower pH (3–3.5), which reduces the virus titer by a 4 log range in the first 5 min of the incubation, this acidic pH resulted in the viral capsid dissociation into the pentameric subunits (mainly 12S subunits) (Hong et al., 2015; Sangar et al., 1976). The use of sonication for removal of FMDF particles was avoided as it was found to cause complete damage of the electrode surface, as well as, a complete removal of VIP components, mainly due to the very thin coating formed on the SPE surface.

Besides, the removal time needed for the FMDV was optimized through testing different intervals ranging from 0 to 45 min. In terms of that, the adequate time for the virus removal was achieved at 25 min. As a control, the same conditions were applied on the NIP electrode which showed no change in the current signals over the different time intervals due to the stability of the POAP film towards citric acid. While, in case of VIP electrodes, the faraday current arises after washing due to removal of the virus molecules with citric acid from the polymer matrix creating empty cavities with exposed gold surface for electrochemical interactions in the film resulting in the current increase as given in Fig. 2B.

3.3. Morphological characterization of the VIP

The VIP formation and FMDV knocking out for creating the specific recognition sites were tested using AFM to explore the different morphological changes for the VIP before and after the removal of FMDV (Fig. 3). The figure demonstrates the difference between the smooth lining layer of POAP (NIP) (Fig. 3B) and the roughness of the same layer when it was polymerized with the virus (MIP) (Fig. 3C). As also apparent from the figure, the gaps-like structures were clear after the virus removal by citric acid (Fig. 3D) as compared to the bare electrode which showed only the gold grains morphology (Fig. 3A).

The numerical analysis of the AFM images for the MIPs and their height profiles is given in the supplementary information file (Figure SI-1A), and it illustrated a difference in heights ranging from 3 nm to 45 nm. The polymerization of the virus on the lining layer of the POAP in the cluster form, as FMDV serotype O is about 23–25 nm in its diameter therefore the value 23.7 nm is attributed to a single virus particle. On the other hand, the presence of unified cavities in the MIP layer confirms the effectiveness of the removal procedure. Besides, the formation of these cavities in the lining layer of the POAP with different depth dimensions such as 4.03 × 123 nm, 6.1 × 289 nm, 3.17 × 123 nm and 14.23 × 273 nm indicated the high efficiency of the FMDV removal (Supplementary information file, Figure SI–1B).

The field-emission scanning electron microscopy (FE-SEM) images of the topographical morphology of the gold electrode with its different modifications (bare gold, POAP film, viral imprinted layer (VIP), and the washed VIP shown in Supplementary information file, Figure SI–1C. POAP thin film arranged regularly on the electrode surface with an average size 19–60 nm on the polymerized electrode while FMDV appeared arranged on/within the surface of the POAP layer with the average size 20–30 nm and the virus cluster agglomerates in the VIP has an average size 40–50 nm and the holes appear on the surface of the VIP after washing with citric acid represent the binding sites of the template virus and it has average size 16–35 nm.

The spectrum of the POAP shows 2 peaks at 3360 cm^{-1} and 1600 cm^{-1} due to the (N–H) stretching vibration and the axial stretching of the (C═O) functional groups in the POAP. The peak at region 2900 cm^{-1} attributed to (C–H) symmetrical stretch, while the

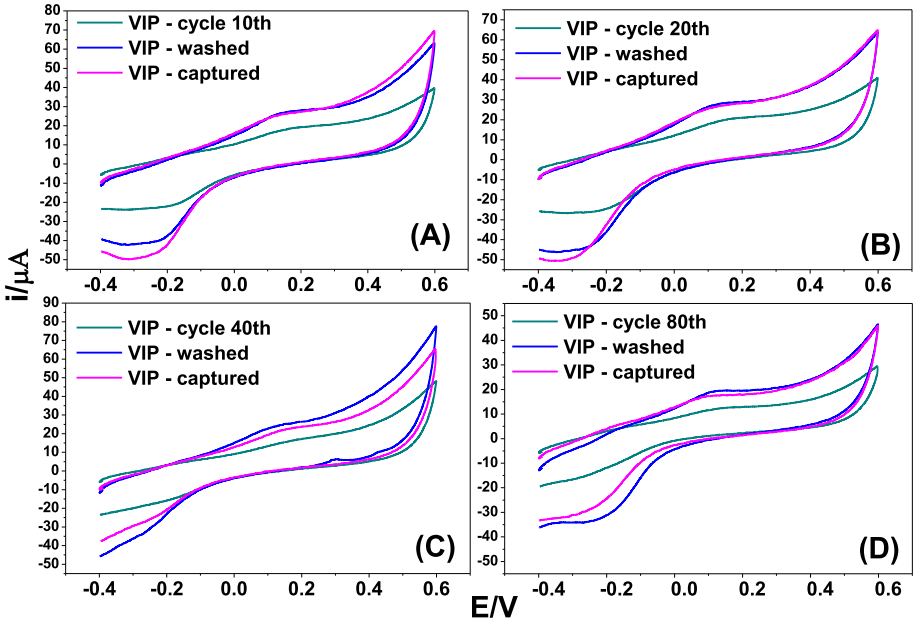

Fig. 1A. Applied number of cycles for the electrochemical polymerization of *O*-AP with FMDV particles (VIP). Voltammograms recorders for (A) 10 cycles, (B) 20 cycles, (C) 40 cycles (ideal) and (D) 80 cycles.

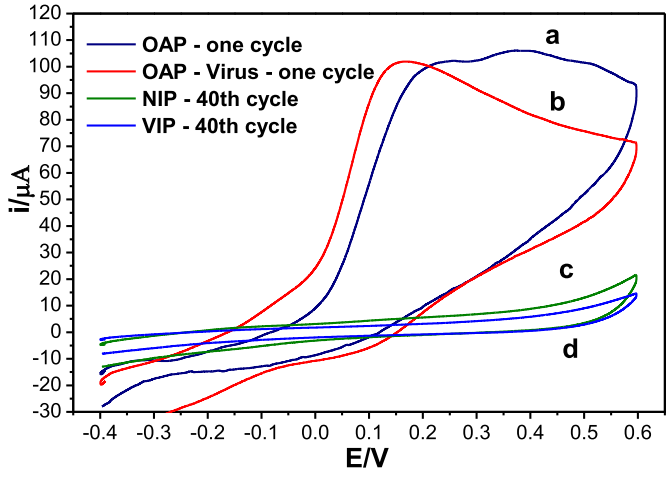

Fig. 1B. Cyclic voltammetry records the declined signal of the proposed electrode from first cycle polymerization (a) NIP and (b) VIP to the complete polymerization (after 40 cycles) (c) NIP and (d) VIP.

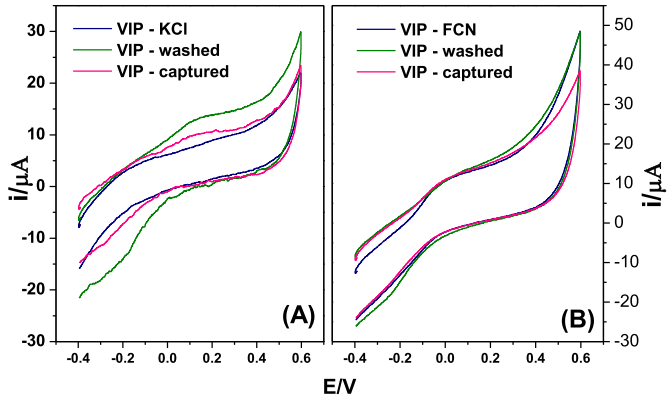

Fig. 2A. The VIP responses in (A) 0.1 M KCl and (B) 1 mM FCN towards the FMDV capturing.

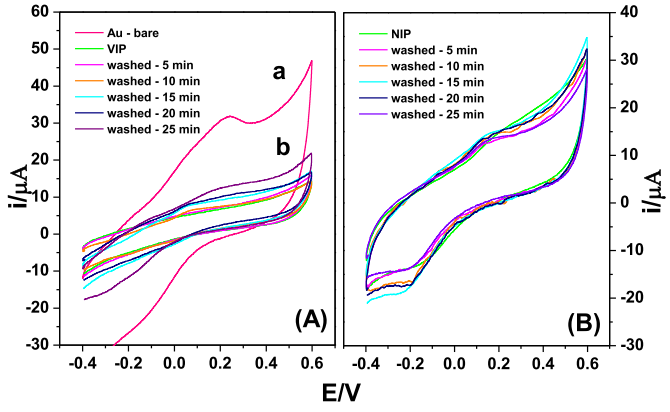

Fig. 2B. The investigated washing intervals using citric acid for the developed (A) VIP and (B) NIP.

peaks 1600-1400 cm-1 due to (C–H) stretching and (C═C) group. The other peak at 1380 cm^{-1} attributed to stretching vibration of secondary aromatic amine group (C–N). The characteristic band at 1100 cm-1 (at the finger print region) is due to (C–O–C) linkage for the changing of the *O*-AP to POAP. The spectrum of the *O*-AP and POAP are the same but they are different in their intensity as it has a low intensity in case of the POAP (NIP) than of the drop casted *O*-AP on the electrode (Ehsani et al., 2012).

3.4. Calibration curve

At optimal conditions, the biosensors limitation (sensitivity and its limit of detection, LOD) were identified with the aid of a calibration curve from the estimated equation (3.3)*(standard deviation of intercept/slope of the calibration curve). A linear progressive relationship was obtained for the tested virus concentrations ranged from 4 ng/mL to 75 ng/mL (R value = 0.990, n = 5 and P = 0.001) with an identified LOD of 1.98 ng/mL, while the limit of quantification (LOQ equally estimated from 10*(standard deviation of intercept/slope of the calibration curve)) found to be 3 ng/mL, as shown in Fig. 4. It is noteworthy to mention that, the LOD value of the developed virus biosensor is 50 fold and 60 fold lower than the antigen detection by ELISA

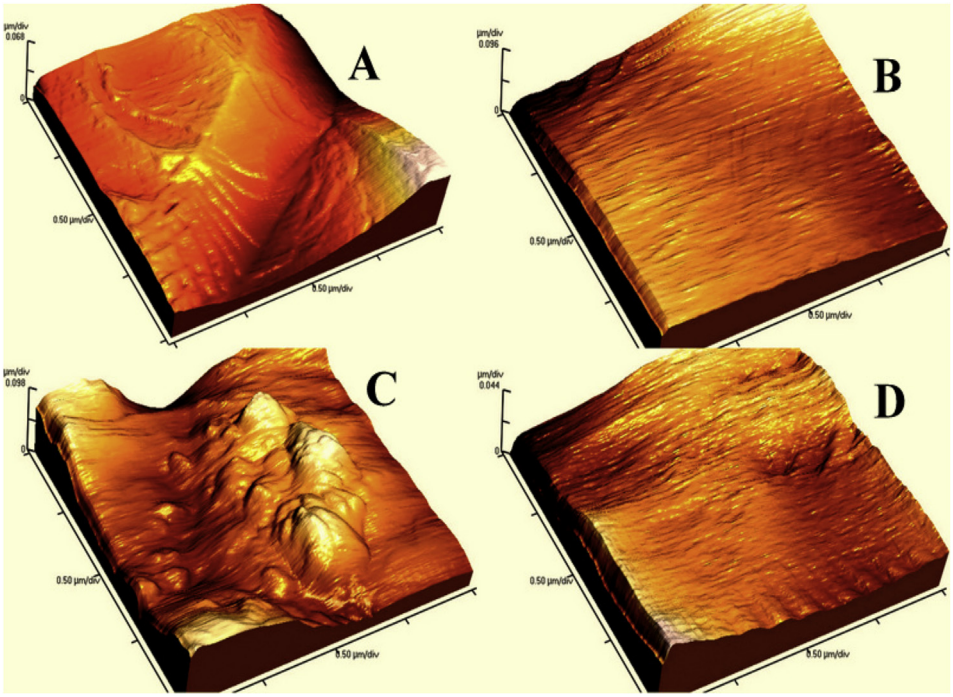

Fig. 3. AFM images (non-contact mode) of (A) bare gold electrode, (B) NIP, (C) VIP and (D) washed VIP.(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

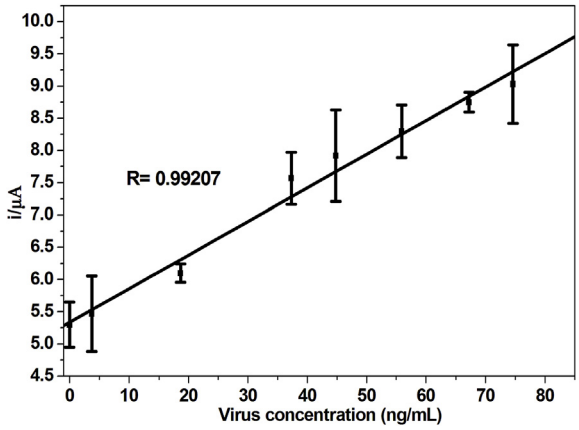

Fig. 4. Calibration curve of different concentrations of FMDV serotype O. The plot records were investigated using LSV in 0.1 M PBS.

treated with gold nanoparticles (LOD = 100 ng/mL) and the ordinary ELISA (Ding et al., 2011), respectively.

3.5. Selectivity testing

The selectivity of the developed electrodes for FMDV serotype O was compared with the cross reactivity of serotypes A, SAT-2 and inactivated serotype O through acid treatment and also with another bovine virus named Lumpy skin disease virus (LSDV). The tested incubation time covered over 5–15 min and the 5 min incubation of the virus suspension with the washed VIP matrix was enough for the saturation of the binding sites as the investigated signals were the same over the 5–15 min.

The resulting data shown in Fig. 5A illustrates that the proposed virus biosensor was selective to FMDV serotype O as the current signal was significant and attained about 9 μA, whereas the signal declines to 3.4 μA when the FMDV serotype A was tested. A sharp decline in the bioelectrochemical signals was also noted in the case of FMDV serotype SAT-2 and the inactivated serotype O where the signals dropped to 1.8 μA and 2.33 μA, respectively.

The electrode had almost no response to the LSDV and that was clear from the obtained response of 1 μA. FMDV serotype A and SAT-2, which belong to the same genus *Aphtho*-virus, reported to have a cross reactivity with serotype O. The produced acid-inactivated virus had an inhibition effect on the current signal with a slight response of 2.5 μA. This value is due to virus capsomers reacting with the hallow gaps which are complimentary to the capsid of the target virus in the polymerized layer. Whereas, there is no interaction for LSDV and this was clear from the produced voltammetric signal. This virus belongs to *poxviridae* family, which includes the largest viruses' size. LSDV is twelve times bigger in size than FMDV. Furthermore, the outer coated part of LSDV is an envelope, brick-shaped with surface arranged in a tubular manner, which differ completely from FMDV morphology which involves a simple arrangement of capsomers in icosahedral pattern.

In order to provide more evidences on the structural analytical selectivity of the fabricated VIP biosensor besides its applications on these viruses' suspension, either a mixture of these viruses in the presence or in absence of the target virus were tested. It was found that the presence of other viruses did not interfere with the selective action of the developed electrode to the target virus, and the mixture (excluding the target virus) showed inhibition values in current from 8 μA to 3.5 μA, respectively. This can be attributed to the slight interference reaction of serotype A and the inactivated serotype O in the case of the mixture that contains the target virus, as the expressed current signal was 8.5 μA which differs from the current signal of the proposed electrode incubated with the target virus. The attained current of 3.5 μA is the same current response of the proposed virus electrode investigated towards serotype A, and this proves that the response value towards the mixture, excluding the target virus, is mainly due to the cross reactivity of serotype A, as given in Fig. 5.

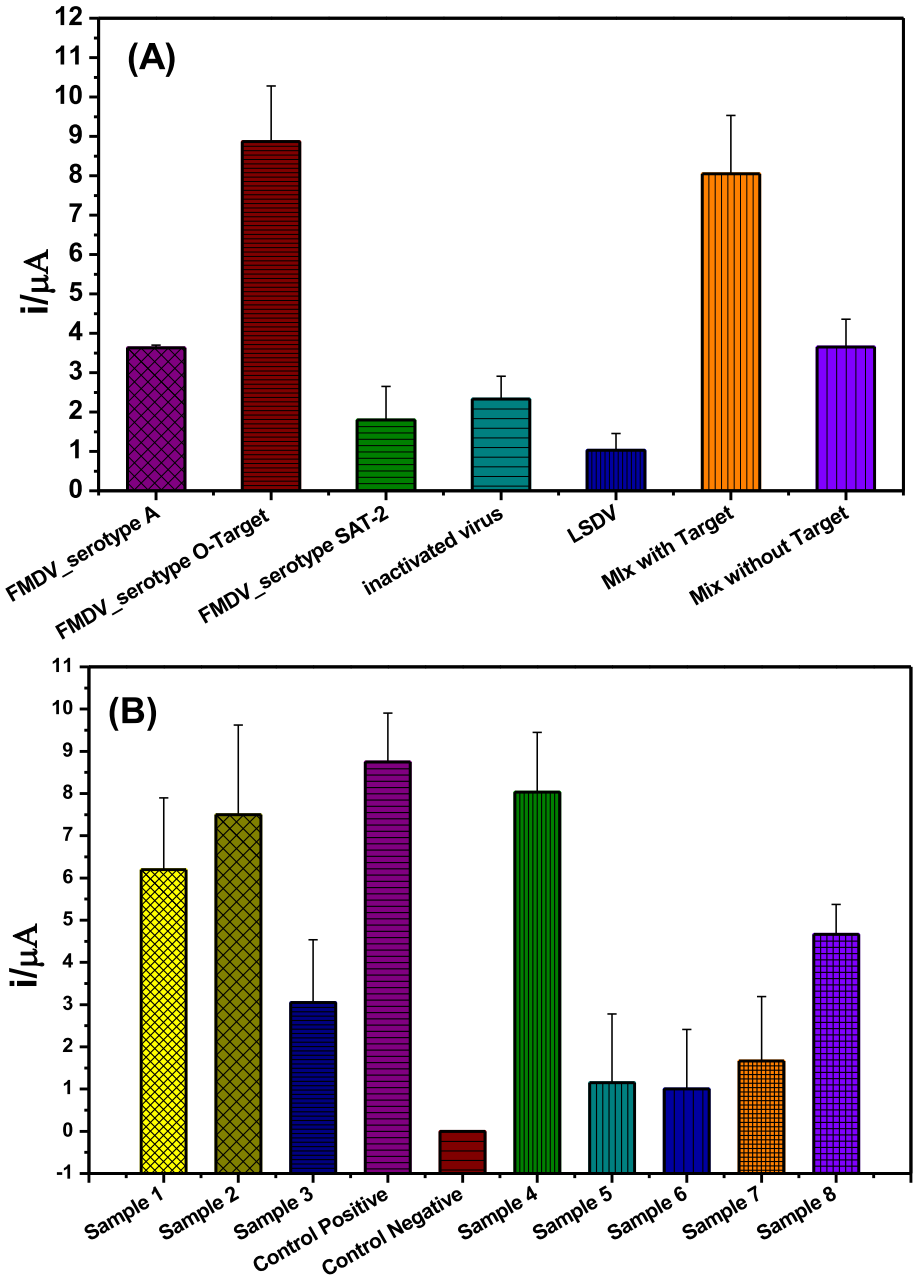

Fig. 5. (A) Selectivity test of the developed VIP biosensor, (B) Application of the VIP biosensor on real field samples, with justification of the control negative and a control positive of a known target virus concentration (56 ng/ml).

3.6. Reproducibility, repeatability, stability and the life span of the fabricated biosensors

The reproducibility of the fabricated virus biosensor has been estimated by testing a known concentration of the virus (9, 37 and 56 ng/mL) using five different fabricated electrodes for three consecutive days. The resulting relative standard deviation (RSD= (SD*100/average)) ranged from 1.7 to 3.5%, indicating high precision of the proposed FMDV biosensor. On the other hand, the repeatability of the assay has been determined for two concentrations of the virus (45 and 67 ng/mL), where they gave almost the same values as given in the calibration curve. The fabricated electrodes were used many times without a marked change in the signal response for the selective capturing feature towards the target virus especially in the first 2 weeks. Only 5% decrease in the reading value was observed during the second two weeks. Overall, the performance of the developed VIP biosensor was almost stable after 2 months.

3.7. FMDV biosensing in real samples

The fabricated electrode was applied for sensing field samples and the results were compared with that obtained by the ordinary laboratory diagnostic techniques for FMDV (ELISA for antigen detection and Real-Time PCR) as can be noted in Table 1. From the table, the obtained results were qualitative in the case of ELISA and PCR (screening only), while quantitative results were obtained in the case of the proposed FMDV biosensor.

Samples (1) and (2) were diagnosed as positive serotype O on applying antigen detection test by ELISA, and sample (3) was positive serotype A with a cross reactivity with serotype O, but these samples were positive FMDV serotype O on applying Real-time PCR with CT = 27th, 24th and 32nd, respectively. It is remarkable that, when

Table 1
Application of the proposed FMDV serotype O biosensor in comparison with standard diagnostic tools.

Sample	Standard tests[a]				Proposed FMDV biosensor (ng/ml)
	Antigen detection by ELISA with OD results•			Real-Time PCR•	
	O	A	SAT2		
1	+	–	–	+ serotype_O (CT = 27th)	18.9
2	+	–	–	+ serotype_O (CT = 24th)	36.9
3	suspected	+	–	+ serotype_O (CT = 32nd)	2.08
4	–	–	–	+ serotype_O (CT = 20th)	54.08
5	–	–	–	+ serotype_O (CT = 38th)	1.14
6	–	–	–	–	0.6
7	–	–	+	+ (SAT-2)	0.8
8	–	–	+	+ (O) CT = 25th	3.19

[a] The routine diagnostic tool (ELISA & PCR) were qualitative procedures in comparison to the quantitative results in the case of the proposed FMDV biosensor.

these samples were tested by the proposed FMDV biosensor, the current signals were quantified and subsequently, the obtained virus concentrations were 18.9 and 36.9 ng/mL in the case of sample (1) and (2), respectively, while a very low virus concentration (2.08 ng/mL) was noticed in the case of sample (3) as shown in Table 1.

It is noteworthy to mention that, the developed sensor shows a highly sensitive and accurate investigated current signal for the tested sample (4) with a quantifying virus payload about 54 ng/mL, while in case of sample (5), the VIP sensor shows a positive current response with a very low concentration of virus concentration detected in this sample. On the same manner, these samples were positive FMDV serotype O using PCR at CT 20th, and 38th for the samples (4) and (5) respectively. Regarding, they all were negative antigen detection by ELISA test. Sample (6) was negatively tested for the serotype O by the reference diagnostics and the proposed biosensor, and showed to be positive in case of applying the tested biosensor, indicating the high sensitivity for the sensor as an early detection tool for the viral infection.

Both samples (7) and (8) showed weak positive results for serotype SAT-2 by ELISA test, but sample (8) was found to be serotype O positive using PCR. The recorded value of these samples using the proposed VIP sensor confirmed the results obtained via ordinary diagnostic tools but with higher sensitivity and selectivity, and the virus payload in sample (8) was measured to be 3 ng/mL.

Moreover, all the investigated samples were compared by setting a negative control and a positive control of known payload of FMDV serotype O (67 ng/mL) with its analytical value using LSV and a response of 9 μA, which is the repetitive and reproducible value of the target virus relative to its standard calibration curve. Samples were tested using the developed VIP biosensor with a small volume concentration (1:60) compared to the volume required in ELISA (1:1) and (1:2) for PCR.

A very important factor to be highlighted related to the proposed biosensor is the time required for performing the analysis. ELISA-based analysis consumed about 5 h to be performed and around 24 h were required for PCR but the developed VIP biosensor took only 5 min not only to diagnose but also to quantify the virus payload in the tested sample. The delays in quantification upon using traditional techniques might lead to higher propagation of the viral infection in more animals and higher mortality rates which would subsequently affect the economy of infected areas.

Furthermore, the laboratory sensitivity and specificity are important factors that affect the diagnostic procedures and confidence. From all the previous results, it can be concluded that the fabricated virus biosensor has 100% confidence for its sensitivity and specificity as compared to the lateral flow devices (LFDs), ELISA and PCR giving 87.3, 87.7 and 95.0% confidence in the predication of the virus concentration in the tested samples, respectively (King et al., 2006a).

4. Conclusion

A new simple VIP biosensor was developed for the early detection of FMDV serotype O virus based on electropolymerization of O-aminophenol (*O*-AP) film imprinted with the target virus on a gold screen-printed electrode (SPE). The results showed high selectivity of the biosensor to the serotype O over all other genus serotypes A, SAT2 and Lumpy skin disease virus (LSDV), as well as, the inactivated serotype O with limits of detection (LOD) and quantification (LOQ) were around 2 ng/mL and 6 ng/mL, respectively that is 50 folds lower compared to traditionally adapted methods (ELISA and PCR). The presented biosensor is superior over these methods in terms of not only being a quantitative rather than a qualitative tool for viral detection but also based on its low required detection time, just 5 min compared to 5 h as in case of ELISA-based analysis and around 24 h on applying PCR.

The fact of the very fast response taking around 5 min in addition to the re-usability of the biosensor developed in the current study for qualitative and quantitative detection of FMDV, presents a promising, affordable and economic tool that can be integrated with a portable electrochemical potentiostat to be used in field without any need to perform complicated and expensive laboratory experiments.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Heba A. Hussein: Data curation, Methodology, Formal analysis. **Rabeay Y.A. Hassan:** Data curation, Methodology, Formal analysis. **Rasha Mohamed El Nashar:** Project administration, Writing - review & editing. **Samy A. Khalil:** Supervision. **Sayed A. Salem:** Supervision. **Ibrahim M. El-Sherbiny:** Supervision, Validation, Visualization, Writing - review & editing.

Acknowledgement

The authors would like to thank Mr. Muhamad Fayez for his kind help during the practical work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.111467.

References

Aidaros, H.A., 2002. Regional status and approaches to control and eradication of foot and mouth disease in the Middle East and North Africa. Rev. Sci. Tech. 21 (3), 451–458.
Amaral-Doel, C.M., Owen, N.E., Ferris, N.P., Kitching, R.P., Doel, T.R., 1993. Detection of foot-and-mouth disease viral sequences in clinical specimens and ethyleneimine-inactivated preparations by the polymerase chain reaction. Vaccine 11 (4), 415–421.
Blanco-López, M.C., Lobo-Castañón, M.J., Miranda-Ordieres, A.J., Tuñón-Blanco, P., 2004. Electrochemical sensors based on molecularly imprinted polymers. Trac. Trends Anal. Chem. 23 (1), 36–48.
Bolisay, L.D., Culver, J.N., Kofinas, P., 2006. Molecularly imprinted polymers for tobacco mosaic virus recognition. Biomaterials 27 (22), 4165–4168.

Callens, M., De Clercq, K., 1997. Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. J Virol Methods 67 (1), 35–44.
Chen, H.T., Zhang, J., Liu, Y.S., Liu, X.T., 2011. Detection of foot-and-mouth disease virus RNA by reverse transcription loop-mediated isothermal amplification. Virol. J. 8, 510.
Chianella, I., Guerreiro, A., Moczko, E., Caygill, J.S., Piletska, E.V., De Vargas Sansalvador, I.M., Whitcombe, M.J., Piletsky, S.A., 2013. Direct replacement of antibodies with molecularly imprinted polymer nanoparticles in ELISA–development of a novel assay for vancomycin. Anal. Chem. 85 (17), 8462–8468.
Ding, Y.Z., Liu, Y.S., Zhou, J.H., Chen, H.T., Zhang, J., Ma, L.N., Wei, G., 2011. A highly sensitive detection for foot-and-mouth disease virus by gold nanopariticle improved immuno-PCR. Virol. J. 8, 148.
Ehsani, A., Mahjani, M.G., Jafarian, M., 2012. Electrosynthesis of poly ortho aminophenol films and nanoparticles: a comparative study. Synth. Met. 162 (1), 199–204.
Gast, M., Kuhner, S., Sobek, H., Mizaikoff, B., 2018. Understanding the viral load during the synthesis and after rebinding of virus imprinted particles via real-time quantitative PCR. Analyst 143 (11), 2616–2622.
Gramsbergen, J.B., Leegsma-Vogt, G., Venema, K., Noraberg, J., Korf, J., 2003. Quantitative on-line monitoring of hippocampus glucose and lactate metabolism in organotypic cultures using biosensor technology. J. Neurochem. 85 (2), 399–408.
Hong, J.K., Lee, K.N., You, S.H., Kim, S.M., Tark, D., Lee, H.S., Ko, Y.J., Seo, M.G., Park, J.H., Kim, B., 2015. Inactivation of foot-and-mouth disease virus by citric acid and sodium carbonate with deicers. Appl. Environ. Microbiol. 81 (21), 7610–7614.
King, D.P., Ferris, N.P., Shaw, A.E., Reid, S.M., Hutchings, G.H., Giuffre, A.C., Robida, J.M., Callahan, J.D., Nelson, W.M., Beckham, T.R., 2006a. Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time reverse transcription-polymerase chain reaction assays. J. Vet. Diagn. Investig. 18 (1), 93–97.
King, D.P., Ferris, N.P., Shaw, A.E., Reid, S.M., Hutchings, G.H., Giuffre, A.C., Robida, J.M., Callahan, J.D., Nelson, W.M., Beckham, T.R., 2006b. Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time reverse transcription-polymerase chain reaction assays. J. Vet. Diagn. Investig. 18 (1), 93–97.
Longjam, N., Deb, R., Sarmah, A.K., Tayo, T., Awachat, V.B., Saxena, V.K., 2011. A brief review on diagnosis of foot-and-mouth disease of livestock: conventional to molecular tools. Vet. Med. Int. 2011 905768-905768.
Malik, N., Kotecha, A., Gold, S., Asfor, A., Ren, J., Huiskonen, J.T., Tuthill, T.J., Fry, E.E., Stuart, D.I., 2017. Structures of foot and mouth disease virus pentamers: insight into capsid dissociation and unexpected pentamer reassociation. PLoS Pathog. 13 (9), e1006607.
Mayes, A.G., Whitcombe, M.J., 2005. Synthetic strategies for the generation of molecularly imprinted organic polymers. Adv. Drug Deliv. Rev. 57 (12), 1742–1778.
Menon, S., Jesny, S., Girish Kumar, K., 2018. A voltammetric sensor for acetaminophen based on electropolymerized-molecularly imprinted poly(o-aminophenol) modified gold electrode. Talanta 179, 668–675.
Moczko, E., Poma, A., Guerreiro, A., Perez de Vargas Sansalvador, I., Caygill, S., Canfarotta, F., Whitcombe, M.J., Piletsky, S., 2013. Surface-modified multifunctional MIP nanoparticles. Nanoscale 5 (9), 3733–3741.
Moniwa, M., Clavijo, A., Li, M., Collignon, B., Kitching, R.P., 2007. Performance of a foot-and-mouth disease virus reverse transcription-polymerase chain reaction with amplification controls between three real-time instruments. J. Vet. Diagn. Investig. 19 (1), 9–20.
Ramakrishnan, M.A., 2016. Determination of 50% endpoint titer using a simple formula. World J. Virol. 5 (2), 85–86.
Rasmussen, T.B., Uttenthal, Å., de Stricker, K., Belák, S., Storgaard, T., 2003. Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of Foot-and-mouth disease virus. Arch. Virol. 148 (10), 2005–2021.
Reid, S.M., Ferris, N.P., Hutchings, G.H., Samuel, A.R., Knowles, N.J., 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J Virol Methods 89 (1–2), 167–176.
Reid, S.M., Ferris, N.P., Hutchings, G.H., Zhang, Z., Belsham, G.J., Alexandersen, S., 2002. Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay. J. Virol Methods 105 (1), 67–80.
Sangar, D.V., Rowlands, D.J., Cavanagh, D., Brown, F., 1976. Characterization of the minor polypeptides in the foot-and-mouth disease particle. J. Gen. Virol. 31 (1), 35–46.
Sangma, C., Lieberzeit, P.A., Sukjee, W., 2017. H5N1 virus plastic antibody based on molecularly imprinted polymers. Methods Mol. Biol. 1575, 381–388.
Tancharoen, C., Sukjee, W., Thepparit, C., Jaimipuk, T., Auewarakul, P., Thitithanyanont, A., Sangma, C., 2019. Electrochemical biosensor based on surface imprinting for Zika virus detection in serum. ACS Sens. 4 (1), 69–75.
Vangrysperre, W., De Clercq, K., 1996. Rapid and sensitive polymerase chain reaction based detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. Arch. Virol. 141 (2), 331–344.
Vestergaard, M., Kerman, K., Tamiya, E., 2007. An overview of label-free electrochemical protein sensors. Sensors (Basel) 7 (12), 3442–3458.
Wu, Z., Zhang, X., Yang, Y., Shen, G., Yu, R., 2006. A sensitive nicotine sensor based on molecularly imprinted electropolymer of o-aminophenol. Front. Chem. China 1 (2), 183–187.
Xu, X.-D., Lin, B.-B., Feng, J., Wang, Y., Cheng, S.-X., Zhang, X.-Z., Zhuo, R.-X., 2012. Biological glucose metabolism regulated peptide self-assembly as a simple visual biosensor for glucose detection. Macromol. Rapid Commun. 33 (5), 426–431.
Yang, B., Gong, H., Chen, C., Chen, X., Cai, C., 2017. A virus resonance light scattering sensor based on mussel-inspired molecularly imprinted polymers for high sensitive and high selective detection of Hepatitis A Virus. Biosens. Bioelectron. 87, 679–685.
Yang, Y.J., Li, J.Y., Liu, X.W., Zhang, J.Y., Liu, Y.R., Li, B., 2013. A non-biological method for screening active components against influenza virus from traditional Chinese medicine by coupling a LC column with oseltamivir molecularly imprinted polymers. PLoS One 8 (12), e84458.