Solvation Effects on Molecules and Biomolecules

# CHALLENGES AND ADVANCES IN COMPUTATIONAL CHEMISTRY AND PHYSICS

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# Solvation Effects on Molecules and Biomolecules

## Computational Methods and Applications

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# CONTENTS

# PREFACE

The incessant development of quantum chemistry since the appearance of the Schrödinger equation has turned this area into a respectable branch of science with unprecedented capabilities. It is now a well-recognized field of research with predictive power that is an important component in physical–chemical laboratories. Very important developments were conducted in the early days by bright theoretical scientists that were ready to absorb the incredible and unpredicted computer revolution which was only just beginning. Isolated medium-size molecular systems can now be accurately studied theoretically by quantum chemical methods. However, it was also long recognized that all biomolecular phenomena necessary to obtain and sustain living systems take place in solution, as well as the vast majority of chemical processes. Indeed solvent and liquid systems are germane in chemistry experiments. In physics, a constant concern is the description of the role played by the environment in modifying the properties of the system as compared to the isolated situation. Hence, the importance of studying atoms, molecules and biomolecules in the solvent environment can hardly be denied. The quantum chemical studies of molecular systems affected by the interaction with a solvent had its own turning point before the end of the 1970s, when some pioneering work was done, including the dielectric properties of the medium in an effective nonlinear Hamiltonian. This naturally led to the development of the so-called continuum models that are important and now popular. Continuum models can be implemented from the simplest to the most sophisticated quantum chemical methods.

The same computer revolution that started in the middle of the last century also plays an important, in fact crucial, role in the development of methods and algorithms to study solvation problems. Dealing, for instance, with a liquid system means the inclusion of explicit molecules, in different thermodynamic conditions. The number of possible arrangements of atoms or molecules is enormous, demanding the use of statistical mechanics. Here is where computer simulation, Monte Carlo (MC) or molecular dynamics (MD), makes its entry to treat liquid systems. Computer simulation is now an important, if not central, tool to study solvation phenomena. The last two decades have seen a remarkable development of methods, techniques and algorithms to study solvation problems. Most of the recent developments have focused on combining quantum mechanics and statistical mechanics using MC or

MD simulations. This led naturally to the so-called QM/MM methods that combine quantum mechanics and molecular mechanics. In the original idea, a part of the system is treated by quantum mechanics and the remaining part by molecular mechanics. Variants of the QM/MM have also been developed adopting the central idea to the particular interest of studies. It is perhaps correct to say that the study of solvation effects in general is the area of physical chemistry research that has recently seen the most spectacular and constant advancements. As such, there is a need for a source material where the important developments and applications are described and directed not only to the specialists, but also for those beginning in this field.

This is the aim of this book. In 18 separate chapters different aspects of the solvation effects in molecules and biomolecules are presented and discussed and applications are shown. Some of the most internationally prominent groups in this field have joined in this project to produce this book that describes some of the important developments that are underway, as well as the achievements that have already been made. Different aspects of the solvation problem are presented, the theoretical methods to solve them are discussed and some perspectives are outlined.

I warmly thank all contributing authors for enthusiastically adhering to the effort to make this book a reality.

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January 2008

CHAPTER 1

# SOLVATION MODELS FOR MOLECULAR PROPERTIES: CONTINUUM VERSUS DISCRETE APPROACHES

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**Abstract:** A comparative description of two different hybrid solvation models is presented, both of them describe the solute at quantum-mechanical level while the solvent is treated either through a polarizable molecular mechanics force field or as a polarizable continuum dielectric. The theoretical framework of the two methods is analyzed in terms of common features as well as of eventual differences. An application to the study of solvent effects on NMR properties of solvated molecular systems is used to analyze the relative performances as well as to underline the differences in the corresponding descriptions

## 1.1. INTRODUCTION

The importance of understanding environment effects on chemical systems is evident in almost all the most active fields of research, such as condensed phase chemistry, biochemistry or material chemistry just to quote a few.

It is thus not strange that, in the years, many different phenomenological approaches and theoretical models aimed at describing the effects of a given surrounding medium on the properties of a selected system have been formulated. However, only with the recent progress achieved in the computational technology, a real step forward has been done in the modelization of the phenomenon giving origin to such effects (which in the following we shall indicate as solvation or embedding). In particular, two have been the successful strategies; on the one hand the availability of fast computers has suggested to try to get a detailed microscopic description of a representative sample of the whole system. This first strategy has led to molecular dynamics (MD) or Monte Carlo (MC) simulations in which a necessarily simplified description of the intra- and intermolecular interactions is introduced in terms of the selected force field.

On the other hand, the development of computers with increasing memory (besides speed) has allowed to increase the realism and the accuracy of the description



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in spite of the dimension of the studied system which now is reduced to a by far smaller part of the whole system, generally called the "solute" even when formed by distinct molecular units. Within this second strategy (which we shall indicate as focussed), the surrounding molecules become the secondary subsystem ("the solvent") and they will be modeled using a less accurate description, in most cases also completely neglecting their microscopic nature. Examples of this second strategy are the hybrid approaches using a quantum-mechanical (QM) description for the solute and a simplified (either Molecular Mechanics, MM [1,2,3,4,5,6] or continuum [7,8,9,10,11]) description for the solvent. In the latter case, the secondary system completely disappears and it is substituted by a macroscopic continuum medium having suitable properties.

It is obvious that the two strategies have different advantages and limits and that their applications are necessarily of different nature. In particular, the first strategy is preferable if a statistically representative picture of the whole system (including its dynamic) is required while the second strategy is necessary if an accurate description of the electronic nature of the solute and how it is changed by the environment is important. The present chapter, devoted to the study of the effects the environment has on the response properties of solvated molecular systems, will thus mainly be focussed on the second strategy even if the importance of an interplay between the two will be made evident.

## 1.2. FOCUSSED MODELS

There are several approaches to be classified in the family of focussed models. The common characteristic of all of them is that the system is divided into two (or even more) parts (or layers) which are described at different levels of accuracy. The target layer (the solute) is generally described at QM level (either ab initio or semiempirical) while the rest (the solvent) is approximated using an MM description as that used in force fields or a continuum description or both of them in case of more than two layers.

In all cases, the formalism of the in vacuo QM molecular calculations is maintained, including in the solute Hamiltonian an explicit expression of the solute–solvent potential. What distinguishes the different approaches is exactly the form of this potential.

In standard QM/MM approaches, the solvent is treated by assigning partial point charges to the atomic sites and the potential due to these point charges is then introduced into the solute Hamiltonian. However, in such a procedure polarization of the solvent is neglected, i.e., only the solute is polarized. This may be refined, for example, by assigning polarizable sites to the solvent giving rise to induced electrical moments and including their effects in the solute Hamiltonian as a further solvent term.

In the continuum models the solvent is also polarizable and its effect on the solute is again represented by the so-called reaction potential part of the Hamiltonian.

In both cases we can introduce a similar picture in terms of an effective Hamiltonian giving rise to an effective Schrödinger equation for the solvated solute. Introducing the standard Born–Oppenheimer approximation, the solute electronic wavefunction $|\Psi\rangle$ will satisfy the following equation:

$$\hat{H}_{\text{eff}}\,|\Psi\rangle = \left(\hat{H}_0 + \hat{H}_{\text{env}}\right)|\Psi\rangle = E\,|\Psi\rangle \tag{1-1}$$

where $\hat{H}_0$ is the Hamiltonian of the solute system when in absence of the rest, and the operator $\hat{H}_{\text{env}}$ introduces the coupling between the solute and the solvent.

The form of the operator $\hat{H}_{\text{env}}$ depends on the particular method used, here, in particular, two alternative schemes are analyzed:

$$\hat{H}_{\text{env}} = \begin{cases} \hat{H}_{\text{QM/MM}} + \hat{H}_{\text{MM}} & \text{QM/MM} \\ V_{\text{cont}} & \text{QM/continuum} \end{cases} \tag{1-2}$$

The details on the operators introduced in the two schemes will be given below, here we only want to add that the addition of $\hat{H}_{\text{env}}$ to the solute Hamiltonian automatically leads to a modification of the solute wavefunction which has now to be determined by solving the effective Eq. (1-1). This can be done using exactly the same methods used for isolated molecules; here in particular we shall mainly focus on the standard self-consistent field (SCF) approach (either in its Hartree–Fock or DFT formulation). Due to the presence of $\hat{H}_{\text{env}}$ the modified SCF scheme is generally known as self-consistent reaction field (SCRF). Historically the term SCRF has been coined for the QM/continuum approach but here, due the parallelism between the two schemes which will be made clear in the following sections, it will be used indistinctly for both.

### 1.2.1. QM/MM

In QM/MM approaches, the MM system is represented through atomic point charges and (if a polarizable force field is used) atomic or molecular polarizabilities at selected points in the solvent molecules, we thus have

$$\hat{H}_{\text{QM/MM}} = \hat{H}^{\text{el}} + \hat{H}^{\text{pol}} \tag{1-3}$$

We note that the $\hat{H}_{\text{MM}}$ introduced in Eq. (1-2) is the classical MM energy, this term, however, is a contribution only to the energy and not to the wavefunction.

The first term in Eq. (1-3) is the electrostatic interaction between the QM system and the point charges in the MM part of the system, namely

$$\hat{H}^{\text{el}} = \sum_m q_m(\mathbf{r}_m)\hat{\mathbf{V}}(\mathbf{r}_m) \tag{1-4}$$

where $\hat{\mathbf{V}}(\mathbf{r}_m)$ is the electrostatic potential operator due to solute electrons and nuclei at the MM charges $q_m$. This term is directly included in the one-electron part of the vacuum Hamiltonian.

The second term in Eq. (1-3) is the polarization interaction between the induced dipole moments and the electric field from the QM system. In the following exposition, the approach proposed by Mikkelsen, Kongsted and coworkers will be used [12,13,14]; for such a version of the polarizable QM/MM scheme the acronym DPM (discrete polarizable method) has been introduced and will be used here. In the DPM $\hat{H}^{\text{pol}}$ can be expressed as

$$\hat{H}^{\text{pol}} = \frac{1}{2} \sum_a \mu_a^{\text{ind}} \, \hat{\mathbf{E}}_a^{\text{solute}} \tag{1-5}$$

$$\mu_a^{\text{ind}} = \alpha_a \left( \mathbf{E}_a^{\text{solute}} + \mathbf{E}_a^{\text{solvent}} \left\{ \mathbf{q}; \mu^{\text{ind}} \right\} \right) \tag{1-6}$$

where the summation runs over all the selected polarizability points of the MM molecules and $\hat{\mathbf{E}}^{\text{solute}}$ is the electric field from the electrons and the nuclei in the QM system at those points. To define the induced dipole moments, $\mu_a^{\text{ind}}$, in Eq. (1-6) we have assumed a linear approximation, neglected any contribution of magnetic character related to the total electric field and used an isotropic polarizability ($\alpha_a$) for each selected point in the MM part of the system. Distributed polarizabilities should be introduced, however, for small solvent molecules with low anisotropies in the molecular polarizability, the use of monocenter polarizabilities is usually sufficient; here, for the sake of simplicity we have used a single point for each MM molecule, its center of mass.

In Eq. (1-6), $\mathbf{E}_a^{\text{solvent}}$ refers to the total solvent electric field and it contains a sum of contributions from the point charges and the induced dipole moments in the MM part of the system. Such a field (and hence the induced dipole) depends on all other induced dipole moments in the solvent. This means that Eq. (1-6) must be solved iteratively within each SCF iteration. As an alternative, Eq. (1-6) may be reformulated into a matrix equation

$$\mu^{\text{ind}} = \mathbf{B}\mathbf{E} \tag{1-7}$$

where the matrix $\mathbf{B}$ is of dimension $3N \otimes 3N$, $N$ being the number of polarizable sites, and the vector $\mathbf{E}$ collects the electric field from the solute and the solvent permanent charge distribution, $\left( \mathbf{E}^{\text{solute}} + \mathbf{E}^{\text{solvent}}\{\mathbf{q}\} \right)$. The form of matrix $\mathbf{B}$ will be determined uniquely by the position of the polarizable sites and the polarizability values.

### 1.2.2. QM/continuum

Moving now to QM/continuum approaches, we shall limit our exposition to the so-called apparent surface charges (ASC) version of such approaches, and in particular to the family known with the acronym PCM (polarizable continuum model) [11]. In this family of methods, the reaction potential $V_{\text{cont}}$ defined in Eq. (1-2) has a form completely equivalent to the $\hat{H}^{\text{el}}$ part of the $\hat{H}_{\text{QM/MM}}$ operator defined in Eq. (1-4), namely:

$$V_{\text{cont}} \rightarrow \hat{V}^{\text{PCM}} = \sum_{s} q_s^{\text{PCM}}(\mathbf{r}_s)\hat{\mathbf{V}}(\mathbf{r}_s) \tag{1-8}$$

Now, however, the point charges $q_s^{\text{PCM}}$ are no longer centered on the solvent nuclei as in the MM description but they are placed on selected points placed on the surface of the molecular cavity containing the solute. In addition, such charges are not fixed but they are "apparent" in the sense that they exist only when the solute exists. As the induced dipoles of the polarizable MM description, the PCM charges are determined by total field acting at the selected points on the surface (i.e., the field due to the solute and the charges themselves) but now they also depend on the dielectric properties of the solvent, on the geometry of the cavity and on the number and position of the points chosen to map the cavity surface. In general, these points are determined by partitioning the surface into finite elements (called tesserae) and identifying a representative point for each tessera. The equations giving the charges can be solved iteratively within each SCF cycle or similar to what is done for the induced dipoles (see Eq. 1-7), a single matrix equation can be solved instead:

$$\mathbf{q}^{\text{PCM}} = -\mathbf{K}\mathbf{f}^{\text{solute}} \tag{1-9}$$

where now the field is only that due to the solute. The effect of the self-polarization, in fact, has been reformulated into the matrix **K** exactly as done before in Eq. (1-7) introducing the **B** matrix for the MM-induced dipoles.

Once again, **K** is a square matrix (the dimension being equal to Nts $\otimes$ Nts where Nts is the number of tesserae) and it depends on the geometrical cavity parameters and the dielectric constant of the solvent. It is to be noted that in Eq. (1-9) we have substituted the electric field $\mathbf{E}^{\text{solute}}$ with the more general vector $\mathbf{f}^{\text{solute}}$; in fact different versions of the PCM approach use different electrostatic quantities to define the charges (and correspondingly different forms of the **K** matrix) [11], namely the normal component of the electronic field in its original version (now called DPCM) or the electrostatic potential in its reformulation known as integral equation formalism (IEFPCM) [15,16].

As for the QM/MM description also for PCM, non-electrostatic (or van der Walls) terms can be added to the $V_{\text{cont}}$ operator; in this case, besides the dispersion and repulsion terms, a new term has to be considered, namely the energy required to build a cavity of the proper shape and dimension in the continuum dielectric. This further continuum-specific term is generally indicated as cavitation. Generally all the non-electrostatic terms are expressed using empirical expressions and thus their effect is only on the energy and not on the solute wavefunction. As a matter of fact, dispersion and repulsion effects can be (and have been) described at a PCM-QM level and included in the solute-effective Hamiltonian $\hat{H}_{\text{eff}}$ as two new operators modifying the SCRF scheme. Their definition can be found in Ref. [17] while a recent systematic comparison of these contributions determined either using the QM or the classical methods is reported in Ref. [18]

The brief descriptions given here for QM/MM(pol) (in its DPM formulation) and QM/continuum (in its PCM formulation) should make clear the parallelism of the two formulations both from a quantum-mechanical and a computational point of view. There are, however, fundamental differences which are worth being recalled here.

The picture given by a QM/MM approach automatically includes a microscopic description of all the components of the system (solute + solvent) even if with different levels of accuracy (a QM charge distribution for the solute and a set of classical charges and dipole moments for the solvent). A preliminary knowledge of the position in the space of all these components (i.e., the configuration) is thus required. A liquid solution, however, is a very dynamic system which cannot be properly represented in terms of a single (or few) configuration: many different configurations obtained from a correct statistical analysis should thus be introduced and used to get the final averaged picture. These configurations can be obtained as snapshots of a MD (or MC) simulation.

By contrast, the description given by a continuum description does not require any knowledge of the solvent configuration around the solute as a structureless continuum dielectric is introduced instead. The response of such a dielectric to the presence of the solute is determined by its macroscopic properties (namely the dielectric constant and the refractive index) and thus it will be implicitly averaged. Contrary to what happens in a QM/MM approach, here a single calculation on a given solute contained within the continuum dielectric will be sufficient to get the correct picture of the solvated system.

It is evident, however, that this enormous gain in terms of computational time (and simplicity of the protocol) will involve some disadvantages. The main one is the loss of the microscopic nature of the solvent molecules. This issue becomes particularly delicate when solute–solvent specific interactions such as hydrogen bonds are present in the liquid solution: in these cases, the picture obtained using a continuum-only description will be incomplete as it misses an important part of the solute–solvent interactions.

In the following section we shall show how all these specificities of the QM/MM and QM/continuum approaches will affect the quality of the description one can obtain applying them to the study of solvent effects on molecular response properties.

## 1.3. MODELING SOLVENT EFFECTS ON PROPERTIES

In the previous sections we have briefly summarized the basic theory of QM/MM and QM/continuum methods showing their differences and similarities, now we can move on to describe their applications to the calculation of molecular response properties and the related spectroscopies for a generic solvated system.

As shown above, in both DPM and PCM versions of these schemes, an appropriate interaction operator between the solute and solvent is added to the Hamiltonian of the isolated molecule. Such an operator is the term $\hat{H}_{\mathrm{env}}$ defined in Eq. (1-2) which is obtained by combining one-electron operators and thus no significant increase in

computational effort is introduced. The electronic density is obtained by including self-consistently the polarization of the solvent. Along the same lines as for an isolated molecule, the use of response theory may in the context of solvation be used to study a variety of molecular properties other than the molecular energy. Introducing the concepts of response theory into the PCM or DPM approach leads to a compact and powerful method to calculate molecular properties of a molecule subjected to an environment. In particular, both properties related to external or internal perturbations may be considered. In addition to this, the specific properties may be of either electric or magnetic origin.

Within the DPM the specific contributions due to the polarizable and structured environment will lead to two different sorts of corrections: (i) contributions due to the static multipole moments (here partial charges) and (ii) contributions due to the induced polarization in the environment. In contrast, for the PCM only contributions due to the induced polarization in the solvent are relevant.

The different characteristics of the two solvation models make it interesting to compare their performances when describing the effects of the solvent on given molecular response properties. A very good candidate for such a comparative analysis is the NMR spectroscopy. This is in fact one of the most important techniques available for investigating molecular structures, molecular interactions and the solvation problems. Most NMR measurements are performed on liquid samples and they yield isotropic chemical shifts (related to the nuclear magnetic shielding constants) and scalar spin–spin coupling constants modified by the solvent. NMR parameters (in particular NMR chemical shifts) are, in fact, extremely sensitive to the molecular environment, and especially hydrogen-bonding effects.

### 1.3.1. QM Evaluation of NMR Nuclear Shieldings

The effects of solvent on nuclear magnetic shielding parameters derived from NMR spectroscopy have been of great interest for a long time. In 1960 Buckingham et al [19] suggested a possible classification in terms of various additive corrections to the shielding arising from (i) the bulk magnetic susceptibility of the solvent, (ii) the magnetic anisotropy of the solvent molecules, (iii) van der Waals interactions and (iv) long-range electrostatic interactions. In the original scheme, strong specific interactions, such as those acting in intermolecular hydrogen bonds, were not specifically dealt with but just mentioned as a possible extreme form of the electrostatic, or, more generally "polar", effect; in the numerous applications that followed Buckingham's classification, however, this further effect has been always included as a separate contribution.

On the basis of such a classification an empirical approach based on the so-called solvent empirical parameters was formulated to evaluate solvent effects on nuclear shieldings. In brief, this approach, originally proposed by Kamlet, Taft and co-workers [20] for electronic excitations, does not involve QM or other types of calculations but introduces a numerical treatment of experimental data obtained for a given reference system to obtain an estimate of solvent effects on various properties.

An extensive study of this type was conducted by Witanowski et al. to interpret the solvent effects on the nitrogen shielding in a large set of compounds (see Ref. [21] and references cited therein).

In the last years, these semiclassical analyses have been substituted by (or supported with) explicit descriptions of the electronic aspects of the solvent effects on NMR properties and in particular on the nuclear shielding. This change of perspective has been made possible by the large development of QM solvation models which have been coupled to QM methodologies initially formulated for isolated systems.

The QM theory of chemical shielding was originally developed many years ago [22,23], but only later have ab initio methods and density functional theories (DFT) been reliably used for the prediction of NMR properties of isolated molecular systems, and finally of solvated systems. The latter step has been achieved by extending the gas-phase theoretical methods to continuum solvation models (see Ref. [11] for a sufficiently updated list of papers).

Here we shall focus on a specific NMR property, namely the nuclear shielding tensor, defined as the second derivatives of the energy with respect to the Cartesian components of the magnetic induction $\mathbf{B}$ and of the nuclear magnetic moment $\mathbf{m}_X$ of nucleus $X$. Using a SCF description of the solute wavefunction with molecular orbitals (MO) expressed as a linear combination of atomic orbitals (AO) and treating the magnetic field perturbation in an analogous way to the perturbation produced by changes in the nuclear coordinates, the components of the nuclear magnetic shielding tensor are obtained as

$$\sigma_{ij}^{X} = \sum_{\mu\nu} P_{\mu\nu} \frac{\partial^2 h_{\mu\nu}}{\partial B_i \partial m_{X_j}} + \sum_{\mu\nu} \frac{\partial P_{\mu\nu}}{\partial B_i} \frac{\partial h_{\mu\nu}}{\partial m_{X_j}} \tag{1-10}$$

where $P_{\mu\nu}$ is an element of the density matrix in the AO basis and $h_{\mu\nu}$ is a matrix element of the effective one-electron Hamiltonian. The second term in Eq. (1-10) (the paramagnetic contribution) is determined by solving a set of response equations for the three components of the magnetic induction. In order to ensure origin-independent results for the nuclear magnetic shielding constants, *gauge including atomic orbitals*(GIAOs)[24,25,26,27] are generally used, that is, the AO basis functions depend explicitly on the magnetic induction through

$$\chi_\mu(\mathbf{B}) = \exp\left[(-\mathrm{i}/2)(\mathbf{B} \times \mathbf{R}_\mu) \cdot \mathbf{r}\right] \chi_\mu(0)$$

where $\mathbf{R}_\mu$ is the vector giving the position of the nucleus to which the field-dependent basis function is attached relative to the global gauge origin, and $\chi_\mu(0)$ indicates a conventional AO basis function not depending on $\mathbf{B}$.

Equation (1-10) applies both to the case of a molecule in vacuo and in solution. The (polarizable) environment makes contributions both through the density matrix, which is obtained self-consistently including the perturbation from the surroundings, and through the derivative of the density matrix with respect to the magnetic induction, i.e., both terms in Eq. (1-10) contain the effect of the environment.

The first derivative of the density matrix with respect to the magnetic induction ($\partial P_{\mu\nu}/\partial B_i$) is obtained by solving the coupled-perturbed Hartree–Fock (or Kohn–Sham) equations to which the first derivative of the effective Fock (or Kohn–Sham) operator with respect to the magnetic induction contributes. Due to the use of GIAOs, specific corrections arising from the effective operator $\hat{H}_{\text{env}}$ describing the environment effects will appear. We refer to Ref. [28] for the PCM model and to Ref. [29] for the DPM within either a HF or DFT description of the solute molecule.

### 1.3.2. An Application to Solvated Systems: N Nuclear Shieldings of Diazines

In this section we shall present and compare different computational strategies one can adopt to simulate the effect of the environment on spectroscopic properties of solvated systems. In particular, as a representative example, we shall summarize the results of two studies [30,31] we have published in the last years on the environment effects on the nitrogen nuclear shieldings of a specific class of molecular systems containing $sp^2$-type nitrogens: three diazines, also known as pyridazine (1,2-diazine), pyrimidine (1,3-diazine) and pyrazine (1,4-diazine).

We have selected these specific studies among the many others we have performed in the years on the effects of the solvent on molecular properties (see for example Ref. [32] for a review published in 2002 and Ref. [33,34,35] for a selection of more recent papers) as they allow to present and discuss all the most important aspects which contribute to define the complex phenomenon of solvation.

Important solvent effects have been observed on the nuclear shielding of diazine nitrogens, for which an increase of up to 40–50 ppm has been measured passing from an apolar solvent to water [36]; we note that this is one of the largest solvent-induced shifts so far observed in nitrogen NMR. Such a sensitivity to the polarity and possible H-bonding properties of the solvent can be easily explained by looking at the electronic charge distribution of diazines in which very polarizable (and H-bonding acceptor) sites are available for the solvent to strongly interact with (see also Figure 1-1 in which the electron density surface painted according to the value of the electrostatic potential is reported for the three diazines).

To try to reproduce the solvent effects on nitrogen nuclear shieldings of diazines and understand the physics beyond them we have applied the QM/continuum (PCM) and QM/MM(pol) (DPM) approaches described in the previous sections.

All QM calculations both in vacuo and in the various solvents have been performed on the basis of the density functional theory (DFT) using the hybrid functional which mixes the Lee, Yang and Parr functional for the correlation part and Becke's three-parameter functional for the exchange (B3LYP) [37,38]. Calculations of nuclear shieldings have been performed exploiting the GIAO method. Within the PCM, the cavities of all the diazines have been obtained in terms of interlocking spheres: in particular, a united atom approach in which hydrogen atoms are inside the sphere centered on the linked carbon atom has been used; the corresponding radii are 1.9 Å for CH and 1.6 Å for N. A cavity scaling factor ($f$) is introduced to enlarge

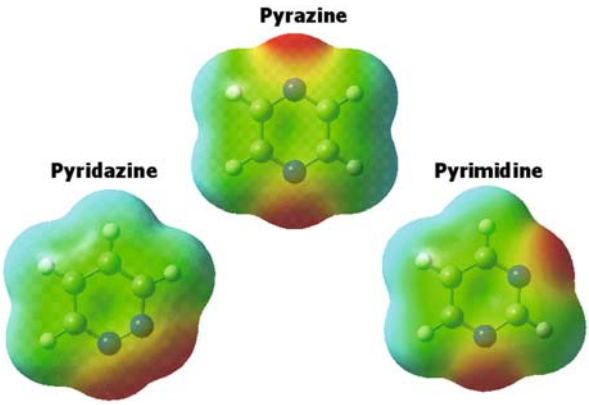


*Figure 1-1.* Graphical representation of the three diazines, pyridazine (1,2-diazine), pyrimidine (1,3-diazine) and pyrazine (1,4-diazine), in terms of their structure and the electron density surface colored according to the value of the electrostatic potential

the basic radii before the individual spheres are defined; when not explicitly indicated the standard value of 1.2 has been used for *f*.

All calculations on the isolated and the PCM solvated systems have been performed using the Gaussian code [39] while the DPM calculations have been performed using the development version of the Dalton Quantum Chemistry Program [40].

### *1.3.2.1. QM/continuum: Polarity Versus H-bond*

A preliminary description of solvent effects on the nitrogen nuclear shielding of diazines can be obtained by applying a continuum-only description and thus assuming that each diazine is contained in a proper cavity inside an infinite polarizable continuum dielectric. As a result, both the geometry and the electronic charge distribution of each diazine will be modified with respect to the gas-phase case, as well as its response equations determining the NMR properties will be changed. These three effects will give rise to the net solvent effect on the property of interest, namely the nitrogen nuclear shielding. These effects are evaluated here by applying the PCM as described above.

In Figure 1-2 we summarize in a graphical way the results obtained in three different environments, namely cyclohexane, acetone, dimethyl-sulfoxide (DMSO), and water. The data are reported here as a correlation plot between calculated and experimental isotropic nuclear shieldings.

From the plot it is evident that for non-protic solvents, PCM gives gas-to-solution shifts in very good agreement with experiments for all molecules (the regression line presents a $R^2$ value of 0.998). In contrast, for water such an agreement significantly worsens: in this case, specific H-bonds between water hydrogens and diazine nitrogens are clearly present while the continuum description cannot properly take into

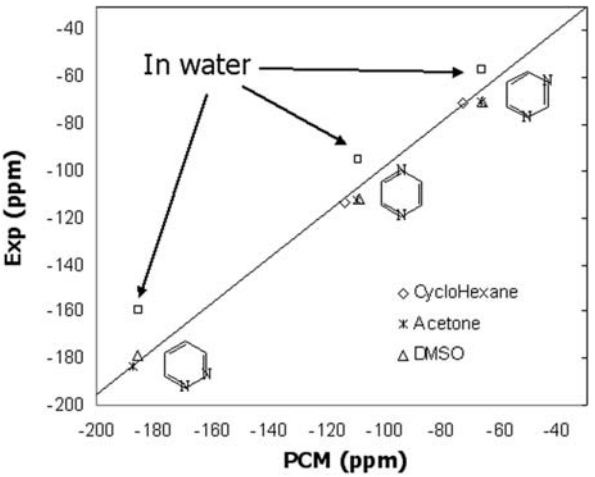


*Figure 1-2.* Correlation plot between calculated and experimental N nuclear shieldings of diazines obtained in three different environments, namely cyclohexane, acetone, DMSO and water. Calculated shieldings have been obtained at B3LYP/GIAO/6-311+G(d,p) while the geometries were optimized at B3LYP/6-31+G(d,p) in all phases

account their possible effects on the property. To test this analysis, we have optimized H-bonded clusters containing one and two water molecules, without and with an external continuum dielectric; in the latter case an enlarged cavity also containing the water molecules has been introduced using radii equal to 1.2 Å for H and 1.53 Å for O (an example of the cluster structure and of the corresponding PCM cavity is shown in Figure 1-3 for pyridazine). On such optimized geometries we have computed nuclear shieldings at the same level used for the single diazines.

In Figure 1-4 we report the same correlation presented in Figure 1-2 but this time limited to water as a solvent: both isolated and "solvated" (i.e., PCM) diazine–2 w clusters are reported.

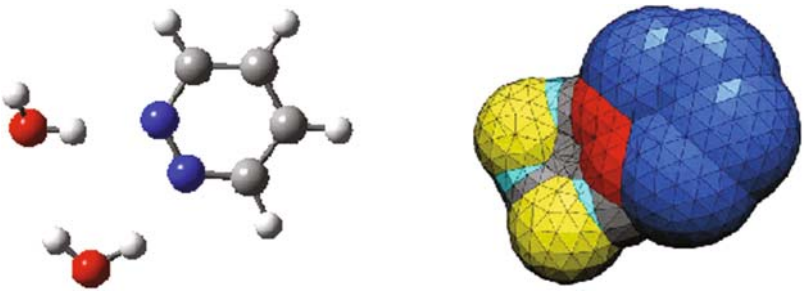

*Figure 1-3.* Example of diazine–water cluster: pyrimidazine+2 w and the corresponding PCM cavity

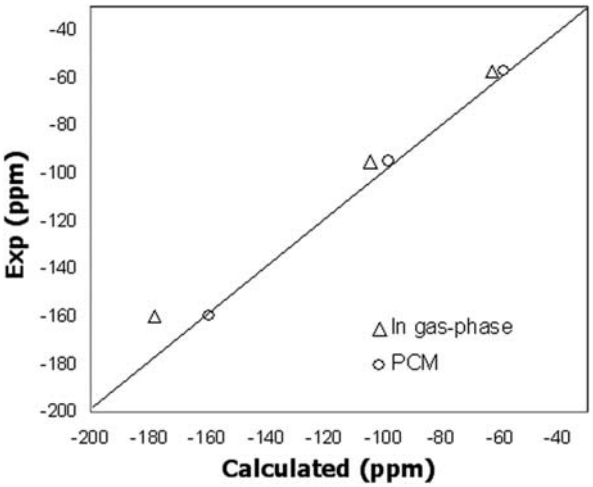


*Figure 1-4.* Correlation plot between calculated and experimental N nuclear shieldings of diazines in water. Calculated shieldings refer to diazine–2 w clusters in gas-phase and within a PCM continuum. All calculations are at B3LYP/GIAO/6-311+G(d,p) level

From the results obtained for the isolated clusters, it seems evident that even by taking into account the two H-bonds, an important portion of the observed shift is still missing. For all molecules, in fact, the isolated "diazine + 2 waters" clusters seem unable to describe the complete effect due to the whole liquid. Only by adding an external continuum, i.e., including also long-range non-specific polarization effects, the missing gap is filled and a very good agreement with experiments is obtained.

The data reported in Figures 1-2 and 1-4 suggest a possible interpretation of the physics beyond the observed solvent effect on nitrogen nuclear shielding but they still prevent a detailed explanation of the competitive/synergic action of short-range and specific interactions on one side, and long-range and mediated effects on the other side. To do that it is useful to recall that the nuclear magnetic shielding can be partitioned into diamagnetic and paramagnetic terms. This partition is here achieved by considering an NBO analysis [41] and relating the changes in the two contributions to increase or depletion of the population in the nitrogen lone pair due to H-bond and polar effects.

In Table 1-1 we thus report the N nuclear shielding (with its paramagnetic and diamagnetic contributions) and nitrogen lone pair natural population (NP(lp)) for the diazine showing the largest solvent effect, i.e., pyridazine, and its clusters in vacuo (VAC) and in the presence of an external PCM continuum.

To first investigate the effects of H-bonding, we compare lone pair occupancies of monomers and clusters in gas phase: a significant decrease is found passing from the "free" pyridazine to either the one-water or the two-water cluster. This decrease, which indicates that electron population has been removed from the lone pair orbital, is accompanied by an increase of the diamagnetic contribution.

*Table 1-1.* Nitrogen lone pair natural population (NP(lp)), nuclear shielding (with its paramagnetic and diamagnetic contributions) (ppm) and absorption energies (eV) for pyridazine and water–pyridazine clusters in vacuo (VAC) and in the presence of an external continuum (PCM). For 2 w clusters two values corresponding to the two nonequivalent nitrogens are reported. Calculations are at B3LYP/6-311++G(2d,2p) level for populations and nuclear shielding and at TDB3LYP/6-31+G(d,p) level for absorption energies

| | VAC | | | | PCM | | | |
|---|---|---|---|---|---|---|---|---|
| | Free | +1 w | +2 w | | Free | +1 w | +2 w | |
| NP(lp) | 1.939 | 1.921 | 1.922 | 1.933 | 1.943 | 1.912 | 1.912 | 1.924 |
| $\sigma^{\text{par}}$ | –513.52 | –513.09 | –496.41 | –504.49 | –482.44 | –487.2 | –474.83 | –475.21 |
| $\sigma^{\text{dia}}$ | 296.58 | 325.07 | 318.31 | 313.2 | 296.9 | 324.8 | 319.33 | 313.95 |
| $\sigma$ | –216.94 | –188.02 | –178.1 | –191.29 | –185.54 | –162.4 | –155.5 | –161.26 |
| $n\pi^*$ | 3.58 | 3.74 | 3.85 | | 3.87 | 4.00 | 4.13 | |

A different analysis involves the comparison between isolated and "solvated" (PCM) systems. For the monomer, the inclusion of a continuum dielectric induces a significant increase in the nitrogen lone pair population, which, however, does not lead to significant changes in the diamagnetic term but instead lead to a less negative $\sigma^{\text{par}}$. A similar change in the paramagnetic contributions is obtained passing from isolated to solvated clusters, but what is different now is that the introduction of the continuum reduces the lone pair population (indicating a stronger charge transfer from nitrogen lone pair to H-bonded waters). This apparently opposite correlation between orbital population and paramagnetic term reveals that a complex combination of different factors is occurring. Going back to the Ramsey formulation we have that the paramagnetic contributions depend on both ground state and excited states while the diamagnetic contributions are determined by the ground state only. Thus, on passing from isolated to solvated clusters, we can assume that the dominant term in determining $\sigma^{\text{par}}$ is not the lone pair population but the low-lying excited states. If we assume that all other electronic transition energies are much larger than $\Delta E(n\pi^*)$ and therefore make a negligible contribution, the observed decrease in the paramagnetic shielding in the presence of the external continuum can be explained in terms of the parallel changes in the $n\pi^*$ transition energy. In particular, an increase in the transition energy should lead to a less negative $\sigma^{\text{par}}$ the latter being inversely proportional to the former according to the Karplus and Pople model [42].

The $n\pi^*$ transition is a well-known example of H-bond-sensitive property. In such transitions, the electronic density on the heteroatom (either oxygen or nitrogen) decreases upon excitation. This results in a decrease in the capability of this heteroatom to form hydrogen bonds. The effect on absorption should then be similar to that resulting from a decrease in dipole moment upon excitation, and a blue shift of the absorption spectrum is expected; the higher the strength of hydrogen bonding, the larger the shift. From the TDB3LYP results reported in the table an increasing blue shift with respect to the gas-phase-free diazines is observed passing from the

isolated to the solvated clusters; this increase is reflected in a significant decrease in the absolute value of $\sigma^{\text{par}}$ as predicted.

To conclude this first part of the analysis, all the results presented show that H-bond effects and long-range non-specific interactions can combine and give rise to a synergic (or cooperative) action and that the complete picture can only be obtained by taking into account both of them introducing solvated clusters.

It is now interesting to check if this picture is either confirmed or modified by changing the description of the solvent. This check is here realized introducing diazine–water clusters extracted from classical MD simulations and comparing their NMR properties calculated once again with PCM or with the QM/MM(pol) model we have introduced in the previous section with the acronym DPM.

#### *1.3.2.2. QM/MM Versus QM/continuum*

In order to generate an appropriate number of solute–solvent clusters to be used in the NMR calculations a series of classical MD simulations of pyrazine, pyrimidine or pyridazine in aqueous solution has been carried out. All the details of the force fields used for the diazines and water as well as computational details of the MD simulation can be found in Ref. [31]. Every 1 ps an MD configuration was dumped so as to obtain 600 different molecular configurations. Then, a spherical cut-off distance equal to 12 Å was applied so as to obtain the final cluster including $\sim$ 230–240 water molecules together with the solute.

In the following electronic calculations, the solute (and potentially a number of the closest water molecules) is treated using DFT/B3LYP while the rest of the solvent is treated either using the same polarizable potential as in the MD simulations (DPM) or introducing an external continuum (PCM). Acronyms like DFT(X)/DPM or DFT(X)/PCM are used here to indicate calculations where X water molecules have been included into the part of the system treated using DFT. The final molecular property in solution is evaluated as a statistical average over all these molecular clusters.

A preliminary necessary check in this kind of calculations is on the convergence of the calculated properties with respect to the number of solute–solvent configurations included in the statistical procedure. Canuto and coworkers have extensively made use of the autocorrelation function of the energy in order to extract uncorrelated solute–solvent configurations to be used in combined QM/MM calculations [43]. The minimum number of solute–solvent configurations to be included in the statistical analysis may, however, depend on the nature of the molecular property in question. The NMR shielding appears to be converged based on around 100 configurations. In this study we have chosen to use 200 configurations in the statistical averaging since this number of configurations clearly provides statistically converged molecular properties. Also, the effect of using a larger number of configurations is to obtain a smaller statistical error in the mean values.

Before moving to the comparison between QM/continuum and QM/MM(pol) results, we briefly comment on differences between the previously QM-optimized clusters and the present MD-derived clusters. The hydrogen coordination number to

the nitrogen site for each solute is almost constant for all the three diazines and is, by spherical integration of the RDFs, found to be around 2: this can be considered as a confirmation of the previous analysis in terms of the diazine–2 w QM clusters. However, in contrast to the QM results, the hydrogen bond distances between the nitrogen site of the solute and the hydrogen of water are all longer in MD-derived configurations. This is not surprising since the outcome from the geometry optimizations are equilibrium structures at $T$=0 K representing the lowest energies on the potential energy surfaces which physically might be different from the true liquid at finite temperatures. These differences can also be due to possible deficiencies in the underlying force field used in the MD simulations to accurately describe the effect of hydrogen bonding. Due to these geometrical differences, the H-bond effects we shall obtain in the nuclear shielding will be different in the two descriptions. One could thus be suggested to use the comparison between the results presented in the previous section, the present ones obtained from MD-derived clusters and experiments to select the best description. Unfortunately, this is not possible as in all calculations we have neglected other aspects, such as rovibrational averaging, which may be important for accurate evaluation of the solvent-induced shifts and thus represent a potential source of intrinsic uncertainty.

On the basis of these considerations, in the following analysis we shall mainly focus on an internal comparison between the continuum (PCM) and the polarizable MM (DPM) descriptions, so as to explore in more detail their similarities and differences when applied to the evaluation of solvent effects on molecular properties.

The comparison begins with the reaction field produced by either models at specific atomic sites. Both models in fact produce at the solute an electric field which perturbs the solute electronic density and thereby change the properties of the solute. In the case of PCM, the reaction field at site $\mathbf{R}_n$ is a true mean field produced by the ASC distribution represented by point charges, i.e.,

$$\mathbf{E}^{\mathrm{RF}}_{\mathrm{PCM}}(\mathbf{R}_n) = \sum_{k=1}^{\mathrm{Nts}} \frac{q_k^{\mathrm{PCM}}(\mathbf{R}_n - \mathbf{r}_k)}{|\mathbf{R}_n - \mathbf{r}_k|^3} \tag{1-11}$$

On the other hand, for the DPM model the reaction field is calculated by the expression

$$\mathbf{E}^{\mathrm{RF}}_{\mathrm{DPM}}(\mathbf{R}_n) = \sum_{s} \frac{q_s^{\mathrm{DPM}}(\mathbf{R}_n - \mathbf{r}_s)}{|\mathbf{R}_n - \mathbf{r}_s|^3} + \sum_{a} \mu_a^{\mathrm{ind}} \mathbf{T}_{na} \tag{1-12}$$

where the first contribution in Eq. (1-12) is due to the point charges representing the permanent charge distribution of the solvent molecules and the second term represents the contribution from the induced dipoles at the polarizable sites in the solvent region. The symbol $\mathbf{T}$ is the dipole interaction tensor. The reaction field in Eq. (1-12) is calculated for each solute–solvent configuration and thus the MM(pol) includes directly the fluctuations in the reaction field and the consequences this might have for the calculated properties.

*Table 1-2.* Nonzero components of the reaction field at the nitrogen nuclei in pyrimidine calculated using either DPM or PCM. All calculations are at B3LYP/6-311++G(2d,2p) level. Results are in $10^3$ au

| Method | $E_y^{\mathrm{RF}}$ | $E_z^{\mathrm{RF}}$ |
|---|---|---|
| DPM | $-8.7 \pm 0.4$ | $7.3 \pm 0.3$ |
| PCM ($f = 1.1$) | –7.6 | 8.6 |
| PCM ($f = 1.2$) | –5.5 | 6.8 |
| PCM ($f = 1.4$) | –2.9 | 4.3 |

In Table 1-2 we report the reaction field at the nitrogen sites of a selected diazine, here pyrimidine, calculated using either DPM or PCM. In the first case the results refer to averaging over 200 solute–solvent configurations; in the second case the reaction field has been calculated for different cavity dimensions obtained using different scaling factors. The molecular coordinate system is defined so that the pyrimidine molecule is confined to the *yz* plane with the internal $C_2$ axis along the *z*-axis (the two nitrogen sites are indistinguishable).

As seen from Table 1-2 the PCM reaction field depends quite drastically on the cavity scaling factor. Choosing this to be equal to 1.4, which in the literature has been recommended in case of less polar solvents [44,45], clearly underestimates the reaction field as compared to the DPM. This is also expected since water is to be considered as a high dielectric. Choosing the cavity scaling factor to be equal to 1.2 (the standard value) improves very much the results but on average a scaling factor of 1.1 gives the best results. We note, however, that it is not possible to obtain a common scaling factor that reproduces all the DPM reaction field components.

These results provide some information between the differences/similarities in the physics within the PCM and DPM models. Since the bare radii of the nitrogen atom is $\sim$1.5 Å the solvent will in case of the PCM be placed either 1.65 ($f = 1.1$) or 1.80 ($f = 1.2$) Å away from the nitrogen site. In contrast, in the MD simulations, and therefore also in the DPM calculations, the solvent is on average placed 1.96 Å from the nitrogen site as inferred from the nitrogen–hydrogen RDF in pyrimidine. Furthermore, the oxygen site of water will be placed on average around 0.96 Å (the OH bond length in water) even further from the nitrogen site. This means that on average the solvent is placed (much) further away from the solute within the DPM as compared to the PCM. Choosing the cavity dimensions so as to reproduce the structural data from the MD simulation would lead to very underestimated results for the solvent shifts in the NMR shielding constants as compared to either the DPM or experimental data. Thereby, the PCM properly works by effectively placing the solvent closer to the solute as compared to MD data.

An underestimation of the reaction field should lead to an underestimation of the solvent shift in the NMR shielding. In fact, the relation between the reaction field and $\sigma^{\mathrm{N}}$ is in this regime linear. Thereby, choosing the cavity scaling factor to be 1.1

*Table 1-3.* Diagonal components, and the corresponding isotropic value, of the nitrogen nuclear shielding tensor in pyrimidine calculated using either DPM or PCM. All calculations are at B3LYP/6-311++G(2d,2p) level. Results are in ppm

| Method | *xx* | *yy* | *zz* | iso |
|---|---|---|---|---|
| PCM ($f$ = 1.4) | 275.3 | –221.8 | –250.6 | –65.7 |
| PCM ($f$ = 1.2) | 275.5 | –216.1 | –239.0 | –59.9 |
| PCM ($f$ = 1.1) | 275.9 | –212.2 | –230.7 | –55.7 |
| DFT(2)/PCM | 274.2 | –211.3 | –235.1 | –57.4 |
| DPM | 273.6 | –211.5 | –231.3 | –56.4 |
| DFT(2)/DPM | 273.2 | –212 | –236.3 | –58.4 |

should result in an improved agreement between the DPM and PCM results. This prediction is confirmed by the data reported in Table 1-3 in which the three components of the NMR shielding tensor and the corresponding isotropic values are reported for the two different solvation models. As for the analysis of the reaction field, in the PCM we have used three different cavity scaling factors ($f$ = 1.1, 1.2 or 1.4).

From the first three entries in Table 1-3 we observe that changing the cavity scaling factor has quite different outcome for the three diagonal components of the NMR shielding tensor. The *xx* component (out of plane) is almost unchanged whereas the magnitudes of the *yy* or *zz* components increases around 10 or 20 ppm, respectively, by changing the cavity scaling factor from 1.1 to 1.4. As already predicted on the bases of the reaction field data in Table 1-2, we find that using $f$ =1.1 gives the best results for the shielding components as compared to DPM. If we also introduce two explicitly treated water molecules (the DFT(2)/PCM entry) the agreement becomes even closer.

Turning to the DPM results we find that introducing two water molecules into the DFT-treated region leads to an average change in each tensor components of around 2 ppm. More evidently than with PCM, the origin of this change is to be found almost entirely due to changes in the *zz* component (along the $C_2$ axis in pyrimidine). Thus within the DPM model a faster convergence is observed with respect to the number of solvent molecules treated using DFT for both the isotropic value and diagonal components of the NMR shielding tensor.

It is evident that for both the isotropic and each tensor component the DFT(2)/MM results are better reproduced by DFT(2)/PCM than DFT/MM. This clearly illustrates that special (QM) treatment of the solvent molecules very close to the solute may be important whereas the bulk solvent is described equally well using either a PCM or a DPM. It is also interesting to observe that a good agreement is found instead between DFT/PCM ($f$ =1.1) and DFT/MM. Such an agreement might be related to the fact that both models introduce "artificial" short-range solute–solvent interactions (one in terms of a smaller cavity and the other in terms of purely classical dipoles) while an important part of these interactions (charge-transfer, dispersion and other QM effects) is not taken into account.

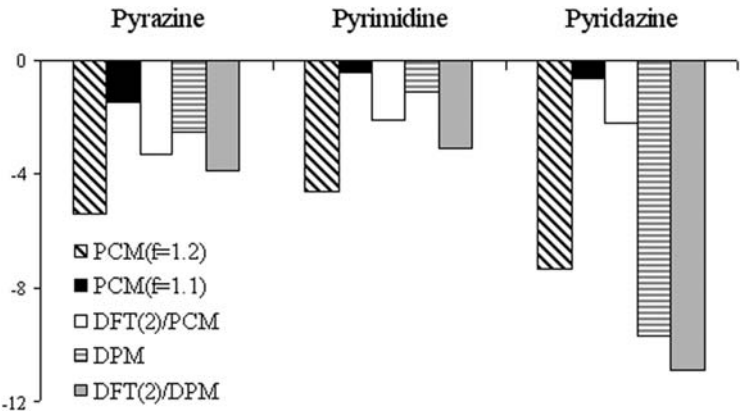


*Figure 1-5.* QM/continuum and QM/MM(pol) errors with respect to experiments for the N nuclear shielding of the three diazines

We now move to consider the comparison between QM/continuum and QM/MM(pol) results. The results are presented in Figure 1-5 for the error with respect to experiments.

As already observed in the previous section, by combining the explicit consideration of the hydrogen bonded water molecules using the DFT(2) approach, in which the two nearest water molecules are included in the system, with an external PCM (the DFT(2)/PCM entry) a very good agreement with experimental data is found. This shows that for chemical shieldings, both specific and bulk effects are important. Moving to the DPM model, the obtained results are of comparable accuracy as the DFT(2)/PCM model. It also has to be noted that small changes are generally observed passing from completely classical water molecules (DPM) to a description in which explicit water molecules are introduced into the region treated using DFT (DFT(2)/DPM).

## 1.4. CONCLUSIONS

We have presented and compared different solvation models (continuum, discrete, continuum + discrete) to study solvent effects on molecular properties. In particular, the nitrogen nuclear shielding, which is known to be very sensitive to even small modifications of electronic and/or nuclear charge distributions, has been analyzed. Such alternation/combination of different models has been required to study the complex nature of solute–solvent interactions when both long-range "polar" and shorter-range specific H-bond effects are active.

The study can be summarized as a two-step procedure. The first step is an analysis of the solvent-induced modifications on the property of interest, when obtained through a solvation continuum model. At this level it is fundamental that the continuum model is as accurate as possible: in our case this is realized through the PCM approach. On the basis of the results obtained in this continuum framework

(and, in particular, of possible failures), the following step is defined so as to include all those aspects of the solvation phenomenon which are missing (or are only partially accounted for). In the numerical practice, this means to introduce different approaches and to combine them in order to get an accurate evaluation of the solute response properties and of the way these are modified by the environment.

A single protocol cannot be found. However, some general rules seem to come out: (1) solute–solvent systems not showing strong specific interactions can be reliably described introducing QM/continuum approaches; (2) strongly interacting (and especially H-bonding) solute–solvent systems require a combination of different solvation approaches which has to be chosen in relation to the nature of the interactions on one hand, and to the type of analysis to be done on the other hand; (3) if this analysis is focussed on molecular response properties, then the solute system has to be described at an accurate electronic level, otherwise a complete confidence in the results cannot be obtained; (4) the solvent (or a part of it) can be treated at a lower level but only if all the interactions have been included in the model in a balanced way and including polarization effects; (5) H-bonding effects, to be correctly described, need the inclusion of the long-range interactions due to solvent molecules far beyond the first solvation shells (i.e., the bulk).

An approach which satisfies all these rules is a statistical analysis based on QM/MM(pol) calculations on large clusters obtained through MD simulations. An evident disadvantage is, however, present: the method is quite computationally expensive requiring first a MD simulation and successively many QM calculations on different configurations so as to obtain a statistically reliable result. Here, we have shown that two simplifications are possible.

The first simplification can be introduced any time we have strongly interacting solute–solvents systems (like diazines in water); in these cases in fact, an alternative and still accurate description can be obtained with a single set of small clusters (namely the solute plus the few solvent molecules forming the first solvation shell) obtained in terms of QM geometry optimizations. The second simplification, by contrast, is valid in all cases (i.e., for strongly or weakly interacting systems) and it implies to simulate the bulk effect through a polarizable continuum model. In such a way, the long-range effects of the solvent will be taken into account in an automatically averaged way using a single calculation.

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