K 120075 Zárójelentés

The main subject of our project was the investigation of unusual structure formation in liquids, in particular by hydrogen bonded molecules in aprotic solvents. The research idea came from the observation of unusual conductometric titration curves of "prepolymerization" mixtures. The latter are used for making molecularly imprinted polymers (MIP). It is expected that understanding of the association phenomena in prepolymerization mixtures will enhance our capabilities to make better MIPs. Beyond this application there are, however, many other practically relevant systems where structure formation in solution is relevant.

Experimental studies and practical applications

Conductivity titrations have been carried out in acetonitrile and acetone, respectively. Acetonitrile is a typical solvent for MIP preparation, while acetone has been chosen because for the molecular dynamics (MD) calculations (see later) this solvent for more suitable. The acids tested included methacrylic acid (MAA), propionic acid, lactic acid, chloroacetic acid and phenylpropionic acid. These represent substantial variety in terms of structure and acid strength. MAA is also the acid important for MIP preparation as monomer. The tested bases were dibutylamine, trietylamine and dibenzylamine. These again represent variability in structure and base strength. Titrations have been made both as titration of the base with acid and vice versa. The great asymmetry between the two titration directions, known for a few other systems from the literature, has been confirmed. Important differences were observed according to the acid/base strength.

The character of the titration curves of the weaker acids with the weaker bases (typical for MIP prepolymerization mixtures) has been found to be very different (in both titration directions) from systems of stronger partners. When titrating a weaker base with a weaker acid, the conductivity increased very long after the equivalence point. The curve remained nearly linear up to 4-5-fold excess of acid. The ionic dissociation in these systems being far from complete, it is not easy to interpret these phenomena quantitatively, but the observations show that in these systems asymmetric complexation occurs between acid and base, with more acid than base molecules in the complexes, probably as BAn type complexes (where B is the base and A is the acid).

Other experimental techniques have also been tested. Here we mention only the liquid-liquid distribution experiments which have confirmed and extended the above conductometric results. The distribution of different acid-base pairs was studied as a function of concentration, using various solvents. The acid was always in excess in the system over the base. In equilibrium there was acid excess in both phases. In the aqueous phase this results in acidic pH and thus the complete ionization of the base. Analytical measurements of the two equilibrated phases provided the excess acid concentration in both phases. In independent experiments the distribution curve of acid alone, between the two solvents, was determined. This allowed that in the presence of base we could calculate the concentration of the uncomplexed acid in the organic solvent, which is in equilibrium with the acid concentration in water. All the remaining amount of acid in the organic phase had to be bound then to the base in the organic phase. This gave us the apparent stoichiometry of the acid-base complex in the organic phase.

By this method we have found that in a typical MIP prepolymerization mixture the complex is BA_2. In other systems we could show that n may increase to about 4-5, where it "saturates". This confirms the conductivity results. It is also probably the first instance of clear experimental evidence for BAn complexes with n being 4 or higher.

Two new experimental methods have been developed (see attached paper) for testing the validity of the computational results in molecular imprinting systems. The first method consisted of the displacement of amines adsorbed on acidic MIPs with the help of a quaternary ammonium ion salt. Due to the macroscopic electroneutrality rule, the quaternary ammonium ion can displace only adsorbed ions, but cannot replace neutral amines. In the experiment a large part of the originally adsorbed amine could be displaced from the MIP's acidic binding sites by the quaternary ammonium ion. This has proved the (at least partial) ion pair character of amine binding on the acidic groups, in agreement with our computational results. The displacement experiments were done in toluene, which is an aprotic solvent. This had proved the existence of ion pairs also in non-polar systems, as predicted by our computations. Our earlier conductivity experiments could prove this only in the polar acetonitrile solvent.

The second novel experiment involved adsorption isotherm measurements on acidic polymer mixtures. The goal was to test if the predictions of the computational work about the stronger binding of amines by acid clusters than by individual acid molecules are valid. The computations had shown that amines in the prepolymerization mixtures of MIPs should preferentially interact with two or more acid molecules, i.e., with an acid cluster generated by the presence of the amine.

The conductivity experiments have been extended to the prepolymerization mixtures themselves, and had confirmed the presence of stronger complexes. We have expected that this stronger interaction is preserved in the imprinted polymer. One cannot expect, however, that all acidic monomer (MAA) molecules participate in such clusters. Therefore the MIP should partly bind amines by weaker forces, exerted by lonely acid groups. To test these predictions, we compared the adsorption of an amine on the MIP with its adsorption on a mixture of two nonimprinted acidic polymers. One of these was the nonimprinted control polymer (NIP), the other was a commercially available acidic polymer. Preliminary experiments had shown that the commercial polymer binds the amine much more strongly than the NIP. The decisive experiments had shown then, that the adsorption of the MIP can be very closely reproduced by a mixture of the NIP with the commercial polymer. This has proven that the acidic binding sites of the MIP may be indeed described as a mixture of NIP type (monoacidic) sites and some stronger acidic sites, which are very likely the acid clusters.

An important aspect of these results is that when acid-base type MIPs are used, the adsorption of bases on the MIP is likely to occur on HAn type sites, which are formed during acid treatment of the BAn complexes on the freshly made MIPs. When these abundant sites rebind a small quantity of the base, B, from a solution, ionic dissociation can again occur, as in our conductivity experiments. We have also shown, that the formation of the acid base complexes elucidated here, can also explain why acidic MIPs are only moderately selective against basic interferents, but highly selective for their basic template against many non-basic compounds.

Work to understand the selectivity of acid-base interactions in molecular imprinting has been successful and was published. We have shown that although the definition of MIP selectivity is ambiguous, there exist important practical situations where selectivity may be demonstrated on “selectivity ladders”, introduced by us. It was also demonstrated that very efficient imprinting, in terms of high imprinting factors, does not necessarily result in improved selectivity when compared with the NIP. We could also show that in certain solvents the MIP’s selectivity for the template against “similar” compounds may be due to other factors than the acid-base interaction, e.g., to differences in hydrophobicity. These results are in agreement with our theoretical work presented above. The higher acid-base adducts in the MIP are stronger than the 1:1 complex in a NIP, but not necessarily more selective. Also, the interactions between template and binding site can be very different in aprotic media from those in aqueous conditions.

Based on our experiments, we have developed a novel, plausible model to explain the imprinting and selectivity pattern of the most studied MIP, the propranolol (PR) MIP. The experiments had shown that the non-imprinted control polymer (NIP) has already a substantial PR/DBA selectivity (DBA=dibenzylamine), and this PR/DBA selectivity is surprisingly not improved by imprinting with PR (i.e., by making a MIP for PR). We explain this by showing that PR can be bound by two nearby carboxylic groups of the NIP by using the amino and OH groups of PR, respectively. DBA with its only amino group can be bound only by one carboxyl group of the NIP. The MIP binds PR also on two points, but with increased strength due to acid homoconjugation (homoassociation), which arises due to the acid-base interaction. But the binding of DBA by the MIP is also stronger than on the NIP, also due to the acid homoconjugation. Thus the PR/DBA selectivity of the MIP is not better than that of the NIP.

We have summarized and extended our results, concerning the role of unusual acid-base interactions, as related to molecular imprinting. In the published paper we have proved that 1/ Imprinting the acidic monomers with amines induces homoassociation of the acidic monomer; 2/ The homoassociated acid binding site is stronger acid than the single acid site; 3/ Homoassociation increases the template rebinding and the class selectivity; 4/ Selectivity towards related compounds is only modestly changed by imprinting. We have also shown that the high selectivity of some imprinted polymers against compounds “similar” to the template, as had been observed by many workers, may be an artefact, due to overlooking large differences in certain relevant physicochemical properties of the apparently similar compounds. We have also clarified the confusion about the often praised high selectivity of MIPs. While the class selectivity of MIPs is indeed often very high, their selectivity against truly similar compounds is in many cases only modest.

Our above described discoveries about MIP selectivity lead us to investigate a MIP which had been found in the literature extremely selective. This was the MIP for theophylline, which had high selectivity against caffeine in so-called competitive binding assays (also called pseudo immunoassays or biomimetic binding assays). We hypothesized that this observation was due to the very different acid dissociation constants of the two compounds. We prepared theophylline imprinted MIPs and confirmed the high selectivity in the binding assay, but also found that the selectivity of adsorption (measured as distribution coefficient ratio) and the selectivity improvement against the NIP was not particularly high.

These observations gave a new direction to our research, in which we compared the selectivity measures in binding assays with those derived from distribution coefficients. We have surprisingly found, that in the literature of competitive immunoassays the measures used for selectivity characterization are inadequate. (One should note here, that such assays have been in routine use for decades in clinical laboratories all around the world.) We realized that the selectivity measures of these assays depend on the assumed chemical model of the binding reaction. In the most studied model, a tracer compound, the analyte and the interferent compete for a limited amount of binding sites (antibody sites or MIP sites). Earlier workers could not calculate the analytical selectivity of these assays because the respective equations were too complicated. We have found a simple mathematical way to go around this difficulty. Thus we could give a simple formula for the selectivity of the assays. Using this formula, we have shown the size of error arising by the use of the conventional measures.

This work about selectivity was also extended to a comparative study of MIP selectivity in different real-life applications (batch adsorption, chromatography, solid phase extraction, sensors, membranes, and catalysts). We have shown that the selectivity of the same MIP (in the same medium, at the same temperature, etc.) depends on the particular experiment and the application for which the MIP is employed.

In accordance with our original plans, we have also moved to studying acid-base interactions of Lewis bases with Bronsted acids. It has been found that a Lewis base can also increase the extraction of a Bronsted acid into an organic solvent. We have hypothesized that weak Lewis acid – Lewis base systems can also be used in molecular imprinting, e.g., when templates with alcoholic OH group are imprinted using an ester monomer in an aprotic solvent. We tried to imprint with the template cholesterol (an alcohol) in a polymer consisting exclusively of a diester monomer. This attempt remained unsuccessful both in acetonitrile and chloroform as porogenic solvents. Surprisingly though, imprinting was successful with isopropanol as porogen. In this system acid-base (Lewis, H-bonding) interaction between template and monomer is practically excluded. This result sheds new light on all imprinting systems, and shows that imprinting for molecular shape is possible without functional group interactions. The applied method represents a hitherto undiscovered way for imprinting with templates and with monomers, which practically cannot interact during polymerization, except by dispersive forces. We have shown this technique to be useful in further examples, beyond cholesterol.

On a more applied side, we have found that certain Lewis bases can extract large quantities of weak acids from their aqueous solutions. This phenomenon has been successfully tested for a technical improvement of a catalytic system. These results have not been published to protect eventually arising IP rights.

We had studied acid-base systems relevant for molecular imprinting mainly with basic templates. We have also began to study the reverse systems, where acidic templates (e.g. the herbicide 2,4D) are imprinted. Such MIPs had been typically described in the literature by using vinylpyridine (VP, either 2VP or 4VP) as functional monomer. We observed that in buffered aqueous solutions, where such MIPs had been mainly used according to the literature, the supposed acid-base binding reactions are unlikely. Therefore, we began studies of this imprinting system, and also of the interactions of weak acids with weakly basic polymers, in general.

As this work (described in the diploma thesis of A. Sváb-Kovács) was in progress, we noted a very unusual observation which had been made with some commercially available solid phase extraction (SPE) sorbents. Weak acids and weak bases were found to be strongly adsorbed on these neutral sorbents from dilute aqueous solutions of pH about 7. At this pH the tested acids and bases are expected to be completely ionized, and therefore only very little adsorbed. The phenomenon has practical relevance because it is often used in environmental analysis, despite its unclear background. We have experimentally tested some of these neutral sorbents, which contain amide groups in an apolar, aromatic matrix. A variety of neutral, acidic and basic compounds was adsorbed at different pH values. The trend of the pH effect was found to be as expected, but the size of the effects was definitely counterintuitive. Addition of methanol reduced the binding, thus showing that binding has an important hydrophobic component. We have also found that the adsorption isotherms of many compounds are highly nonlinear. This may prove to be very important in environmental analytical applications, because the apparent binding capacity is strongly concentration-dependent. This work is close to be finished and published.

According to the original plans, we have attempted to make a polymer from MMA with imidazolium counterions, with the hope that the resulting structure will have carbene-activity. Unfortunately, however, the resulting material did not show any organocatalytic activity in the benzoin condensation, thus this research direction had to be abandoned.

Computational studies and their applications

For a detailed understanding of the effect of hydrogen bond formation, and to evaluate the role of the basicity, we have started with calculations on the amine formic acid systems, using ammonia, methylamine, dimethyamine and trimethylamine, respectively, as bases, with up to four formic acid molecules. The network of the hydrogen bonds shows large variations between these systems.

We have realized from the systematic study that our previous results based on the Pople type basis sets change significantly, by using the correlation consistent cc-PVDZ basis, and even the cc-PVTZ results exhibit some (albeit smaller) changes. Accordingly we use the cc-PVDZ basis even for the largest systems, where the M06-2X functional was used to account for the dispersion effects, while for the amine-formic acid systems we used the cc-PVTZ basis throughout.

The systematic study on the adducts formed from different bases and formic acid revealed several previously unknown or overseen results:

1./ With two or more acid molecules there are several hydrogen bonded structures, and some of them are relatively close in energy. In the most stable structures the number of hydrogen bonds is maximized, forming a cyclic arrangement with the nitrogen in the ring. In case of the base + two acid system there are two low energy structures which are close in energy, and these two structural motifs can be found in the two lowest energy structures of the base + three acid system as well. Clearly these structures should be considered in the future works on more extended systems.

2./ More importantly, in the presence of more than one acid molecule, there are several structures in which both the neutral and the ion pair structures could be optimized. These two structures look very similar, the difference between them is in the position of one hydrogen, which is bound to oxygen in case of the neutral, while to nitrogen in case of the ion pair structure. In general, the stability of the ion pair structures is increasing with the increasing number of acids. While in the 1:1 adduct no ion pair structure could be optimized, in case of the one secondary amine base : three acid structures the ion pair is the most stable one. The relative stability of the two structures depends also on the polarity of the solvent. Using the PCM solvent model, solvents with high dielectric constant stabilize the ion pair structures, allowing the stability of the ion pair even in case of one base : two acid systems (acetonitrile PCM). Gradual substitution of the hydrogens of ammonia with alkyl groups results in stronger bases and in accordance, the relative stability of the ion pair adducts is increasing with respect to the neutral ones. In case of the tertiary amines the nitrogen can bear only a single hydrogen involved in hydrogen bonding in case of the ion pair structure, and as a consequence the most stable cyclic hydrogen bonded arrangements, observed with primary and secondary amines, cannot be formed. These results are compiled in a diploma thesis work (Orsolya Panna Kovács), and a publication after repeated checking of the results is under preparation.

To investigate the interaction of secondary amines with methacrylic acid (MAA), we have calculated adducts (complexes) between dibenzyl-amine and 1 to 4 MAA molecules. This has been done at first by optimizing structures, which we have obtained from classical molecular dynamics calculations as snapshots. The optimizations were carried out in a PCM solvent model, using acetone and acetonitrile as solvent. We have established that the most stable optimized structures are contact ion pairs for this system, from two up to four acid molecules, while for the dibenzyl-amine MAA 1:1 ratio the hydrogen bonded adduct turned out to be the most stable structure at this level of the theory. The stability of the adducts is increasing with the number of the MAA molecules.

From the calculations on the DBA + 2MAA system we were able to locate both ion pair and neutral adducts, which have comparable energies. These adducts have a stability of 5 kcal/mol in terms of Gibbs free energy (standard conditions). It is noteworthy that the calculations with propranolol, which is a secondary amine containing also an OH side group, predict a structure which has a stability of 9 kcal/mol, exceeding significantly the stability of the DBA adduct, in accordance with a stabilizing extra interaction with the OH side group. These results are in accordance with the observed selectivity of the different MIPS made with DBA or propranolol template.

Upon further investigation of the complexes formed between weak acid clusters and amines as weak bases we were able to locate several clusters with different hydrogen bonding networks. We have established that depending on the relative acidity and basicity of the constituents and the number of the reactant acid molecules there are certain acid-base complexes, where both the neutral and the “isomeric” ion pair complexes can be optimized. In these cases, the energy difference between the two structures is very small, furthermore, the barrier of the proton transfer reaction also allows a fast interconversion between these structures. Unfortunately, the corresponding double well potential energy surface does not allow for the description of the vibrational frequencies within the harmonic approximation, thus a direct correspondence between experimentally observed vibrational spectra (IR spectra) and calculated spectra is rather difficult.

Nevertheless, with the increasing basicity of the base, and the increasing polarity of the solvent (and also the increasing acidity of the acid), the ion pair structures are getting favored. Most importantly, the tendency to form a stable ion pair cluster increases with the increasing number of acid molecules. The structure of the complexes formed is stabilized by an extended hydrogen bonding network, and the structure of this network is also influenced by the type of the amine. While for primary amines the formation of the possible hydrogen bonded network allows several and highly complicated structures, in case of secondary amines two stable hydrogen bonded cyclic networks can form between one amine and two acid molecules, with nearly identical energy.

We have studied also the effect of adding a weak base or a hydrogen bonding liquid to the non H-bonding, weak acid, CO2. Supercritical CO2 (scCO2) is an important non-toxic solvent, but its solvation power is not sufficient in many applications. The solvation ability of scCO2 can be improved by adding other, co-solvent molecules to it. The use of methanol as a co-solvent is of particular importance. Therefore, we have studied this system by computer simulation. We have found that in the studied parameter range mixtures with high CO2 content become inhomogeneous, and different CO2 models show rather different performance.

Our work has also been extended to studies of unusual acid-base effects in important chemical reactions. In the framework of a project in the field of organosilicon chemistry, we had noticed an interesting effect caused by variation of the acidity of the system. This can be explained by the presence of a weak base/acid system (triethylamine/triethylamine-hydrochloride), which results in a controllable tuning of the product of a chemical reaction. In the reaction of (EtO)3SiCl (1) with PhCO-CH2CO-Ph (2) in the presence of the weak base trimethylamine, not only the expected (EtO)3Si-O-C(Ph)=CH-COPh (3) product is formed (together with triethyalmine-hydrochloride), but also two hexacoordinate silicon compounds, (EtO)2SiL2 (4) and SiL3+ (5) were obtained (where L denotes the ligand 2), with the gradual replacement of the ethoxy units of 3 by one and two molecules of 2, yielding the neutral 4 and ionic 5, respectively. While the formation of the ionic hexacoordinate 5 is the thermodynamic sink of the reaction cascade, the replacement of the two OEt groups of 4 by one ligand 2 can only be accomplished at a proper pH range, which can be regulated by the presence of the triethylamine/triethylamine-hydrochloride system.

In another example we studied the carbon-nitrogen bond formation, which is one of the most important organic reactions. In a combined experimental/theoretical work together with colleagues from EGIS, we found an aza-Wittig reaction, which could selectively be tuned, by the amount of the added base, from an anionic to a dianionic mechanism, yielding different pharmaceutically interesting ring systems – each with a high yield. With the computed reaction mechanism we were able to show the effect of the acidity not only during the reaction, but also in case of the workup.

Acid-base interactions at interfaces are also important, but their study (by molecular dynamics in our case) requires much fundamental work. One step in this direction is to better understand the molecular level structure of interfaces and to develop the methods for calculating interfacial thermodynamic quantities from simulation data. We have shown that alternative methods for calculating the surface entropy yield different results. Studies have also been made to investigate the thermodynamics of gas expanded liquids, a modern solvent medium where acid-base interactions are less understood yet.

Our work related to acid-base interactions at interfaces has been extended to the investigation of the interface of the Lewis base acetone with the acid anhydride carbon dioxide. The vapor-liquid interface of mixtures of these two components have been studied in the vicinity of their critical point. Since experimental data in this respect exist only in limited ranges of compositions (at low acetone mole fractions) and temperatures (data above about 335 K are scarce), our study largely extended the range of thermodynamic conditions in which we have reliable information on the liquid-vapor equilibrium, including the surface tension of acetone−CO2 mixtures.

A special case of hydrogen bonded (mainly acid-base type) interactions in relation to aprotic solvents is the behavior of the hydrogen bonded structure of water at the interface with an immiscible, aprotic liquid. We have found that at the interface between water and CCl4, the presence of CCl4 slowed down the dynamics of the surface water molecules, and it restricted also the orientational freedom of the surface water molecules, changing thereby the hydrogen bonded structure of water at the interface and resulting in fewer inlayer and more out-of-layer hydrogen-bonded neighbors.

We had shown earlier that the surface tension of liquids can be broken up into contributions along the interface normal. We have continued this work by investigating a variety of surfactants. We have found that the surface tension of surfactant solutions (which is of course much lower than that of water) has very different contributions from the different types of headgroups. The neutral alcoholic headgroups, which adapt to the hydrogen bond network of water, have little contribution, while the ionic headgroups (anions of acids and cations of bases, with their counterions) have a large contribution. This work was continued by systematically studying the surface tension contribution of the counterions in the series of alkali dodecyl sulfate solutions. Unfortunately, we have found that the chosen model combination fails in describing the surface tension decrease induced by the surfactant. However, despite this failure of the model, we have observed a clear relation between the soft-hard character (in the sense of the Hofmeister series) and the surface tension contribution of the counterions. We studied also the artificial system, where the counterion and surfactant charges are inverted in sign, and the counterions become considerably harder. This charge inversion changes considerably the surface tension contributions of the counterions, surfactant headgroups, and water molecules, stressing the key role of the hardness of the counterions in this respect. Further work in this direction is in progress.

Remarks

In March 2017 Ms Zsanett Dorko defended successfully her PhD dissertation (https://repozitorium.omikk.bme.hu/handle/10890/5343), which includes results achieved partly in the preparation for the present project, partly in the framework of the project. Part of the experimental work has been described in an MSc thesis (Anett Nagy-Szakolczai) and in a BSc thesis (Anikó Sváb-Kovács). Part of the computational work is in the BSc thesis of Orsolya Panna Kovács.