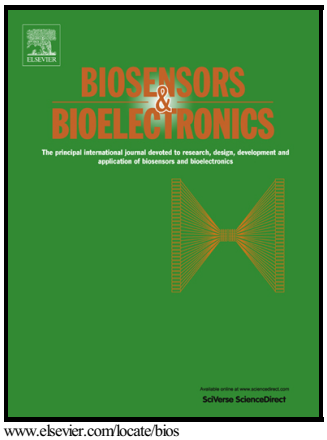

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Smartphone-based portable biosensing system using impedance measurement with printed electrodes for 2, 4, 6-trinitrotoluene (TNT) detection

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Abstract

Rapid, sensitive, selective and portable detection of 2,4,6-trinitrotoluene (TNT) is in high demand for public safety and environmental monitoring. In this study, we reported a smartphone-based system using impedance monitoring for TNT detection. The screen-printed electrodes modified with TNT-specific peptides were used as disposable a biosensor to produce impedance responses to TNT. The responses could be monitored by a hand-held device and send out to smartphone through Bluetooth. Then, the smartphone was used to display TNT responses in real time and report concentration finally. In the measurement, the system was demonstrated to detect TNT at concentration as low as 10^{-6} M and distinguish TNT versus different chemicals in high specificity. Thus, the smartphone-based biosensing platform provided a convenient and efficient approach to design portable instruments for chemical detections such as TNT recognition.

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Keyword: Peptide, Smartphone, Portable instrument, Screen-printed electrode, Impedance, 2,4,6-trinitrotoluene (TNT)

1. Introduction

Over last couple of decades, explosive detections, especially for 2,4,6-trinitrotoluene (TNT), attracted worldwide interests because of their threats to public security and human health as pollutants in natural water, soil and air (Caygill et al. 2012; Habib 2007; Steinfeld and Wormhoudt 1998). The most conventional method for explosive detection is to use animals such as sniffer dogs. Due to excellent abilities of biological olfactory perceptions, the well-trained sniffer animals can discern explosive molecules sensitively and selectively in complex environment. But, it has to cost a great amount of money and time to train and maintain sniffer animals, which are difficult to thoroughly control and can only be deployed for a very limited period. Thus, significant efforts were made to develop electronic sensors with techniques, such as ion spectrometer, electrochemistry, surface plasmon resonance and quartz crystal microbalance, to detect explosive compounds rapidly, selectively and sensitively (Mäkinen et al. 2011; Smith et al. 2008). And, employing complex bio-inspired elements such as Escherichia coli, fluorescent proteins and polymers, some kinds of biosensors were also developed by more and more groups to mimic biological specific sensing for explosives in recent years (Gingras et al. 2013; Yagur-Kroll et al. 2014; Yatabe et al. 2013).

Smartphone is the most widely used mobile device in the world, having great built-in functions such as touch-screen display, advanced computing capability and powerful data storage. Recently, it played increasingly important roles in portable sensor systems as platforms to receive, analyze and display detecting signals (Lillehoj et al. 2013; Vashist et al. 2014; Zangheri et al. 2015). These portable systems often used sensor devices on smartphones or added smartphone-linked sensor accessories to sense different types of information ranging from optical spectroscopy to electrochemical current. In several biosensing cases, smartphones were also used to replace scientific instruments to detect biomolecular bindings (Boero et al. 2014; Liu et al. 2014; Roda et al. 2014). Various sensor techniques such as fluorescence, electrochemistry and plasmonic resonance was linked to smartphones progressively and used in biodetections for targets such as bacteria and biomarkers (Jiang et al.

2014; Rajendran et al. 2014; Roda et al. 2014; Souza Filho et al. 2014).

Electrochemical impedance spectroscopy (EIS) was one of the most common detecting methods in biosensor studies (Grieshaber et al. 2008). In electrochemical measurement, three-electrode system was a conventional fabrication which employs gold electrode, platinum electrode and Ag/AgCl electrode as working electrode, counter electrode and reference electrode respectively. However, the sizes of traditional electrodes were often large and give difficulties to integrate electrodes into portable instruments. Recently, screen-printed electrodes were fabricated and used in electrochemical analyses (Piermarini et al. 2013; Sekretaryova et al. 2014; Teixeira et al. 2014). Working electrode, reference electrode and counter electrode were printed with multi layers of printed inks on surface of flat substrates such as paper and plastic materials. Printed electrodes were always small in size, lightweight and low-cost, allowing their wider applications in portable and disposable biosensors (Rafiee and Fakhari 2013; Ravalli et al. 2013). Thus, printed electrodes were a good choice to develop the transducers for miniaturized and portable detecting systems.

As one kind of new biosensing elements, peptides are short amino acid chain which can be designed in specific sequence and synthesized artificially with chemical methods. It provides an easy way to design and obtain artificial receptors to mimic biosensitivities. Thus, peptides were applied as biosensing elements in several recent biosensor designs for healthcare diagnosis, environment monitoring and food analysis (Cui et al. 2012; Li et al. 2013; Pavan and Berti 2012). Without chain folding, robust structures of peptides can also be used in more extreme conditions and had long-term storage, compared to natural biomaterials such as proteins with complex folding structures. Thus, it is a high sensitive and selective biosensing component which can work in simple and portable instruments without complicated environmental controlling.

Here, a portable smartphone-based biosensing platform for TNT detection was developed with impedance monitoring on screen-print electrodes. The screen-print electrodes were modified by TNT-specific peptide and showed sensitivity to TNT in alternating current (AC) impedance around 20 kHz. A hand-held device that contained AD5933 impedance analyzer chip and Arduino microcontroller was developed to detect AC impedance of the modified electrodes and deliver impedance data to smartphone through Bluetooth module. An

Android application program (App) was also designed to control impedance measurement and visualize recording data in real time. Combined with smartphone, the portable biosensing platform successfully detected TNT as low as 10^{-6} M and recognized TNT versus other explosives and several common odorants.

2. Methods and materials

2.1. Chemicals and reagents

The peptide was designed into TNT-specific sequence (WHWQRPLMPVS) based on reports about TNT-specific amino acids (Jaworski et al. 2008; Kuang et al. 2010). The polypeptide was from GenScript (>95% purity) and stored in the form of freeze-dried powders. It was dissolved in phosphate buffer solution (PBS, 0.1 M, pH 7.2) at 250 μg/ml for electrode modification. TNT was diluted into eight different concentrations with methanol solution, while concentration of the standard TNT solution was 1 mg/ml. β-ionone, isoamyl acetate, acetic acid and butanedione of 1 mM were used as the negative control for selective test. 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ (1:1) was employed as redox couple in aqueous solution for electrochemical measurements. All chemical regents were of analytical grade and purchased from Sigma-Aldrich.

2.2. TNT-specific peptide modified electrodes

As shown in Figure.1a, peptides could be immobilized on surface of the electrodes and specifically bind to TNT molecules, which inhibited electron transfer on surface of the electrode and increased interface impedance. The printed electrodes were provided by GSI Technologies (Burr Ridge, IL, USA) and fabricated by continuous web-fed screen printing press (Lupo et al. 2013). The carbon electrode was printed on 3 cm×1 cm polyethylene glycol terephthalate (PET) substrate as working electrode and counter electrode, while reference electrode was printed by silver (Figure.1b). The diameter of the working electrode was 3 mm. The electrical connects on the bottom of the printed electrode were also made of silver and could be linked to sensor interface of the smartphone-based system with special socket.

The modification of the electrodes with peptides utilized the direct physical absorption, which was an easy and low-cost way to fabricate of disposable biosensors with printed

electrodes (Liu and Crooks 2012; Nie et al. 2010). 30 μl peptide solutions at 250 μg/ml were spotted on the surface of working electrodes. The solutions of peptides were diffused and distributed evenly on the electrodes due to the surface tension. The electrodes were incubated and dried for 12 h at 4 °C. After the solutions dried, the peptides were uniformly absorbed on the surface of the electrodes, which could be used for TNT detections. The modified electrodes could be stored under 4 °C and kept bioactivity more than 1 week.

Fig 1

2.3. Determination of the impedance properties using EIS

The EIS was performed by electrochemical workstation (CHI660E, Chenhua, China) to evaluate peptide immobilization and frequency-impedance properties of binding between peptide and TNT, respectively. In the EIS measurement, the working electrodes of the printed electrodes were connected to input of the electrochemical workstation, while the reference electrode and counter electrode were connected together to the output. The frequency was scanned from 10 kHz to 1000 kHz with 200 mV alternating voltage. 10 μl TNT at different concentrations were added to the electrodes for recording the electrochemical impedance spectroscopy, when methanol solution was used as blank control. EIS was recorded and calculated into normalized impedance change (NIC), which was described as:

$$NIC = \frac{Z_a - Z_b}{Z_b} \qquad (1)$$

where Z_a and Z_b were impedance of electrodes with and without TNT. The printed electrodes were all disposable in the measurements.

2.4. Impedance monitoring based on smartphone

The impedance monitoring system was designed to record time-impedance scanning. As schematic diagram shown in Figure.1c, the smartphone-based system included two devices: a hand-held wireless device and a smartphone. The hand-held device had bio-modified electrodes, impedance converter network analyzer (AD5933 and LM358 circuit), micro

controller (Arduino board) and Bluetooth module (HC-06 shield). The impedance analyzer sent out sinusoidal signals into the working electrode of the bio-modified electrodes as AC stimuli, and monitored feedback signals from the reference and counter electrodes of the printed electrodes. In presence of TNT, impedance signals could be sent back to the smartphone through Arduino board and Bluetooth module, and displayed on smartphone immediately. The microcontroller, Arduino board, could receive commands from smartphone through Bluetooth module and control impedance parameters of AD5933 circuit, such as starting, finishing point and AC frequency of the stimuli signals. AD5933 circuit and Bluetooth module was integrated into an expansion printed circuit board, which could snap on the top of Arduino board (Figure.1d).

An App was developed on smartphone to control impedance measurement, receive real-time data and plot impedance changes on screen. As shown in Figure.1e, there were four buttons on welcome screen of the App. The 'Connect' button was used to search and link the portable impedance monitoring device with the smartphone through Bluetooth. The 'Calibrate' button was set up to calibrate the measurement device with reference sample resistance. The 'Start' and 'Exit' button had functions to enter real-time monitoring user interface and terminate the program respectively. In the measurement, impedance monitoring curve could be plotted in real time and impedance values were given synchronously. Finally, the concentration of TNT could be reported on the screen of the smartphone.

2.5. TNT Detection using smartphone-based system

The time-impedance scanning was carried out by the smartphone-based system at frequency of 20 kHz for performance evaluations and TNT detections, respectively. The alternating voltage was also fixed at 200 mV and scanning interval was 1 s. In the performance test for the system, sensor interface was connected to resistances ranging from 100 kΩ to 1 MΩ. The resistances were measured at different frequencies for 120 s. The coefficient of variation (CV) was the ratio of the standard deviation to the mean value, which represented detecting stability of the system. The difference was defined using following equation:

$$Difference = \frac{Z_m - Z_r}{Z_r} \qquad (2)$$

where, Z_r represents the impedance value measured the electrochemical workstation, which could be regarded as real value of resistive and capacitive components, Z_m represents the impedance value measured by the smartphone based system. The maximum difference was calculated in the group containing 120 samples to evaluate the detecting accuracy of the system. In TNT detection, two ports of the sensor interface in the system were connected to working electrode and reference electrode respectively, when the reference electrode and counter electrode were linked together by electrical contacts. The whole recording kept 120 s, and TNT was added in the same way as that of EIS measurement. The impedance monitoring curve could be plotted in real time when impedance values were normalized with the initial recording point. The NIC values were given to calculate the TNT concentrations. All of the impedance detections were performed at room temperature (22 °C).

3. Results

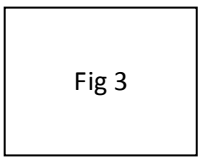
3.1. Impedance properties of the TNT and peptide binding

To determine peptide modification, EIS of the electrodes were also measured in the presences of 10 μl $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$. As shown in Figure.2a, the impedance of the electrodes increased significantly in Nyquist plot after the modification. It suggested that the printed electrodes were modified by TNT-specific peptides successfully and could be used as impedance transducers in TNT detection. In biosensing cases, impedance readout was often frequency-dependent. Thus, EIS measurement was used to determine sensitive frequency range and optimal frequency point for impedance monitoring of binding between TNT and peptides on the surface of the electrodes, when methanol solvent of TNT solution was used as supporting electrolyte. The frequency-dependent impedance properties of the peptide modified electrodes could be observed in Figure.2b. In presence of TNT, the impedance spectroscopy showed significant increase in low frequency range from 10 kHz to 30 kHz, while no changes could be observed in high frequency range. The impedance had the most significant increase around 20 kHz in the whole frequency range of 10 - 1000 kHz (Figure.2c). Especially, the AC impedance change of electrodes at 20 kHz was larger than that of 15 kHz

and 25 kHz, although they all increased significantly with concentrations of TNT (Figure.2d). Thus, the optimal frequency point of impedance measurement for TNT detection was at 20 kHz, which could be used in fixed frequency impedance monitoring based on smartphone.

3.2. Impedance test using the smartphone-based system

Before TNT detections, impedance test of the smartphone-based impedance monitoring system was carried out to determine whether the system could detect impedance changes accurately and stably. The measurement frequency of the system was fixed at 20 kHz, which was the optimal frequency point for TNT detection. As shown in Figure.3a and b, the performance of the system were respectively tested with resistances and capacitances. The tested resistances ranging from 100 kΩ to 1 MΩ were used to mimic the impedance of the electrodes in TNT detection. The system showed stable impedance recording for resistances, although having slight fluctuations for large resistances of 1 MΩ. Similarly, the system could also measure different capacitive impedance accurately and stably for long-term recoding. Table.1 further illustrated the detecting stability and accuracy of the system in the recording of 120 s. The CV values for resistive and capacitive measurements were all around 1% - 2%. The detecting differences of resistance measurements were often less than 2%, while those of capacitances were around 5%. Thus, the accuracy and stability of the smartphone-based system satisfied the demand for impedance monitoring in TNT detections.



Beside stability and accuracy, the efficient working range in frequency was also an important parameter of the system. Thus, performance of the system was tested at different frequency points (Figure.3c and Table.S1). The system could detect resistive impedance accurately from 10 kHz to 100 kHz, without significant changes with frequency. In contrast,

capacitive impedance decreased with frequency, keeping inverse relationship with value of frequency. These results suggested good detecting accuracy of the system in frequency range from 10 kHz to 100 kHz.

Tab 1

3.3. TNT detection using the smartphone-based system

The smartphone-based system was used to detect TNT through real-time impedance monitoring with the peptide modified electrodes. As shown in Figure.4a, the real-time impedance change was normalized and plotted on the smartphone screen, when the impedance values and detection reports were displayed on the bottom. Figure.4b showed the impedance change recorded by the system without and with TNT. All data were normalized with the impedance values of initial recording points. The whole impedance monitoring lasted for 120 s. TNT was added on surface of the modified electrodes at the point of 50 s after beginning of the recording. After a slight and very rapid decrease, the binding of TNT to peptides on the electrodes would increase the impedance dramatically into a high plateau phase. Ultimately, significant impedance increase could be observed. To explore relationship between the responses and concentrations of TNT, NICs were calculated from average value of recording points in high plateau phase and plotted with the concentrations (Figure.4c). A dose-dependent curve of the smartphone-based system was fitted with NIC from responses to TNT at different concentrations as follow:

$$NIC = 0.1491\log(C) + 0.9517 \qquad (3)$$

where C represents the concentrations of TNT. The curve showed a high similarity to calibration curve from measurements by CHI660E electrochemical workstation in same conditions, which indicated reliability of the smartphone-based system for TNT detections. To further test accuracy of the system for TNT detection, a measurement of prepared TNT solution at 5×10^{-5} M was performed. The NIC value of the response was 0.2981, which was plotted as blue cross in Figure.4c. According to the fitting curve, the measured concentration

was calculated into 4.13×10^{-5} M, which showed the relative error of about 17.4 % with respect to the actual concentration.

Fig 4

Besides sensitivity, different kinds of chemicals were used to test the selectivity of the system for TNT detection. These chemicals included some analogues of TNT, such as 2,4-dinitrotoluene (DNT) and 2-nitrotoluene (1NT), and several common odorants usually used in olfactory sensor study, such as isoamyl acetate, acetic acid, β-ionone and butanedione. The NIC responses to these chemicals were obtained in same way as that of TNT detections and shown in Figure. 5. It was obvious that the NIC elicited by TNT was significantly larger than those of common odorants, isoamyl acetate, acetic acid, β-ionone and butanedione, which almost had no impedance changes. However, the system showed responses to DNT and 1NT, which both shared similar benzene ring with TNT. In selective test for DNT, the system offered NIC value of 0.1875±0.0964 in statistics, which was about one third of the response to TNT. The system also showed slight response to 1NT. The results might attribute that molecular structure of DNT was more similar to TNT than that of 1NT. Moreover, the electrodes without peptide modification had no significant responses to all chemicals, even including TNT. It indicated that the selectivity and sensitivity of the smartphone-based system was really from the specific conjunctions between peptides and TNT.

Fig 5

4. Discussions

4.1. Performance of the smartphone-based system for TNT detection

Electrochemical impedance is useful to monitor changes in electrical properties arising from binding events at surfaces of electrodes. For instance, AC impedance changes of electrodes often can be measured as results of protein immobilization and antigen-antibody

reactions on electrode surfaces (Chikkaveeraiah et al. 2012; Lu et al. 2014). In this study, the printed electrodes were used as impedance transducer, while TNT-specific peptides were employed as biosensitive components. Compared to traditional large electrodes, the printed electrodes were low-costing and easy-to-use. Thus, the biosensor for TNT detection could be developed into disposability and portability. On the other hand, peptides had more excellent robust bioactivities which could be less affected by environmental conditions such as temperature and humidity. The biosensor with peptides could work outside the laboratory controlled environment and had mobile detection abilities. It showed a great advantage than biosensors using complex proteins, cells and organs from animals (Gingras et al. 2013; Radhika et al. 2007; Smith et al. 2008). Furthermore, the hand-held device, recording the impedance change from the biosensor was also much smaller than commercial electrochemical systems in size and could be easily integrated into miniaturized systems. Ultimately, the combination of the TNT-specific peptides, printed electrodes and the device using Arduino controllers provided a low-cost portable smartphone-based system for mobile detection of TNT.

The smartphone-based system showed a good performance in the test of TNT. Similar to several biosensors using impedance detection (Dastider et al. 2013; Lin et al. 2012; Wang et al. 2014), the impedance signals recorded by the system had linear relationship with concentrations of TNT. The detection limit of the system was as low as 7.09×10^{-7} M with 3δ/slope calculation for the dose-dependent fitting curve. As shown in Table.S2, the detection limit was lower than reported portable sensor with glassy carbon electrodes，while kept in same order of magnitude as that using similar screen-printed electrodes. But, it was not excellent in comparison to several other methods using other different electrodes (Casey and Cliffel 2015; Caygill et al. 2013; Goh and Pumera 2011). Thus, more different format of electrodes could be attempt in our system to improve its sensing performance. Moreover, by utilizing the fitting curve, the system could also measure the concentrations of prepared TNT with recorded NIC value, although having certain relative errors. In the selective test, the system could specifically distinguish TNT versus other chemicals, when having slight responses to DNT and 1NT. Thus, the smartphone-based impedance monitoring system could be successfully used for TNT detection.

4.2. Potential biosensor applications of the smartphone-based system

In recent years, a lot of portable sensor instruments were developed for different applications such as pH sensing, cell monitoring and food component analysis (Dos Santos et al. 2013; Tsai et al. 2014; Yunus et al. 2011). The portable sensor instruments integrated with miniaturized sensors and detecting circuits were designed into smaller and smaller size, which made higher request for mobility of storage and display equipment. Smartphones, as the most widely used device in the world, was a good choice to control measurement and display consequences because of its powerful functions. It was also easy to connect with internet, which was helpful to deliver and store data to cloud server for further analysis. Thus, several groups had linked fiber-optic sensor array, microfluidic chip and electrochemical sensors with smartphones to extend electrochemical and optical applications (Bishara et al. 2011; Kim et al. 2013; Lillehoj et al. 2013). In our study, the smartphone-based system using impedance monitoring could also be extended to perform other electrochemical analyses of EIS and cyclic voltammetry. Thus, a versatile electrochemical lab on smartphone would be built and used for various biosensing in fields such as point-of-care diagnosis, environment monitoring and food safety evaluation in the future.

5. Conclusion

In summary, we presented the design, fabrication and test of a smartphone-based impedance monitoring system which could detect TNT. Peptide modified screen-printed electrodes were used as biosensor for TNT. The smartphone-based system, consisting of hand-held impedance monitoring device and smartphone, could detect TNT as low as 10^{-6} M by real-time monitoring with a high specificity. Thus, the smartphone-based biosensing system provided a low-cost, potable and efficient platform to detect explosives such as TNT sensitively and selectively.

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References

Bishara, W., Sikora, U., Mudanyali, O., Su, T.-W., Yaglidere, O., Luckhart, S., Ozcan, A., 2011. Lab on a Chip 11(7), 1276-1279.

Boero, C., Casulli, M.A., Olivo, J., Foglia, L., Orso, E., Mazza, M., Carrara, S., De Micheli, G., 2014. Biosens. Bioelectron. 61(0), 251-259.

Casey, M.C., Cliffel, D.E., 2015. Anal. Chem. 87(1), 334-337.

Caygill, J.S., Collyer, S.D., Holmes, J.L., Davis, F., Higson, S.P., 2013. Analyst 138(1), 346-352.

Caygill, J.S., Davis, F., Higson, S.P.J., 2012. Talanta 88, 14-29.

Chikkaveeraiah, B.V., Bhirde, A.A., Morgan, N.Y., Eden, H.S., Chen, X., 2012. ACS Nano 6(8), 6546-6561.

Cui, Y., Kim, S.N., Naik, R.R., McAlpine, M.C., 2012. Acc. Chem. Res. 45(5), 696-704.

Dastider, S.G., Barizuddin, S., Dweik, M., Almasri, M., 2013. RSC Advances 3(48), 26297-26306.

Dos Santos, C.A.T., Lopo, M., Páscoa, R.N., Lopes, J.A., 2013. Appl. Spectrosc. 67(11), 1215-1233.

Gingras, A., Sarette, J., Shawler, E., Lee, T., Freund, S., Holwitt, E., Hicks, B.W., 2013. Biosens. Bioelectron. 48, 251-257.

Goh, M.S., Pumera, M., 2011. Anal. Bioanal.Chem. 399(1), 127-131.

Grieshaber, D., MacKenzie, R., Voeroes, J., Reimhult, E., 2008. Sensors 8(3), 1400-1458.

Habib, M.K., 2007. Biosens. Bioelectron. 23(1), 1-18.

Jaworski, J.W., Raorane, D., Huh, J.H., Majumdar, A., Lee, S.-W., 2008. Langmuir 24(9), 4938-4943.

Jiang, J., Wang, X., Chao, R., Ren, Y., Hu, C., Xu, Z., Liu, G.L., 2014. Sens. Actuat., B 193, 653-659.

Kim, U., Ghanbari, S., Ravikumar, A., Seubert, J., Figueira, S., 2013. IEEE Journal of Translational Engineering in Health and Medicine 1, 1-7.

Kuang, Z., Kim, S.N., Crookes-Goodson, W.J., Farmer, B.L., Naik, R.R., 2010. ACS Nano 4(1), 452-458.

Li, M., Cushing, S.K., Zhang, J., Suri, S., Evans, R., Petros, W.P., Gibson, L.F., Ma, D., Liu, Y., Wu, N., 2013. ACS Nano 7(6), 4967-4976.

Lillehoj, P.B., Huang, M.-C., Truong, N., Ho, C.-M., 2013. Lab on a Chip 13(15), 2950-2955.

Lin, Z., Chen, L., Zhang, G., Liu, Q., Qiu, B., Cai, Z., Chen, G., 2012. Analyst 137(4), 819-822.

Liu, H., Crooks, R.M., 2012. Anal. Chem. 84(5), 2528-2532.

Liu, X., Lin, T.-Y., Lillehoj, P.B., 2014. Ann. Biomed. Eng., 1-13.

Lu, Y., Li, H., Zhuang, S., Zhang, D., Zhang, Q., Zhou, J., Dong, S., Liu, Q., Wang, P., 2014. Sensors and Actuators B: Chemical 193, 420-427.

Lupo, D., Clemens, W., Breitung, S., Hecker, K., 2013. OE-A Roadmap for Organic and Printed Electronics. In: Cantatore, E. (Ed.), Applications of Organic and Printed Electronics, pp. 1-26. Springer US.

Mäkinen, M., Nousiainen, M., Sillanpää, M., 2011. Mass Spectrom. Rev. 30(5), 940-973.

Nie, Z., Nijhuis, C.A., Gong, J., Chen, X., Kumachev, A., Martinez, A.W., Narovlyansky, M., Whitesides, G.M., 2010. Lab on a Chip 10(4), 477-483.

Pavan, S., Berti, F., 2012. Anal. Bioanal.Chem. 402(10), 3055-3070.

Piermarini, S., Migliorelli, D., Volpe, G., Massoud, R., Pierantozzi, A., Cortese, C., Palleschi, G., 2013. Sens. Actuat., B 179(0), 170-174.

Radhika, V., Proikas-Cezanne, T., Jayaraman, M., Onesime, D., Ha, J.H., Dhanasekaran, D.N., 2007. Nat. Chem. Biol. 3(6), 325-330.

Rafiee, B., Fakhari, A.R., 2013. Biosens. Bioelectron. 46, 130-135.

Rajendran, V., Bakthavathsalam, P., Jaffar Ali, B., 2014. Microchim. Acta 181(15-16), 1815-1821.

Ravalli, A., dos Santos, G.P., Ferroni, M., Faglia, G., Yamanaka, H., Marrazza, G., 2013. Sens. Actuat., B 179, 194-200.

Roda, A., Michelini, E., Cevenini, L., Calabria, D., Calabretta, M.M., Simoni, P., 2014. Anal. Chem. 86(15), 7299-7304.

Sekretaryova, A.N., Vagin, M.Y., Beni, V., Turner, A.P.F., Karyakin, A.A., 2014. Biosens. Bioelectron. 53(0), 275-282.

Smith, R.G., D'Souza, N., Nicklin, S., 2008. Analyst 133(5), 571-584.

Souza Filho, C.A.d., Lima, A., Neff, H., 2014. Smartphone based, portable optical biosensor utilizing surface plasmon resonance. Instrumentation and Measurement Technology Conference (I2MTC) Proceedings, 2014 IEEE International, pp. 890-895. IEEE.

Steinfeld, J.I., Wormhoudt, J., 1998. Annu. Rev. Phys. Chem. 49(1), 203-232.

Teixeira, S., Conlan, R.S., Guy, O.J., Sales, M.G.F., 2014. Electrochim. Acta 136(0), 323-329.

Tsai, S.L., Chiang, Y., Wang, M.H., Chen, M.K., Jang, L.S., 2014. Electrophoresis 35(16), 2392-2400.

Vashist, S.K., van Oordt, T., Schneider, E.M., Zengerle, R., von Stetten, F., Luong, J.H., 2014. Biosens. Bioelectron. 67, 248-255.

Wang, H.-B., Zhang, H.-D., Xu, S.-P., Gan, T., Huang, K.-J., Liu, Y.-M., 2014. Sens. Actuat., B 194, 478-483.

Yagur-Kroll, S., Lalush, C., Rosen, R., Bachar, N., Moskovitz, Y., Belkin, S., 2014. Appl. Microbiol. Biotechnol. 98(2), 885-895.

Yatabe, R., Onodera, T., Toko, K., 2013. Sens. Mater 25, 45-56.

Yunus, S., Attout, A., Vanlancker, G., Bertrand, P., Ruth, N., Galleni, M., 2011. Sens. Actuat., B 156(1), 35-42.

Zangheri, M., Cevenini, L., Anfossi, L., Baggiani, C., Simoni, P., Di Nardo, F., Roda, A., 2015. Biosens Bioelectron 64, 63-68.

Illustration of the figures

Figure.1 The detection principle and design of the smartphone-based impedance monitoring system for TNT detection. (a) Binding of TNT to peptides on the surface of the electrodes, which can block electron transfer and increase interface impedance of the electrodes. Peptides were immobilized on electrodes by physical absorption. (b) Photo of the printed electrodes, containing working electrode, reference electrode and counter electrode. (c) Basic diagram of the smartphone-based system, which includes a hand-held monitoring device and a smartphone. (d) Picture of the impedance monitoring device with Arduino board and expansive board. The expansive board consists of Bluetooth module (1), AD5933 chip (2), LM358 chip (3) and sensor interface (4) connecting to the working and reference electrodes. (e) Welcome window of the App in smartphone for TNT measurement.

Figure.2 EIS measurement for the peptide modified electrodes. (a) Nyquist plot of the electrodes before (red) and after (blue) the peptide modification. (b) Impedance recording for the modified electrodes from 10 kHz to 1000 kHz in presence of TNT. (c) Statistics for NIC with TNT at different frequency points. (d) NIC increasing with concentrations of TNT at several frequency points in sensitive frequency range around 20 kHz. (mean ± SD,n=5).

Figure.3 Impedance test of the smartphone-based monitoring system. (a) Performance of the system for resistance measurement. (b) Performance of the system for capacitance measurement. Both resistances and capacitances are recorded at 20 kHz in 120 s. (c) Performance test of the system at frequency ranging from 10 kHz to 100 kHz for both resistive and capacitive components.

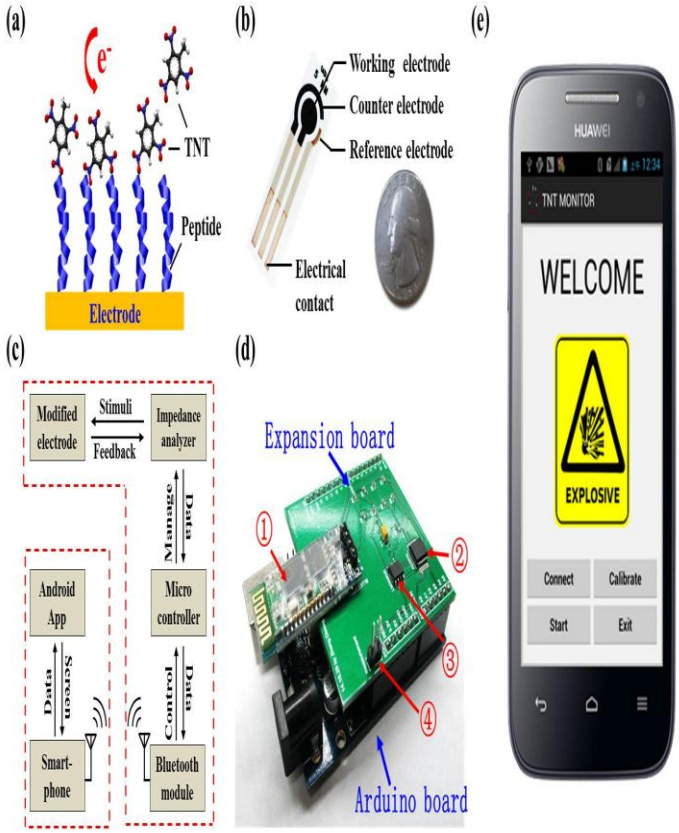
Figure.4 Measurement for TNT using the smartphone-based system. (a) The smartphone screen showing the measurement for TNT. The top half plots real-time impedance monitoring curve, when the bottom half gives information about real time impedance values and finally calculates concentrations. (b) The real-time monitoring recorded by the system in presence of TNT at different concentrations. (c) The dose-dependent fitting curve from meaurements of the smartphone-based system (green circle) and CHI660E electrochemical workstation (red

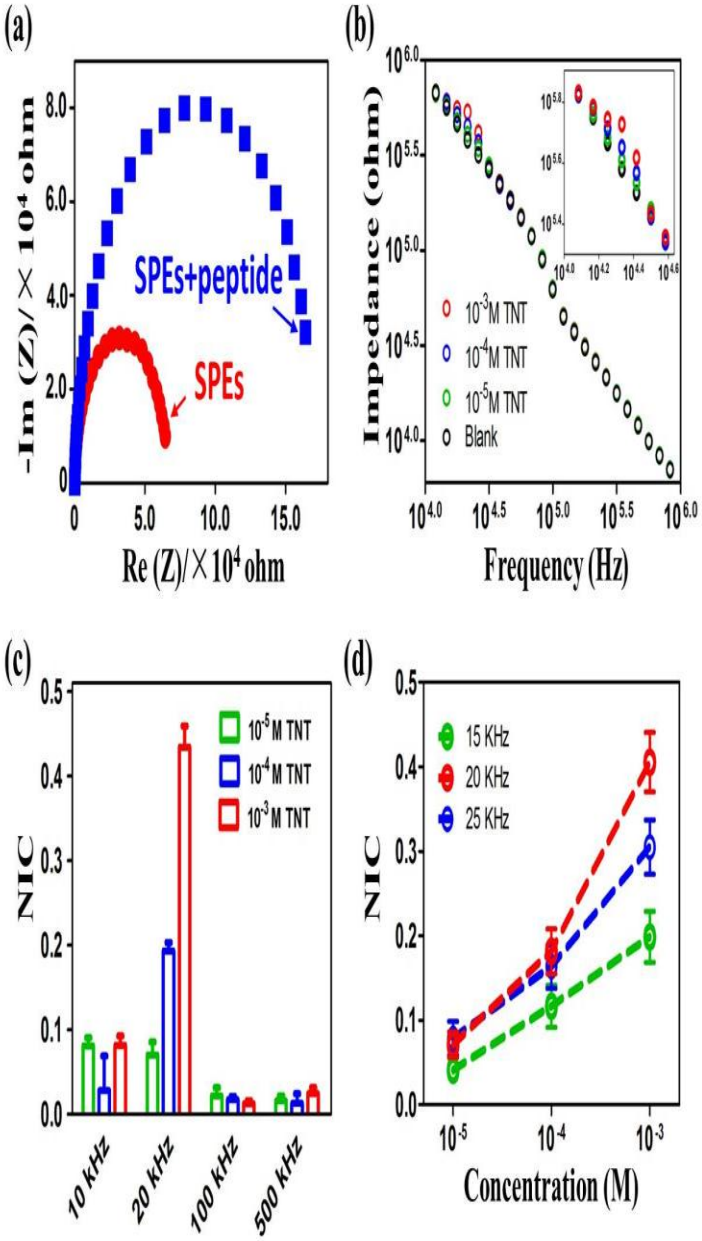
square) for NIC versus concentrations. The NIC response to prepared TNT solution at 5×10^{-5} M was shown as blue cross.

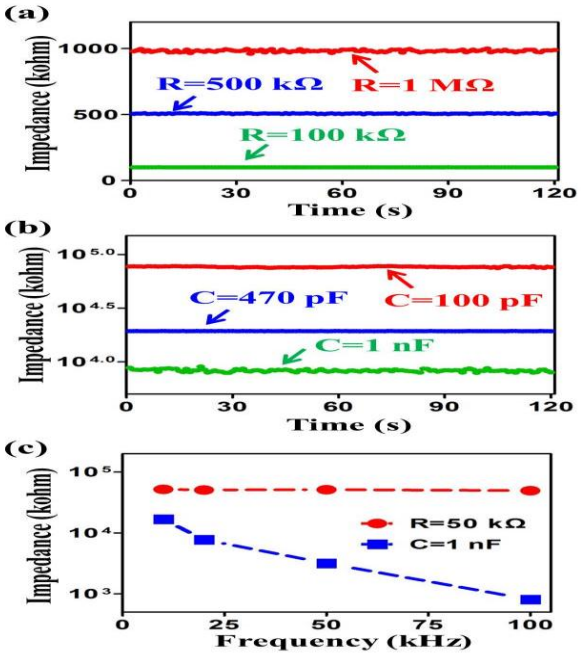
Figure.5 NIC responses to different chemicals detected by the smartphone-based impedance monitoring system. The concentrations of DNT, 1NT, isoamyl acetate, acetic acid, β-ionone and butanedione are all fixed at 1 mM (mean ± SD, n=10).

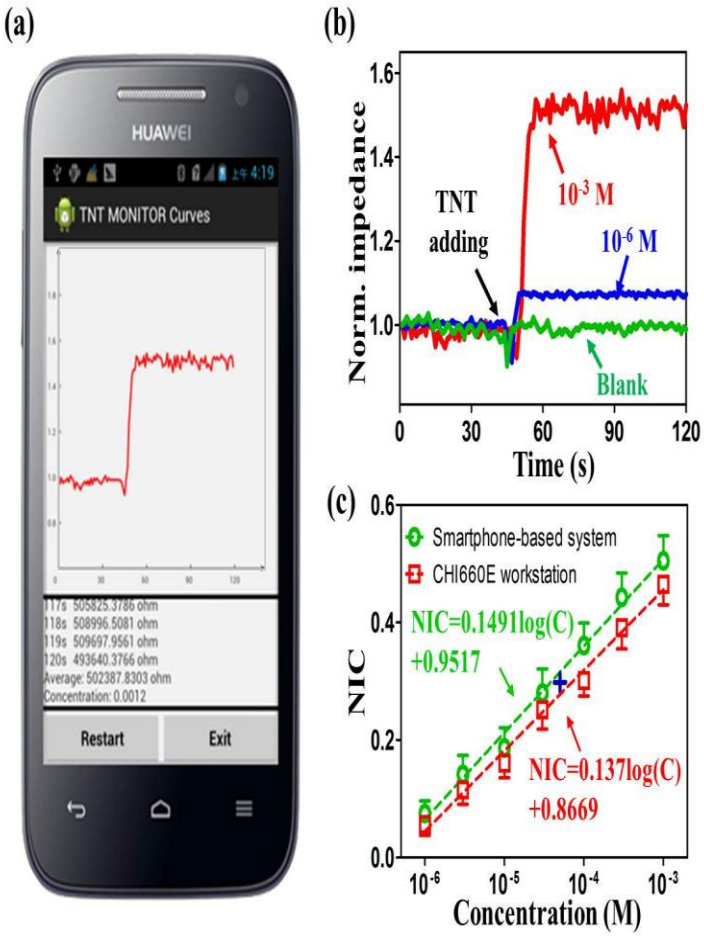
Illustration of the table

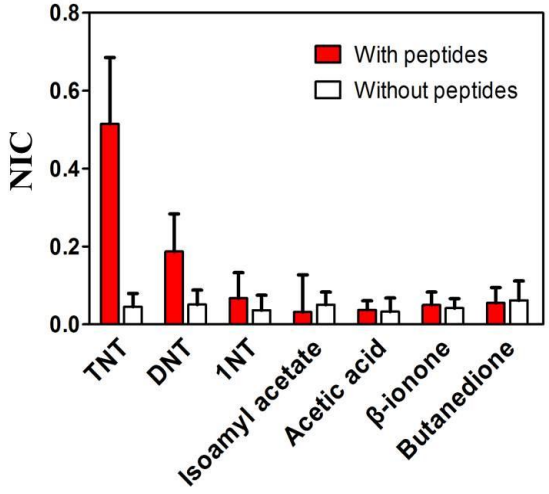
Table.1 Performance test for the smartphone-based system with resistances and capacitances at 20 kHz.






(a)
Impedance (kohm)
1000
500
0
R=500 kΩ
R=1 MΩ
R=100 kΩ
0
30
60
90
120
Time (s)
(b)
Impedance (kohm)
10^5.0
10^4.5
10^4.0
C=470 pF
C=100 pF
C=1 nF
0
30
60
90
120
Time (s)
(c)
Impedance (kohm)
10^5
10^4
10^3
R=50 kΩ
C=1 nF
0
25
50
75
100
Frequency (kHz)


(a)
HUAWEI
TNT MONITOR Curves
117s 505825.3786 ohm
118s 508996.5081 ohm
119s 509697.9561 ohm
120s 493640.3766 ohm
Average: 502387.8303 ohm
Concentration: 0.0012
Restart
Exit
(b)
Norm. impedance
1.6
1.4
1.2
1.0
TNT
adding
10-3 M
10-6 M
Blank
0
30
60
90
120
Time (s)
(c)
NIC
0.6
0.4
0.2
0.0
Smartphone-based system
CHI660E workstation
NIC=0.1491log(C)
+0.9517
NIC=0.137log(C)
+0.8669
10-6
10-5
10-4
10-3
Concentration (M)



Highlights

- A smartphone-based biosensor for 2,4,6- trinitrotoluene (TNT) detection.
- Screen-printed electrodes were modified with peptides and used as biosensor.
- Impedance monitoring device was developed using Arduino microcontroller and AD5933 analyzer chip.
- TNT as low as 10^{-6} M was detected by the smartphone-based system.
- The study provides a low-cost, potable and efficient approach to detect explosives.

Component	Resistance (n=120)			Capacitance (n=120)		
	100 kΩ	510 kΩ	1 MΩ	100 pF	470 pF	1 nF
Real value	100105.4	510243.6	987683.5	75819.2	18956.6	8451.8
Average	100515.9	507362.4	982390.4	76718.7	19337.8	8295.8
SD	122.4	2111.8	7621.7	769.3	24.2	213.0
CV	0.12%	0.42%	0.78%	1.01%	0.13%	2.57%
Maximum	100989.4	511778.5	1000087.1	78154.3	19404.1	9029.1
Minimum	100047.6	501311.5	960953.4	74943.8	19276.0	7901.6
Difference	< 0.89%	< 1.7%	< 2.7%	< 3.1%	< 2.4%	< 6.8%

CV, coefficient of variation; SD, standard deviation.