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Polymer dots for quantifying the total hydrophobic pathogenic lysates in a single drop

Hani Nasser Abdelhamid[a,b], Hui-Fen Wu[a,c,d,e,*]

[a] Department of Chemistry, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
[b] Department of Chemistry, Assuit University, Assuit 71516, Egypt
[c] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 806, Taiwan
[d] Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, Kaohsiung 804, Taiwan
[e] Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, Kaohsiung 804, Taiwan

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ABSTRACT

A selective, rapid, small sample load (2–4 μL), and sensitive quantification method for the hydrophobic cellular biomolecules of pathogenic bacteria and their biosensing application were reported. The present approach is based on using polythiophene polymer dots (2.5 nm), which were prepared via the oxidation/polymerization reactions and then were characterized using transmission electron microscopy (TEM), Fourier transform infrared (FT-IR), ultraviolet spectroscopy (UV), and matrix (surface) assisted laser desorption/ionization mass spectrometry (M(S)ALDI-MS). The present method requires only gentle agitation for a single drop of aqueous bacteria suspension (10 μL, *Pseudomonas aeruginosa* (1×10^4 cfu/mL) and *Staphylococcus aureus* (1×10^5 cfu/mL)) with 1 mL of polythiophene (0.5 mg/mL) in chloroform, and the time required for quantifying the total hydrophobic was significantly reduced to less than 3 min. The polythiophene polymer dots is also a quantitative assessment of bacteria for aqueous and blood samples if exposed to more than 4–5 μL of pathogenic bacteria and thus, it is a new biosensor for quantitative hydrophobic portions. The fluorescence intensity of polythiophene was enhanced after adding different volumes of pathogenic bacteria with low colony units. The standard bacteria suspensions of *P. aeruginosa* and *S. aureus* have low LOD (limits of detection) for 2 μL (1×10^4 cfu/mL) and 4 μL (1×10^5 cfu/mL), respectively. Further, the pathogenic bacteria were spiked into mouse blood and the total hydrophobic biomolecules were quantified. This method is extremely rapid as it does not require any culture steps prior to analysis and also no need for any separation or post sample treatments.

1. Introduction

Conducting polymers (CPs) or conjugated polymers are organic-multifunctional materials with highly π–π conjugated polymeric chains; so, it has been received intensive research attention nowadays [1–5]. It possesses various interesting properties such as extraordinary stability, photostability, high electric conductivity, biocompatibility and simplicity of synthesis from an inexpensive monomer. Semiconducting polymer dots (Pdots) represent a new class of conducting polymer, which can be applied as effective fluorescent probes. It is a promising material because of their exceptional brightness and their nontoxic features comparing to the inorganic quantum dots (QDs) [1–6]. Nowadays, CPs have been attracted to a considerable interests due to its simplicity, sensitivity, and selectivity towards different analytes rely on the changes in their fluorescence intensities upon binding analytes [7–17]. The optical application of CP-based has been published in Ref. [7–17]. The recent progress and new applications of CPs in microextraction [18], health care, food industries, environmental monitoring [19], and as tools in clinical diagnosis and surgical interventions [20] were reviewed.

Among various CPs, the polythiophene derivatives exhibit intensive applications in separation [21–24], increase stability of carbon nanomaterials [25], eradicate bacterial biofilms via conjugation with gold nanoparticles [26], biosensing [27], and inhibit the growth of tumor cells [28]. It has been successfully applied for DNA, [29] proteins [30], nucleotides [31], and amino acids [32,33] analyses based on the analyte-induced aggregation of polythiophene backbones through electrostatic and hydrophobic cooperative interactions. Because of the high conductivity of CPs, the conductometric electronic noses based on CPs were applied for detecting bacteria and fungi [34–40]. Polythiophene (PTH) is a promising class of organic semiconductors, because it is simple in synthesis and its structure is easily to be modified [27,41–48]. Recently, the

* Corresponding author at: Department of Chemistry, National Sun Yat-Sen University, 70 Lien-hai Road, Kushan District, Kaohsiung 804, Taiwan.
Tel.: +886 7 5252000 3955; fax: +886 7 525 3908.
E-mail address: hwu@faculty.nsysu.edu.tw (H.-F. Wu).

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application of cationic polythiophene for microbial biosensing in water was reported [48], and it is also used as a new photodynamic agent with anticancer and antimicrobial activity [49,50]

The aim of this study is to develop a novel, rapid, ultra small sample loading, and label-free quantification method for the cellular hydrophobic lysates of pathogenic bacteria as a new biosensing method using fluorescence spectroscopy and matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS). To our best knowledge, this is the first study that can successfully quantify the total cellular hydrophobic biomolecules for pathogenic bacteria in a single drop with volume <2–4 μL. The novelty of the present work is to use a miniaturized extraction technique, which can greatly reduce the sample volume, and direct biosensing pathogenic bacteria based on their hydrophobic moieties for effectively and highly sensitive biosensing technique.

2. Materials and methods

Thiophene monomer was purchased from Sigma–Aldrich, USA. Anhydrous ferric chloride ($FeCl_3$) and 2,5-dihydroxy benzoic acid (DHB) were purchased from Riedel de Häen, Germany. Methanol and chloroform ($CHCl_3$) were purchased from J.T. Baker, USA. High pure water obtained by a Milli-Q water purification system (18.25 MΩ cm, Millipore, Bedford, USA).

2.1. MALDI-MS

MALDI-MS spectra were obtained with MALDI-MS (Microflex, Bruker Daltonics, Bremen, Germany) equipped with nitrogen laser (wavelength 337 nm). The spectra were recorded in positive and reflectron mode using an acceleration voltage of 20 kV and 10 ns delay time. 2,5-dihydroxy benzoic acid (DHB) and gold nanoparticles were used as the matrices to characterize the polymer dot while the former was used for biomolecules detection after separation.

2.2. UV, fluorescence, FT-IR, and TEM

All chemical structures and mass calculations were performed by the software program ACD-Chemsketch V12. The UV measurements were undertaken in UV spectrophotometer (Lambda 25, PerkinElmer, German). The fluorescence spectra were performed in fluorescence spectrophotometer (F-2700 Hitachi Co., Japan), equipped with xenon arc lamp (150 W). The scan speed was set at 120 nm min^{-1}. All spectra were drawn and fitted by using the software of Origin V6.0. Fourier transform infrared (FT-IR) spectra of polythiophene were recorded on a FT-IR spectrometer (Spectrum 100, PerkinElmer, USA). The size distributions of nanoparticles were determined by transmission electron microscopy (TEM, Philip CM200, Switzerland).

3. Experimental

3.1. Bacteria cultivation

Both bacteria were cultivated using the conventional method [51,52]. *S. aureus* (BCRC 10451) and *P. aeruginosa* (BCRC 10303) standard cultures were purchased from Bioresource Collection and Research Center (BCRC, Hsin-Chu, Taiwan). Both bacteria were cultivated in agar medium plate (Gene Chain Scientific, GCS, USA) and Difco nutrient broth (USA). After incubation the bacteria plate (24 h), bacteria harvest were suspended into 1 mL deionised and sterilized water and used as stock solution. The bacteria forming colony (cfu/mL) was determined using plate counting method.

3.2. Plate counting method

Pour plate method was used to calculate bacteria forming colony (cfu/mL). Briefly, a diluted bacteria suspension (0.1–1.0 mL) is pipetted into a sterile Petri plate, and then melted agar is poured in and mixed with the suspension. Caution must be taken with this method to ensure that the organism to be counted can withstand the temperatures associated with the melted agar. After 24 h, bacteria colons were counts in triplicate of the same dilution to confirm the results.

3.3. Preparation of polythiophene

Polythiophene was synthesized by chemical oxidative polymerization method, in which the anhydrous ferric chloride ($FeCl_3$) was used as oxidant; thiophene was used as the monomer; and $CHCl_3$ was used as the solvent [53]. Briefly, 10 g of anhydrous $FeCl_3$ was dissolved into 150 mL of $CHCl_3$, and then 10 mL of thiophene was added into the solution drop by drop. The mixture was stirred for 24 h with N_2 as the protection (inert) gas. After the process of polymerization, the solvent was evaporated. The obtained polythiophene was washed with MeOH (50%) and water. The washing step by using methanol is critical for removing Fe(II) and Fe(III). Polythiophene (0.5 mg/mL) was re-suspended into 15 mL of $CHCl_3$ using ultrasonication water bath for 2, 3 h.

3.4. Preparation of gold nanoparticles

The gold nanoparticles (Au NPs) were prepared according to Frens's approach [54]. Briefly, $HAuCl_4$ aqueous solution (100 mL, 2.43 mM) was heated to boiling with vigorous stirring; then a trisodium citrate solution (0.75 mL, 1%) was added into the solution. The mixture was kept boiling for 15 min. Afterwards, the solution was allowed to cool to room temperature with continuous stirring.

3.5. Fluorescence procedure

About 8.0 mg of the prepared polythiophene were dispersed in $CHCl_3$ (15.0 mL) via ultrasonication for 1 h. For 1.0 mL of the previous solution, different volume (10–100 μL) of pathogenic bacteria were added and vortexes (1 min for *P. aeruginosa* (1×10^4 cfu/mL) and 3 min for *S. aureus* (1×10^5 cfu/mL). The mixture solution was measured at emission wavelength of 520 nm at the absorption excitation wavelength. The experiments were repeated more than 3 times of the same/different suspension of pathogenic bacteria to confirm their reproducibility and repeatability.

3.6. Fluorescence measurement for mice blood sample

The previous procedure is applied for real sample such as mouse blood. About 200 μL of blood sample were diluted 50 times with deionized and sterilized water. The prepared solution was used instead of deionized water. The pathogenic bacteria were dispersed in the previous suspension and were used as stock suspension of the bacteria. Then we followed the same procedure that describe above.

3.7. MALDI-MS analysis of standard and blood suspensions

From the same suspension that previously used in fluorescence analysis, about 10.0 μL were diluted with 10.0 μL of DHB matrix (50.0 mM in aqueous acetonitrile in v/v equal 50:50). About 2.0 μL of the mixture was spotted onto the MALDI plate; air dried and then subject to MALDI-MS analysis.

For mice blood samples, we used the same protocol for bacteria that dispersed in blood sample.

3.8. Replication of batch experiments

Each experiment was conducted in triplicates to confirm the repeatability. The error bars represent the standard deviation of all the three trial experiments.

4. Result and discussion

The hydrophobic portions of pathogenic bacteria play a significant role in the bacteria toxicity. Cellular hydrophobic biomolecules such as lipid, lipoprotein, lipopolysaccharide are important components of cells. For instance, the endotoxin (lipopolysaccharide) of Gram-negative bacteria "*P. aeruginosa*" contributes greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack [55]. Therefore, direct measurements of bacteria hydrophobic biomolecules can provide information about infection, biofuel yield and immune responds of human body toward bacteremia [56]. Thus, we propose a new approach for bacteria sensing via quantifying the total hydrophobic portion of pathogenic bacteria (*P. aeruginosa* and *S. aureus*).

The goal of the presented work is to provide a direct, simple and low cost methodology for the quantification of hydrophobic biomolecules from bacterial suspension to facilitate physiological studies and to use it for bacteria biosensing. Note that the described method can quantify the total mass of hydrophobic biomolecules of pathogenic bacteria directly based on fluorescence results. The change of fluorescence signal is directly proportional to the bacteria cell number.

4.1. Characterization of polythiophene polymer dots

Generally, conducting polymers (CPs) have been prepared by chemical or electrochemical methods. Comparably, chemical synthesis not only provides many possible routes to synthesize a variety of conducting polymers but also permits the scale-up preparation of these materials, which is currently not available with electrochemical synthesis. Chemical synthesis of the conducting polymer is usually performed by such chemical oxidants as $FeCl_3$ of the monomer solutions. However, the new research trends are to use another oxidant for better application [57]. Polymerization of the thiophene monomer was initiated using anhydrous $FeCl_3$ as an oxidant in $CHCl_3$. The TEM image (Fig. 1A) of the prepared polythiophene reveals that the polymer morphology is spherical dots with average size 2.5 nm as shown in the distribution diagram in Fig. 1B. To evaluate the molecular weight of polythiophene, DHB and Au nanoparticles were used as the matrix for MALDI-MS and surface assisted laser desorption/ionization mass spectrometry (SALDI-MS), respectively. The spectra (Fig. 1C) indicated that the ionization/desorption in the case of Au NPs (TEM image, Fig. 1D, average size 7 nm, inset represent the size distribution) are higher than that of the conventional organic matrix (DHB, Fig. 1C). The reason may be due to the self assembles of the polymer on the Au nanoparticles surface via forming sulfur–Au bonds (Fig. 1E); thus, higher ionization/desorption can be achieved without generating any interferences. The Au nanoparticles are superior for polymer analysis than the conventional organic matrices as gold assisted laser desorption/ionization mass spectrometry (GALDI-MS) offers low interference. GALDI-MS spectra produce peaks at 1382.6 Da, corresponding to polythiophene molecular weight, which is absent in the conventional organic matrix spectrum (DHB, Fig. 1C). The UV spectra (Fig. 1F) display absorption at 310 and 320 nm for thiophene monomer and polythiophene, respectively. Fourier transform infrared (FI-IR) spectra of thiophene and polythiophene were investigated (Fig. 1G). The FT-IR spectra (Fig. 1G) offer salient characteristic peaks, among them, 1450 cm^{-1} that assigned as the stretching vibration of —C=C— of aromatic thiophene ring. Peaks at 1035, 788 and 701 cm^{-1} were the C—H plane vibration of thiophene ring, C—H out of plane, C—H of plane vibration of the mono-substituted thiophene ring, respectively. Peaks at 1411 and 632 cm^{-1} were assigned as the bending vibration and the stretching vibration of C—S bond, respectively. Two weak bands at 2981 and 2889 cm^{-1} are corresponding to the methylene moieties of the resultant polythiophene polymers. The FT-IR results indicated that polythiophene was successfully synthesized.

The polythiophene has been reported in various applications due to their exceptional brightness and their nontoxic features compared with quantum dots [1–6]. Furthermore, the substituent of polythiophene would not influence the band gap of polythiophene, as it has metallic nature and also called 'synthetic metals' [58–60]. However, the major drawback of polythiophene or other conventional conducting polymers is their poor solubility. Many potential applications of conducting polymers are limited because of their insolubility in common organic solvents. Here, we try to use this disadvantage to be a merit. Thus, we dissolve it in an organic solvent such as chloroform ($CHCl_3$) in order to quantify the hydrophobic cellular biomolecules and their application for biosensing.

4.2. Fluorescence analysis of standard bacteria suspensions

In conventional methods [57,61], the accurate quantity of hydrophobic biomolecules can only be obtained after the microorganism culture is harvested; so, the adjustment of cultivation parameters require an experience and numerous numbers of experiments to obtained the accurate data. Therefore, it is important to propose a sensitive, effective separation, and detection methodology in order to analyze the hydrophobic biomolecules of pathogenic bacteria.

Among various analytical techniques, fluorescence method is high sensitivity due to a bright signal appears against a dark background. It probes tiny changes in the fluorescence intensity or wavelength of any fluorophore in response to its environment. Due to the excellent brightness of polymer dots, it was used as a fluorophore. The conducting polymer such as polythiophene is a water-insoluble polymer. Thus, the polyelectrolyte conjugated polymer (PEC) was proposed to solve these limitations [50]. In our experiments, we dissolved polythiophene in chloroform (organic solvent). The organic phase ($CHCl_3$) can increase the fluorescence yield of polymer dots, due to the absence of soluble oxygen and water molecules, which can quench the fluorescence emission. Furthermore, selectively hydrophobic biomolecules of pathogenic bacteria can be only transferred from the aqueous layer. The present approach depends on mass transfer of hydrophobic biomolecules from aqueous bacteria suspensions to the organic phase. The types of these biomolecules could be lipid, chlorophylls, phospholipid, and lipoprotein. The general procedure to quantify the total hydrophobic cellular biomolecules of pathogenic bacteria is shown in Fig. 2A. Bacteria were cultivated in the agar medium and re-suspended in de-ionized water prior to use. The suspension solution was used as a stock solution of bacteria. Small drops (10–100 μL) of bacteria suspension were added to 1 mL of polythiophene (0.5 mg/mL $CHCl_3$). The mixture was vortexed 1–3 min until the microdrop was invisible (>3 min). This approach doesn't require tedious separation procedures or complicated pretreatment techniques such as ultracentrifugation and it can significantly reduce the sample loading to only several microliters.

Upon addition of different volume (10–100 μL) of *P. aeruginosa* (Fig. 2B) and *S. aureus* (Fig. 2C), the fluorescence emission of polymer dots are enhanced. Fig. 2(D, E) presents good linear

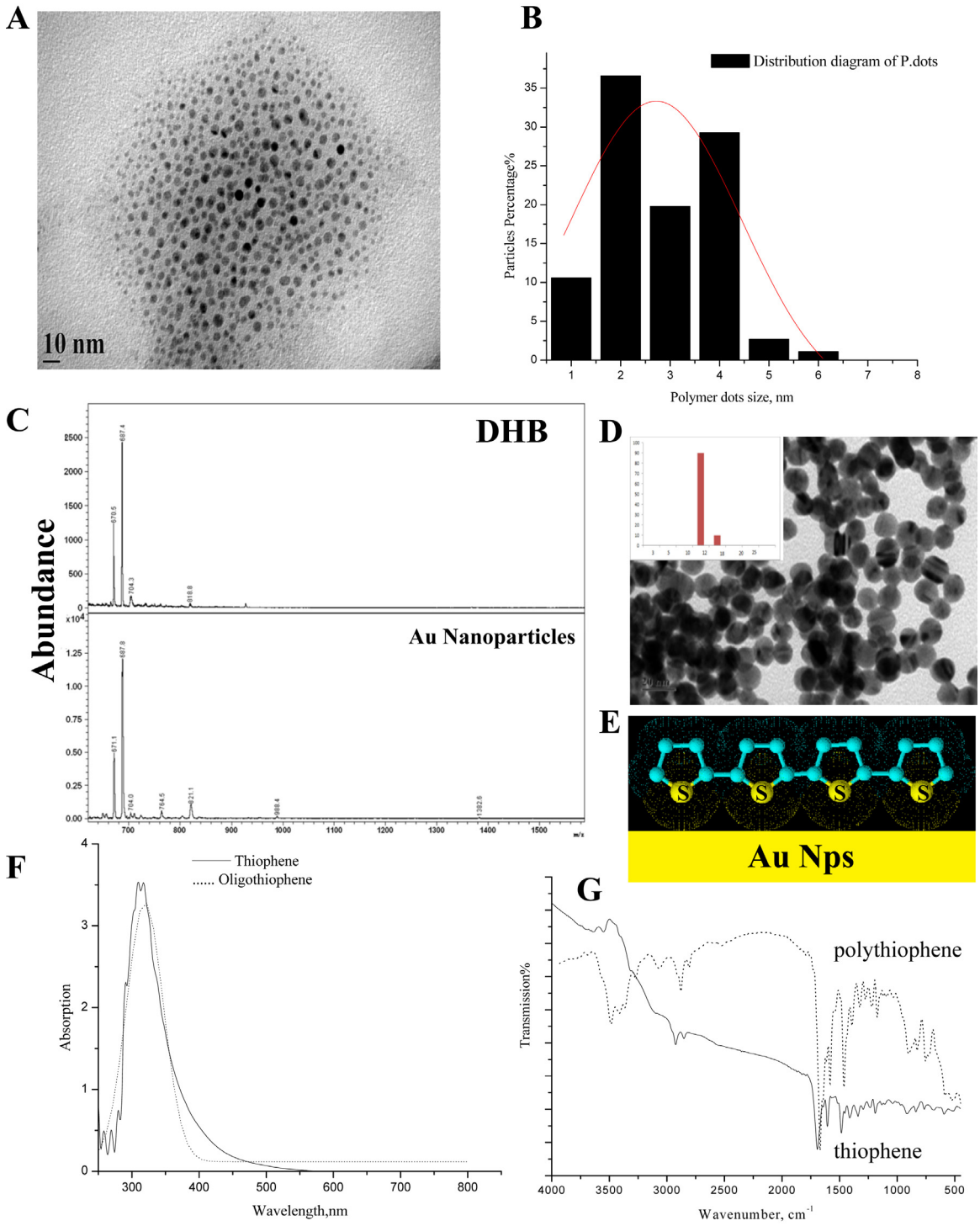

Fig. 1. Characterization of polythiphene polymer dots using (A) TEM image, (B) distribution diagram, (C) MALDI-MS using DHB and gold nanoparticles (Au), (D) TEM image of Au NPs (inset represent the size distribution), (E) self-assembly of polythiophene on Au nanoparticles, (F) UV spectra, and (G) FT-IR spectra.

relationship between fluorescence and microliters addition of *P. aeruginosa* (1×10^5 cfu/mL) (Fig. 2D) and *S. aureus* (1×10^6 cfu/mL) (Fig. 2E). The spectra reveal significant enhancement of signals from the polymer dots background, however, bacteria have only small amount of hydrophobic biomolecules. These enhancements may be due to the strong aggregation that induced emission enhancement (AIEE).

The conducting polymers exhibit multi-functionality, which can assist biomolecules adsorption by different mechanisms such as hydrophobic, acid–base interaction, π–π interaction, polar functional groups interactions, hydrogen bonding, and electrostatic interactions. The bacterial cells are negatively charged due to the presence of teichoic acid in the Gram-positive bacteria and lipopolysaccharide in Gram-negative bacteria [62]. Fig. 2 reveals that the interactions between the polythiophene and the pathogenic bacteria lysates do not cause the shift in the fluorescence wavelength but can enhance the fluorescence intensity indicating a biophysical phenomenon (such as aggregation), which

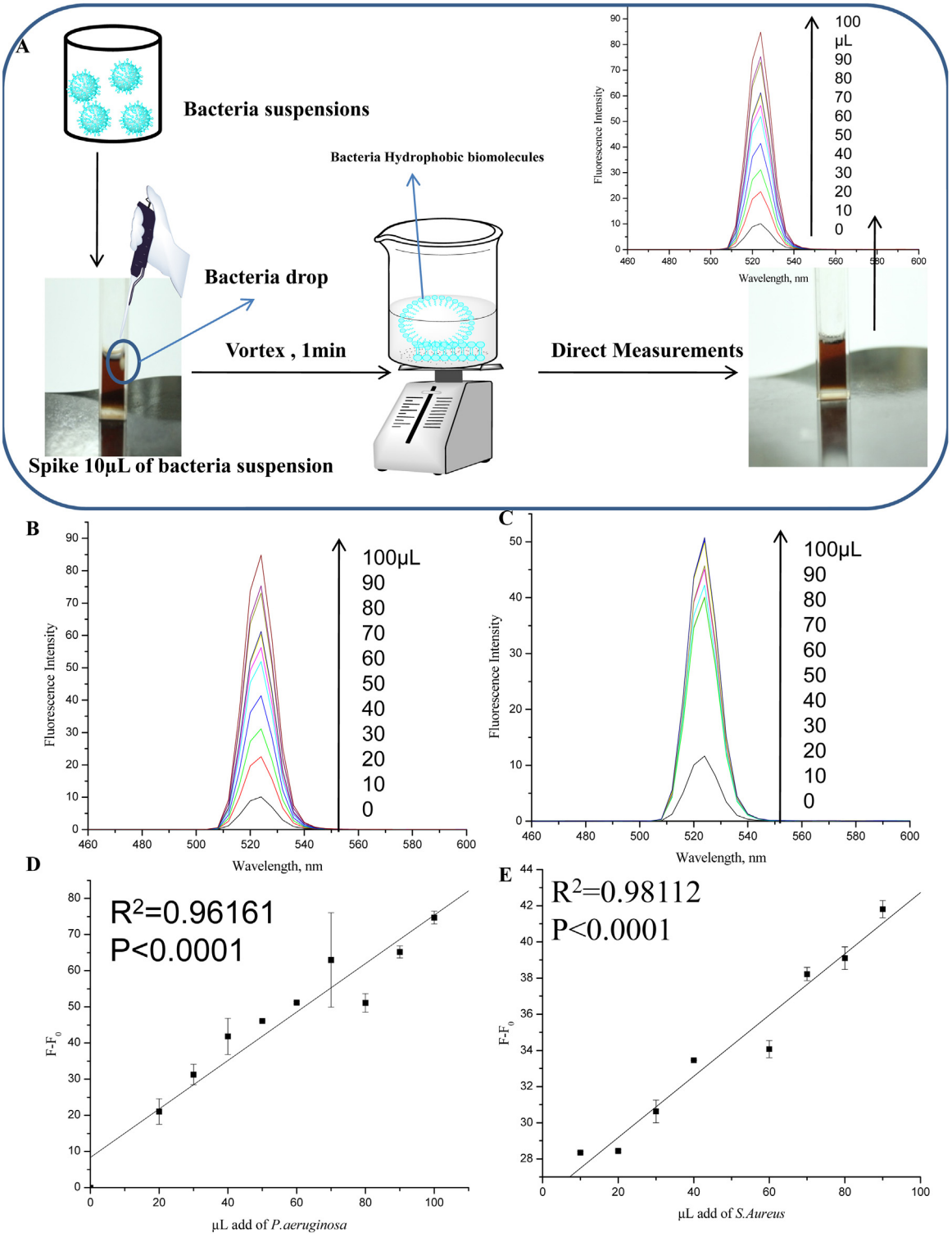

Fig. 2. (A) Schematic representation of quantification of total hydrophobic content of pathogenic bacteria. From a suspension solution, different volume of bacteria are added to 1 mL of polymer dots in organic layer, then mixture vortexed 1–3 min and fluorescence were measured. Fluorescence responds upon addition different volume of (B) *P. aeruginosa* and (C) *S. aureus* and the linear relationship (D, E), respectively.

enables biosensing. For example, adsorption of Lewis bases on the surface enhanced photoluminescence intensity; while, Lewis acids produced a photoluminescence decrease. This indicates that the extracted crude may be having Lewis bases character. Therefore, highly fluorescent conjugated polymers have been used for detection of electron-deficient by efficient fluorescence quenching [63,64]. The question should be mentioned is the types of biomolecules that were transferred from the bacterial suspension to the organic layer of the polymer dots. Thus, we investigated the separated biomolecules using mass spectrometry.

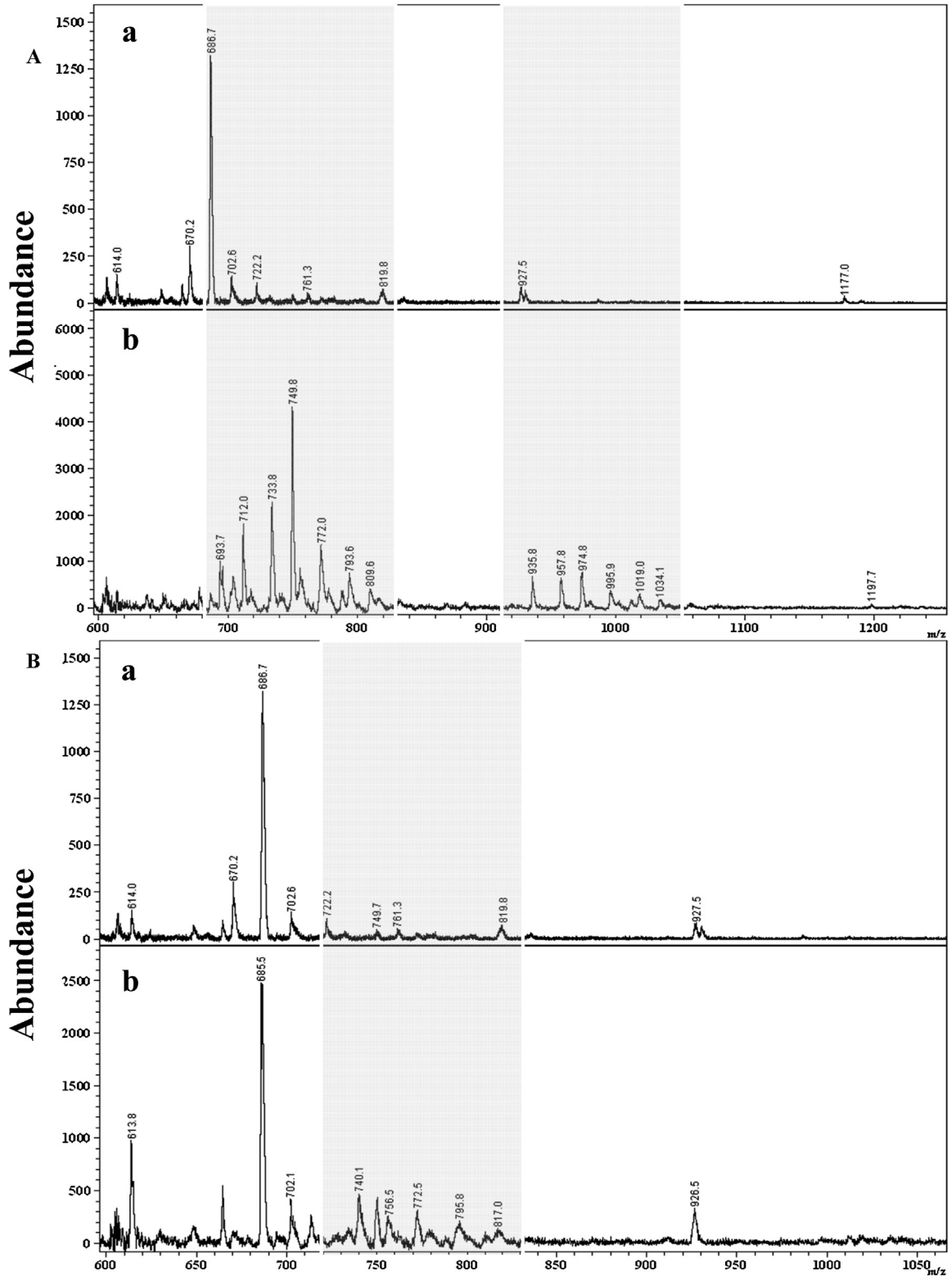

Fig. 3. MALDI spectra of thiophene polymer, *P. aeruginosa* (A) and *S. aureus* (B) using DHB as matrix (a) before and (b) after separation. The highlight part shows the biomolecules that undergoes mass transfer from the bacteria solution to the organic phase that contain polymer dots.

4.3. Matrix assisted laser desorption/ionization analysis of extracted biomolecules from pathogenic bacteria

Our current approach can quantify the total hydrophobic biomolecules of pathogenic bacteria such as lipid, lipoprotein, and lipolysaccharide (endotoxin). We try to detect these biomolecules that undergo mass transfer from the bacteria cells to the organic solvent ($CHCl_3$) using mass spectrometry.

Among various clinical-related techniques, MALDI-MS has been widely used for bacterial studies [65,66]. The hydrophobic portions of pathogenic bacteria may be lipid, lipoprotein, and lipopolysaccharide "endotoxin". These biomolecules can influence on the

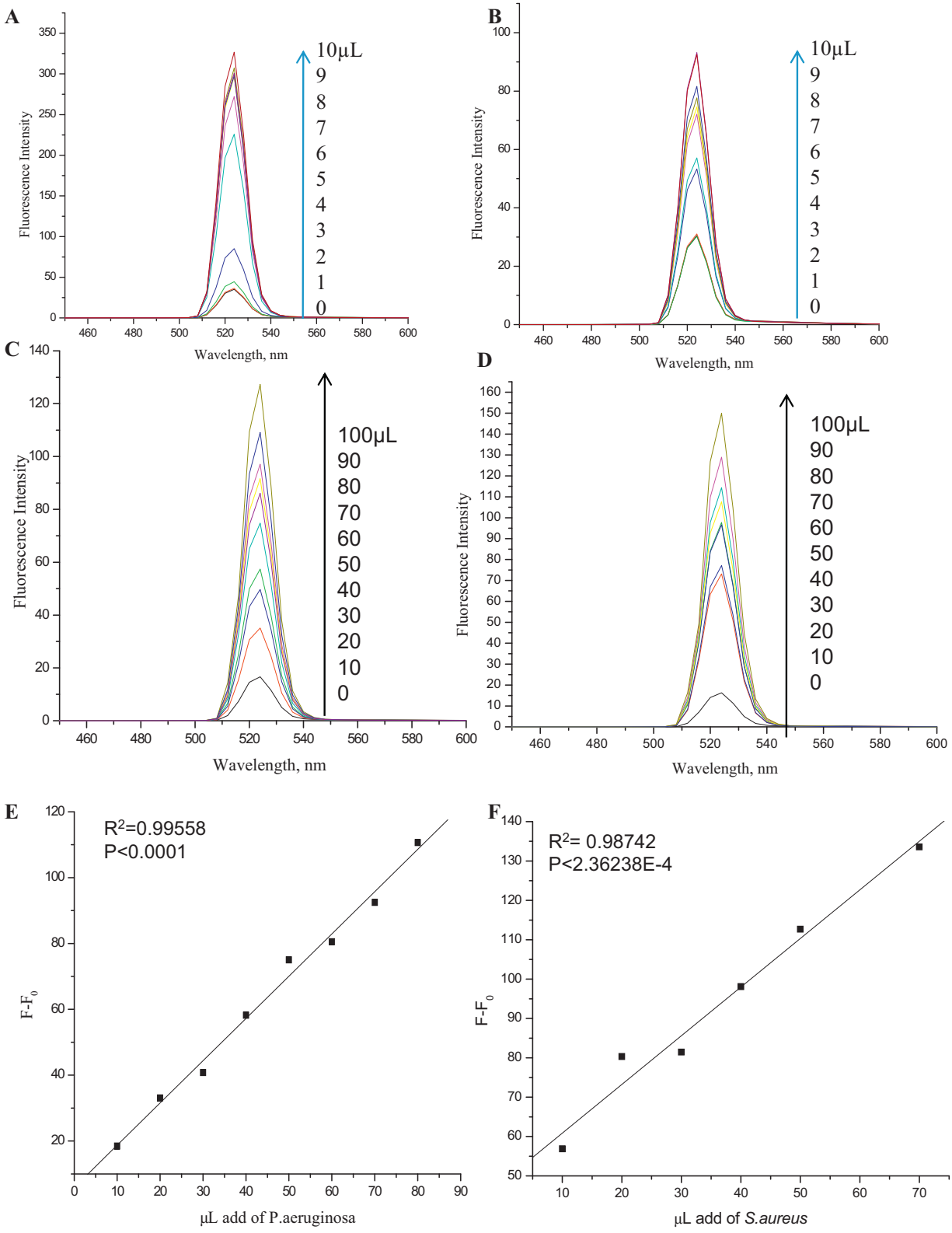

Fig. 4. Limit of detection of (A) *P. aeruginosa* and (B) *S. aureus*. Blood analysis of (C) *P. aeruginosa* and (D) *S. aureus* that give linear relationship at (E, F), respectively.

human health. For instance, endotoxin, or lipopolysaccharide of Gram-negative bacteria "*P. aeruginosa*" contributes greatly to the structural integrity of the bacteria, and protecting the membrane from certain kinds of chemical attack. The biological activity of an endotoxin is associated with the lipopolysaccharide (LPS), while the toxicity is associated with the lipid component (lipid A), and the immunogenicity is related to the polysaccharide components. MALDI-MS spectra of *P. aeruginosa* (Fig. 3A) and *S. aureus* (Fig. 3B) indicated change of polythiophene mass profile (*m/z*) due to transferring the hydrophobic bacteria lysates. The extracted crude of *P. aeruginosa* (Fig. 3A) indicates change in the range 700–800 and 900–1100 Da. While the *S. aureus* (Fig. 3B) generated mass signals

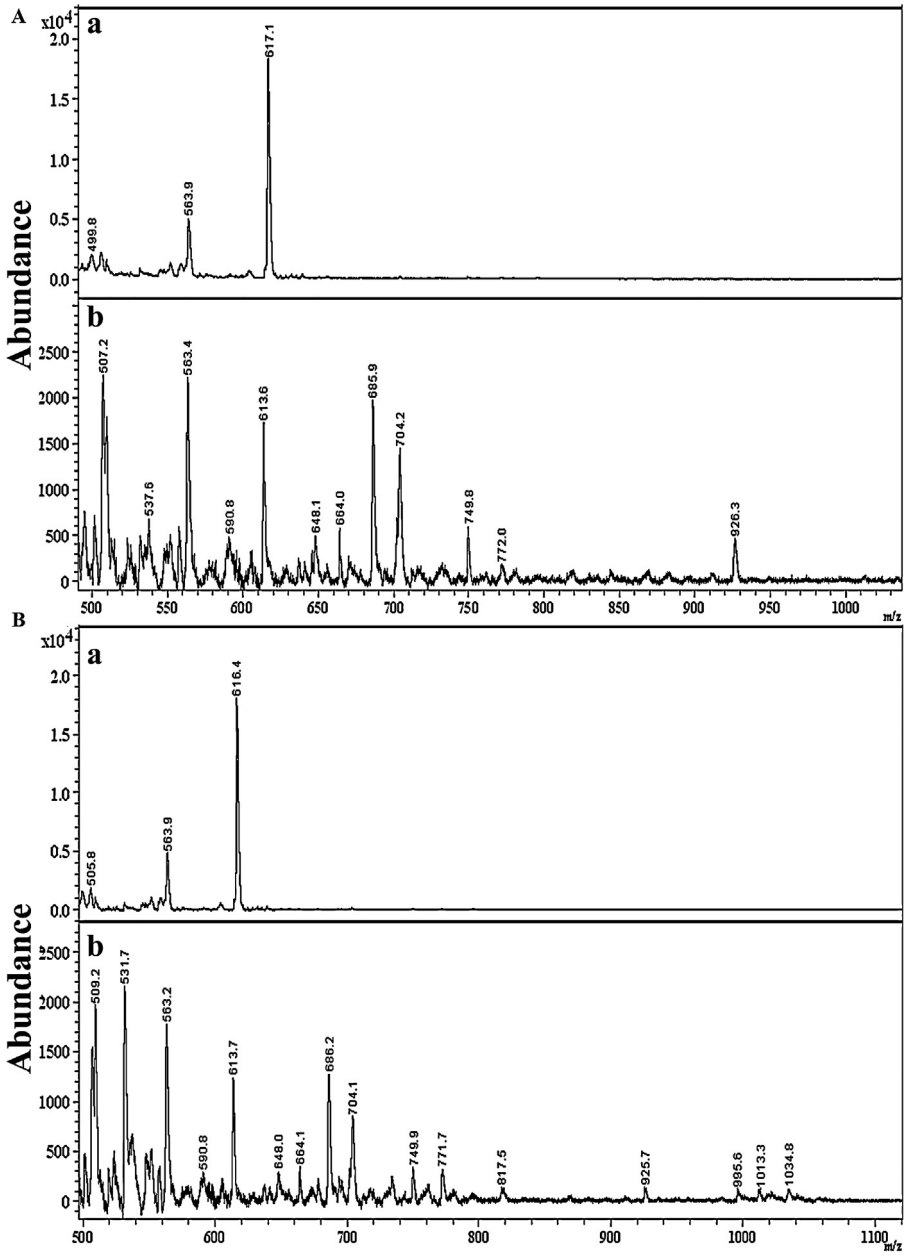

Fig. 5. MALDI spectra of blood analysis for (A) *P. aeruginosa* and (B) *S. aureus* using DHB as matrix (a) before and (b) after separation.

at 700–850 Da. The extracted biomolecules were highlighted in gray mark (35% Darker). The results reveal a pattern of lipolysaccharides with difference of 24 Da, which is corresponding to the endotoxin moieties (Fig. 3A) [55]. Fig. 3B (*S. aureus*) shows only three peaks at 740.0, 772.0 and 7985.8 Da. Comparing MALDI-MS with fluorescence, the former is a destructive technique and cannot perform quantification, thus fluorescence spectroscopy was proposed as a complementary instrument. Recently, a non-invasive Raman method has been proposed to quantify cellular lipid of fungus [67].

4.4. Fluorescence biosensing on blood samples

Generally, the biological samples are complex mixtures with extremely low analyte contents and abundance. To evaluate the fluorescence sensitivity, small volumes (1–10 μL) of bacteria suspensions were detected. Fig. 4A (*P. aeruginosa*, 1×10^4 cfu/mL) shows lowest volume of detection is 2 μL, while Fig. 4B (*S. aureus*, 1×10^5 cfu/mL) is 4 μL. The lower limits of detection of Gram-negative bacteria (*P. aeruginosa*) than that of the Gram-positive bacteria (*S. aureus*) is due to the higher contents of lipopolysaccharide or other hydrophobic biomoleules in *P. aeruginosa*. Moreover, the Gram-positive bacteria have thick peptidoglycan layers than the Gram-negative bacteria. The presence of peptidoglycan can increase the hydrophobic interactions, if the bacteria directly interact with polymers via hydrophobic interactions [62,68].

Direct analysis of blood sample is a challenge task due to the intensive interferences originated from blood cells, proteins, peptides, and high salt contents. Bacteria in blood "bacteremia" can cause serious health problems such as sepsis, septic shock, and inflammation. Patients need blood transfusion typically face a fatal problem, if the blood is infected by bacteria. Because the recipients of blood transfusions are usually critically sick and seriously has immunity suppression. Thus, analysis of bacteria in blood sample is extremely important. Biosensing the hydrophobic biomolecules can provide the information for bacterial infection, and it is also

a rapid quantitative methodology for bacteria analysis. Traditional method for bacteria determination requires minimum 2–8 days for confirmation [52], but it is low sensitivity, selectivity, and tedious in procedures. The blood content, such as mineral salts, plays a significant role on bacteria growth rate such as suppression due to mineral salts. Furthermore, dead bacteria cells cannot be detected due to lack of fluorescence of the excreted biomolecules in the blood. Thus, we introduce a new methodology of bacteria quantification based on detecting the hydrophobic bacteria biomolecules, which were transferred from the aqueous bacterial suspension to the polymer dots in organic solvent. It can also give indication for dead cells and respond on why there is increase in the immune system.

Pathogenic bacteria were spiked into mice blood samples. Different volumes (0–100 μL) of blood suspension were added to 1 mL of polymer dot (0.5 mg/mL) suspension. Upon addition of different volumes of *P. aeruginosa* (Fig. 4C) and *S. aureus* (Fig. 4D), the polythiophene fluorescence offer signals enhancement due to the transferring hydrophobic biomolecules to the organic phase and aggregation via hydrophobic interactions. Different volume of *P. aeruginosa* (Fig. 4E) and *S. aureus* (Fig. 4F) offer linear relationship with differential fluorescence signal (F–F_0), where "F" is the fluorescence signals after extraction and "F_0" fluorescence represent the signals of polymer dots in blood. The linear regression (R^2) values of both plots were inserted into each figure (Fig. 4).

Comparing the current approach to the traditional methods, the polymer dots approach is rapid, sensitive, extremely low sample consumption, and no complicated procedures or treatment is required. Traditional approaches for quantifying hydrophobic biomolecules such as lipids from pathogenic bacteria rely on labor work, expensive equipment, and intensive methods, such as gas chromatography–mass spectrometry (GC–MS). Analysis of these biomolecules requires also derivatization due to lack of volatility and thermo-stability. In the conventional methods [55,62], the accurate quantity of hydrophobic biomolecules can only be obtained after the growth of the microorganism; so, the culture steps requires experience and also need large number of experiments to obtain accurate data. Besides, large volumes of biological samples are required due to low amounts of hydrophobic biomolecules. In contrast, our approach only needs extremely low amount of biological samples (several microliters) for quantification and detection of the bacteria. Because the sample volume is very small, no need to separate the aqueous layer. The present approach is considered more selective for bacteria than the conventional methods due to two reasons: (1) the interactions between the bacteria with the polymer dots depending on the hydrophobic portions, which were transferred to the organic layer. (2) The hydrophilic biomolecules and salts in the blood samples have been excluded. Nanomaterial such as quantum dots (QDs), graphene (G) applied for bacteria biosening display disadvantages such as aggregation, cytotoxicity, expensive, and requires functionalization [1–6,52,66,68,69].

4.5. Matrix assisted laser desorption/ionization analysis of extracted biomolecules from pathogenic bacteria for mice blood sample

To further identify the compounds that undergoes mass transfer from the blood sample contaminated bacteria to organic layer (Pdots), MALDI-MS was used (Fig. 5). Referring to the standard solution, we found that these changes were due to biomolecules that originated from bacteria. MALDI-MS data (Fig. 5) revealed that the mass transfer of bacteria biomolecules from aqueous solution to organic layer. Note that mass peaks may be shifted due to the bacteria related biomolecules with the blood interferences such as metals or other molecules during mass analysis. These interferences were excluded in fluorescence analysis as we draw the linear relationship between the fluorescence in presence and absence of bacteria (F–F_0) and bacteria concentration. Furthermore, these materials (Pdots) suppress all the autofluorescence that come from the cells as it has long life time.

5. Conclusion

The growing interest in conducting polymer both scientifically and economically is not surprising due to the remarkable physicochemical properties that have been reported during the last two decades. Thus, we reported a novel and low cost biosensor and MALDI-MS analysis method for detection and quantification of hydrophobic biomolecules in pathogenic bacteria from biological samples. The novel quantitative assessment does not require cultivation and only require tiny volume (2–4 μL) of samples. Due to the high brightness of polymer dots, small concentration of polymer is required (0.5 mg/mL). To measure the hydrophobic moieties of pathogenic bacteria for biosensing have some advantages than those techniques deal with whole cells as it is rapid, simple, and sensitive. Furthermore, it can also provide information about dead infected cells that adds new knowledge to our understanding of pathogenic infection and provides insight into developing new biosensing approaches, and ultimately our approaches can be effective in pathogenic bacteria diagnosis after treatment. These novel and effective platforms for pathogenic bacteria analysis certainly will make significant contribution to medicine and biomedicine.

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Notes

The authors declare no competing financial interest.

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