Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/analyst

Paper

Supramolecular interaction of labetalol with cucurbit[7]uril for its sensitive fluorescence detection

Changfeng Li[a,b], Jianxia Feng[b] and Huangxian Ju[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

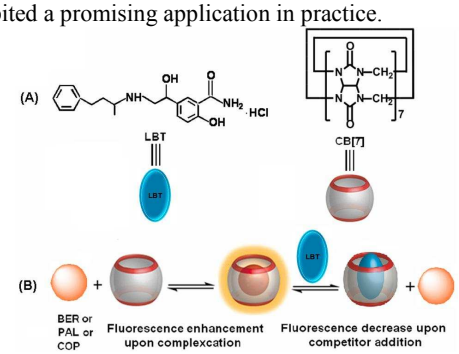
This work studied the host-guest interaction between cucurbit[7]uril (CB[7]) and labetalol in acidic aqueous solution and proposed a simple competitive method for fluorescence detection of labetalol. The binding constant of labetalol-CB[7] was $(1.83\pm0.22)\times10^6$ M^{-1}, which was greater than those of palmatine-CB[7], berberine-CB[7], and coptisine-CB[7] complexes. The fluorescence intensity of palmatine-CB[7], berberine-CB[7], and coptisine-CB[7] complexes linearly decreased with the increasing concentration of labetalol ranging from 0.014 to 2.06, 0.014 to 1.15, and 0.034 to 1.23 μM, respectively. Based on the competitive interaction, the proposed detection method for labetalol showed a limit of detection of 4.9 nM, 4.9 nM, and 12.0 nM, respectively, and was successfully applied for the determination of labetalol in human urine samples with good precision and the recovery from 95.4% to 102.5%. Moreover, it could be employed to monitor the time-dependent concentration of labetalol in urine from a healthy volunteer after oral medication. The superstructure-based competitive mode provided a promising fluorescence assay strategy for various potential applications.

Introduction

Labetalol hydrochloride (LBT), 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino) ethyl] salicylamide hydrochloride, as a non-cardiovascular β-blocker, is reported to possess some intrinsic sympathomimetic and membrane stabilizing activity. It can reduce heart rate and tremor.[1] Hence, it has been added to the list of forbidden substances issued by the International Olympic Committee.[2] Many methods have been reported for the determination of LBT in biological and pharmaceutical samples, including solid phase extraction-high performance liquid chromatography,[3] liquid chromatography-tandem mass spectrometry,[4,5] capillary electrophoresis,[6] fluorescence optosensing,[7] spectrophotometry,[8,9] and resonance light-scattering method.[10] However, the reported liquid chromatographic analysis requires complicated extraction process and time-consuming operation steps.[3-5] Though easier to operate, the low sensitivity of the reported spectrofluorometric method limits its practical application[8] due to the low level of LBT in urine. Therefore, the sensitive and simple detection method is an urgent need for the detection of LBT in real samples.

Over the past decade, the cucurbit[*n*]uril (CB[*n*], n = 5, 6, 7, 8, 10) family of molecular containers has emerged as a premiere platform for basic and applied studies of molecular recognition in water.[11-13] The CB[*n*] hosts feature two symmetry-equivalent ureidylcarbonyl portals, which are electrostatically negative and guard the entrance to a hydrophobic cavity. Among these hosts, CB[7] displays a favorable combination of a sufficient cavity size and water solubility (ca. 5 mM), which turns it into the most promising CB[*n*] host for binding of organic molecules.[14-18] This work studied the host-guest interaction between CB[7] and labetalol in acidic aqueous solution and proposed a simple method for sensitive fluorescence detection of labetalol through the competitive inclusion of CB[7] with labetalol and other three molecules to change the fluorescent emission.

In order to achieve the fluorescent detection of LBT, palmatine (PAL), berberine (BER) and coptisine (COP), a series of very weak fluorescence molecules that can form inclusion complexes with CB[7] to greatly enhance their fluorescence emission, were used as signal probes (Scheme 1). Due to the stronger binding ability of LBT-CB[7] than PAL-CB[7], BER-CB[7], and COP-CB[7] complexes, the presence of LBT significantly decreased the fluorescence intensity of these complexes, which led to a fluorescence method for detection of LBT. The proposed method could be successfully utilized to detect LBT in pharmaceutical dosage forms and urine samples, and exhibited a promising application in practice.



Scheme 1 Competitive recognition of CB[7] to LBT against PAL, BER, or COP

[a] *State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093 PR China,* [b]*School of Chemistry and Materials Science, Shanxi Normal University, Linfen 041004, PR China. E-mail: hxju@nju.edu.cn.*

Experimental

Materials and reagents

PAL hydrochloride, BER hydrochloride, COP, and LBT hydrochloride were obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing). CB[7] was prepared and characterized according to the reported procedure.[19,20] The stock standard solutions of CB[7] are stable for several weeks at room temperature. Britton-Robinson buffer solutions were prepared using 0.01 M boric acid, acetic acid and phosphoric acid, and their pH values were adjusted using 0.05 M sodium hydroxide or hydrochloric acid. All other chemicals were of analytical grade, and doubly distilled water was used thoroughly.

Instrumentation

The fluorescence measurements were performed on a F97XP spectrofluorometer (Shanghai, China) equipped with a xenon lamp in the fluorescence mode at the slit widths of 10.0 nm and 5.0 nm for excitation and emission in a standard 10 x 1 mm path-length quartz cell at 25.0 ± 0.5 °C. Calorimetric experiment was performed using a thermostated and fully computer operated ITC-200 calorimeter purchased from GE Instruments Corporation. ^{1}H NMR spectra were obtained using a Bruker AV-600 MHz spectrometer (Switzerland) in D_2O. Molecular modeling calculations were optimized at the B3LYP/6-31 G(d) level of density functional theory with the Gaussian 03 program.

Analysis of human urine

The fluorescence measurements of PAL, BER and COP and their complexes were carried out with the excitation wavelength of 344, 348, or 356 nm in the absence or presence of LBT or sample, respectively. Urine samples were handled according to the previous protocol.[21] Briefly, the urine samples were collected from healthy volunteers at a certain period over 12 h to monitor the time dependent concentration of LBT in the urine after the oral administration of 80 mg of LBT medication, and immediately frozen and stored at -20□ until analysis. Prior to detection, the amino acids in the urine samples were firstly eliminated by adding 0.5 mL of 4 M sodium hydroxide and 5.0 mL dichloromethane to 1.0 mL urine to vortex-extract for 3 min and centrifuge at 4000 rpm for 10 min. A total of 4.0 mL of the dichloromethane layer was then evaporated to dryness under N_2, and the residue was dissolved in 1.0 mL water for fluorescent detection.

Result and discussion

Spectral characteristics

In acidic aqueous solution, PAL, BER, and COP showed undetectable or very weak fluorescent emission, while LBT exhibited a weak native fluorescence (Fig. 1). In the presence of CB[7], the fluorescence emission of LBT did not show obvious change. However, a dramatic increase in fluorescence intensity was observed upon addition of CB[7] in PAL, BER, and COP solutions. This should be attributed to the inclusion of PAL, BER and COP by CB[7] to change their space structure or conformation and produce fluorescent complexes. Interestingly, the addition of LBT to the mixture of CB[7] and PAL, BER, or COP led to significant decrease of fluorescence intensity, which suggested a fluorescence method for detection of LBT.

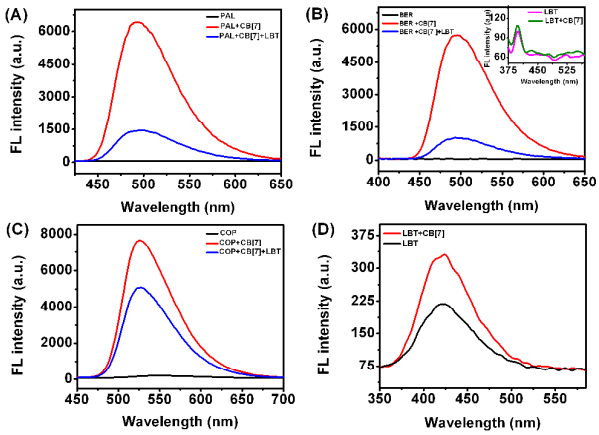


Fig. 1. Fluorescence spectra of different marked solutions (pH 2) with excitation wavelength of 344 (A), 348 (B), 356 (C) and 301 nm (D) at 2.50 μM.

Influence of pH

The influence of pH on fluorescence intensity of formed inclusion complex in the absence or presence of LBT was examined in pH 1.0-7.0 Britton-Robinson buffer solutions. Upon the addition of LBT all inclusion complexes showed the maximum change at pH 2.0 (Fig. 2). Therefore, the Britton–Robinson buffer with pH 2.0 was used for all subsequent experiments.

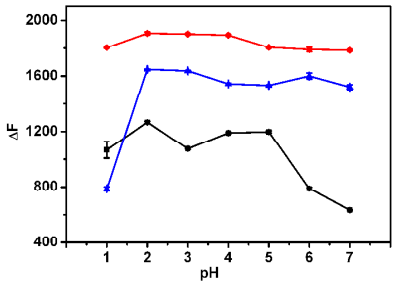


Fig. 2. Influence of pH on fluorescence intensity change of 2.5 μM BER-CB[7] (●), PAL- CB[7] (▼), and COP-CB[7] (■) upon addition of 0.685 μM LBT.

Interaction mechanism of CB[7] with PAL, BER, COP, and LBT

Although the fluorescent emission of pure PAL, BER, and COP was very weak, the fluorescence intensity greatly enhanced after they entered the hydrophobic cavity of CB[7], which showed a good linear relationship between $1/(F-F_0)$ and $1/c_{CB[7]}$ (Fig. 3), indicating the existence of a 1:1 complex.[22] From the plots the binding constants (K) for these complexes could be determined to be 1.10×10^5, 1.77×10^5, and 1.28×10^4 M^{-1}, respectively.

According to above results, the interaction of CB[7] with LBT formed a inclusion complex, which decreased the amount

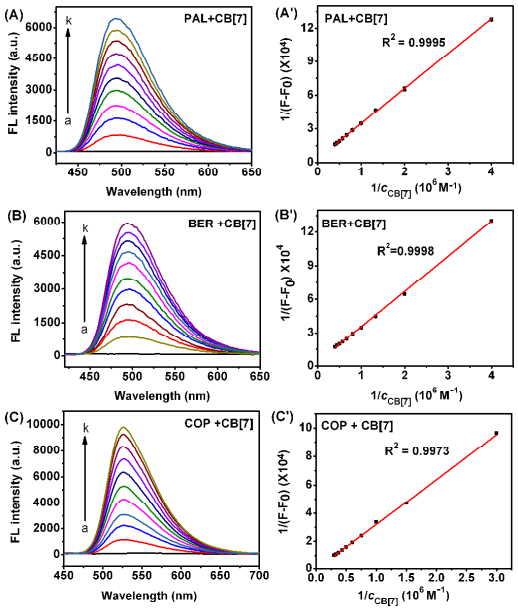


Fig. 3. Fluorescence spectra of 2.50 μM PAL (A), BER (B), and COP (C) upon addition of 0, and 0.25 to 2.50, 0.25 to 2.50 and 0.33 to 3.33 μM CB[7]. (A'), (B') and (C'): plots of $1/(F\text{-}F_0)$ vs $1/c_{CB[7]}$.

of formed PAL-CB[7], BER-CB[7] and COP-CB[7], thus decreased the fluorescence intensity of these complexes. However, it was difficult to obtain the K value of this complex using the same method as that for other three complexes due to the negligible change of fluorescence intensity upon addition of CB[7] to LBT solution (Fig. 1D). Thus the isothermal titration calorimetric (ITC) experiment, which is a powerful tool for investigating the host-guest complex interactions, was applied to determine the binding constant (K) and the thermodynamic parameters (enthalpy and entropy changes ΔH° and ΔS°) of LBT-CB[7] complex.[23] From the ITC data (Fig.4), K value for the formation of 1:1 LBT-CB[7] complex was calculated to be $(1.83\pm0.22)\times10^6$ M^{-1} with a "N" value of 1.02 by the curve fitting. The $K_{LBT\text{-}CB[7]}$ value was more than 10 times greater than those of PAL-CB[7], BER-CB[7] and COP-CB[7], which meant the stronger binding of LBT with CB[7].

The formation of LBT-CB[7] inclusion complex could be confirmed using ^{1}H NMR spectroscopy (Fig. 5). Compared with the proton resonance of the unbound LBT molecule (Fig. 5a), the resonance of protons H_1, H_2, H_3, H_4, H_5, H_6 and H_7 of the bound LBT in the ^{1}H NMR spectrum of LBT-CB[7] complex experienced a progressively up-field shift (Fig. 5b), indicating that CB[7] bound selectively the protonated phenylpropylamino residues due to cooperative hydrophobic and ion-dipol interactions and the well-matched size and morphology. The resonance of protons H_8, H_9, H_{10}, H_{11} and H_{12} of LBT experienced a slightly down-field shift, indicating this part of the molecule was located just outside the carbonyl portal of the CB[7] host.[13]

Molecular modeling calculation was optimized at a B3LYP/6-31G(d) level of density functional theory[24-26] using Gaussian 03 program. The results confirmed partial inclusion of

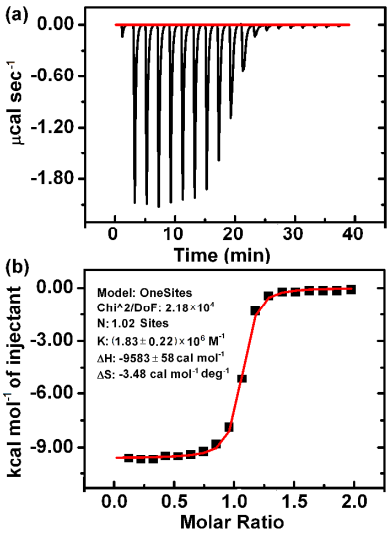


Fig. 4. Microcalorimetric titrations of LBT with CB[7] in Britton–Robinson buffer solution (pH 2.0) at 298.15 K. (a) Raw ITC data for 20 sequential injections (2.0 μL per injection) of LBT solution (2.0 mM) into the CB[7] solution (0.2 mM). (b) "S-type" heat effect of the complexation between LBT and CB[7] for each injection, obtained by subtracting the dilution heat from the reaction heat, which was fitted by computer simulation using the "one set of binding sites" model.

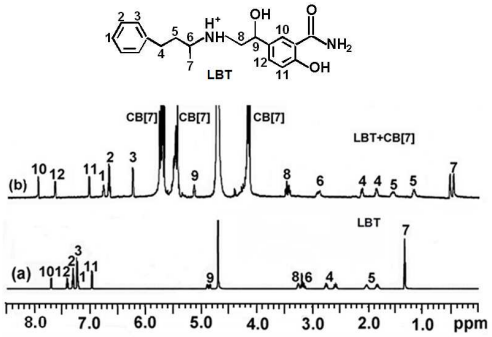


Fig. 5. ^{1}H NMR spectra of (a) LBT and (b) LBT + CB[7].

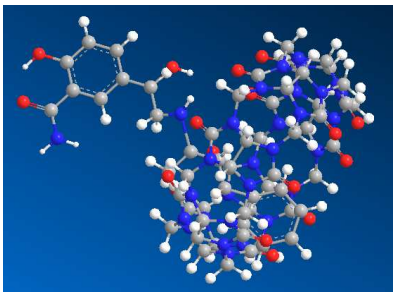


Fig. 6. Energy-minimized structure of LBT-CB[7] complex in the ground state using balls and tubes for rendering the atoms. Color codes for LBT and CB7: oxygen, red; nitrogen, blue; carbon, dark gray; hydrogen, white.

LBT in the hydrophobic cavity of CB[7] (Fig. 6). It can be seen from molecular simulation that the phenylpropylamino group was protonated in acidic solution, giving the cationic form of LBT. This indicated that, in the energy-minimized structure, the phenylpropylamino group of the molecule located inside the

host, however, the salicylamide part of the molecule located just outside the carbonyl portal of the CB[7] host.

Analytical performance

As shown in Fig. 7, with the increasing concentration of LBT, the fluorescence intensity of PAL-CB[7], BER-CB[7] and COP-CB[7] linearly decreased, and then trended to a minimum value at 2.64, 1.62 and 1.50 μM LBT, respectively. The linear range was 0.014-2.06 μM, 0.014-1.15 μM, and 0.034-1.23 μM, respectively. The linear regression equations were F = -2409.3 c + 6732.4, F = -2408.8 c + 5593.6, and F = -1761.9 c + 7523.0 (c denotes the concentration of LBT in μM) with the correlation coefficients of 0.9989, 0.9914, and 0.9963, and the detection limits of 4.9 nM, 4.9 nM, and 12.0 nM at 3δ, respectively. The

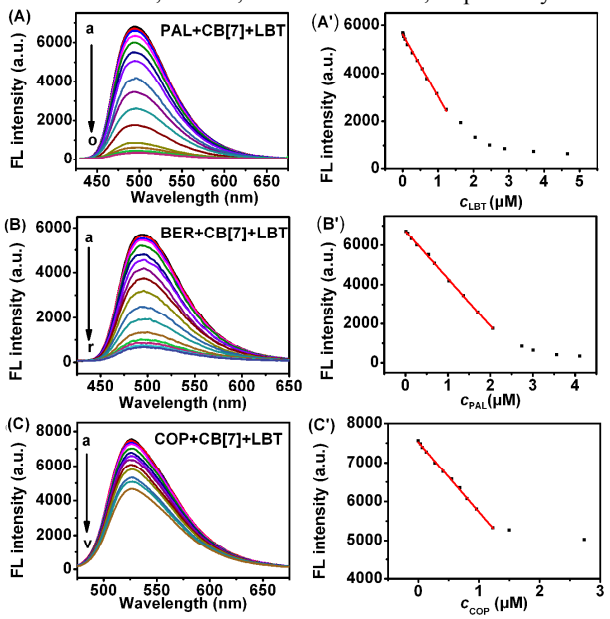


Fig. 7. Fluorescence spectra of 2.50 μM PAL-CB[7] (A), BER-CB[7] (B) and COP-CB[7] (C) (pH 2) in presence of 0, and 0.014 to 4.66, 0.014 to 4.11 and 0.034 to 2.74 μM LBT. (A'), (B') and (C'): Plots of FL intensity vs LBT concentration.

Table 1 Comparison of detection methods for LBT

Technique	Linear range (μM)	Limit of detection (μM)	Detection wavelength (nm)	Reference
SPE-HPLC	0.027-2.74	0.0033	220	3
LC-MS-MS	---	0.55	---	4
SPME-LC-ESI-MS	0.003-0.27	0.0003	---	5
CE	63.6-318.5	0.33	---	6
Fluorescence optosensing	0.027-0.68	0.009	435	7
Spectrofluorimetry	2.74-41.1	2.16	432	8
Spectrophotometry	2.74-27.4	2.13	410 or 456	9
Resonance light scattering	0.4-240.0	0.21	356	10
Spectrofluorimetry				
PAL-CB[7]	0.014-2.06	0.0049	495	This work
BER-CB[7]	0.014-1.15	0.0049	497	
COP-CB[7]	0.034-1.23	0.012	527	

proposed method proved to have higher sensitivity and selectivity than other spectrofluorimetric methods for detection of LBT reported in the literature, as presented in Table 1.

Analytical application

Prior to the application of the proposed fluorescence method in drug analysis of human urine samples, the effects of commonly used tablet excipients and common ingredients in human urine on the determination of LBT were examined. The criterion for interference was fixed at a ±5% variation in the average fluorescence intensity calculated for the established level of LBT. Because PAL, BER and COP showed the same trends, the following examination used BER as an example (Table 2). The results did not show any interference of the common ingredients in tablet and urine samples. However, the components in urine samples, such as cysteine, alanine, phenylalanine and valine, could change the fluorescence intensity to a certain degree. Hence, they should be separated prior to the determination. Accordingly, the separation of LBT from the interfering substances could be achieved through extraction method with organic solvent such as dichloromethane.

The determination results of LBT with the proposed fluorescent method were listed in Table 3. The standard deviations for commercial tablets were less than 0.73%, and the recoveries examined with a standard addition method were in the range of 98.2-99.7%. The detection of LBT in urine samples

Table 2 Effect of interferents on the determination of 1.0 μM LBT (tolerance error ± 5.0%)

Tolerance ratio in mass	interferents
3000	Starch, glucose, sucrose, lactose, sorbitol, mannitol, boracic acid, hexane diacid, urea
2000	Methyl cellulose, Cl^-, I^-, CO_3^{2-}, NO_3^-, SO_4^{2-}
1500	Gelatin, glycin, uric acid
1000	Sodium hydroxymethyl cellulose, gum acacia power, tryptophan
500	Sodium carboxymethyl cellulose
100	NH_4^+, Na^+, K^+
50	Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Mn^{2+}
6	Atenolol, bopindolol, acebutolol, metoprolol
0.5	Alanine, cysteine, cystine, phenylalanine, valine

Table 3 Fluorimetric determination of LBT in commercial tablets and spiked urine samples (n = 5, p = 95%).

Samples	Amount added or spiked	Amount found	Recovery (%)±S.D[a]
Drug 1	80 mg/grain	79.78 mg	99.7± 0.52
Drug 2	80 mg/grain	78.56 mg	98.2± 0.73
Urine 1	0.8 μg mL^{-1}	0.82 μg mL^{-1}	102.5±0.17
Urine 2	1.60 μg mL^{-1}	1.57 μg mL^{-1}	98.1±0.25
Urine 3	2.40 μg mL^{-1}	2.29 μg mL^{-1}	95.4 ±0.30

showed the satisfactory recoveries from 95.4 to 102.5%.

This method could be employed to monitor the time-dependent concentration of LBT in the urine of healthy volunteers after the oral administration of the LBT medication. The volunteers were premedicated with 80 mg of LBT, and the urine was collected over 12 h at various times. The concentration of LBT in the urine increased with the metabolic time from 0 to 4 h, and reached the maximum in a period of 4.0-4.5 h. Afterward the concentration of LBT in the urine decreased. The result indicated that this method was promising as a cost-effective, sensitive, and selective technique for study of the pharmacokinetics of LBT.

Conclusions

This work designs a novel method for the determination of LBT through the supramolecular interaction of CB[7] with LBT and PAL, BER, or COP and their fluorescence characterization. The formation of PAL-CB[7], BER-CB[7], and COP-CB[7] complexes greatly enhances the fluorescent emission of PAL, BER, and COP due to their strong coplanar and rigidity. The LBT-CB[7] complex possesses greater binding constant than PAL-CB[7], BER-CB[7], and COP-CB[7] complexes, thus the PAL, BER, or COP in CB[7] cavity can be replaced by LBT to decrease the fluorescent emission of PAL-CB[7], BER-CB[7], and COP-CB[7]. Based on the competitive mode, the fluorescence method for the determination of LBT shows high sensitivity and good selectivity, and has successfully been applied in the analysis of LBT in pharmaceutical preparations and biological fluids. The CB[7]-based fluorescence method provides a robust tool for monitoring the drug metabolism in pharmaceutical treatment.

Acknowledgemetns

We gratefully acknowledge National Basic Research Program (2010CB732400) and National Natural Science Foundation of China (21135002, 21121091). We are grateful to Dr. Jiahuang Li from Nanjing University for their kind help in the ITC experiments.

References

1 P. Hemmersbach, R. De La Torre, *J. Chromatogr. B*, 1996, 687, 221-238.
2 H. Maurer, O. Tenberken, C. Kratzsch, A. Weber, F. T. Peters, *J. Chromatogr. A*, 2004, 1058, 169-181.
3 W. Boonjob, H. Sklenářová, F. J. Lara, A. M. García-Campaña, P. Solich, *Anal. Bioanal. Chem.* 2014, 4064, 207-4215.
4 M. Gergov, J. N. Robson, E. Duchoslav, I. Ojanperä, *J. Mass. Spectrom.* 2000, 35, 912-918.
5 J. C. Whoq, H. L. Lord, J. Pawliszyn, H. Kataoka, *J. Microcolumn Sep.* 2000, 12, 255–266.
6 R. Nehmé, A. Lascaux, R.Delépée, B. Claude, P.Morin, *Anal. Chim. Acta*, 2010, 663, 190-197.
7 E. J. Llorent-Martínez, D. Šatínský, P. Solich, *Anal. Bioanal. Chem.*, 2007, 387, 2065-2069.
8 F. Belal, S. Al-Shaboury, A. S. Al-Tamrah, *J. Pharm. Biomed. Anal.,* 2002, 30, 1191-1196.
9 F. Belal, S. Al-Shaboury, A. S. Al-Tamra, *IL Farmaco*, 2003, 58, 293-299.
10 Q. Sun, X. C. Jing, J. Ma, J. Anzai, B. Z. Wang, X. Y. Du. *Mat. Sci. Eng. C*, 2009, 29, 271-274.
11 Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem. Int. Ed.*, 2005, 44, 4844-4870.
12 S. Moghaddam, C. Yang, M. Rekharsky, Y. H. Ko, K. Kim, Y. Inoue, M. K. Gilson, *J. Am. Chem. Soc.*, 2011, 133, 3570-3581.
13 L. P. Cao, M. Šekutor, P. Y. Zavalij, K. Mlinarić-Majerski, R. Glaser, L. Isaacs. *Angew. Chem. Int. Ed.*, 2014, 53, 988-993.
14 V. N. Sueldo Occello, A. V. Veglia, *Anal. Chim. Acta*, 2011, 689, 97-102.
15 H. Tootoonchi, S. Yi, A. E. Kaifer, *J. Am. Chem. Soc.,* 2013, 135, 10804-10809.
16 C. F. Li, L. M. Du, W. Y. Wu, A. Z. Sheng, *Talanta*, 2010, 80, 1939-1944.
17 C. J. Li, J. Li, X. S. Jia, *Org. Biomol. Chem.*, 2009, 7, 2699-2703.
18 Y. X. Chang, Y. Q. Qiu, L. M. Du, C. F. Li, M. Guo, *Analyst*, 2011, 136, 4168-4173.
19 Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.,* 2000, 122, 540-541.
20 A. Day, A. P. Arnold, R. J. Blanch, B. Snushall, *J. Org. Chem.,* 2001, 66, 8094-8100.
21 G. Q. Wang, Y. F. Qin, L. M. Du a, J. F. Li , X. Jing , Y. X. Chang, H. Wu, *Spectrochim. Acta A*, 2012, 98, 275-281.
22 M. Megyesi, L. Biczók, I. Jablonkai, *J. Phys. Chem. C,* 2008, 112, 3410-3416.
23 Q. Li, D. S. Guo, H. Qian, Y. Liu. *Eur. J. Org. Chem.* 2012, 962-3971.
24 C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B.* 1988, 37, 785-789.
25 A. D. Becke, *J. Chem. Phys*., 1988, 88, 2547–2553.
26 A. D. Becke, *Phys. Rev. A*, 1988, 38, 3098–3100.