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Experimental and theoretical investigation of the complexation of methacrylic acid and diisopropyl urea

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Graphical Abstract:

Abstract

The present paper explores the complexation ability of methacrylic acid which is one of the most abundant functional monomer for the preparation of molecularly imprinted polymers. Host-guest interactions and the mechanism of complex formation between methacrylic acid and potentially genotoxic 1,3-diisopropylurea were investigated in the pre-polymerization solution featuring both experimental (NMR, IR) and *in silico* density functional theory (DFT) tools. The continuous variation method revealed the presence of higher-order complexes and the appearance of self-association which were both taken into account during the determination of the association constants. The quantum chemical calculations – performed at B3LYP 6-311++G(d,p) level with basis set superposition error (BSSE) corrections – are in agreement with the experimental observations, reaffirming the association constants and justifying the validity of computational investigation of such systems. Furthermore, natural bond orbital analysis was carried out to appraise the binding properties of the complexes.

1. Introduction

Molecular imprinting is a versatile technique for the preparation of intelligent materials exhibiting selective molecular recognition towards a template.¹ The self-assembly of the template molecule and the functional monomer in the pre-polymerisation solution yields selective binding cavities with complementary functionalities and topographies to the template. Imprinting is usually based on reversible non-covalent interactions, and one of the most common functional monomers is methacrylic acid.^{2,3,4,5} This article deals with the pre-polymerisation complex of methacrylic acid (MAAH) and potentially genotoxic 1,3-diisopropylurea (IPU) template which were previously used for molecular imprinting as functional monomer and template, respectively (Figure 1).⁶ In order to better understand the complexation mechanism, multiple methods were applied to investigate the system (NMR, IR and DFT).

Figure 1. Structure and atom numbering of IPU template (guest), MAAH functional monomer (host).

NMR titration, a relatively common method in host-guest chemistry, was used to determine binding constants where host and guest are mixed in different ratios and the NMR spectrum of the mixture is measured. NMR titration is often used to understand complexation phenomena in macrocycle chemistry^{7,8,9}, molecular imprinting^{10,11} and receptors^{12,13}. Due to fast-exchange correlation the NMR signals of the complexes and the initial compounds do not appear separately but as their average. Both 1:1 and 1:2

complexes and self-association of the initial compounds were considered. Although the application of IR in the investigation of binding properties of weak complexes is limited, it can provide information about the vibrations at the binding site, thus giving additional proof for complexation.

In recent years parallel to the experimental methods, the interactions between the template and the functional monomer or the polymer were explored by various *in silico* methods.^{14,15,16,17,18,19} In the earlier studies the statistical and the molecular mechanics/classical molecular dynamics studies are predominant and quantum chemical methods have only been applied in recent years due to rapid development in computational power. Most of the computational studies in the imprinting literature focus on rapid functional monomer screening. In this method semiempirical or simple Hartree-Fock techniques are employed in order to choose the most stable complexes, which are recalculated with density functional theory (DFT) methods. The *ab-initio* and DFT methods, which are applied in present article, are suitable for modelling the chemical interactions between the monomers and templates, describing their binding properties and the stability of the investigated complexes. The exact determination of equilibrium constants in condensed phases is, however, presently beyond the limit of these methods.

2. Experimental

2.1. Materials

1,3-diisopropylurea (IPU) was kindly provided by Hovione FarmaCiencia SA (Portugal). The chemicals were of reagent grade or higher and used as received from Sigma-Aldrich. Anhydrous solvents were stored over molecular sieves.

2.2. Analytical methods

2.2.1. NMR and IR methods

The ¹H NMR spectra were recorded at 25 °C within a deviation of 0.2 °C. Chemical shifts (δ) are reported in ppm, with respect to the solvent peak of deuterated chloroform being 7.26 ppm²⁰. The following abbreviations are used in the discussion: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, bs = broad singlet. The solvent was kept under molecular sieves and handled under inert atmosphere in order to avoid contamination of the system with water which disrupts the intramolecular hydrogen bonds. For the self-association NMR experiments 1 mM, 2.5 mM, 5 mM, 7.5 mM, 10 mM, 20 mM, 30 mM, 40 mM IPU working solutions were prepared in CDCl₃. Additionally, Continuous Variation Method (CVM) was also performed for obtaining the stoichiometry of the complex. During the CVM, the host/guest molar ratio was varied between 0 and 1 while their total concentration was kept constant at 20 mM. In CVM the host was MAAH. Furthermore, two sets of NMR titrations were carried out where IPU was titrated with MAAH: T1 (5 mM IPU) and T2 (10 mM IPU). In order to study the effect of concentration, T1 was performed from 0-2 equivalents of MAAH with respect to 5 mM IPU, while T2 was performed from 0-5 equivalents of MAAH with respect to 10mM IPU. The solutions were agitated to dissolve the IPU and homogenise the solution and

were always freshly prepared just prior to analysis. The room temperature Fourier transform infrared (FTIR) spectra of MAAH and IPU compounds and corresponding 20 mM IPU:MAAH (1:1) complexes were measured in DCM, in the 4000-400 cm^{-1} region at a resolution of 4 cm^{-1} .

2.3. Quantum chemical methods

Quantum chemical computations were carried out using Gaussian 03 suite of programs²¹, using the Becke3-Lee-Yang-Parr exchange-correlation functional^{22,23} in conjunction with 6-311++G(d,p) basis set. The B3LYP functional is a general functional was chosen to be accurate with more calculated properties and this functional was used in most of the previous DFT studies^{24,25}. Diffuse functions in the basis set (denoted with ++) were chosen to obtain better results for the hydrogen-bonded systems^{26,27}. The minimum character of the stationary points obtained by geometry optimizations was checked by vibrational frequency calculations. The solvent effects were considered implicitly by the polarizable continuum model (PCM)^{28,29,30}. The solvation energies could be calculated only on the optimised geometries of the isolated structures, since the starting unequilibrated structures in most cases failed to converge with PCM. The other two investigated compounds – 1,3-diisopropyl-urea (IPU) and methacrylic acid (MAAH) – were built up manually. Three structures were considered both for IPU^{a,b,c} and *trans*-MAAH and *cis*-MAAH (Figure 2) for the further calculations determining the lowest energy geometrical structure of the complexes. Due to the high number of possible conformers 24 initial IPU-MAAH complex structures were built up and optimised both for 1:1 and 1:2 complexes. Natural bond orbital (NBO) analysis³¹ was performed to explain the binding properties of the complexes. The calculation of anharmonic frequencies as well as the usage of scaling factors was omitted, since the agreement between the measured and the calculated spectra was sufficiently good for most of the spectra even without considering these corrections. Due to the appearance of weak interactions (H-bonds), the calculated reaction energies were corrected utilizing the a posteriori counterpoise method of BSSE³². This error arises due to the finite basis sets which are centered on the atoms: the number of basis functions is different for the complex and the two initial compounds and the ghost orbitals can contribute to a larger decrease in the energy in the complex compared with the initial compounds. The computed geometries have been visualised using the GaussView 3.09 program.

3. Results and discussion

3.1. Effect of the deprotonating agent on the complexation mechanism

The investigation started with Continuous Variation Method (CVM) experiments, where IPU molefraction $\times \Delta\delta$ (chemical shift change) is plotted as a function of IPU mole fraction. The points on this curve are proportional to the concentration of the complex assuming solely 1:1 complexation (for details see Supporting Information 3.4). The results of the CVM are shown in Figure 2. The global maximum of the fitted curve occurs at about 0.57 mole fraction of IPU. This corresponds to a complex ratio of IPU:MAAH

(1.33:1). This result shows may indicate: (i) the formation not only of 1:1 but 1:2 and 2:1 binary (ii) the possible self-association of IPU and (iii) the possible self-association of MAAH. Given the proportion of IPU:MAAH (1:4) in the prepolymerization solution⁶ the presence of IPU:MAAH(2:1) complex can be considered as negligible. Thus the present study focuses only on IPU:MAAH of 1:2.

Figure 2. Job plot of the IPU-MAAH complex, the blue dots and green squares are attributed to the H1 and H3 signals of IPU, respectively (for the numbering of atoms see Figure 1); the total concentration IPU+MAAH was 20 mM.

IPU (the guest) is the observed species for the NMR titration experiments. In case of titration and Job's method its H1 (NH) and H3 (CH) protons were chosen, while in case of self-association experiments its H1 (NH) and H4 (CH₃) protons were chosen to be followed throughout the corresponding NMR studies since significant shifts were observed for these hydrogens. The explanation of the discrepancy of the chosen hydrogens between the different reactions was aided by DFT calculations. In case of the IPU self-association, in the most stable dimers the methyl group (H4 hydrogen) is towards the other IPU carbonyl group, whereas the H3 proton is usually on the other side, making the creation of intermolecular hydrogen bond much more difficult to H3 than H4. In contrast, in the IPU:MAAH complexes in some non-ground state but minimum-type complex structures the MAAH O3 can form weak H-bonds with H3 and H4 (CH₃) of IPU, indicating also that the effect is much weaker for H4 (whose change of chemical shift was insignificant in the experiments) than for H3. These structures have energies in the range of 20-50 kJ.mol⁻¹ above the ground state. Since the proportion of these higher-energy complex structures is assumed to be small, the total chemical shift difference within the NMR titration of H3 is expected to be smaller than that of H1 (ca. 0.5 ppm and 0.05 ppm for H1 and H3, respectively – vide infra: dataset T2).

To confirm that the observed chemical shifts are due to complexation between IPU and MAAH, two additional studies were carried out: (i) self-association of IPU; (ii) self-association of MAAH were assessed. Note that both for the IPU dimers and the most stable IPU:MAAH complexes the two NH signals (H1a and H1b signals) are not equivalent on molecular scale (one is H-bonded the other is not). The two NH signals of IPU split at lower concentrations when performing IPU:MAAH titration, at higher concentrations the broadening of the signal covers this difference. In case of the self-association the two distinct NH signals can be seen, however, both their chemical shift changes with concentration (see Figure 3). In case of the complex

the more flexible MAAH groups will cause a very fast exchange and therefore the differences between the two NH will disappear. Whereas in the dimer due to the isopropyl groups the IPU is more rigid, thus the NH will be slightly split.

Figure 3. Self-association of IPU (top panel) and MAAH (bottom panel). Concentration range of 1-40 mM in CDCl_3 indicating the (A) downfield shift of H1, the (B) no-shift of H3 and the (C) upfield shift of H4 in case of IPU and the (D) downfield shift of H6, the (E) no-shift of H5 and the (F) upfield shift of H7 in case of MAAH. For the numbering of atoms see Figure 1.

3.2. The self-association of the guest (IPU) and the host (MAAH)

Figure 3 shows the downfield shift (0.201 ppm, Fig. 3A) and splitting of the H1 signals as well as the upfield shift (0.009 ppm, Fig. 3C) of the H4 doublet. Due to the low solubility of IPU (50 mM in CDCl_3 at r.t.) the saturation of the self-association curve was not reached, however the self-association constant was estimated to be $0.502 \text{ dm}^3\text{mol}^{-1}$ (the self-association calculations are described in the Supporting Information). As predicted, the association is unfavourable and it is only significant at high IPU concentrations or in the absence of MAAH, i.e. at the initial points of the NMR titrations. This is also supported by the complexation studies. If MAAH is present, the NH signal splitting can be noticed only at

low MAAH concentrations. At higher MAAH concentrations the two H1a and H1b will become indistinguishable. The bottom panel of Figure 3 shows the downfield shift of H6 (0.034 ppm, Fig. 3D) and the upfield shift of H7 (0.007 ppm, Fig. 3F) of MAAH. The self-association constant of MAAH was estimated to be $360 \text{ dm}^3 \cdot \text{mol}^{-1}$. This value corresponds well with the previously determined value³³ of $330 \text{ dm}^3 \cdot \text{mol}^{-1}$.

3.3. Effect of the deprotonating agent on the complexation strength

In order to investigate the binding strength between IPU and the functional monomer, two sets of NMR titrations were carried out at concentrations of 5 and 10 mM IPU. The observed chemical shifts are shown in Figure 4 whilst a typical spectrum indicating all the chemical shifts of the studied system can be found in the Supporting Information in Figure S2. Figure 4 shows the two investigated datasets: with 5 mM IPU (T1) and with 10 mM IPU (T2) starting concentration, which was titrated with MAAH until 2 equivalents and 5 equivalents for datasets T1 and T2, respectively. For both measurements the IPU:MAAH (1:2) complex and the self-association constant of both IPU and MAAH were considered. The association constants and chemical shifts of the investigated compounds obtained are summarised in Table 1.

Figure 4. Measured chemical shifts of H1 (filled signs) and H3 (empty signs) and the fitted curves (lines) for the NMR titration of IPU-MAAH system where the red rectangular and green circular data sets represent titration T1 and T2 respectively where T1 (5 mM IPU) and T2 (10 mM IPU).

Table 1. Calculated association constants and observed chemical shifts of the H1 of IPU during the titration experiments. s^2 means standard deviation of the fit.

	T1 (H1)	T1 (H3)	T2 (H1)	T2 (H3)
K_1	506	560	322	678
K_2	6.02	1.54	5.94	14.98
δ_{C_1}	4.508	3.774	4.656	3.775
δ_{C_2}	11.269	3.019	11.640	3.747
s^2	1.20×10^{-3}	3.35×10^{-4}	1.74×10^{-3}	1.05×10^{-4}
K_1 average	516 ± 148			

According to the conclusions drawn from the Job plot, the IPU-MAAH complexation is multi-step. Due to the complexity of the system – two step complexation and dimerization – a Python script was written (available as a Supporting Information). The calculated average values for the association constants and their standard deviations are summarised in Table 1.

The uncertainties of the equilibrium constants are relatively high partly due to the complex nature of the system, the low concentrations and thus the small changes in the chemical shift values and also because the water could be not completely removed from the system. The very high uncertainty of K₂ can be explained by the fact that this association constant lies beyond the range of 10^{-10} to 10^5 dm³.mol⁻¹ in which the conventional NMR titrations are performed. In order to prove the importance of considering the higher order complexes and the self-association, the T1 and T2 NMR data representing the 5 and 10 mM IPU titrations were, in addition, evaluated in a conventional fashion. Assumptions are often made in the literature during the evaluation of complexation studies of MIPs wherein higher order complexes and self-association are neglected. Following this approach and the widely applied Rose-Draco method^{34,35} the calculated 1/K is depicted as a function of the chosen set of $\Delta\delta_{\text{MAX}}$ values. At different $\Delta\delta_{\text{MAX}}$ values different lines are obtained which have a common intersection point giving the value of 1/K (for further details see equations S17-21 in the Supporting Information). Association constants of 525 dm³.mol⁻¹ was obtained for the T1 dataset. In case of the T2 dataset due to both the higher initial IPU concentration the self-association and the higher final MAAH concentration the formation of 1:2 complexes are more pronounced the Rose-Draco plot gave an the standard deviation of the association constant in the same order of magnitude as the K itself. The Rose-Draco plot can be found in the Supporting Information in Figure S3. Evaluation of this data hence demonstrates that for IPU-MAAH system one has to consider self-association as well as higher order complexes.

3.4. Geometrical parameters of the complexes

Figure 5. Schematic representation of the initial conformers of IPU and MAAH for further DFT calculations

The theoretical calculations of the IPU and MAAH showed that the most stable conformers of these two compounds are IPU^a and *trans*-MAAH (the different conformers of IPU and MAAH considered in the calculations are depicted in Figure 5), both for gas phase, implicitly treated DCM and CHCl₃ solutions. *Trans*-MAAH has already been established by previous articles^{36,37}. The difference of the ground state structure and the other two IPU conformers is significant with a value of 23 kJ.mol⁻¹ in gas phase, 25 kJ.mol⁻¹ in DCM and 35 kJ.mol⁻¹ in CHCl₃ for IPU^b and 8 kJ.mol⁻¹ in gas phase, 13 kJ.mol⁻¹ in DCM and 10 kJ.mol⁻¹ in CHCl₃ for IPU^c. However, the energy difference between *trans*-MAAH and *cis*-MAAH is only marginal, being 1-2 kJ.mol⁻¹ for gas phase and both solvents. The complexes were constructed considering all the sterically different structures.

Figure 6. The most stable conformers of IPU-MAAH complexes with the H-bonds (depicted with green lines, distances from top to bottom: gas phase, DCM and CHCl₃, respectively) given in angstroms: (A) 1:1 complex of IPU and MAAH (IPU^c + *trans*-MAAH, except DCM, where IPU^c + *cis*-MAAH), (B) 1:2 complex of IPU and MAAH (2×*trans*-MAAH + IPU^b, except DCM, where *trans*-MAAH + *cis*-MAAH + IPU^b)

The differences in the observed order of stability of the conformers between gas phase and CHCl₃ solution are insignificant. Figure 6 shows the most stable structures of the complexes. The black arrows indicate that while in gas phase and chloroform the methacrylic acid is always present as *trans*, in dichloromethane for structures on Figure 6A and 6B one MAAH is present as *cis*.

To explain the differences between gas phase and the investigated solvents during complexation natural bond orbital analysis (NBO) was applied. Table 2 shows the NBO charges and Table 3 the charge transfers for the host and guest molecules and the 1:1 IPU:MAAH complex. The NBO charges in the gas phase differ significantly from those in which the solvents were considered. The interactions within the

IPU:MAAH (1:1) complex are relatively heterogeneous. The charge of the N1b did not change significantly with the complexation, whereas O2 of MAAH moiety and O1 of IPU which both take part in hydrogen bonding became more negative (together $-0.129 e$, $-0.046 e$ and $-0.056 e$ in gas phase, DCM and CHCl_3). H1b became more positive ($0.044 e$, $0.012 e$, $0.020 e$), whereas H3 became more positive in gas phase and less positive in DCM and CHCl_3 ($0.032 e$, $-0.013 e$, $-0.004 e$ for gas phase, DCM and CHCl_3 , respectively). The charge transfer for this complex shows strong delocalization of electrons in $(\text{HN})\text{-C=O}\dots\text{H-O-C=O}\dots\text{H-N}(\text{CO})$ ring. According to the two intermolecular charge transfers: $\text{C1-O1} \rightarrow \text{O3-H3}$ of ca. 170 kJ.mol^{-1} and $\text{C4-O2} \rightarrow \text{N1b-H1b}$ of ca. 50 kJ.mol^{-1} a bigger change of the charge for O3 would be expected than for O2 and that the N1b would be expected to become more negative. However, due to the strong delocalization, O2 got charge from the lone pair of O3 and the negative charge excess (compared to the unbound guest) of N1b disappeared with a charge transfer from the lone pair of N1b to C1-O1 antibonding orbital. The $\text{O1}\dots\text{H3}$ hydrogen bond is stronger in solutions than for the isolated molecule. Since both for DCM and CHCl_3 the charge transfer to the antibonding orbital of O3-H3 from C1-O1 bonding and O1 lone pair is with ca. 20 kJ.mol^{-1} higher than in gas phase whereas the differences in backdonation are within 3 kJ.mol^{-1} , this can indicate the reason of the opposite change of the charge for H3.

Table 2. Selected computed natural charges (e) of IPU^c and 1:1 complex of IPU and MAAH. For the numbering of atoms see Figure 1 and Figure 6.

	gas phase		DCM		CHCl_3	
	$\text{IPU}^c/trans\text{-MAAH}$	$\text{IPU:MAAH=}(1:1)$	$\text{IPU}^c/cis\text{-MAAH}$	$\text{IPU:MAAH=}(1:1)$	$\text{IPU}^c/trans\text{-MAAH}$	$\text{IPU:MAAH=}(1:1)$
C1	0.816	0.835	0.820	0.836	0.820	0.836
O1	-0.684	-0.742	-0.758	-0.778	-0.746	-0.767
N1b	-0.674	-0.677	-0.672	-0.673	-0.672	-0.672
H1b	0.393	0.437	0.416	0.428	0.412	0.432
O2	-0.597	-0.668	-0.657	-0.683	-0.640	-0.675
O3	-0.695	-0.703	-0.701	-0.711	-0.705	-0.714
H3	0.486	0.518	0.528	0.515	0.520	0.516

Table 3. Main charge transfer interactions^a in IPU:MAAH 1:1 complexes.

Charge transfer (kJ.mol^{-1})	gas phase	DCM	CHCl_3
	$\text{IPU:MAAH}=(1:1)$		
BD C1-O1 $\rightarrow$ BD* O3-H3 LP O1 $\rightarrow$ RY* O3, H3	165.9	182.1	183.7
BD C4-O2 $\rightarrow$ BD* N1b-H1b LP O2 $\rightarrow$ RY* N1b, H1b	57.9	41.3	45.9
LP O3 $\rightarrow$ BD* C4-O2	278.3	270.7	273.2
LP N1b $\rightarrow$ BD* C1-O1	258.1	269.3	277.6

^a – Second-order perturbation energies ($E(2)$ donor $\rightarrow$ acceptor, kJ.mol^{-1}). The abbreviations LP, BD, BD* and RY* mean lone pair, bonding, anti-bonding and Rydberg orbitals, respectively. For the numbering of atoms see Figure 1 and Figure 6.

IPU:MAAH (1:2) complex was also calculated and its structure is depicted in Figure 6B, which has a C_2 symmetry in gas phase and $CHCl_3$. In DCM solution one of the MAAH moieties is changed from *trans* to *cis* therefore the symmetry is broken in this case. The energy differences between this most stable DCM complex – featuring one *trans*-MAAH and one *cis*-MAAH conformers – and the less stable structures having two *trans*-MAAH or two *cis*-MAAH conformers are 1 $\text{kJ}\cdot\text{mol}^{-1}$ and 2-3 $\text{kJ}\cdot\text{mol}^{-1}$ higher in energy, respectively. It is interesting to note that the most stable IPU:MAAH (1:2) complexes incorporate IPU^b conformer while the IPU:MAAH (1:1) complexes feature IPU^c conformer. Despite of the fact that IPU^b is higher in energy than IPU^c by a value of 15-24 $\text{kJ}\cdot\text{mol}^{-1}$, the former conformer allows a better access to the NH and C=O moieties which are the active sites of the complexation.

Table 4. Internal energies of complex or dimer formation reactions and the calculated Gibbs free energies from the measured association constant at 25 °C (the values are in $\text{kJ}\cdot\text{mol}^{-1}$). Multiple values are given as without/with BSSE correction.

	$\Delta E(\text{calculated})/\text{kJ}\cdot\text{mol}^{-1}$			$\Delta G(\text{measured})/\text{kJ}\cdot\text{mol}^{-1}$
	gas phase	DCM	$CHCl_3$	$CDCl_3$
IPU (dimer)	-38.7/-35.9	13.0/15.6	3.2/5.7	1.7
MAAH (dimer)	-66.9/-63.6	-17.4/-14.0	-23.6/-20.2	-14.6
IPU:MAAH (1:1)	-59.0/-55.8	-11.3/-8.2	-17.7/-15.9	-15.5
IPU:MAAH (1:2)	-96.4/-89.9	-11.2/-4.9	-27.0/-23.3	-20.3

The reactions leading to complex formation were also investigated and the corresponding reaction energies are summarised in Table 4. Note that in solution, due to both the existence of configurations with close lying energy levels and the presence of thermal perturbation, the simultaneous appearance of more complex structures is expected. Hence, the measured association constants cannot be assigned to one, well-defined structure. This is the main reason why the theoretically calculated energies cannot be directly compared to the Gibbs energies (ΔG) derived from the measurements. Nevertheless, trend is expected to be found between these theoretical and experimental values. The reaction energies related to the formation of the most stable conformers, were calculated for dimers, 1:1 and 1:2 complexes as shown in equation 1-3 (X stands for MAAH or IPU). For all compounds we considered the ground state structures obtained after geometry optimization. The associated Gibbs free energies were calculated from the measured association constants as given in equation 4.

$$\Delta E(\text{dimer}) = E(X_2) - 2 \cdot E(X) \quad (\text{eq. 1})$$

$$\Delta E(1:1) = E(C_1) - E(\text{IPU}^a) - E(\text{trans-MAAH}) \quad (\text{eq. 2})$$

$$\Delta E(1:2) = E(C_2) - E(\text{IPU}^a) - 2 \cdot E(\text{trans-MAAH}) \quad (\text{eq. 3})$$

$$\Delta G = -RT \ln(K) \quad (\text{eq. 4})$$

Interestingly, a good agreement between ΔE values from theoretical calculations and ΔG values computed from the measured association constants were found, despite their different theoretical meaning. Both from the theoretical investigations and the measurements concluded that the IPU dimerization is disfavoured ($K < 1$; $\Delta G > 0$), showing that the monomer concentration in the chloroform solution is higher than the dimer concentration for the used concentration ranges. For the rest of the investigated data $\Delta G < 0$ and thus $K > 1$ was found. Both the complex formations and the MAAH dimerization were found to be favourable. From the calculated results it can be concluded that the complexes and dimers are more stable in gas phase than in solutions. This is a consequence of the effect of the solvent which decreases the strength of the interactions between the parts of the complex³⁸Error! Bookmark not defined. The stability of the complexes is higher for CHCl_3 than DCM solutions. Therefore it would be expected that the equilibrium constants will be somewhat smaller in DCM solutions as those found in CHCl_3 .

Table 5. Measured and calculated IR bands of the complex compared to the measured spectra of MAAH and IPU.

No	V_{meas} [cm^{-1}]	Int_{meas} ^a	V_{MAAH} ^b [cm^{-1}]	V_{IPU} ^b [cm^{-1}]	V_{calc} [cm^{-1}]	Int_{calc} [km.mol^{-1}]	Assignment ^c
1	951	m	951	-	983	53	$\delta_r\text{CH}_2$, $\nu\text{C-C}$
2	1007	w	1009	-	1026	26	$\delta_r\text{CH}_3$, $\delta_r\text{CH}_2$
3	1128	w	-	1128	1142	29	$\gamma_s\text{CH}_3$, $\nu_{\text{as}}\text{CC}$
4	1177	sh	1178	(1167)	1181	83	δCNC , $\gamma_s\text{CH}_3$, $\beta_{\text{sci}}\text{CCC}$
5	1204	s	1204	-	1207	256	<i>trans</i> : $\nu\text{C-O}$, βOH , $\delta_r\text{CH}_3$
6	1221	m	1221	-	1229	281	<i>cis</i> : $\nu\text{C-O}$, βOH , $\delta_r\text{CH}_3$
7	1300	m	1300	-	1308	281	βOH , $\delta_r\text{CH}_2$, $\nu\text{C-C}$
8	1317	sh	1320	-	1326	206	$\nu\text{C-C}$, βOH , $\nu\text{C-O}$, $\beta_s\text{OCO}$
9	1375	w	1375	(1368) (1385)	1378	53	$\delta_s\text{CH}_3$, $\beta_s\text{CH}$
10	1423	m	(1431)	1423	1413	150	$\delta_s\text{CH}_3$
11	1454	s	1454	-	1480	118	$\delta_{\text{as}}\text{CH}_3$, $\delta_{\text{sci}}\text{CH}_2$
12	1541	m	-	1524	1573	664	$\beta_r\text{NH}\dots\text{O}$, $\beta_{\text{sci}}\text{NCN}$, βOH
13	1636	s	1636	-	1675	556	$\nu\text{C}=\text{C}$, $\delta_s\text{CH}_2$
14	1697	vs	1697	(1670)	1650	498	$\nu\text{C}=\text{O}$
15	1732	w	1730	-	-	-	overtone/combination
16	2930	m	2930	(2932)	3019	25	$\nu_s\text{CH}_3$
17	2972	s	-	2972	3027	22	$\nu_{\text{as}}\text{CH}_3$
18	3053	w	-	3429	3437	707	$\nu_s\text{NH}$

^a Measured intensity of the complex. The abbreviations vs, s, m, w and sh mean very strong, strong, medium, weak and shoulder, respectively.

^b Measured bands of MAAH and IPU. The bands in parentheses cannot be detected due to overlap.

^c The abbreviations s, as, ν , δ , γ , β , sci, r mean symmetric, asymmetric, stretch, deformation, out-of-plane bending, in-plane bending, scissoring and rock, respectively.

The IR experiments confirmed the existence of these binding sites. The observed IR spectra of IPU, MAAH and IPU-MAAH system can be found in the Supporting Information in Figure S1. Detailed IR studies of MAAH in chloroform solution can already be found in the literature^{36,37} performed at level (MP2 6-31G(d,p), B3LYP 6-31G(d,p) and B3LYP 6-311+G(d,p)) which were used in the data evaluation. The measured IR spectra for the MAAH and both measured and calculated IR spectra for the IPU can be found in the Supporting Information (Tables S1 and S2, respectively). According to these results dimer formation was observed for MAAH, however the calculated IR spectra with the employment of scaling factors could describe the system. During the calculations the self-association of the IPU was not put into the model since

according to the determined self-association constant (vide infra) the dimer concentration is around 1% at the concentration of measurements (20 mM). The calculated harmonic spectra could be assigned to the measured with an average difference between the measured and calculated frequencies of 37.8 cm^{-1} for IPU. If CH_3 stretching vibrations are not considered, which were overestimated by the calculations this difference is reduced to 19 cm^{-1} for IPU. The lack of significant hydrogen bonding in the spectra of IPU is supported by the quite narrow NH stretching vibrations in the region of $3330\text{--}3500\text{ cm}^{-1}$. However, for MAAH a quite broad signal can be found around $\sim 3000\text{ cm}^{-1}$, showing a significant hydrogen bonding.

Table 5 comprises the most important bands of the measured IR spectrum of 1:1 mixture of IPU:MAAH. In case of the most bands, either the IPU or the MAAH signals can be seen, which appear mostly at identical wavenumbers as on the spectrum of the two separate compounds. The $\beta_r\text{NH}$ band of the complex appears on the spectrum, however, there are two major differences compared with the same band of IPU: (i) the band is blue-shifted (it appears at 1541 cm^{-1} , while the same band is at 1524 cm^{-1} in case of IPU) and (ii) the band is broadened. These two changes indicate the appearance of hydrogen bond in the system. Interestingly, the carbonyl band (between $1660\text{--}1700\text{ cm}^{-1}$) of the complex cannot be seen, instead only the carbonyl band of MAAH appears (at 1697 cm^{-1}). The possible reason why we cannot observe the shifted carbonyl band of the complex is the broadening in complex compared to free IPU. Another important difference is the disappearance of the NH stretching vibrations of IPU (at 3339 cm^{-1} and 3429 cm^{-1}). These NH vibrations should red-shift and broaden when hydrogen bonds form. Therefore the band at around ~ 3000 and the peak at 3053 cm^{-1} in the spectrum of the complex can be assigned to the NH stretching vibration.

It is important to mention that the band at 1221 cm^{-1} appearing both in the complex and MAAH spectrum was not discussed in previous investigations^{36,37}. We found that the 1204 cm^{-1} and 1221 cm^{-1} are showing the different conformers of MAAH, while the band at 1204 cm^{-1} belongs to *trans*-MAAH, the one at 1221 cm^{-1} belongs to the *cis*-MAAH conformer. This is consistent with the calculations. Firstly, the energy difference between the two conformers is around 1 kJ.mol^{-1} which is sufficiently small to have both conformers present in the solutions at normal temperatures. Secondly, the calculated band intensity is 1.5 times larger for *cis*-MAAH than *trans*-MAAH (bands: 1137 cm^{-1} with intensity of 396 km.mol^{-1} and 1148 cm^{-1} with intensity of 547 km.mol^{-1} for *trans* and *cis*-MAAH, respectively) and *cis*-MAAH since being the less stable, is present in slightly smaller amount. Thus the two bands belong to the same vibrations of the two conformers.

4. Conclusions

The MAAH and IPU complexation was investigated by NMR, IR and theoretical calculations. MAAH is one of the most abundant functional monomer for the preparation of molecularly imprinted polymers. The model system mimics the self-assembly taking place in the pre-polymerization solution of IPU imprinted scavengers. The NMR and quantum chemical investigation revealed a two-step complex formation mechanism in the absence of the deprotonating agent. The binding strengths were derived through quantum chemical calculations and NMR titrations for both 1:1 and 1:2 complexes. The quantum chemical

calculations are in good agreement with the experimental observations, reaffirming the association constants. The theoretical calculations showed only minor differences in complexation between DCM and CHCl₃. Due to the lack of information on higher-order complexes in the literature and in order to facilitate the complex data treatment thereof, a Python script treating both the 1:2 complex and self-association is provided in the Supporting Information.

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Supporting Information: Python script for handling NMR titration data of higher-order complexes; Chemical shifts of IPU and MAAH; Rose-Drago plots; IR spectra; Mathematical framework of the NMR titration and the association constant. This material is available free of charge on the website of the publisher.

References

- ¹ B. Sellergren (Ed.), *Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry*, Elsevier, Amsterdam, 2001.
- ² G. Szekely, I.B. Valtcheva, J.F. Kim, A.G. Livingston, *React. Funct. Polym.*, **86**, **2015**, 215-224.
- ³ M. Razali, J.F. Kim, M. Attfield, P.M. Budd, E. Drioli, Y.M. Lee, G. Szekely, *Green Chem.*, **17**, **2015**, 5196-5205.
- ⁴ I. Dolak, R. Keçili, D. Hur, A. Ersoz, R. Say, *Ind. Eng. Chem. Res.*, **54**(19), **2015**, 5328-5335.
- ⁵ J. Kupai, E. Rojik, P. Huszthy, G. Szekely, *ACS Appl. Mater. Interfaces*, **7**(18), **2015**, 9516-9525.
- ⁶ G. Szekely, J. Bandarra, W. Heggie, F.C. Ferreira, B. Sellergren, *Sep. Purif. Technol.* **86**, **2012**, 190-198.
- ⁷ G. Szekely, B. Csordas, V. Farkas, J. Kupai, P. Pogany, Z. Santa, Z. Szakacs, T. Toth, M. Hollosi, J. Nyitrai, P. Huszthy, *Eur. J. Org. Chem.*, **2012**, 3396-3407.
- ⁸ Laurent Caron, Caline Christine, Sebastien Tilloy, Eric Monflier, David Landy, Sophie Fourmentin, Gheorghe Surpateanu, *Supramol. Chem.*, **14**, 2002, 11-20
- ⁹ Christiane Dethlefs, Jens Eckelmann, Hauke Kobarg, Thomas Weyrich, Stefan Brammer, Christian Näther, Ulrich Lüning, *Eur. J. Org. Chem.*, **2011**, 2011, 2066-2074
- ¹⁰ Y. Zhang, D. Song, L.M. Lanni, K.D. Shimizu, Importance of functional monomer dimerization in the molecular imprinting process, *Macromol.*, **43**, 2010, 6284-6294.
- ¹¹ A.J. Hall, P. Manesiotis, M. Emgenbroich, M. Quaglia, E. De Lorenzi, B. Sellergren, *J. Org. Chem.*, **70**(5), **2005**, 1732-1736.
- ¹² Diane M. Kneeland, Katsuhiko Ariga, Vincent M. Lynch, Chia-Yu Huang, Eric V. Anslyn, *J. Am. Chem. Soc.*, **115**, 1993, 10042-10055
- ¹³ P.A. Gale, J.R. Hiscock, C.Z. Jie, M.B. Hursthouse, M.E. Light, *Chem. Sci.*, **1**, **2010**, 215-220.
- ¹⁴ B. Liu, L. OU, F. Zhang, Zh. Zhang, H. Li, M. Zhu, Sh. Wang, *J. Sep. Science*, **37**(23), **2014**, 1615-9314
- ¹⁵ T.-P. Huynh, B. K. C. Chandra, M. Sosnowska, J. W. Sobczak, V. N. Nesterov, F. D'Souza, W. Kutner, *Biosens. Bioelectr.*, **64**, **2015**, 657-663.
- ¹⁶ K. K. Tadi, R. V. Motghare, *J. Mol. Model.*, **19**(8), **2013**, 1610-2940.
- ¹⁷ J. Liu, Zh. Dai, B. Li, Sh. Tang, R. Jin, *J. Mol. Model.*, **20**(10), **2014**, 2456.
- ¹⁸ S. A. Piletsky, K. Karim, E. V. Piletska, C. J. Day, K. W. Freebairn, C. Legge, A. P. F. Turner, *Analyst*, **126**, **2001**, 1826-1830.
- ¹⁹ S. Shoravi, G. D. Olsson, B. C. G. Karlsson, I. A. Nicholls, *Int. J. Mol. Sci.*, **15**(6), **2014**, 10622-10634.
- ²⁰ H.E. Gottlieb, V. Kotlyar, A. Nudelman, NMR, *J. Org. Chem.*, **62**, **1997**, 7512-7515.
- ²¹ Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.;

- Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision E.01, Gaussian, Inc., Wallingford CT, **2004**.
- ²² A. D. Becke, *J. Chem. Phys.*, **98**, **1993**, 5648-5652.
- ²³ C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B*, **37**, **1988**, 785-789.
- ²⁴ J. Tonannavar, Y. B. Chavan, J. Yenagi, *Spectrochim. Acta A*, **149**, **2015**, 860-868.
- ²⁵ J. Tonannavar, Y. B. Chavan, J. Yenagi, *Spectrochim. Acta A*, **160**, **2016**, 19-25.
- ²⁶ J. B. Foersman, Æ. Frisch, Exploring Chemistry with Electronic Structure Methods (2)Gaussian, Inc, Pittsburgh, PA, **1996**
- ²⁷ Amarendra Kumar, Vijay Narayan, Onkar Prasad, Leena Sinha, Monomeric and dimeric structures, electronic properties and vibrational spectra of azelaic acid by HF and B3LYP methods, *J. Mol. Struct.*, **1022**, **2012**, 81-88.
- ²⁸ M. Cossi, V. Barone, R. Cammi, J. Tomasi, *Chem. Phys. Lett.*, **255**, **1996**, 327-335.
- ²⁹ R. Cammi, B. Mennucci, J. Tomasi, *J. Phys. Chem. A*, **104**, **2000**, 5631-5637.
- ³⁰ M. Cossi, G. Scalmani, N. Rega, V. Barone., *J. Chem. Phys.*, **117**, **2002**, 43-54.
- ³¹ E. D. Glendening, J. K. Badenhoop, A. E. Reed, J. E. Carpenter, J. A. Bohmann, C. M. Morales, F. Weinhold, NBO 5.0; Theoretical Chemistry Institute, University of Wisconsin: Madison, **2001**.
- ³² S. F. Boys, F. Bernardi, *Mol. Phys.*, **19**, **1970**, 553-566.
- ³³ Richard J. Ansell, Dongyao Wang, Janice K. L. Kuah, *Analyst*, **133**, **2008**, 1673-1683.
- ³⁴ Norman J. Rose, Russell S. Drago, *J. Am. Chem. Soc.*, **81**, **1959**, 6138-6141.
- ³⁵ H. N. Wachter, V. Fried, *J. Chem. Educ.*, **51**, **1974**, 798-799.
- ³⁶ J. Saloni, S. S. R. Dasary, Y. Anjaneyulu, H. Yu, G. Hill Jr., *Struct. Chem.*, **21**, **2010**, 1171-1184.
- ³⁷ H.M. Badawi, M.A.A. Al-Khaldi, S.S.A. Al-Abbad, Z.H.A. Al-Sunaidi, *Spectrochim. Acta A*, **68**, **2007**, 432-442.
- ³⁸ A. Bhaskarapillai, S. Chandra, N.V. Sevilimedu, B. Selligren, *Biosens. Bioelectr.*, **25**, **2009**, 558-562.