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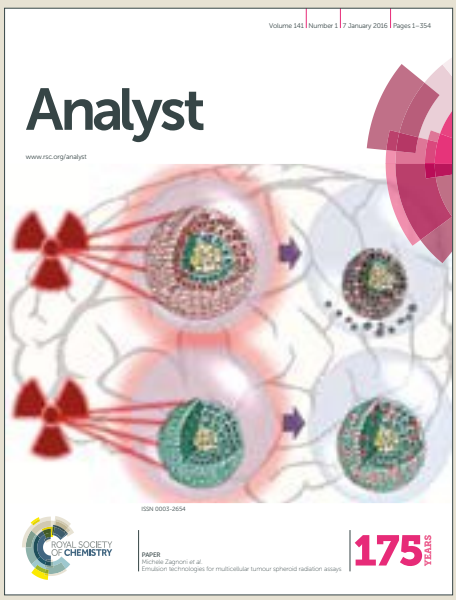

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ARTICLE

Simultaneous label-free screening of G-quadruplex active ligands from natural medicine with a microfluidic chip electrophoresis-based energy transfer multi-biosensor strategy

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Abstract: Rapid screening of active compounds plays a crucial role in the research and application of complex natural medicine. A new method of simultaneous label-free multi-drug screening based on selective aptamer-carboxyfluorescein/graphene oxide energy transfer optical sensor combined with microfluidic chip electrophoretic separation was reported here. Seven traditional Chinese medicinal monomers were chosen as targets for the screening of G-quadruplex ligands. The screening results of G-quadruplex active ligands including daidzein, berberine hydrochloride, jatrorrhizine hydrochloride and fangchinoline, and non-active ligands including geniposide and oxymatrine were consistent with literature. At the same time, one new potential G4DNA active drug, jujuboside A, was identified. Molecular simulation of the interaction between G4DNA and drugs with Hyperchem and Autodock was also carried out to verify the results of the experimental screening. It further demonstrated the reliability of our strategy. This novel separation based multi-sensor strategy provides a simple, rapid, and sensitive tool for simultaneous multi-drug screening which is very meaningful for the drug screening and bio-interaction analysis.

Introduction

G-quadruplexes are repetitive, guanine-rich nucleic acid sequences in the human genome presenting at the ends of telomeres, centromeres, and introns or in the regulatory regions of genes, it has been demonstrated that the stability of G-quadruplex structures potentially plays a crucial role in telomere maintenance[1,2], and in the regulation of gene expression[3]. There is a growing recognition for its profound impacts on a wide spectrum of diseases, including cancer, diabetes, and cardiovascular disease. Therefore, quadruplex stabilizing compounds, i.e., quadruplex ligands, have become a focus of attention, as they may interfere with the telomere elongation/replication and proliferation of cancer cells, and they become a potential target for cancer therapy[4]. Indeed, quarfloxin, a first-in-class G-quadruplex interactive compound, which progressed to Phase II clinical trials for cancer proved the potential therapeutic viability of targeting G-quadruplexes. However, the compound was withdrawn from further trials due to bio-availability problems[5]. Drug discovery from natural products has gained renewed interest in recent years. As a result, the demand for an efficient drug-screening tool is growing in order to facilitate the discovery of novel natural medicine compounds from complex mixtures of natural resources.

There are several techniques used to screen active ligands in native resources, such as temperature melting fluorescence assays[6], electrospray ionization mass spectrometry[7], telomeric repeat amplification protocol[8], and nuclear magnetic resonance (NMR)[9-10]. But techniques available require vast amount of time and labor, as well as special equipment and personnel to perform the analysis. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE)-based affinity chromatography exhibited high efficiency in multi-screening of active components. HPLC was also used to study the interaction of thrombin-binding aptamer G-quadruplex and ligand TmPyP4 according to the change in retention time and absorption intensity before and after the interaction[11]. However, the screened ligands must be detectable by UV or fluorescence end detector equipped by HPLC and CE. This requirement restricted the application of separation based methods for drug screening because of the low sensitivity of UV detector and time-consuming preparation for fluorescence labelling. In addition, with non-selective UV detector or fluorescence labelling, it is difficult to discriminate the peaks of target compounds from the fingerprint of complex mixtures. Thus Mass spectrometry (MS) in combination with chromatographic or electro-kinetic separation was usually used to further characterize and confirm the eluents[12]. Therefore, a new efficient method

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should be developed to accurately detect the bioactive compounds in a complex mixture.

Fluorescence resonance energy transfer (FRET) is a phenomenon of non-radioactive energy transfer between two fluorescent molecules occurred when the emission spectrum of donor fluorescent molecule and the absorption spectrum of acceptor fluorescent molecule overlap, and the distance between two molecules is within 10 nm. This phenomenon formed the basis of elaborately designed FRET spectroscopic technique with pronounced selectivity and sensitivity for medical diagnostics and DNA analysis. There were also considerable interests in designing a FRET system to identify the molecules that stabilize G-quadruplexes or to investigate the interaction between a quadruplex-binding ligand and the human telomeric G-quadruplex[13–18]. Nevertheless, it is noteworthy that most of the FRET-based strategies reported could screen only one target at a time. Multicolor DNA probes were synthesized in order to increase the throughput of this kind of mix-and-detect assay[19,20]. However, the advancement towards high-throughput sensors is hampered by the limited number of color probes available. Although the throughput of the sensor array with ensemble aptamers for a wider range of targets[21] was greatly improved, its limitation was remarkable because of the difficulty to obtain and immobilize pure sensors on the chip. For the first time, separation technique based label-free multiple parallel protein detection with an individual color probe was achieved in our group with isoelectric focusing (IEF) and capillary zone electrophoresis (CZE) coupled two-dimensional microfluidic chip electrophoresis[22]. The principle of the assay was, as a result of p-stacking interactions, graphene oxide (GO) sheets strongly adsorbed single stranded nucleic acids, and then quench the fluorescence of organic dye, which is labelled to ssDNA. Therefore, in the initial mixture of GO and fluorescein-based dye (FAM)-labeled ssDNA of aptamers, the fluorescence of labelled FAM was quenched due to FRET between the fluorophore and quencher GO. With the input of sample including target protein, specific binding of the aptamer probe with the target protein caused the conformation alteration and forced the separation of aptamer from the quencher. Its labelled fluorescence signal was selectively restored. The critical simultaneous multi-protein sensoring was achieved by chip electrophoresis separation. Although capillary electrophoresis and chip electrophoresis had been used in enzyme assays[23] and multiplexed inhibitor detection[24], but the high background interference was serious without the selective fluroscence recovery. In our strategy, FRET was combined with chip-CE to realize selective and sensitive sensoring.

In this study, based on previous work[22], a novel simultaneous label-free multi-drug screening method was established. Graphene oxide was used as quencher in FRET sensor because of its ultrahigh quenching efficiency[25–30]. Severn typical flavonoid or alkaloid natural compounds, including isoflavones aglycone, berberine hydrochloride, jatrorrhizine hydrochloride, fangchinoline, geniposide, oxymatrine, and jujuboside A were chosen as the targets for the screening of ligands. With our new method, all known G4DNA active and non-active ligands in these seven ligands were screened out. What is more, one new potent G4DNA active drug was also found. These drugs were discussed in reference to literature and further verified by computer simulation carried out in this study.

Experimental

Reagents

Oligonucleotide chain (5′-the FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3′) was synthesized by Youbao biotech Co., LTD. (Dalian, China), with a 6-FAM attached to the 5′-end of the chain. Daidzein, berberine hydrochloride, jatrorrhizine hydrochloride, fangchinoline, oxymatrine, gardenin, and jujuboside A were all purchased from China National Institutes for Food and Drug Control. Other reagents were purchased from Beijing Chemical Works, and were analytically pure. Graphene oxide (GO) was synthesized from graphite powder using Hummer's method[31]. All aqueous solutions were prepared with purified water collected from a Millipore Purification System (Billerica, MA). The electrophoresis chip utilized a spin-coated chromium plate (SG2506, UM grade, chromium layer thickness 145 nm; Az-1805 photoresist, 570 nm thickness; Hunan Changsha Shaoguang Microelectronics Co.) as a substrate, and the same type of sheet glass as a cover slip.

Microchip electrophoresis for ligand screening

Sample preparation

Fluorescent-labelled ssDNA (5′-the FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3′) (2 μM) prepared in Tris-HCl buffer (pH 7.5) was mixed with GO (0.3 mg/mL in pure water), followed by incubation at 90°C for 5 min. The mixture was then cooled down to room temperature. After that, a certain volume of solution containing one drug or a mixture of drugs (1 mg/mL in Tris-HCl) was added into the ssDNA solution in the volume ratio of 1:1:1 for GO, fluorescent-labelled ssDNA, and drug solution. They were mixed and incubated for 30 min.

Chip preparation

Glass chips with criss-cross channel structure and linear channel structure respectively were fabricated according to standard procedures involving photolithography, wet chemical etching, hole drilling, and high-temperature bonding[32]. The UV lithography was performed using JKG-2A lithographic device (Photomechanical, Shanghai, China). Fig. 1(a) shows the schematic illustrations of microfluidic chips with a criss-cross structure, including a 263 μm wide, 40 μm deep, and 1.4 cm long sample loading channel intersected by an electrophoresis separation channel 130 μm wide and 3.5 cm long.

Chip electrophoresis

Fig. 1 (a) shows the schematic illustrations of the whole setup for chip electrophoresis based multisensor. The process of electrophoretic separation in criss-cross channel structure was carried out as follows: after the chip channel between S and SW was filled with the sample mixture, four reservoirs at both ends of two channels were filled with buffer. Electric voltages of 200 V, 200 V, 300 V, and ground were applied on reservoirs

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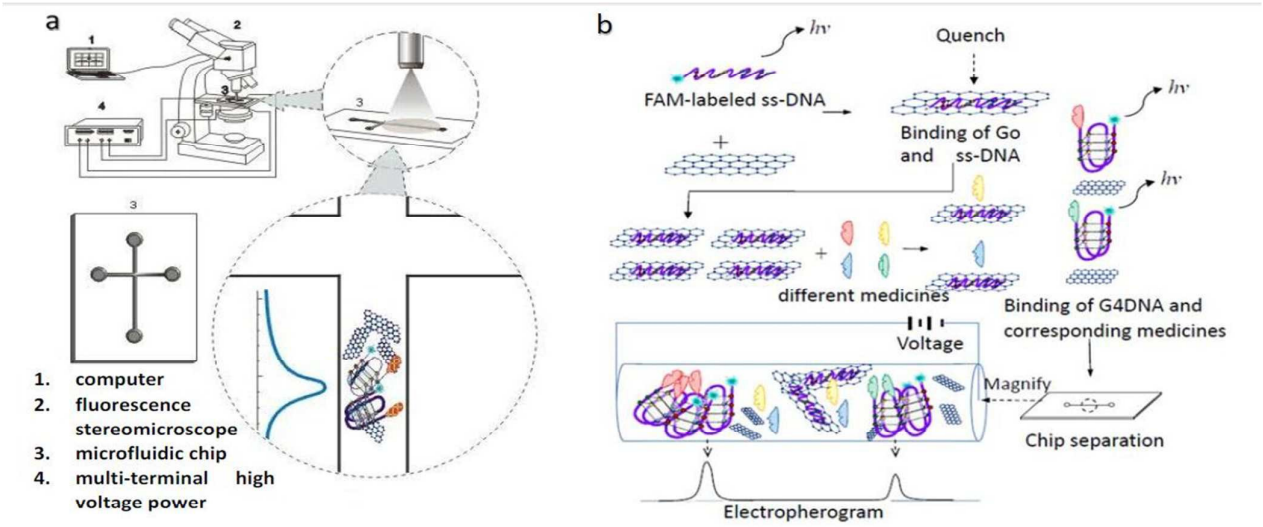

Figure 1 (a) The schematic illustrations of the whole set up for chip electrophoresis; （b） The schematic diagram of graphene-based FRET and chip-CE-assisted biosensor for detection of drug.

of S, SW, BW, and B, respectively, with a computer-controlled high-voltage power supply (XCDY microfluidic chip electrical source, Shandong Normal University, Shandong, China) to initiate electrokinetic sample loading and the following electrophoresis. Every time before chip electrophoresis, the chip channel was a 10-min washing with water, methyl cellulose solution (1.3% w/w in 0.05 mol/L Tris-HCl pH 7.5) and Tris-HCl were injected sequentially into channels using a manual pump. Thereafter, the chip was ready for use.

Data collection and image analysis

The whole process was monitored and recorded with an IX70 inverted fluorescent stereomicroscope equipped with a CCD camera (Olympus, Japan) (Fig. 1(a). The view field of the inverted microscope was aimed at the downstream of the separation channel. The separation behaviour was captured by a microscope. After that, ImageJ software (http://rsb.info.nih.gov/ij) was used to convert the photographs captured from the videos of electrophoresis into electropherograms.

G4DNA – drug interaction simulation with Hyperchem software and Autodock

Hyperchem was used to simulate the interaction between G4DNA and drugs. Molecular mechanics force field of AMBER99 was chosen. The parameters and atom type were set to the default value. The next step was the drawing of the molecule's structure. After the structures of seven drugs and ssDNA were drawn, one drug and ssDNA were merged together and optimized respectively by geometry computation. Polak-Ribiere algorithm in the Hyperchem panel was chosen. Finally, Total energy value for each pair was obtained by computing their single point. The total energy value was gained after performing molecular dynamics with a 0.001 ps step size and 100 ps of heat, run, and cool time. The temperature step was set to 1 K, the starting and final temperatures 0 K and the simulation temperature 500 K.

The steps of AutoDock simulation were as following: (1) coordinate preparation was done to make the ligand and receptor coordinate files including polar hydrogen atoms, partial charges, atom types and information on the articulation of flexible molecule; (2) AutoGrid was run to pre-calculate grid maps, one for each atom type present in the ligand being docked; (3) Using the maps generated by AutoGrid to evaluate the ligand-protein interaction at each point in the docking simulation, docking was carried out to explore the conformational states of a flexible ligand; (4) The docking results were evaluated.

Result and discussion

The principle of the microfluidic chip electrophoresis-based graphene energy transfer biosensor

Two basic steps were involved in this proposed microfluidic chip electrophoresis-based FRET multi-sensory system. The first was similar to classic FRET[33]. FAM-labelled G-quadruplex oligonucleotide was assembled non-covalently onto GO. Due to the strong interaction between oligonucleotide and GO, the fluorescence of labelled FAM was quenched by FRET between GO and FAM. After the introduction of drug analytes, the specific interaction of bioactive drug and oligonucleotide created a relatively rigid hybrid. There was an intramolecular folding from an oligonucleotide with the human telomeric sequence into a G-quadruplex structure. This folding caused the release of oligonucleotides from the vicinity of GO and restoring the fluorescence of FAM. Otherwise, without bioactive drug which interacted with oligonucleotide, no recovery of fluorescence occurred. Secondly, after the selective fluorescence recovery, chip-CE played the critical role in multiplex sensoring by separating every drug-G4DNA binding pair in the mixture. The schematic diagram of

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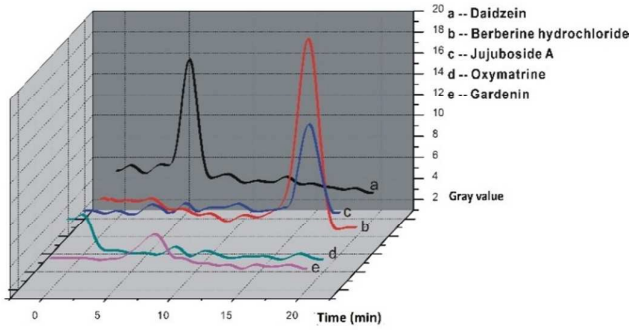
graphene-based FRET and chip-CE-assisted biosensor for detection of drugs is given in Fig. 1(b).

Individual screening of the G4DNA binding ligand

First of all, the binding activity of each Chinese medicinal components as potential ligands was characterized individually using the proposed FRET and chip-CE integrated biosensor.

Figure 2 The chip electrograms of individual drugs.



The electrophoresis mode of chip-CE was zone electrophoresis with criss-cross chip channel structure. The electrophoresis results of some drugs are shown in Fig. 2. Compared with a blank system consisting of only fluorescence-quenched GO and G4DNA, bigger electrophoresis peaks were found in the systems containing daidzein, berberine hydrochloride, and jujuboside A respectively. No new peaks were found in the systems containing geniposide and oxymatrine. In the system of jatrorrhizine hydrochloride and fangchinoline, there was a decrease in fluorescence intensity.

These three different kinds of experimental phenomena were discussed in reference to literature as follows. Firstly, the fluorescence recovery with daidzein, berberine hydrochloride, and jujuboside A could be ascribed to their binding activity with G4DNA. The interaction between G4DNA and the isoflavones, including daidzein, had already been reported in literature[34]. Berberine hydrochloride together with its group of isoquinoline alkaloids had been studied for their interaction with quadruplex DNA[35] and proved for their potential use as therapeutic DNA binders[36].

It is noteworthy that a new potential active ligand, jujuboside A, was found by our novel method. Jujuboside A, isolated from Semen Ziziphi Spinosae (SZS), was used as a hypnotic-sedative medicine for thousands of years in East Asian countries[37,38]. Although the interaction between jujuboside A and G4DNA had not been reported elsewhere, it was reported that SZS had notable neuroprotective activities and they might be served as potential therapeutic agents for the treatment of Alzheimer' disease[39]. Meanwhile, it was reported that the change in telomere length[40] and chromosome instability[41] were all associated with Alzheimer's disease. Furthermore, antitumor activity of jujuboside B and the underlying mechanism via induction of apoptosis and autophagy was reported[42]. While autophagy was thought to act as a safeguard mechanism against G-quadruplex ligand-mediated DNA damage[43]. Therefore, a lot of existing literature supported the possible biointeraction between jujuboside A and G4DNA.

In the analysis of the geniposide and oxymatrine with our method, no obvious peaks appeared. It was reflected that there was no interaction existed between these two drugs with G4DNA. At the same time, no related report was available on the interaction of geniposide and oxymatrine with G4DNA either. Thus, the screening results of geniposide and oxymatrine in our experiment was consistent with literature.

Lastly, we noted that in the system of jatrorrhizine hydrochloride or fangchinoline, there was a remarkable decrease of fluorescence intensity, even of the background fluorescence. It was different with an increase or no obvious change in whole fluorescence occurred in above two cases. This phenomenon was deduced to comprise two processes. The first was the stronger interaction of drugs, jatrorrhizine hydrochloride and fangchinoline with G4DNA. Otherwise, the fluorescence labelled G4DNA would still assemble on the surface of GO and there was no deep quenching of fluorescence. The binding interaction of six alkaloids, including jatrorrhizine hydrochloride and fangchinoline, with G-quadruplex had already been reported[45,46]. Therefore, our drug screening results were consistent with the literature. The reason for the fluorescent quenching in this paper was thought to be the second process of the chemical reaction between drugs and FAM. Fluorescent quenching caused by these two drugs had not been reported. There had been a lot of direct fluorescence quenching based analyses of other drugs. For example, oxymatrine was reported to quench the fluorescence[47]. But in our system, since there was no interaction between oxymatrine and G4DNA. And then, there was no the detaching of fluorescence labelled G4DNA from GO, and the following fluorescent quenching by oxymatrine. This point, on the contrary again supported the interaction of jatrorrhizine hydrochloride and fangchinoline respectively, with G4DNA. In a short, all kinds of experimental phenomena can give us useful information.

Molecular simulation of the interaction between G4DNA and drugs

The binding of drugs with G4DNA was studied by Hyperchem and Autodock. Figure 3 gives the result of free energy value computed with Hyperchem software simulation. It was shown that the binding energy of geniposide and oxymatrine with G4DNA (lighter colour), was lower than that of others, demonstrating that no bio-interaction existed. The higher binding energy of other five drugs, daidzein, berberine hydrochloride, jujuboside A (fluorescent green), and jatrorrhizine hydrochloride together with fangchinoline (gray

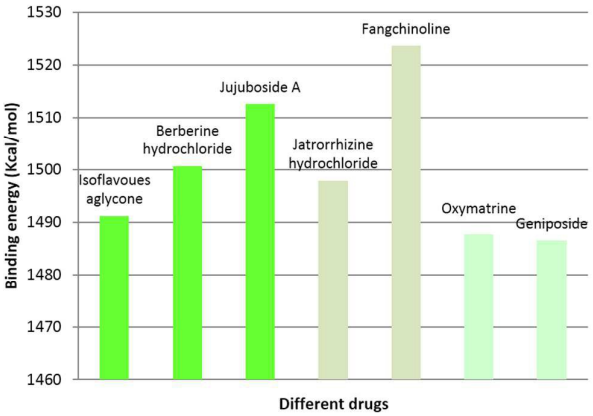


Figure 3 The free energy of docking using Hyperchem software.

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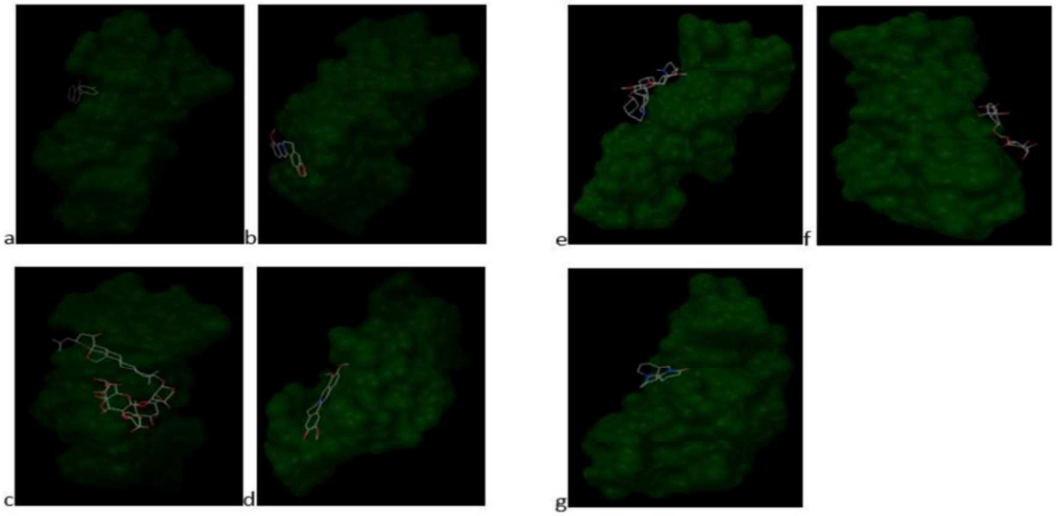
Figure 4 The schematic diagram of the interaction of drugs with G4DNA by molecular docking studies (a) isoflavones aglycone; (b)berberine hydrochloride; (c)jujuboside A; (d) jatrorrhizine hydrochloride; (e)fangchinoline; (f) geniposide; (g) oxymatrine.



colour) confirmed their biointeraction with G4DNA. Especially, simulated binding energy of jujuboside A, jatrorrhizine hydrochloride and fangchinoline with G4DNA again supported the experimental results and analysis above.

Autodock software was used for the molecular docking of G4DNA drugs as well. As shown in Fig.4, different kinds of drugs bound with various sites on the G4NDA. Isoflavones aglycone and geniposide are two kinds of Saponin natural medicine component. It was shown that their binding sites on G4NDA were nearby. But isoflavones aglycone fitted into the groove of G4DNA better than geniposide. Unlike oxymatrine, berberine hydrochloride and jatrorrhizine hydrochloride bound effectively with almost the same site by intercalating into the groove on the G4DNA. The molecular size of jujuboside A, and fangchinoline are bigger than others. Their binding sites were in the same area. Although the relatively larger molecular size prohibited them from fitting into a small groove, their non-rigid structure might be helpful for them to adjust, fit and bind with superficial area of G4DNA. And then, these simulation explained the difference in drug potency to a certain extent. It was shown in docking simulation that isoflavones aglycone, berberine hydrochloride, jujuboside A, jatrorrhizine hydrochloride, and fangchinoline bound more effectively with G4NDA. While geniposide and oxymatrine were unable to intercalate into the gap region of G4DNA well, possibly due to the chemical and structural unsuitability. Therefore, the molecular simulations were in agreement with experimental results, supporting the effectiveness of our proposed screening method.

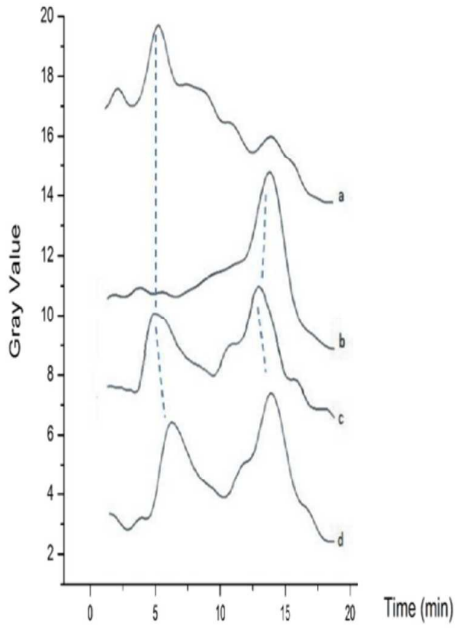


Figure 5 The chip electrograms of (a) daidzein, (b) berberine hydrochloride, (c) their mixture of daidzein and berberine hydrochloride, (d) the mixture of daidzein, berberine hydrochloride, gardenin, and oxymatry.

Simultaneous screening of the G4DNA binding ligand

Multiplex drug screening is particularly appealing in the development of nature medicine. In this paper, the simultaneous screening of multi-ligands was achieved easily by giving full play to the separation ability of microfluidic chip electrophoresis. During the experiment, different drugs were mixed together to simulate real sample mixture, and then reacted with GO bound G-quadruplex. After that, chip electrophoresis-based separation of the reaction mixture was used to perform simultaneous multi-ligand screen.

Firstly, a mixed sample including daidzein and berberine hydrochloride, together with G4DNA bound GO was analysed. An IEF chip with a linear channel structure was used. Two peaks could be observed in the electropherogram of the mixture. Compared with the IEF electropherograms of single drug system (Fig. 5(a) and (Fig. 5(b)), two peaks in the mixture (Fig. 5(c) vs. Fig. 5(c)) could be ascribed to daidzein (Fig. 5(a) vs. Fig. 5(c)) and berberine hydrochloride (Fig. 5(b)), respectively. With the further introduction of geniposide and oxymatrine

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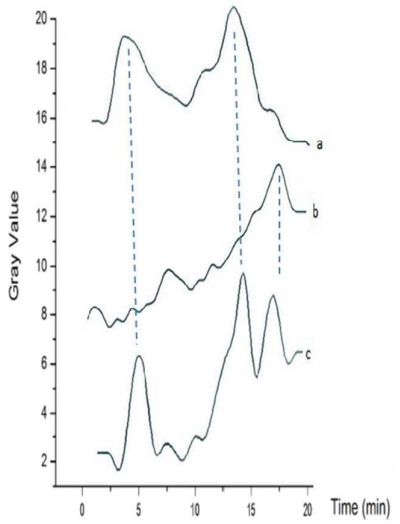
into the mixture, there was no new peak (Fig. 5(d)). It was consistent with experimental results above that geniposide and oxymatrine had no binding with G4DNA. Hence no recovered fluorescence and corresponding peaks were found. While jujuboside A was introduced into the mixture of daidzein, berberine hydrochloride, together with G4DNA bound GO, three peaks were observed in the electropherogram (Fig.6). As compared to the electropherogram of the mixture with daidzein and berberine hydrochloride (Fig. 6(a)), and that of a single jujuboside A system (Fig. 6(b)), there was no doubt that the new peak belonged to jujuboside A (Fig. 6(c)). And then, by comparing the relative retention time of every peak between the electropherograms of mixed drugs and of the single drug, these constituents with G4DNA binding activity were selectively and sensitively revealed.

Figure 6 The chip electrograms of (a) the mixture of daidzein and berberine hydrochloride, (b) single jujuboside A system, (c) the mixture of daidzein, berberine hydrochloride, and jujuboside A.



A brief discussion of the resolution and the detection range in terms of the ligand's molecular weight is given here to ensure a high throughput screening. The impact of chip-CE separation ability on the resolution here and the impact of FRET on the detection range can be analyzed. It had been reported that DNA fragments from the digestion with HaeIIIfrom pBR322 , consisted of 22 fragments sized between 8 and 587 bp could be well separated in native gel[47]. With a kind of N-methyl-2-pyrrolidonium methyl sulfonate acidic ionic liquid inner-wall coating, basic proteins were baseline separated[48]. Meanwhile, with a lot of methods reported to improve the separation resolution, there is no doubt that the chip-CE in this paper can reach the same level of resolving power as other chip-CE, although more work is still needed to learn the actual exact resolution of our methods. In the case of detection range. A variety of graphene enhanced FRET methods had been reported for detection from metal ions[49], DNAs[27], protein[28], virus[50]. Performed in the same way as traditional FRET sensor, except for the following chip-CE to realize separated fluorescence detection, our new method owns the same power of detection as traditional FRET sensor. Therefore, due to the characteristic mechanism of our novel strategy, an easy and direct combination of FRET sensor with chip-CE not only retains the merits of constitutive methods, but also brings new ability of simultaneous multi-sensor.

Conclusions

The therapeutic potential of gene promoter G-quadruplexes has boosted studies in finding small-molecule ligands as G-quadruplex stabilizers[5]. Our strategy brings a novel and efficient method for drug screening. Active and non-active G-quadruplexe binding drugs consistent with literature and one new potential active drug were found. After that, the bindings between G4DNA and drugs were analysed by computer simulation. The molecular simulation further supported our experimental drug screening.

The novel combination of a resonance energy transfer (RET)-based sensing method with high-performance chip electrophoresis brings several striking advantages into drug screening. (i) No sample pre-treatment emphasized in traditional mix-and-detect methods actually presented the challenge in multi-drug sensoring. The combination with chromatography based separation technique opens up a new way to high-throughput and simultaneous optical sensor for drug screening; (ii) With selective fluorescent recovery and fine separation, multi-sensoring can be realized without sample pre-treatment. At the same time, the number of targets that can be detected simultaneously will be constantly increased along with development in the separation technology of chip-CE; (iii) It is extremely easy and convenient without sample pre-labelling here. There is no need to worry whether the analyte itself can be detected by UV or fluorescent detector; (iv) Compared with traditional GO based RET sensor, no multi-colored probes are required here to realize innovative multi-target sensing; (v) Unlike the strategy of sensor array biochip to achieve multi-sensoring, there is no need of sensor immobilization here; (vi) A high detection sensitivity with a low background noise is ensured by the super-quenching ability of GO, selective fluorescence restoring and the high separation resolution of chip-CE; (vii) With selective fluorescent recovery and fine separation, drug screening can be achieved more easily without having to compare the change of retention time before and after the treatment of drugs as in traditional affinity chromatography based drug screening; (viii) This methodology can be used not only in drug screening, but also in bio-interaction study which will be carried out in our following work. The proteins interacted with target directly or indirectly can be simultaneously detected. Furthermore, according to the peak area, the strength of binding activity or the number of active components can also be quantified; (ix) Nowadays, micro- and nano-fluidic devices are becoming the hot focus of investigations. The advantages of chip integration with other upstream and downstream functional modules give our method a wide applicability.

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References

1 K. N. Luu, A. T. Phan, V. Kuryavyi, L. Lacroix and D. J. Patel, *J. Am. Chem. Soc.*, 2006, **128**, 9963–9970.

2 A. T. Phan, Y. S. Modi and D. J. Patel, *J. Am. Chem. Soc.*, 2004, **126**, 8710–8716.

3 Q. He, P. Zeng, J. H. Tan, T. M. Ou, L. Q. Gu, Z. S. Huang and D. Li, *Biochim. Biophys. Acta - Gen. Subj.*, 2014, **1840**, 2222–2233.

4 J.-L. Mergny, L. Lacroix, M.-P. Teulade-Fichou, C. Hounsou, L. Guittat, M. Hoarau, P. B. Arimondo, J.-P. Vigneron, J.-M. Lehn, J.-F. Riou, T. Garestier and C. Helene, *Proc. Natl. Acad. Sci.*, 2001, **98**, 3062–3067.

5 S. Balasubramanian, L. H. Hurley and S. Neidle, *Nat. Rev. Drug Discov.*, 2011, **10**, 261–75.

6 F. Koeppel, J. F. Riou, a Laoui, P. Mailliet, P. B. Arimondo, D. Labit, O. Petitgenet, C. Hélène and J. L. Mergny, *Nucleic Acids Res.*, 2001, **29**, 1087–1096.

7 F. Rosu, E. De Pauw, L. Guittat, P. Alberti, L. Lacroix, P. Mailliet, J. F. Riou and J. L. Mergny, *Biochemistry*, 2003, **42**, 10361–10371.

8 D. Gomez, J. L. Mergny and J. F. Riou, *Cancer Res.*, 2002, **62**, 3365–3368.

9 Q. Zhou, L. Li, J. Xiang, H. Sun and Y. Tang, *Biochimie*, 2009, **91**, 304–308.

10 H. H. Li and G. Yuan, *Chinese Chem. Lett.*, 2008, **19**, 1108–1110.

11 M. del Toro, R. Gargallo, R. Eritja and J. Jaumot, *Anal. Biochem.*, 2008, **379**, 8–15.

12 G. Bai, X. Cao, H. Zhang, J. Xiang, H. Ren, L. Tan and Y. Tang, *J. Chromatogr. A*, 2011, **1218**, 6433–6438.

13 Y. Jin, H. Li and J. Bai, *Anal. Chem.*, 2009, **81**, 5709–5715.

14 L. Fu, B. Li and Y. Zhang, *Anal. Biochem.*, 2012, **421**, 198–202.

15 A. Bourdoncle, A. E. Torres, C. Gosse, L. Lacroix, P. Vekhoff, T. Le Saux, L. Jullien and J. L. Mergny, *J. Am. Chem. Soc.*, 2006, **128**, 11094–11105.

16 F. He, Y. Tang, S. Wang, Y. Li and D. Zhu, *J. Am. Chem. Soc.*, 2005, **127**, 12343–12346.

17 P. V. Jena, P. S. Shirude, B. Okumus, K. Laxmi-Reddy, F. Godde, I. Huc, S. Balasubramanian and T. Ha, *J. Am. Chem. Soc.*, 2009, **131**, 12522–12523.

18 A. I. H. Murchie, R. M. Clegg, E. von Kitzing, D. R. Duckett, S. Diekmann and D. M. J. Lilley, *Nature*, 1989, **341**, 763–766.

19 S. He, B. Song, D. Li, C. Zhu, W. Qi, Y. Wen, L. Wang, S. Song, H. Fang and C. Fan, *Adv. Funct. Mater.*, 2010, **20**, 453–459.

20 J. H. Kim, S. Chaudhary and M. Ozkan, *Nanotechnology*, 2007, **18**, 195105.

21 H. Pei, J. Li, M. Lv, J. Wang, J. Gao, J. Lu, Y. Li, Q. Huang, J. Hu and C. Fan, *J. Am. Chem. Soc.*, 2012, **134**, 13843–13849.

22 F. Lin, X. Zhao, J. Wang, S. Yu, Y. Deng, L. Geng and H. Li, *Analyst*, 2014, **139**, 2890–2895.

23 Q. Xue, A. Wainright, S. Gangakhedkar and I. Gibbons, *Electrophoresis*, 2001, **22**, 4000–4007.

24 P. Yang, R. J. Whelan, Y. Mao, A. W. M. Lee, C. Carter-Su and R. T. Kennedy, *Anal. Chem.*, 2007, **79**, 1690–1695.

25 W. Yang, K. R. Ratinac, S. R. Ringer, P. Thordarson, J. J. Gooding and F. Braet, *Angew. Chemie - Int. Ed.*, 2010, 49, 2114–2138.

26 Y. Q. Li, L. Y. Guan, J. H. Wang, H. L. Zhang, J. Chen, S. Lin, W. Chen and Y. D. Zhao Yuan-Di, *Biosens. Bioelectron.*, 2011, **26**, 2317–2322.

27 P. Alonso-Cristobal, P. Vilela, A. El-Sagheer, E. Lopez-Cabarcos, T. Brown, O. L. Muskens, J. Rubio-Retama and a G. Kanaras, *ACS Appl. Mater. Interfaces*, 2015, **7**, 12422–12429.

28 X. Sun, J. Fan, W. Ye, H. Zhang, Y. Cong and J. Xiao, *J. Mater. Chem. B*, 2016, **4**, 1064–1069.

29 B. C. Yao, Y. Wu, C. B. Yu, J. R. He, Y. J. Rao, Y. Gong, F. Fu, Y. F. Chen and Y. R. Li, *Sci. Rep.*, 2016, **6**, 23706.

30 J. Guo, E. W. C. Chan, S. Chen and Z. Zeng, *Front. Microbiol.*, 2017, **8**, 8.

31 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339–1339.

32 C. A. Emrich, I. L. Medintz, W. K. Chu and R. A. Mathies, *Anal. Chem.*, 2007, **79**, 7360–7366.

33 H. Wang, T. Chen, S. Wu, X. Chu and R. Yu, *Biosens. Bioelectron.*, 2012, **34**, 88–93.

34 Y. Jin, H. Li and P. Liu, *Biosens. Bioelectron.*, 2010, **25**, 2669–2674.

35 K. Bhadra and G. S. Kumar, *Biochim. Biophys. Acta - Gen. Subj.*, 2011, **1810**, 485–496.

36 J. S. Tomar, *J. Mol. Model.*, 2015, **21**, 193.

37 X. S. Fang, J. F. Hao, H. Y. Zhou, L. X. Zhu, J. H. Wang and F. Q. Song, *Phytomedicine*, 2010, **17**, 75–80.

38 J. X. Cao, Q. Y. Zhang, S. Y. Cui, X. Y. Cui, J. Zhang, Y. H. Zhang, Y. J. Bai and Y. Y. Zhao, *J. Ethnopharmacol.*, 2010, **130**, 163–166.

39 Z. Liu, X. Zhao, B. Liu, A. J. Liu, H. Li, X. Mao, B. Wu, K. S. Bi and Y. Jia, Eur. J. Pharmacol., 2014, 738, 206–213.

40 P. Thomas, N. J. O' Callaghan and M. Fenech, *Mech. Ageing Dev.*, 2008, **129**, 183–190.

41 B. Spremo-Potparević, L. Živković, B. Plećas-Solarović and V. P. Bajić, *Arch. Biol. Sci.*, , DOI:10.2298/ABS1103603P.

42 M. Y. Xu, S. Y. Lee, S. S. Kang and Y. S. Kim, J. Nat. Prod., 2014, 77, 370–376.

43 N. I. Orlotti, G. Cimino-Reale, E. Borghini, M. Pennati, C. Sissi, F. Perrone, M. Palumbo, M. G. Daidone, M. Folini and N. Zaffaroni, *Autophagy*, 2012, **8**, 1185–1196.

44 N. Xu, H. Yang, M. Cui, F. Song, Z. Liu and S. Liu, *J. Mass Spectrom.*, 2012, **47**, 694–700.

45 X. Cui, S. Lin and G. Yuan, *Int. J. Biol. Macromol.*, 2012, **50**, 996–1001.

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DOI: 10.1039/C7AN00692F

46 Z. G. Pang, B. Q. Wang, X. Wang, *J. Xi'an Jiaotong University(Med.Sci.),* 1998, **2**, 201-203
47 Á. Végvári and S. Hjertén, *Electrophoresis*, 2002, **23**, 3479–3486.
48 X. F. Guo, H. Y. Chen, X. H. Zhou, H. Wang and H. S. Zhang, *Electrophoresis*, 2013, **34**, 3287–3292.
49 W. T. Huang, Y. Shi, W. Y. Xie, H. Q. Luo and N. B. Li, *Chem. Commun.*, 2011, **47**, 7800.
50 L. Chen, X. Zhang, G. Zhou, X. Xiang, X. Ji, Z. Zheng, Z. He and H. Wang, *Anal. Chem.*, 2012, **84**, 3200–3207.

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DOI: 10.1039/C7AN00692F

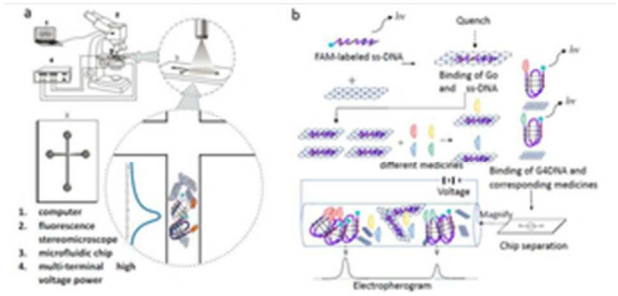

Figure 1 (a) The schematic illustrations of the whole set up for chip electrophoresis; （b） The schematic diagram of graphene-based FRET and chip-CE-assisted biosensor for detection of drug.

25x13mm (300 x 300 DPI)

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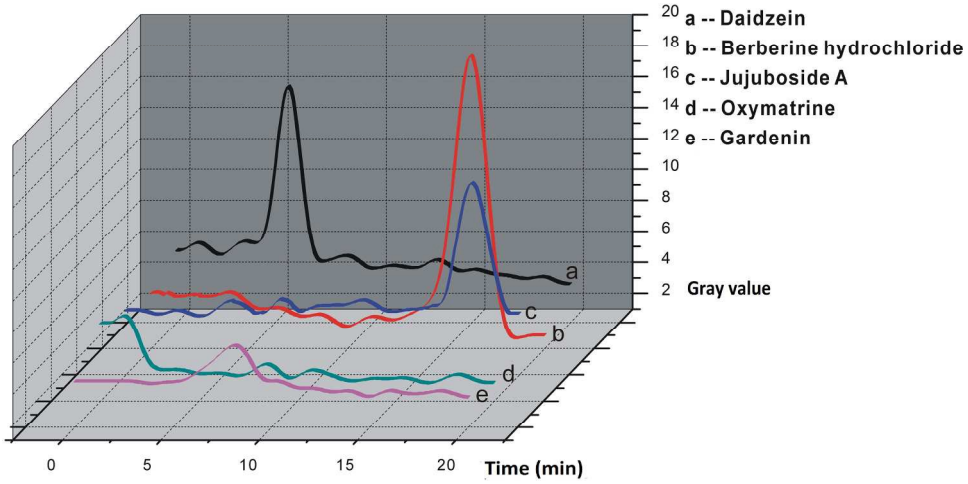

Figure 2 The chip electrograms of individual drugs.

203x115mm (300 x 300 DPI)

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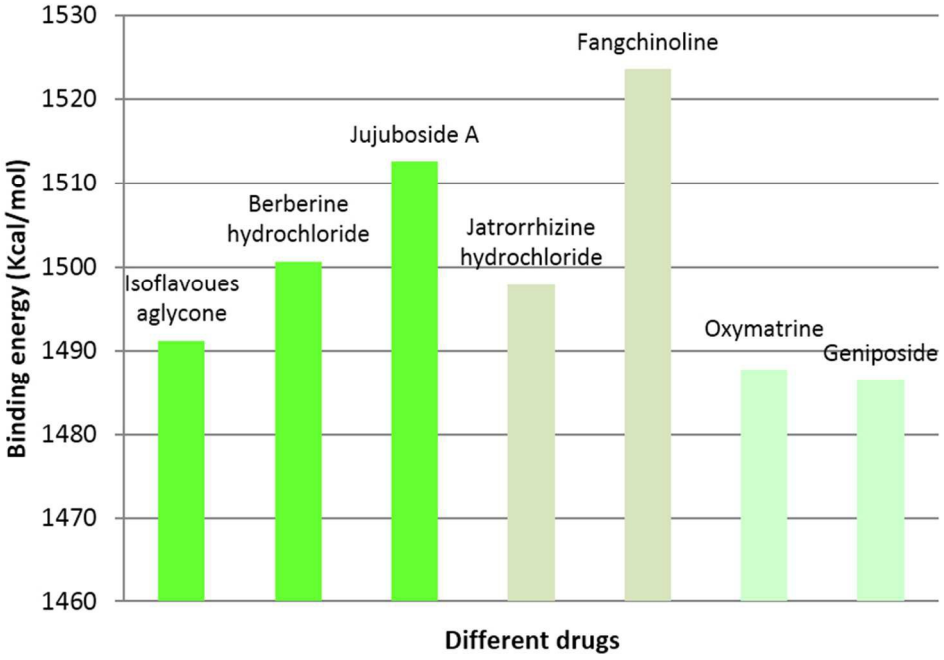

Figure 3 The free energy of docking using Hyperchem software.

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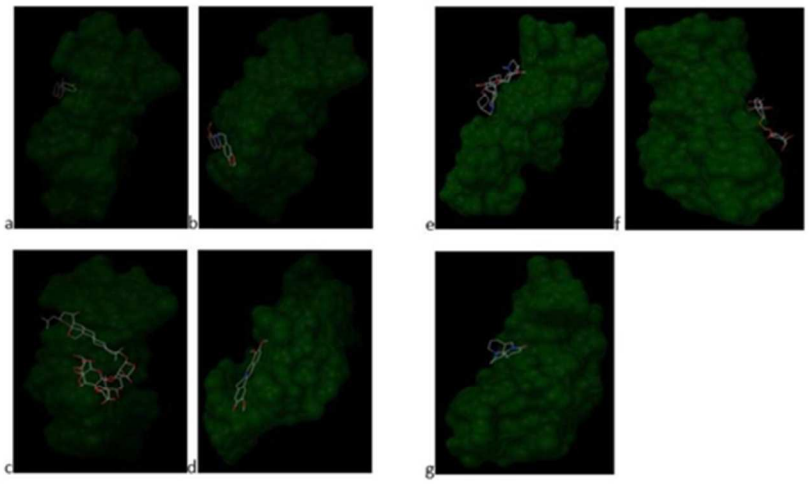

Figure 4 The schematic diagram of the interaction of drugs with G4DNA by molecular docking studies (a) isoflavones aglycone; (b)berberine hydrochloride; (c)jujuboside A; (d) jatrorrhizine hydrochloride; (e)fangchinoline; (f) geniposide; (g) oxymatrine

50x24mm (300 x 300 DPI)

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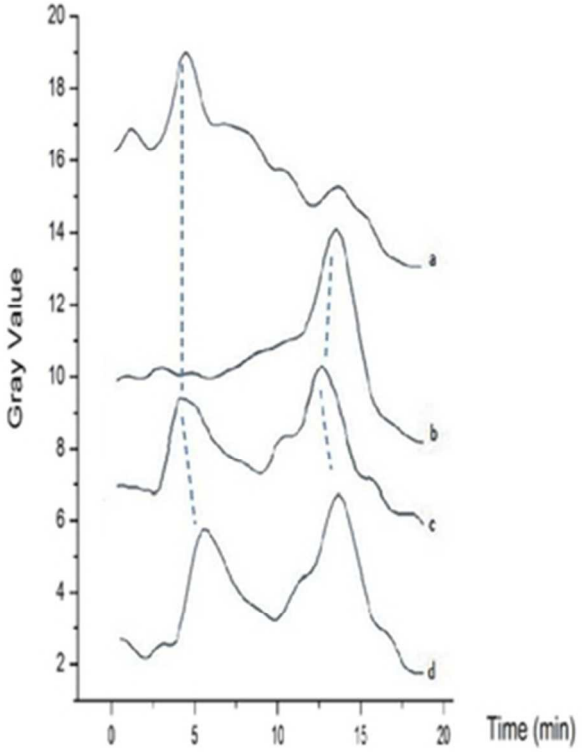

Figure 5 The chip electrograms of (a) daidzein, (b) berberine hydrochloride, (c) their mixture of daidzein and berberine hydrochloride, (d) the mixture of daidzein, berberine hydrochloride, gardenin, and oxymatry.

27x32mm (300 x 300 DPI)

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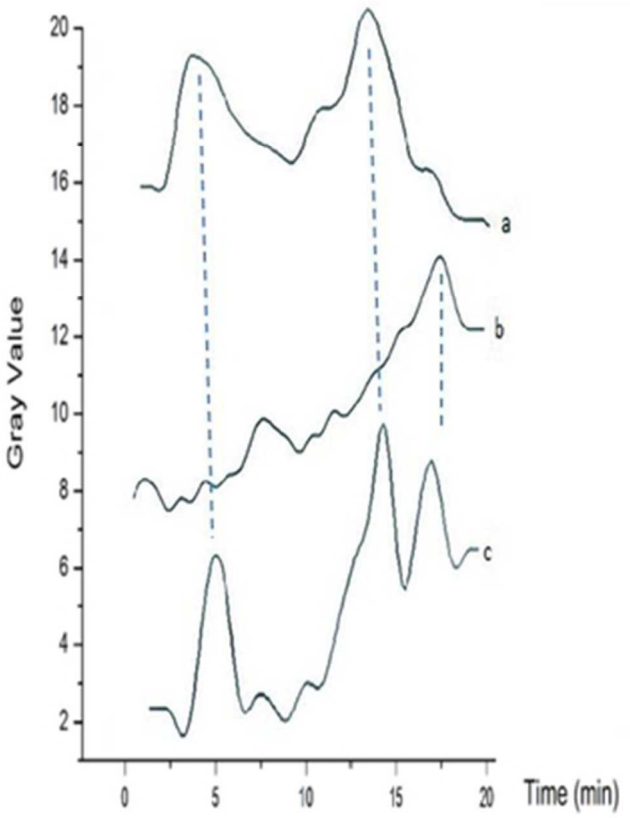

Figure 6 The chip electrograms of (a) the mixture of daidzein and berberine hydrochloride, (b) single jujuboside A system, (c) the mixture of daidzein, berberine hydrochloride, and jujuboside A.

29x38mm (300 x 300 DPI)

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Chip-electrophoresis based separation was combined with RET optical sensor to establish a new sense of simultaneous label-free multi-drug screening method.