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Rational Design and Study on Recognition Property of Paracetamol-Imprinted Polymer

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Abstract The design and study on recognition of paracetamol-imprinted polymer for application in quantification of drugs was reported. Base on our previous work, the promising monomer, itaconic acid (IA), was computationally selected rapidly from the virtual library using the interaction energy (ΔE) between a paracetamol (PR) molecule and four monomer molecules as a measure of their interaction. The possible conformation of PR interacting with IA displayed the nature of the interaction between PR and IA; hydrogen bonds (hbs) mainly contribute to this interaction. UV spectra analysis confirmed the occurrence of the hbs interaction between PR and IA at the polymerization stage. The optimal solvents for porogen and eluant were determined by the strength of hbs interaction between PR and the solvents, which were calculated employing density functional theory. The corresponding molecularly imprinted polymers (MIPs) and non-imprinted polymers were prepared and evaluated. The experimental results were consistent with those calculated, which confirmed the validity of the above-related calculation believed to facilitate the selection of monomers and solvents for the synthesis of MIP at molecular level.

Keywords Molecularly imprinted polymer (MIP) · Design · Interaction energy · Solvent · Hydrogen bond (hb)

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Abbreviations

IA	itaconic acid
ΔE	interaction energy
PR	paracetamol
hbs	hydrogen bonds
DFT	density functional theory
MIP	molecularly imprinted polymer
TRIM	trimethylolpropane trimethacrylate
AIBN	azo-bis(isobutyronitrile)
MAA	methacrylic acid
G_{solv}	solvation energy
AA	acrylamide
IF	imprinting factor
SCIPCM	a self-consistent isodensity polarizable continuum model

Introduction

Molecular imprinting is an effective method for obtaining a synthetic material with a predetermined selectivity for a target molecule by producing recognition sites [1–3]. The shape, size, and positions of the functional groups in the recognition sites generated are complementary to those of the target. Molecular imprinting polymer (MIP) has made rapid progress since 1993 when the MIP with theophylline as template was reported [4]. At present, most of MIPs are performed using non-covalent imprinting due to its facile preparation. In principle, any compound as a template which interacts with monomers through hydrogen bonds or electrostatic, hydrophobic, and van der Waal's forces can be made into a corresponding MIP. In fact, to date, only a limited number of compounds can produce good imprinting effect.

Successful molecular imprinting must accomplish the following three steps: forming imprinted sites, locking the sites, and making the sites accessible to analyte molecule. For a specific template, it is very important to select a promising monomer and optimize the polymeric conditions to maximize MIP performance, which has traditionally relied on trial-and-error and combinatorial approaches involving the mass preparation and screening of individual recipe formulations prepared from a library of would-be functional monomers. With the rapid development of computational chemistry, applying computational approach to molecular imprinting technology has been drawing numerous attentions. Takeuchi's group [5] studied the functional monomer-template conformation before polymerization with a Monte Carlo simulation technique. Piletsky's group [6–9] used a virtual library of functional monomers to assign and screen against the target template molecule. Dumitru's group [10] applied state-of-the-art computational tools to achieve an understanding of intermolecular interactions in molecular imprinting of theophylline into complex polymeric systems. Liqing's group [11] and Wenguo's group [12] selected monomers using density functional theory (DFT) method. Obviously, most effort has been directed towards the choice of monomers; there is no perfect method for designing MIP because imprinting systems are numerous and complicated.

Our interest relates to not only the selection of monomer but also the selection of solvents for porogen and eluant. We paid more attention to paracetamol-imprinted MIP. Paracetamol (acetaminophen) is well recognized as an analgesic and antipyretic agent in replacement of aspirin. However, some side effects of paracetamol overdose has been reported, such as acute liver failure [13], intrauterine growth retardation [14], involvement of brain serotonergic system [15], fever and malaise [16]. Paracetamol poisoning has been a public health issue since the 1970s [17]. It is significant to detect and quantify paracetamol in the human serum and urine and calculate the optimal dose. MIP is promising to be used for the quantification of drugs in real samples using competitive binding assay. Based on our previous work [18], for the given template, paracetamol (PR), we rapidly selected a promising monomer, itaconic acid (IA), from the virtual library using the interaction energy, ΔE, between a template molecule and four monomer molecules as a measure of their interaction and searched the possible conformation, which displayed the nature of the interaction between PR and IA in the molar ratio of 1:4 (template/monomer).

The optimal solvents for porogen and eluant were determined by the strength of hydrogen bonds (hbs) interaction between PR and the solvents, which were calculated using DFT at B3LYP/6-31 + G** level. UV spectra of PR in the presence of IA in the chosen solvent revealed the existence of hbs interaction between PR and IA.

To examine the validity of calculations above, the corresponding MIPs were prepared and evaluated. Comparison of the affinities for paracetamol or its analogues of the MIPs showed that the experimental results were well in agreement with the results calculated.

Material and Method

Chemicals

Paracetamol was kindly donated by the Pharmaceutical Plant of Taixing, Jiangsu, China. Trimethylolpropane trimethacrylate (TRIM) was from Sigma, USA, and phenacetin, 4-hydroxybenzaldehyde and itaconic acid (IA) were purchased from Aldrich, USA. Azo-bis (isobutyronitrile) (AIBN), and methacrylic acid (MAA) were obtained from Shanghai Chemical Company, China and purified before use. Other chemicals were of analytical grade purchased from standard domestic suppliers and were used as received.

Calculation of Interaction Energy and the Search of Conformation

The calculation of interaction energy (ΔE) and the search of conformation were performed as recited in the literature [18]. First, the conformations of the template and the monomers were optimized and the energies of the molecules with the optimized conformations were calculated. Then, the conformation optimization and energy calculation were applied to the complex formed between the template and the monomers in the molar ratio 1:4 (template/monomer), which is generally used in molecular imprinting. Finally, the ΔE was obtained from Eq. 1:

$$\Delta E = E(\text{template} - \text{monomer complex}) - E(\text{template}) - \sum E(\text{monomer}). \qquad (1)$$

The more negative ΔE value is corresponding to the higher ΔE.

We docked PR and IAs to find the possible interaction geometry, in the molar ratio of 1:4, using a simulated annealing algorithm.

Calculation of the Solvation Energy and the Strength of hbs

We adopted Gaussian 03 [19] to calculate the Mulliken charges of the atoms in PR and IA, the dipole moments, the solvation energy (G_{solv}), and the strength of hbs at B3LYP/6-31 +

G** level. B3LYP is a kind of DFT method which takes electronic correlation energy into consideration and usually gives better results of weak interaction system compared with Hartree–Fock method. These simulations were carried out on the Legend PC cluster.

Solubility Experiment

The literature [20] was taken as a reference to do the solubility experiment at ambient temperature and atmospheric pressure.

UV Spectra Analysis

UV spectra were used to study the hb interaction between PR and IA. During a run, a 5-ml volume of a solution of PR (0.05 $\text{mmol}\cdot\text{l}^{-1}$) in acetonitrile was titrated with aliquots of IA (0.05 $\text{mmol}\cdot\text{l}^{-1}$) at 25 °C. After equilibrated for 5 min, the UV spectra of the mixtures were measured using the corresponding pure IA solutions as blank.

Preparation of MIP

The required amounts of template, monomer, cross-linker (TRIM), initiator (AIBN), and porogen were placed into glass tubes (the detailed composition was listed in Table 1). After shaking for homogeneity, the mixture was sparged with nitrogen for 15 min to remove the dissolved oxygen and then sealed under vacuum. The polymerization was allowed to continue in a water bath at 60 °C for 24 h. The resulting polymer was grounded and sieved to collect 75-μm particles. After being washed extensively with eluant, all particles were

Table 1 Polymer composition and polymerization condition.

Polymer	P_1	P_{01}	P_2	P_{02}	P_3	P_3'	P_3''	P_{03}	P_4	P_{04}	P_5	P_{05}	P_6	P_{06}
PR (mmol)	0.25	/	0.25	/	0.25	0.25	0.25	/	0.25	/	0.25	/	0.25	/
MAA (mmol)	1	1	/	/	/	/	/	/	/	/	/	/	/	/
AA (mmol)	/	/	1	1	/	/	/	/	/	/	/	/	/	/
IA (mmol)	/	/	/	/	1	1	1	1	1	1	1	1	1	1
TRIM (mmol)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Acetonitrile (ml)	10	10	10	10	10	10	10	10	/	/	/	/	/	/
Methanol (ml)									10	10	/	/	/	/
THF (ml)	/	/	/	/	/	/	/	/			10	10		
Acetonitrile/chloroform (ml)[a]	/	/	/	/	/	/	/	/	/	/			10	10
AIBN (mg)	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Eluant	–[b]	–[c]	–[b]	–[c]	–[b]	–[d]	–[e]	–[c]	–[b]	–[c]	–[b]	–[c]	–[b]	–[c]

[a] Acetonitrile /chloroform (*v*/*v*=1:1)

[b] The polymers were washed with five repeats of 100 ml of acetic acid/methanol (*v*/*v*=1:5) and then three repeats of 100 ml acetonitrile, all at room temperature to remove template and impurities.

[c] The polymers were washed with two repeats of 100 ml acetonitrile to remove impurities.

[d] The polymers were washed with five repeats of 100 ml of acetone and then three repeats of 100 ml acetonitrile.

[e] The polymers were washed with five repeats of 100 ml of THF and then three repeats of 100 ml acetonitrile.

washed with rebind solvent and dried to constant weight under vacuum and stored at ambient temperature until use.

As a reference, non-imprinted blank polymer was also prepared in the absence of the template.

Evaluation of MIP

To evaluate the imprinting effect, affinity, and selectivity of the MIP, the adsorption of PR was performed at 25 °C in a shaker operating at 150 rpm. Three sets of experiments were conducted. The first set was to determine the time course of adsorption. In this case, samples were taken periodically, and the amount of PR adsorbed was determined by UV spectrometry at 246 nm. The second set of experiment was to determine the affinity for PR of the different MIPs. For this, the adsorption on the MIPs was conducted in 1 $g \cdot l^{-1}$ PR solution for 10 h. The adsorption time of 10 h was determined by the results of the first set experiment. The third set of experiment was to determine the selectivity for PR or its analogues (phenacetin, 4-hydroxybenzaldehyde) of the MIP. The adsorption was performed in 1 $g \cdot l^{-1}$ phenacetin or 4-hydroxybenzaldehyde solution for 10 h, and the amounts of phenacetin and 4-hydroxybenzaldehyde were determined by UV spectrometry at 249 and 270 nm, respectively. One hundred milligrams of polymer was used in every experiment.

The imprinting factor $(\mathrm{IF}) = \frac{K_\mathrm{D}(\mathrm{MIP})}{K_\mathrm{D}(\mathrm{Blank})}, K_\mathrm{D} = \frac{C_\mathrm{p}}{C_\mathrm{S}}$, where C_p and C_s refer to the PR concentration in the polymer and in solution, respectively, at adsorption equilibrium. K_D (MIP) and K_D (Blank) are the partition coefficients of the imprinted polymer and non-imprinted polymer, respectively.

Results and Discussion

Effect of Interaction Energy on Recognition (Affinity) and IF

The recognition ability of MIP is generally evaluated by the affinity for template. Two factors are important for the high affinity for the template of MIP: the strength and quantity of the interactions between the monomers in the polymer network and the template. Therefore, in the computational approach for the rational design of the MIPs, it was assumed that the monomers giving the highest ΔE with the template should also give the polymer with the highest affinity. The ΔEs between PR and the monomers from the virtual library were calculated using the method as described above, identifying IA as a promising monomer (2, 6-bisacylamide pyridine is the most promising monomer, but not commercially available in China; we chose another promising monomer (IA) with the capability of interacting with PR more strongly. To examine the computational method above, MAA and AA generally used as monomers to prepare MIPs were also chosen as monomers to synthesize paracetamol-imprinted polymers as a reference. The correlation between interaction energy and affinity or IF is shown in Fig. 1.

As anticipated, the observed affinity for PR (47.95 mg/g) of P_3 synthesized using IA as monomer is higher than those of P_1 (30.34 mg/g) and P_2 (39.70 mg/g) synthesized using MAA and AA as monomer, respectively (MIP synthesized under the same conditions but with a different monomer; see Table 1). Similarly, the IF (1.49) of P_3 is larger than those of P_1 (1.08) and P_2 (1.25). The order of affinity or IF determined in adsorption is consistent with that of ΔE calculated above.

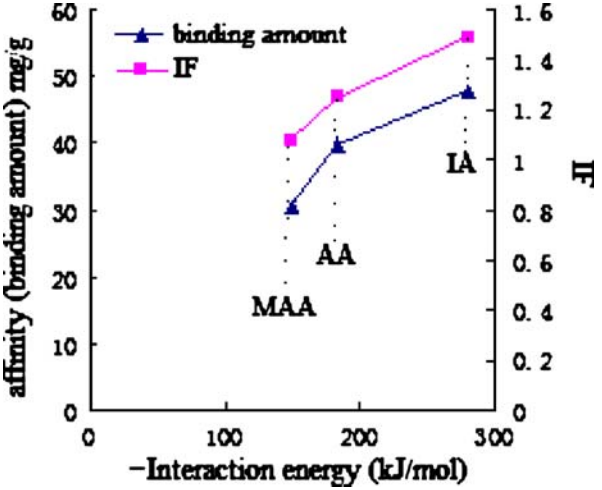

Fig. 1 The relation between the interaction energy and affinity or IF

Effect of Solvent on IF

Computational selection of solvents for MIP is a challenge. The accepted theory behind imprinted polymerization shows the importance of the conformation of a binding 'pocket'. The molecular complex formed between interacting template and functional monomers is 'fixed' into place within a three-dimensional polymer structure made of a suitable cross-linker. A solvent chosen as the porogen for MIP has not only to have the ability to dissolve all of the required components but also to not to interfere with the interaction between the template and the monomer. But a solvent used as an eluant has to disturb the forming of the template–monomer complex as it can.

In order to choose solvent rationally, conformation optimization was performed employing DFT at the B3LYP/6-31 + G** level. The optimized conformations of PR and IA are shown in Fig. 2. The Mulliken charges of the atoms in them and their dipole moments are shown in Table 2.

From the calculated dipole moments of PR and IA, polar solvent can be primarily selected as porogen for paracetamol-imprinted MIP according to the principle of solubility, like dissolving like. The solvation energies (G_{solvs}) of PR and IA in acetonitrile (MeCN),

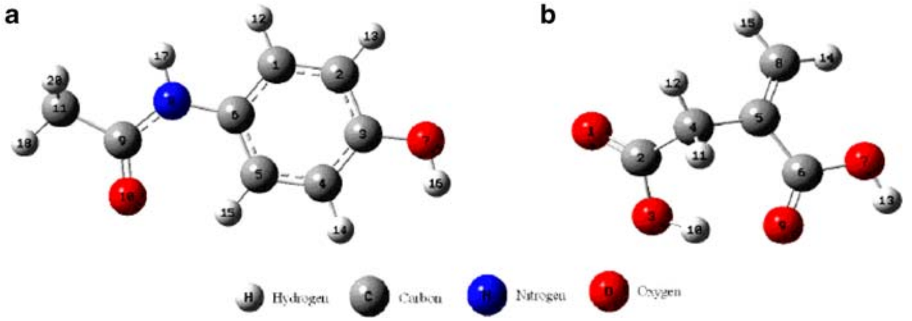

Fig. 2 Optimized conformations of PR (**a**) and IA (**b**)

Table 2 Mulliken charges of the atoms in PR and IA and their dipole moments.

Molecule	Number	Atom	Atomic charges	Dipole moment (Debye)
PR	1	C	−0.760528	
	2	C	0.300594	
	3	C	−0.166257	
	4	C	0.070780	
	5	C	0.326934	
	6	C	−0.088240	
	7	O	−0.539283	
	8	N	−0.393865	
	9	C	0.793231	
	10	O	−0.511630	
	11	C	−0.728926	
	12	H	0.106686	
	13	H	0.134766	
	14	H	0.111761	
	15	H	0.171984	
	16	H	0.353832	
	17	H	0.291419	
	18	H	0.188830	
	19	H	0.167855	
	20	H	0.170056	
				2.3735
IA	1	O	−0.432322	
	2	C	0.308152	
	3	O	−0.435709	
	4	C	−0.457765	
	5	C	0.283804	
	6	C	0.402692	
	7	O	−0.448183	
	8	C	−0.179753	
	9	O	−0.521687	
	10	H	0.398277	
	11	H	0.190883	
	12	H	0.177749	
	13	H	0.383084	
	14	H	0.173951	
	15	H	0.156827	
				5.8296

methanol (MeOH), tetrahydrofuran (THF), and acetone (CH_3COCH_3) were also calculated. In this step, a self-consistent isodensity polarizable continuum model (SCIPCM) was adopted, and the optimized conformations of PR and IA in vacuum (shown in Fig. 2) were transferred into the solvents to optimize and generate the energy in solvent environment [E (solvent)] using the same method at the same level. G_{solv} was obtained by the following Eq. 2:

$$G_{\text{solv}} = E(\text{solvent}) - E(\text{gas}). \tag{2}$$

The results (shown in Table 3) display that there is a good correlation between the solvation energies of PR or IA and the dielectric constants (ε_s; ε is used as a measure of

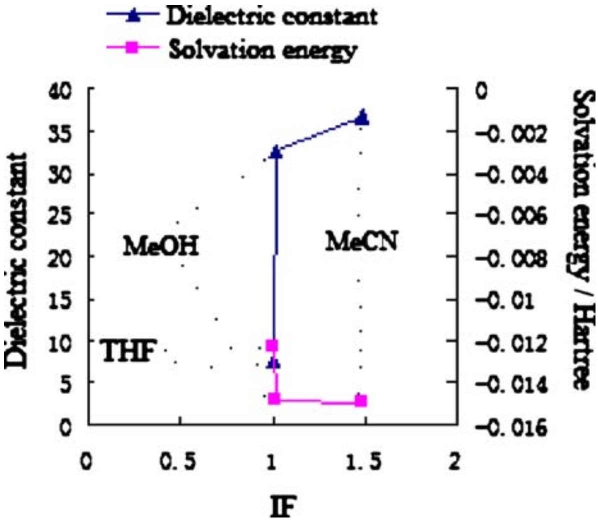

Fig. 3 The relation between IF and dielectric constant or solvation energy

polarity of solvent; generally, low ε means low polarity, and high ε means high polarity) of the solvents. The higher the ε of the solvent, the higher is the G_{solv} of PR or IA in the solvent. It seems that we should choose THF, which can dissolve PR and IA with the lowest G_{solv} (in this case, i.e., with the lowest ε), as porogen for the MIP because it was believed that the solvent with low G_{solvs} of template and monomer interfered with the interaction between template and monomer to a small degree.

To validate the above calculation, the experiments of different solvents dissolving PR and IA were done, and the results were also shown in Table 3. Table 3 validates calculated dipole moments (polarities) of PR and IA; PR and IA cannot be dissolved in the non-polar solvents, but can be in polar solvents. At the same time, Table 3 illustrates the deficiency of SCIPCM model in which only the ε and the solvent molecular size are considered (i.e., herein, $G_{\mathrm{solv}}=\Delta E_{\mathrm{cav}}+\Delta E_{\mathrm{dis\text{-}rep}}+\Delta E_{\mathrm{ele}}$); the degree to which the polar solvent dissolves PR

Table 3 The solubility and the dielectric constant of the solvent and the solvation energies of PR and IA.

Solvent	ε	Solubility[a]		G_{solv} (a.u.)	
		PR	IA	PR	IA
C7H16	1.920	–	–	/	/
CCl4	2.228	–	–	/	/
C6H6	2.247	–	–	/	/
C6H5CH3	2.379	–	–	/	/
CHCl3	4.900	–	–	/	/
THF	7.580	++	++	−0.01222823	−0.0133494
CH2Cl2	8.930	–	–	/	/
CH3COCH3	20.700	++	++	−0.01432708	−0.01546984
MeOH	32.630	++	++	−0.01481570	−0.01595158
MeCN	36.640	+	+	−0.01491074	−0.01604453

[a] – represents the solvent does not dissolve PR or IA; + represents the solvent can dissolve PR or IA; ++ represents the solvent can dissolve PR or IA and the soluble performance (e.g., solubility and velocity) is obviously better than that + represents. This experiment was performed at ambient temperature and atmospheric pressure.

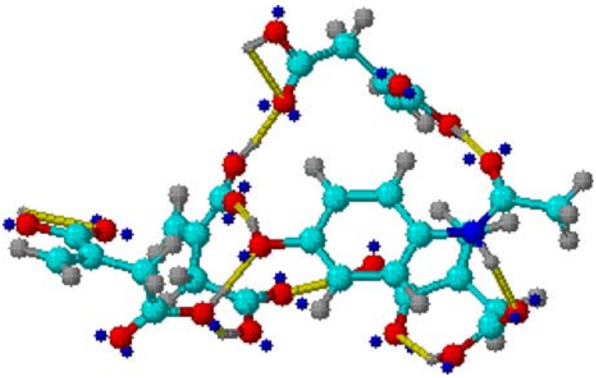

Fig. 4 The possible conformation of PR interacting with IA in the molar ratio 1:4. *Small balls* representing the atoms, *tubular bonds* connecting atoms, *dots* representing lone pairs, and *yellow bonds* representing hydrogen bonds

or IA is not in good agreement with the corresponding ε of the solvent (G_{solv} used as a measure of the interaction between solvent molecule and solute molecule, high G_{solv} meaning good ability of the solvent to dissolve the solute). For example, the ε of THF is 7.580, lower than that (36.640) of MeCN; however, the solubility of PR or IA in THF is higher than that in MeCN. It is interesting that the ε (8.930) of dichloromethane (CH_2Cl_2) is larger than that of THF, but CH_2Cl_2 does not dissolve PR and IA. This suggests that we should not only take G_{solv} (calculated using SCIPCM model) or ε of solvent into consideration when we select solvents for MIP. This is confirmed by the relation between IF and G_{solv} or ε shown in Fig. 3.

From Fig. 3, we can see that the MIP made with THF or MeOH as the porogen shows almost no imprinting effect toward PR, while that with MeCN as the porogen shows a moderate imprinting effect (IF=1.49).

What caused this? It seems that the omitting of the hbs interference of the porogen may introduce a significant error. The possible conformation of PR interacting with IA (shown in Fig. 4) displayed that hbs mainly contribute to the interaction. To confirm the occurrence of the interaction between PR and IA at the polymerization stage and to investigate the nature of the interaction using experimental method, the UV spectra of the mixtures described above were measured (shown in Fig. 5). With the titration of IA, the first

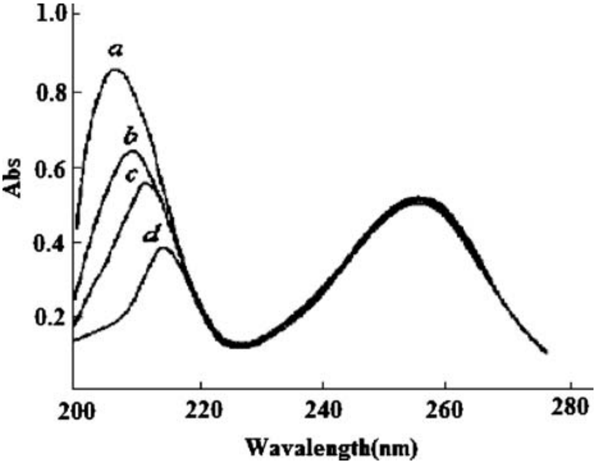

Fig. 5 The UV spectra of PR in the presence of various concentration of IA in MeCN. Concentration of PR, 0.05 mmol·l^{-1}; concentration of IA for lines *a*–*d*, 0, 0.05, 0.1, and 0.20 mmol·l^{-1}; corresponding pure IA solutions as blanks

absorption peak of PR produced obvious red shifts, and the corresponding maximum absorbance was also gradually decreased. It is typical for hb effect on the π–π* absorption band of a molecule whose chromospheres act as a proton donor [21–22]. This indicates the formation of PR–IA complex in MeCN.

In fact, there are three types of inter (not intra) molecular forces, dipole–dipole forces, hydrogen bonding forces, and dispersion forces, that are very important to evaluate the ability of the solvent to dissolve solute [23]. This is the reason why the SCIPCM model underestimates the ability of solvent.

To circumvent the problem, benefited from John [23] who proposed that solvent should be treated like a molecule, we modeled the possible dimeric PR-MeCN or other molecule of solvent complexes formed through hb interaction. From the results shown in Table 2 and the spatial considerations [24], we deduce that the proton donors in PR are H_{16} and H_{17} and the proton acceptor is O_{10}. The possible complexes were optimized, and the ΔE's as a measure of the strength of hbs were obtained from the following Eq. 3 employing the method as described above:

$$\Delta E' = \Delta E(\text{complex}) - \Delta E(\text{PR}) - \Delta E(\text{molecule of solvent}). \qquad (3)$$

The optimized conformations are shown in Fig. 6 and the hb lengths and energies listed in Table 4 for comparison.

Clearly, both $-OH_{16}$ and $-NH_{17}$ group binding sites in PR can bind with MeOH, THF, CH_3COCH_3, and CH_3COOH. Besides $-OH_{16}$ and $-NH_{17}$ sites, CH_3COOH and PR can form hb in $-CO_{10}$ site, but MeCN only interacts with PR in $-NH_{17}$ site (the conformations of PR-MeCN in $-OH_{16}$ site and PR-MeOH in $-CO_{10}$ site optimization did not converge well). PR and CH_3COOH in $-CO_{10}$ site forms strong hb as shown in Table 4; bond length and energy are 2.698 Å and −0.01695 a.u., respectively, while PR and MeCN in $-NH_{17}$ site forms weak hb with large length (3.223 Å) and low energy (−0.00914 a.u.). In $-OH_{16}$ site, the $\Delta E'$ between PR and CH_3COOH or THF (−0.01598 and −0.01409 a.u., respectively) is higher than that between PR and MeOH or CH_3COCH_3 (−0.0122 and −0.01275 a.u., respectively), but in $-NH_{17}$ site, the $\Delta E'$ between PR and CH_3COOH or CH_3COCH_3 (−0.0100 and −0.00973 a.u., respectively) is lower than that between PR and THF or CH_3OH (−0.0107 and −0.01004 a.u., respectively).

The energies and lengths of hbs through which PR and IA may interact are also shown in Table 4. It is evident that hbs that PR and IA formed are stronger than those that PR and solvent molecule formed. However, at the polymerization stage, the concentration of solvent is much higher than that of IA, and the hydrogen bonding interference of solvent should be paid attention to.

Taking the hydrogen bonding interference of solvent into account, if pure solvent is used as porogen, we predict that the MIP synthesized in MeCN should give a larger IF than that made in CH_3OH or THF, but under the precondition of possessing the ability to dissolve PR and IA, the mixture of MeCN and non-protic solvent with lower ε (such as $CHCl_3$) as porogen may be better than pure MeCN due to the smaller degree to which the mixture interferes with hbs interaction between PR and IA. Contrary to the mixture of MeCN and $CHCl_3$, the mixture of CH_3COOH and protic solvent (such as MeOH) or dipolar protophilic solvent (such as THF and CH_3COCH_3), which can disturb the hbs interaction between PR and IA strongly, is expected to be an ideal eluant to remove the template, and the washing effect of the above related mixture may be better than that of pure THF or CH_3COCH_3.

The imprinting effect of MIP prepared using MeCN as porogen is better than that of MIP with MeOH or THF as porogen, which is confirmed by the experiment (shown in Fig. 3).

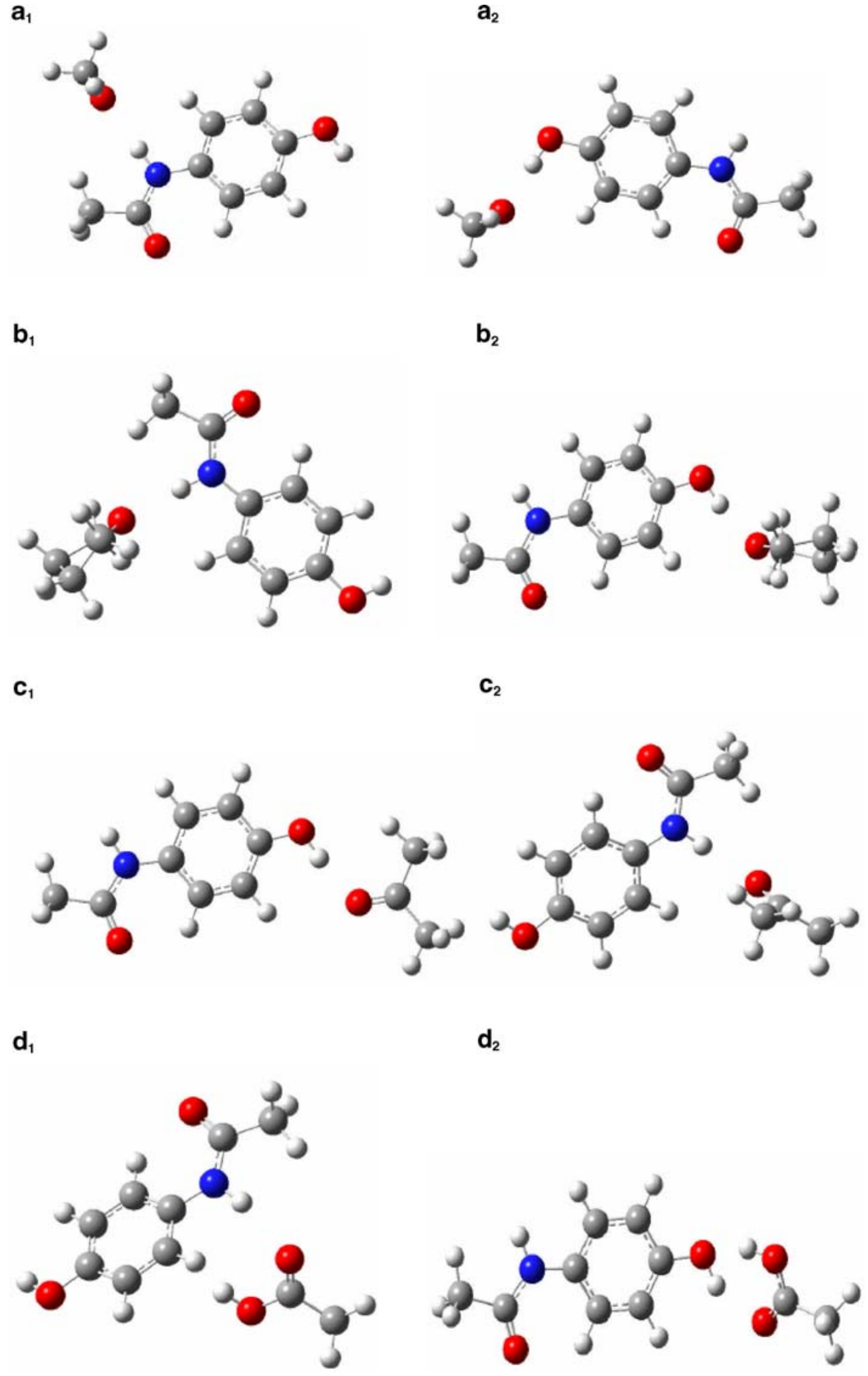

Fig. 6 The conformations of complexes: **a** Complexes of PR–MeOH; **b** complexes of PR–THF; **c** complexes of PR–CH$_3$COCH$_3$; **d** complexes of PR–CH$_3$COOH; **e** complex of PR–MeCN; **f** complexes of PR–IA

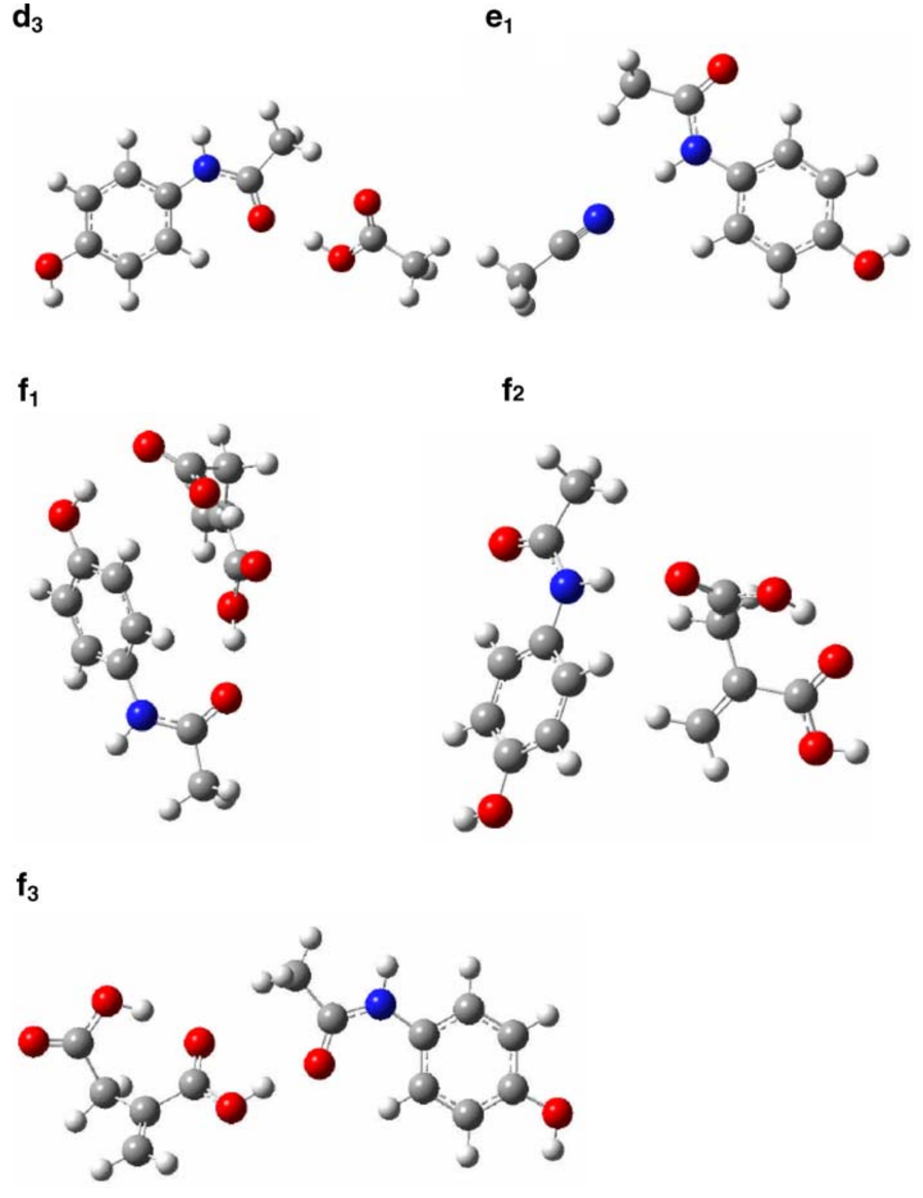

Fig. 6 continued

Further attempts to enhance the imprinting effect (i.e., replacing pure MeCN with the mixture of MeCN and CH_3Cl as porogen) did result in significant improvement (IF=4.50). Interestingly, even after extensive washing, P_3' and P_3'' (see Table 1) showed leaching of PR; P_3' leached more than P_3''. It is obvious that washing effect of pure CH_3COCH_3 or THF is worse than that of the mixture of MeOH and CH_3COOH. It is also consistent with the result predicted using the computational method. This confirms the validity of the above calculations and analysis focusing on the hb interaction between PR and other molecule and also shows that it is not strict, and even wrong, to select solvents for MIP only from the ε or

Table 4 The hb length and interaction energy between PR and molecule of solvent.

Complex	Site of hb formed in PR					
	$-OH_{16}$		$-NH_{17}$		$-CO_{10}$	
	Length[a] (Å)	$\Delta E'$ (a.u.)	Length (Å)	$\Delta E'$ (a.u.)	Length (Å)	$\Delta E'$ (a.u.)
PR-MeOH						
Fig. $6a_1$			3.048	−0.01004		
Fig. $6a_2$	2.813	−0.0122				
PR-THF						
Fig. $6b_1$			3.022	−0.0107		
Fig. $6b_2$	2.751	−0.01409				
PR-CH3COCH3						
Fig. $6c_1$			3.071	−0.00973		
Fig. $6c_2$	2.800	−0.01275				
PR-CH3COOH						
Fig. $6d_1$			3.028	0.0100		
Fig. $6d_2$	2.725	−0.01598				
Fig. $6d_3$					2.698	−0.01695
PR-MeCN						
Fig. 6e			3.223	−0.00914		
PR-IA						
Fig. $6f_1$	2.645	−0.02115				
Fig. $6f_2$			3.0178	−0.01187		
Fig. $6f_3$					2.618	−0.0209

[a] The hb length is defined as the distance between atom D and atom A, where D is hydrogen donor and A is hydrogen acceptor.

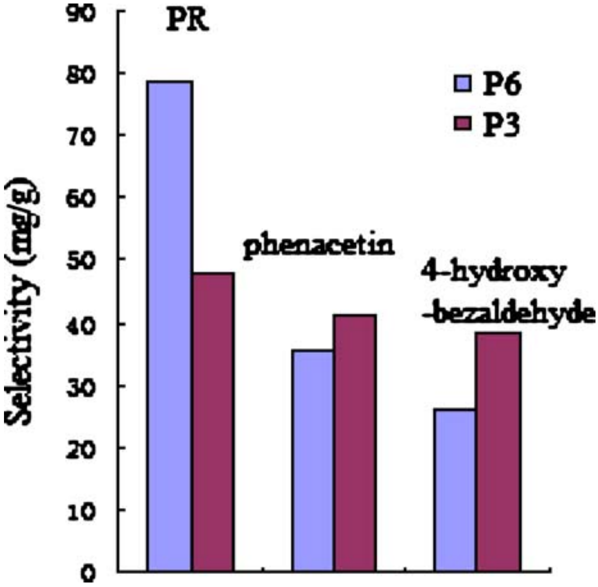

Fig. 7 The selectivity of P_3 and P_6

G_{solv} calculated with SCIPCM model. In this case, if the hydrogen bonding interference of solvents is omitted and we only take the ε of solvent or G_{solv} into consideration, the imprinting effect of P_4 should be larger than that of P_3. However, the experimental result is otherwise.

Effect of Solvent on Selectivity

The capacity of an analogue binding to MIP was defined as its selectivity. Different porogens also had effect on the selectivity of MIP.

The binding amounts of PR or its analogue on P_6 made in the mixture of MeCN and $CHCl_3$ and P_3 made in MeCN are shown in Fig. 7. There is a little difference between the binding amount of PR and that of phenacetin or 4-hydroxybenzaldehyde on P_3. However, the obtained binding amount of PR on P_6 is 1.76 times as high as that on P_{06} (not shown here), being 2.22 and 2.97 times as high as that of phenacetin or 4-hydroxybenzaldehyde on P_6, respectively. This does demonstrate that P_6 exhibiting the enhanced affinity and selectivity is due to the imprinting effect produced in the presence of PR as the template and the mixture of MeCN and $CHCl_3$ as porogen.

Conclusion

Together with our previous work, we have demonstrated that paracetamol-imprinted polymer is promising and can be used in quantification of drugs. For a given template PR, we selected the promising monomer, IA, from the virtual library using the ΔE between PR and monomer as a measure of their interaction strength. Further conformational analysis displayed the nature of interaction between PR and IA; hbs interaction mainly contributed to the interaction between the template and the monomer. The ideal solvents chosen as porogen and eluant were the mixture of MeCN and $CHCl_3$ and the mixture of MeOH and CH_3COOH, respectively, due to the different degree in interference with the interaction between PR and IA.

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