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A comparison report of three advanced methods for drug-cyclodextrin interaction measurements

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ABSTRACT

Three advanced methods, high performance affinity chromatography (HPAC), surface plasmon resonance (SPR) and surface plasmon resonance imaging (SPRi) were compared and evaluated for determining the drug-cyclodextrin (CD) interactions herein. In total, 18 sparingly soluble drugs were selected for this comparative study. The three methods share a unique connection in the working principles and strategies. The same strategies of CD fixation onto solid phase were used in HPAC and SPR for the measurements, whereas, the SPR and SPRi share identical working principles. However, whilst these relationships are evident, no strong correlation was found between kinetic constants obtained from the three methods: Four drugs, namely, prednisolone, pseudolaric acid B, diazepam and gramisetron failed to show any response on SPR, whereas, the kinetics parameters from SPRi and HPAC were successfully measured. From a comparative review of all the kinetic data, random results without any trends were observed (ka, kd and K_A) regardless of the relationships between the three methods: It is apparent that the measurement conditions (volume, flow rate, buffers), non-specific adsorption and experimental procedures had a strong impact on the generated data. The relative advantages and limitations of each method are critically presented on the basis of generated data. This comparative study provides a basis to further upgrade these techniques for confident measurement of drug-CDs interactions.

1. Introduction

Low aqueous solubility is a common hurdle in developing novel small molecules into successful medicines [1]. Molecular inclusion complex formation with CDs remains an attractive approach for insoluble drug candidates [2]. Cyclodextrins have been used successfully in the pharmaceutical formulation field to increase aqueous solubility and bioavailability of poorly soluble drugs through inclusion complex formation [3]. The formation of inclusion complex is totally dependent upon the binding affinity of drug molecules with CDs and therefore, it is critically important to study and understand the affinity behavior and kinetic parameters between the host-guest molecules. The binding constant (K_A) is a useful index to estimate the binding strength between the guest and the host molecules and the disassociation/association stability of the complex. It is also essential for evaluating the influence of CDs on *in vivo* absorption and bioavailability of drugs [4]. In studying these systems, it is assumed that the free and bound drugs exist in a state of equilibrium for the drug-CD supramolecular systems in aqueous solution, which is characterized by the equilibrium binding constant (K_A).

The K_A is widely used to estimate the interaction between drugs and cyclodextrins (CDs) [5]. CDs are able to include wide range of organic and inorganic molecules within the hydrophobic cavity of inner surface [6]. Although, the kinetics for the association and dissociation process are the base for the supramolecular

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system formation and *in vivo* functions of drug-CD complex [7–9], quantitative measurement for the kinetic rate constants has proved challenging [10–12]. The short relaxation time (<1 s) and the requirement of high time resolution is difficult to achieve experimentally [13]. As pointed out by Bohne, kinetic studies are necessary to provide the 'movie' in addition to the 'snapshots' taken from structural and thermodynamic measurements [14]. In addition, the *in vivo* functions (absorption) of free drugs from the CD supramolecules are controlled by the kinetics constants as explained below:

$$D + CD \underset{k_d}{\overset{k_a}{\rightleftarrows}} D - CD$$

$$K_a = \frac{k_a}{k_d}$$

Where k_a and k_d is the association and dissociation rate constants, respectively and also termed as on-rate (k_{on}) and off-rate (k_{off}) constants in kinetic studies for drug-target interactions [8,9].

To date, few studies have been reported focusing on the measurement of kinetic constants of drug-CD supramolecules including conventional techniques of NMR and UV spectroscopy [15,16]. Fluorescence correlation spectroscopy (FCS) [17] has been employed to compare the complexation kinetics of pyronines and analyze the individual steps during association and dissociation, but is limited for selected applications to the drugs with fluorescence properties. Capillary electrophoresis (CE) [18] has also been employed to estimate the rate constants of drug-CD interactions but suffers from poor reproducibility. Previously, there were few reports on the use of an SPR system, where the modified CD was fixed onto a gold sensor chip and drugs were passed over this surface in solution as analytes using the SPR system [19–21]. However, the methodology did not prove productive and efficient due to its non-high throughput nature. We have reported a new approach based on high performance affinity chromatography (HPAC) for determination of kinetic rate constants [22]. Although the method was able to measure the weak affinities and results were in agreement with the capillary electrophoresis method, the HPAC method was also laborious and time consuming. In HPAC, the modified mono-6A-*N*-propargylamino-6A-deoxy-CD was used as a stationary phase in a silica column, which changed the CDs chemically. We have recently combined HPAC with LC–MS/MS to achieve high-throughput efficiency [23].

Therefore, it is of special interest to establish high-throughput methodologies to measure the kinetics of drug-CD supramolecular interactions with extensive, weak binding and fast dissociation. In this aim, we have reported a high-throughput method based on small molecule microarrays (SMMs) in conjugation with surface plasmon resonance imaging technique (SPRi) [24]. SPRi is a label free technique and provides many advantages over classical SPR [25], allowing the parallel evaluation of hundreds or thousands of compounds simultaneously [26].

All reported methods which aim to measure the kinetic rate constants for drug-CD interactions have their own advantages as well as limitations. Therefore, it is necessary to compare the certain methods to evaluate their performance and accuracy. In this study, three advanced methods, HPAC-MS/MS, SPR and SPRi, were compared in measuring the interaction of 18 drugs with β-CD. The chemical structures and aqueous solubility of drugs were presented in Fig. 1. The research was designed to compare the three methods in terms of methodology principles and their effects on kinetic parameters, data quality, ease of measurement and applications.

2. Materials and methods

2.1. Materials

Unless otherwise stated, materials and solvents were obtained from commercial suppliers and used without further purification. Drugs (>99.5% purity) were purchased from the Dalian Meilun Biotech Co., Ltd. The β-CD (mono-6A-*N*-propargylamino-6A-deoxy-CD) bonded columns were kindly gifted by Professor Xinmiao Liang from Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China. HPLC grade ammonium acetate (NH_4Ac) and acetic acid (HAc) were purchased from SigmaAldrich Company (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Merck Millipore (Shanghai, China). Deionized water was purified on a Milli-Q system (Millipore, Bedford, MA, USA). X-Mercaptoundecyl bromoisobutyrate was purchased from HRbio (Beijing, China). Succinic anhydride, 2,2 ethylenedioxy-bis (ethylamine), 2,2-Bipyridyl (Bipy), Copper(II) chloride (CuCl2), 2-Hydroxyethyl methacrylate (HEMA), poly(2-hydroxyethyl methacrylate) (OEGMA), Ascorbic acid (AscA), *N*,*N*-Disuccinimidyl carbonate (DSC) and 4- (Dimethylamino) pyridine (DMAP) were purchased from SigmaAldrich (Beijing, China) for the SPRi experiments. For SPR, the β-CD-NH_2 was purchased from Shandong Binzhou Zhiyuan Biotechnology Co. Ltd (Shandong, China). Biacore®-specific products such as, CM5 Sensor Chip was purchased from GE Healthcare Life Sciences (Uppsala, Sweden). Chemicals required for covalent immobilization, such as *N*-hydroxysuccinimide (NHS), (1-ethyl-3- (3-dimethylaminopropyl) carbodiimide (EDC), ethanolamine were obtained from Sinopharm Biotechnology Ltd (Beijing, China).

2.2. Methods

2.2.1. HPAC-MS/MS method

The procedure followed for this technique is reported in our previous publication [23]. Briefly, a native β-CD stationary phase was prepared by covalently coupling the β-CD (mono-6A-*N*-propargylamino-6A-deoxy-CD) on silica particles via Huisgen [3+2] dipolar cycloaddition between the organic azide and terminal alkyne, which was characterized by FT-IR, 13C NMR and elemental analysis. As for the evaluation of the column performance, the separation efficiency is about 75,000 plates m^{-1}. The asymmetry of peaks is 1.03 calculated from orotic acid on this column and the high column efficiency and good peak shape prove the successful packing. Furthermore, the stability of the column performance still proved to be excellent after the injections of 400 samples. With the advantage of high sensitivity and selectivity, the high-throughput approach will be developed to simultaneously measure the kd values for eighteen drugs through one injection with low sample loading quantity (<10 ng per injection for single compound) by the HPAC-MS/MS technique. Therefore, a modified peak profiling HPAC with mass spectrometry detection (HPAC-MS/MS) method based on the correction of the plate height for the non-retained substance was employed to determine the kd,app values with low sample loading quantity. The mass spectrometry detection conditions were presented in Table S-1.

The studies were performed on a β-CD column (50 × 2.1 mm, 5) at 25 °C. Uracil was used as the non-retained substance. In order to elute the drugs in a reasonable time period, 17% v/v of acetonitrile was added into the mobile phase of NH_4Ac (50 mM, pH = 6.80). The flow rate was 0.3, 0.4, 0.5 and 0.6 mL/min. The peak profiling experiments were performed by injecting 5 μL sample. All experiments were performed in triplicate under each set of test conditions and full method details are provided in the supporting information.

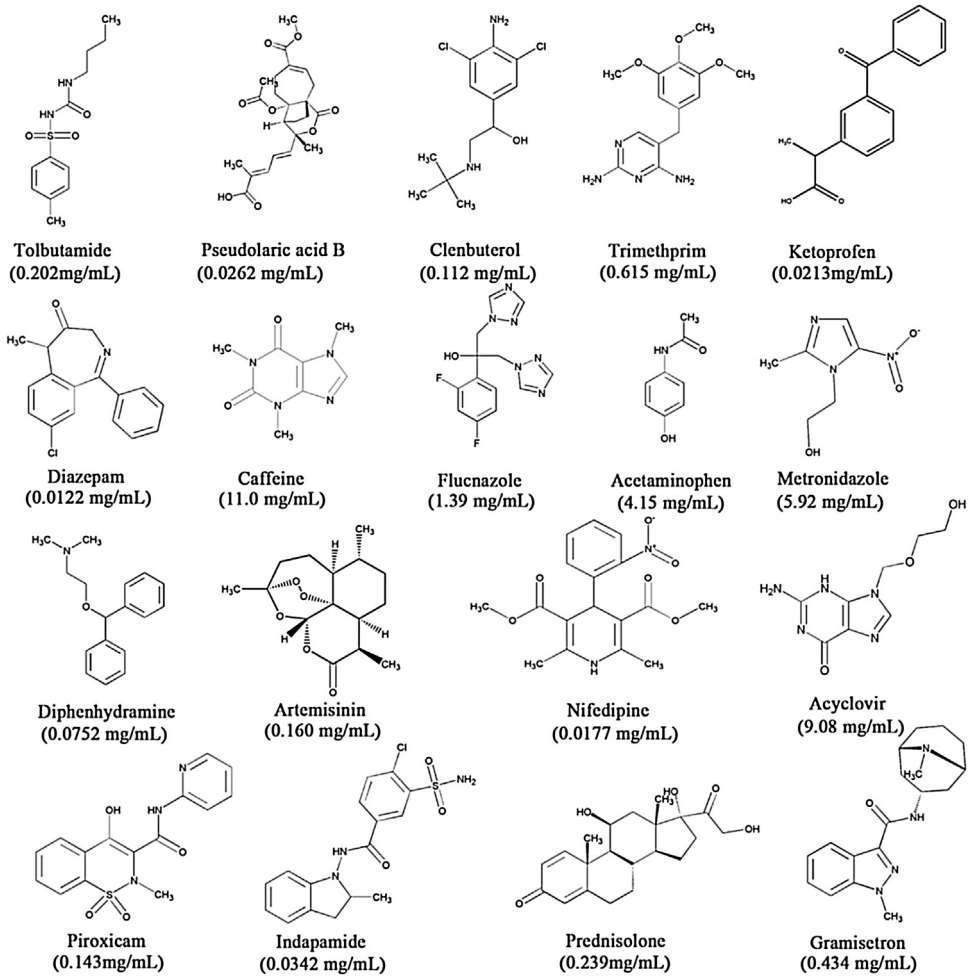

Fig. 1. The chemical structures and aqueous solubility of 18 drugs used in this comparison study (Drugbank).

2.2.2. SPRi method

All the experiments were carried out using the PlexArray® HT system (Plexera, LLC) which is based on surface plasmon resonance imaging [21]. The drugs were immobilized onto a 3D polymer brush surface based on surface initiated polymerization using a photo-cross-linked technique. All samples were injected at the rate of 2 μL/s at 25 °C. Oval regions of interest (ROIs) were set as a 9 pixel × 7 pixel area in imaging area. ROIs of bovine serum albumin (BSA) were used as controls for the measurement of specific signals. CD solutions in PBST (1 mM) containing Tween 20 (0.05% v/v), pH 7.4 at three concentrations (0.25, 0.5 and 1 mM) were used as analytes with association (400 s) and dissociation (300 s) flow rate of 2 μL/s at different concentrations by serial dilution. A solution of glycine HCl (pH 4.2, 10 mM) was used to regenerate the surface and remove bound proteins from the small molecules enabling the sensor chip to be reused for additional analyte injections. For data analysis two software packages, ORIGIN Lab and the Data Analysis Module (DAM) of Plexera were used. The supporting information provides full details of the methodology and analysis.

2.2.3. SPR method

This work was performed on a Biacore® T200 instrument and details are given in the supporting information. All experiments were carried out at 25 °C [17,18]. The β-CD-NH_2 was covalently captured on a carboxymethyl dextran coated gold chip by activation of carboxymethyl groups using EDC/NHS (0.39 M/0.04 M) chemistry The β-CD-NH_2 was dissolved and flowed in PBS buffer and passed over the chip at a flow rate of 5 μL/min. The unreacted ester groups on the sensor chip were inactivated or blocked by injection of 1 M ethanolamine at pH 7.4 for 7 min. Each drug was dissolved in the flowing buffer in five concentrations (0, 2.5, 5, 10, 20 μM) with 2% DMSO and injected into both the β-CD-NH_2 immobilized and reference flow cells at the flow rate of 10 μL/min with association and dissociation phases of 60 s and 100 s, respectively. Sensorgrams were evaluated using BIAevaluation software.

3. Results and discussion

3.1. Operational principals and experimental design of used methods

The three methods were used for the measurement of affinities and kinetic constants of 18 drugs against β-CD. Two methods, SPR and SPRi share similar operational principles but incorporate different experimental designs (molecules fixation strategies). In contrast, SPR and HPAC operate under the same strategies but the principles are very different. These relationships between principals and methodology provided the scientific motivation for this comparative study as well as to provide insight into the uniformity of derived kinetic and interaction data between the different methodologies.

3.1.1. HPAC-MS/MS

The HPAC-MS/MS method relies on the well-known principle of phase separation based on retention time, as in routine chromatography. One ligand is immobilized as the stationary phase and the relevant partner is incorporated into the mobile phase for interaction measurements. In this study, the β-CD column was fabricated by a click chemistry technique. The mono-6A-*N*-propargylamino-6A-deoxy-CD was immobilized onto silica beads by click chemistry [27] and the drug solutions (DMSO) flow as analytes to measure the kinetic rate constants at 25 °C.

3.1.2. SPR

SPR is a technique for detecting changes in refractive index at the sensor surface of a metal, gold or silver. As the analyte begins to flow over the sensing layer and bind to the substrate, the angle of reflectivity that satisfies the resonance condition will change accordingly until it reaches saturation and all the binding sites have been occupied [28]. The dissociation of analyte from the substrate causes the angle of the detector to return back to baseline once all the analyte has been completely removed. Thus, the strategy in this study is similar to that of HPAC. The mono-amino-β-CD was covalently immobilized onto the sensor chip and drug solutions (aqueous; 2% DMSO, v/v) were passed over the substrate as analytes for the measurements.

3.1.3. SPRi

The SPRi working principle is very similar to that of SPR except we can manually select the 'spots' (immobilized ligand location) and print thousands of ligands by spotter/printer before loading into the SPRi instrument [24]. The strategy for the measurement in SPRi technique however is completely different and the drugs were immobilized on the sensor surface and aqueous solution of β-CD (unmodified) is passed over the surface using three different concentrations of 0.25, 0.5 and 1 mM at a flow rate of 2 μL/s. Fig. 2 shows the schematic of drug-CDs interaction measurements of the three methods in comparison.

3.2. Experimental based methodology comparison

The relationships in working principles and strategies of the three experimental methods provide a scientific rationale to compare and discuss the generated data of drug-CD interactions. HPAC-MS/MS analyses provide relatively good reproducibility derived from the ability to reuse the cyclodextrin bonded column for multiple experiments. HPAC exhibits high efficiency in the measurement of the interaction kinetics of charged or uncharged solutes with cyclodextrins, without the addition of surfactants. As explained above, the β-CD was chemically modified with propargylamine and immobilized on silica beads in HPAC. For SPR, the β-CD was chemically modified with amino ($-NH_2$) groups and immobilized on gold chips though a dextran film. Thus, the β-CD was immobilized in both the cases, but the mode of immobilization, spacer length and surrounding environment was totally different and does not provide identical conditions for CD-drug interaction. In SPRi, the drugs were immobilized by a photo-cross-linking technique which allows the drug molecules to attach from different positions due to reactive carbene species and display simultaneously in various orientations. Whilst operating under the same working principles, the mode of measurement was totally different in SPR and SPRi. The dense dextran surface chemistry was used for SPR whereas the polymer brushes based on surface initiated polymerization platform modified with highly reactive, 3-trifluoromethyl-diazarine benzoic acid photo-cross-linker was employed in SPRi experiments. Overall, the three methodologies have elements of interconnectivity and therefore are worthy of comparison to study their impact on the measurement of drug-CD kinetics constants.

Table 1
Association rate constants determined by HPAC, SPRi and SPR experiments.

Drug Name	ka (HPAC) (1/Ms)	ka (SPRi) (1/Ms)	ka (SPR) (1/Ms)
Prednisolone	3.672E+02	1.660E+03	n.d.
Pseudolaric acid B	4.318E+02	1.800E+03	n.d.
Piroxicam	3.662E+02	8.760E+03	6.040E+05
Ketoprofen	1.752E+02	1.710E+03	3.677E+05
Tolbutamide	9.733E+01	3.140E+03	5.014E+05
Diphenhydramine	2.455E+02	6.260E+03	6.252E+05
Artemisinin	1.625E+01	6.610E+03	5.104E+05
Acyclovir	2.842E+00	8.690E+05	6.282E+05
Diazepam	1.757E+01	1.480E+03	n.d.
Indapamide	6.291E+01	6.070E+03	6.088E+05
Nifedipine	4.316E+01	4.420E+03	6.781E+05
Gramisetron	1.097E+02	2.660E+03	n.d.
Caffeine	1.593E+00	4.590E+03	6.200E+05
Clenbuterol	1.571E+01	3.190E+03	6.516E+05
Trimethprim	5.260E+01	9.690E+02	7.197E+05
Metronidazole	4.602E+00	8.540E+00	5.066E+05
Acetaminophen	1.045E+01	6.730E+03	6.136E+05
Fluconazole	1.267E+01	5.580E+04	6.344E+05

n.d. is denoted for not detectable.

Table 2
Dissociationrate constants determined by HPAC, SPRi and SPR experiments.

Drug Name	kd (HPAC) (1/s)	kd (SPRi) (1/s)	kd (SPR) (1/s)
Prednisolone	2.075E-01	1.300E-05	n.d.
Pseudolaric acid B	2.580E-01	9.540E-06	n.d.
Piroxicam	6.705E-01	9.300E-07	1.320E-03
Ketoprofen	7.013E-01	1.390E-05	2.099E-03
Tolbutamide	7.255E-01	9.340E-06	3.067E-03
Diphenhydramine	9.876E-01	1.120E-06	3.120E-03
Artemisinin	1.231E+00	3.850E-06	2.693E-03
Acyclovir	1.276E+00	6.550E-06	1.955E-03
Diazepam	1.330E+00	4.510E-06	n.d.
Indapamide	1.385E+00	3.040E-06	4.555E-04
Nifedipine	1.702E+00	3.410E-05	5.942E-03
Gramisetron	2.300E+00	6.350E-06	n.d.
Caffeine	2.575E+00	1.660E-06	2.518E-03
Clenbuterol	3.875E+00	6.110E-06	6.522E-04
Trimethprim	4.340E+00	3.540E-05	4.380E-04
Metronidazole	5.289E+00	3.410E-05	4.069E-04
Acetaminophen	7.108E+00	7.020E-06	2.774E-03
Fluconazole	3.375E+00	1.540E-03	5.376E-04

n.d. is denoted for not detectable.

3.3. Comparison based upon obtained data

Each of the three methods used for the measurement of drug-CD interaction has been claimed as superior in the literature although only the relative advantages of the strategy has been presented with an absence of discussion on the limitations. A list of the kinetics parameters, ka, kd and K_A from the three methods are shown in Tables 1, 2 and Table S-2, respectively, for 18 drug with β-CD. A marked difference is apparent when the kinetic values from three methods are compared but this large difference and poor correlation can be explained by the alternative experimental methodologies used. Four drugs, Prednisolone, Pseudolaric acid B, Diazepam and Gramisetron, repeatedly failed to show any response on SPR but the kinetics parameters from SPRi and HPAC were measured successfully. Whilst, sharing the same strategies, the marked deviation in the magnitude of kinetics parameters produced by HPAC and SPR is attributed to the different environment and instruments as discussed above. For SPRi, the static and mobile phase conditions were reversed.

The drug-CDs interaction exhibited fast association and dissociation rates. The smaller magnitude of k_a (k_{on}) from HPAC than

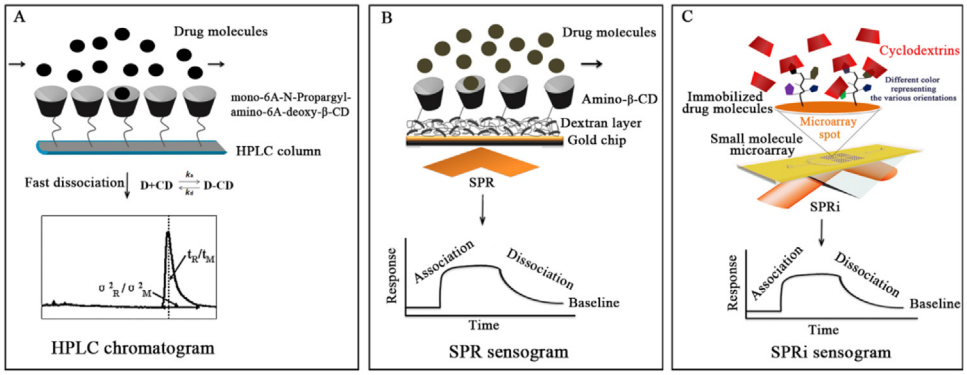

Fig. 2. Schematic representation of the three methods used in comparison study of drug-CD interaction measurements. The schematic of drug-CDs interaction measurements of HPAC (A), SPR (B), and SPRi (C).

SPRi and SPR suggests that the association phase of drug-CDs was slowest in HPAC, intermediate in SPRi and fastest when measured by SPR instrument. This indicates that the immobilized CDs might be sterically hindered due to tight packing of column with limited space for the free movement of drug molecules.

The dissociation constant (k_d) values generated from SPRi and SPR were considerably smaller than those obtained from HPAC, showing the impact of non-specific binding in SPR and SPRi which is supported by the fact that the biosensors are limited by non-specific binding problems.

From data in Tables 1 and 2 the slowest associations and fastest dissociations were recorded from HPAC. These observations are attributed to both the non-specific adsorption of drugs and steric hindrance due to the tight packing of the column. In contrast, the fast association and average dissociation in SPR is due to the free environment and non-specific binding which led to the slow dissociation rates.

The situation in SPRi is quite different and CDs are mobile whilst slower dissociation rates also indicate the high non-specific bindings due to the CDs interaction with photo-cross-linker moieties or blocking agents. Whilst the non-specific signals were subtracted from signals of interest but, the impact on SPR curve shape could not be excluded which affect the kinetic parameters. Hence, the magnitudes of equilibrium association rate constants are also affected, as shown in Table S-2. Apart from the above mentioned factors, the data analysis part could also be one of the reasons which are responsible for the deviation in data correlation. For example, however, the data was fitted using Langmuir model (1:1) in SPRi and SPR case, but the mode of data extraction and software used in analysis were very different which is part of the instrumental procedure. Whereas, the data analysis of HPAC was totally different and analysed using peak profiling method and Van Deemter equation fitting [20]. Last but not the least, the effects of drug structures on generated data should also be taken into account to explain the large deviation. The insoluble drugs are well-known to be interacting with β-CD and hence, the 18 sparingly soluble drugs with diverse structures were selected for this comparison study. However, it is not possible to compare each interaction due to large number of combinations; methodology performance can be easily evaluated on structural basis. Despite the drugs were flowed as analytes in HPAC and SPR at specific diluted concentration in organic solvents which allow them to interact freely with fixed β-CD, a large deviation in kinetic parameters can only be explained through technology differences. On the other hand, the case of SPRi was little special where drugs were chemically fixed through different functional group at given microarray spot and the generated parameters are the average of all possible orientations. Even the difference was much significant in methodology and technology of HPAC and SPRi; the values of kinetic constants are close when compared with those obtained from SPR leads to the minimization of technology gap. Therefore, the attempts were made to probe correlations of the generated parameters between the three methods. As shown in Fig. 3 and Fig. S-1, no strong correlation was found in any of the parameters ka, kd and K_A. However, it is interesting to note that the parameter values obtained from HPAC and SPRi are closer in magnitude when compared to those from HPAC/SPR and SPR/SPRi.

3.4. Relative importance of each method

From the claimed superiority by literature reports HPAC-MS/MS, SPR and SPRi techniques were selected for a comparative study and to discuss the relative advantages and limitations of the individual methods. With advantages of good speed, high precision and ease of automation, especially for its accessibility, HPAC is considered one of the best techniques to study the kinetics of interactions with weak to moderate affinities. HPAC in conjugation with MS/MS (HPAC-MS/MS) improve the performance and create a high-throughput technique. In addition, the method can be further improved by adjusting the spacer length and column packing in order to provide more flexibility to immobilized CDs molecules.

The SPR is a well-established technology used to measure the bio-molecular interactions in real time. However, when addressing drug-CDs interactions, the technology is limited by low drug solubility and small molecular size which affects the signal strength (In SPR, the drugs need to be flow in aqueous solution). As shown in Fig. 4, the signals produced from SPR instrument are of smaller magnitude when compared to those from SPRi. The non-specific binding can be controlled by increasing spacer length for CDs immobilization, different blocking agents and running buffers.

In comparison with the superiority of high throughput kinetics measurement, the SPRi method is a valuable choice for measurement of multiple drug-CD interactions. It is concluded that SPRi was able to generate signals of high magnitudes for weak interactions from high sensitive surface chemistries with the advantage of a photo-cross-linking technique which allows the one molecule to display in various orientations. Each orientation may have different binding affinity to the specific CD and therefore, the kinetic constants obtained from this method reflect the average of different multiple orientations. The other important factor to be considered is the non-specific bindings presented in all systems with different magnitudes, which are likely to contribute to the magnitude of the various kinetic parameters. Non-specific binding is a major challenge in the technology scale up and development which can be controlled by similar methods as used by SPR. The major advantage of SPRi is high-throughput and capacity of measuring thousands

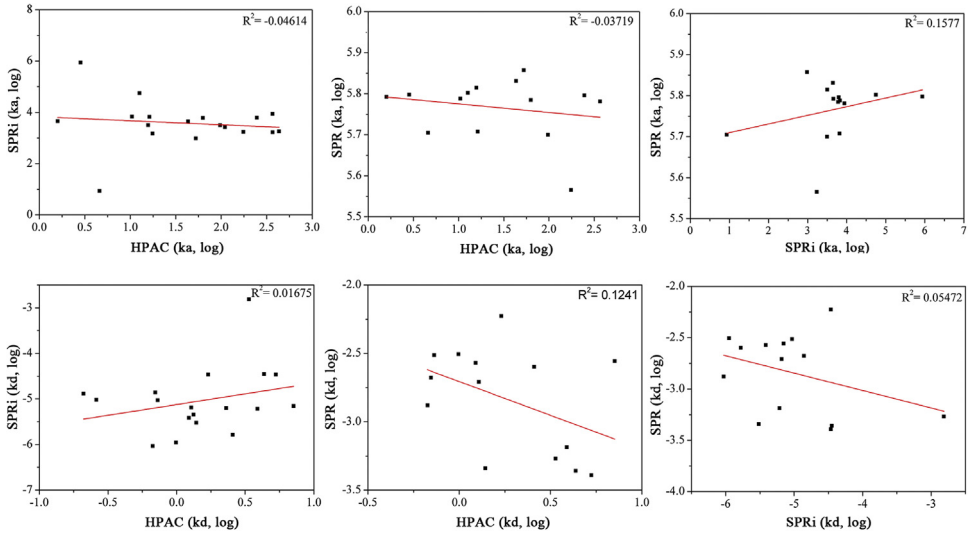

Fig. 3. The correlation graphs of association constant (ka) and dissociation constant (kd) values of three used methods in comparison to each other.

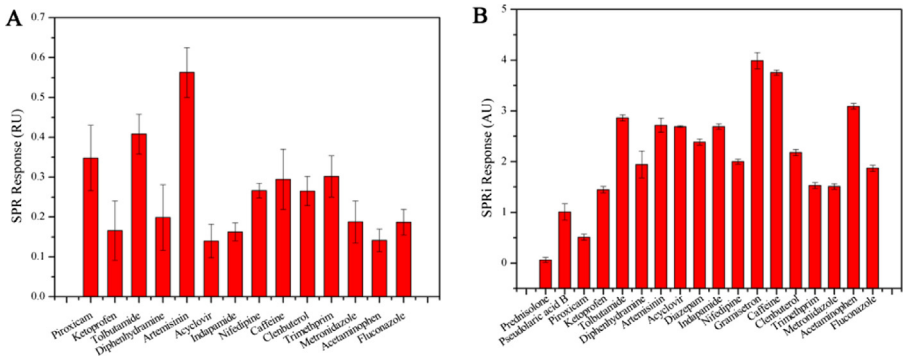

Fig. 4. Signal responses from (A) SPR (14 drugs) and (B) SPRi (18 drugs) showing signal profiles of drug-CD interactions at single highest concentrations of analytes (drugs (20 μM) in case of SPR and CDs (1 mM) in case of SPRi).

of drug-CDs interactions on a single platform. However, the mode of measurement is very reverse and CDs were suing as a mobile phase but drug displays in various orientations provided the more strength to this technique which gives the average affinity profile of different exposures. The non-specific binding refers to indiscriminate binding of the analyte or other component in the sample to either the ligand or the sensor surface matrix which is presented in all systems but quite different and might be contributed in kinetic parameters.

4. Conclusions

This paper reports the comparative study of three advanced methods, HPAC, SPR and SPRi for measuring drug-CDs kinetic constants. Although claims of superiority for a specific method over the others have been made [19,23,24] the relative merits and limitations are thoroughly discussed herein. The lowest degree of non-specific adsorption was observed in HPAC-MS/MS which is capable of measuring the drug-CDs interactions in more precise manner. Using the same measurement strategy, the SPR technique has not provided a marked improvement because of higher non-specific binding and non-high-throughput nature. On the other hand, SPRi could be a good choice due to high-throughput capability and a new mode of interaction measurement which provides the CDs affinity profiles with drug molecules exposed at various orientations due to photo-cross-linking technology. The problem of non-specific adsorption however remains, requires consideration and needs to be considered and potentially exempted by adjusting various parameters. This comparative study provides a basis/platform to direct method selection and improvement in techniques for measurement of drug-CDs interactions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2016.11.037.

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