REVIEW

by Prof. Dr. Bogdan Ranguelov, Institute of Physical Chemistry - BAS

on

PhD DISSERTATION

submitted for the award of the PhD degree in doctoral programme "Theoretical Chemistry/Computational Chemistry" (Chemical sciences),

Title: **MOLECULAR MODELLING OF PHASE TRANSITIONS AT THE WATER-ALKANE INTERFACE: ROLE OF SURFACTANTS AND THE CURVATURE**

PhD candidate: **Stoyan Iliyanov Iliev**, full-time PhD student at the Department of Physical Chemistry, Supervisors: Prof. Dr. Anela Ivanova and Acad. Prof. DSc Nikolay Denkov

I. Proceedings materials:

Stoyan Iliyanov Iliev was enrolled as a full-time PhD student in the professional field 4.2. Chemical Sciences, PhD program "Theoretical Chemistry/Computational Chemistry" at the Department of Physical Chemistry from 10.07.2020 by order RD 20 - 934 of the Rector of Sofia University. He was dismissed with the right to PhD defence as of 10.07.2023, by order of the Rector of Sofia University RD 20 -1285. The PhD candidate has passed all the examinations according to the individual study plan, and the materials submitted in the procedure for the award of the PhD degree meet the requirements of the Law on PhD and the regulations for its application (diplomas, abstract, dissertation, certificate of passed examinations, references and used publications are attached). The report on the fulfilment of the minimal national requirements under Article 2b of the Law on Academic Staff Development in the Republic of Bulgaria for scientific area 4.2. ‚Chemical sciences', shows that the applicant's assets significantly exceed the minimum national requirements for indicator groups D, E and G. It should also be noted that there is an apparent unintentional technical (but still annoying) inaccuracy in the completion of the reference for indicator A, where for Indicator 1: Dissertation for the award of the degree of PhD, the dissertation submitted for defence is listed under the topic 'Porous metals obtained by selective dissolution of alloys - suitable electrode materials in ion batteries', which is linked to past defence in 2023, related to another PhD student, namely Evelina Yordanova Vassileva in the PhD programme "Solid State Chemistry". The rest part of the reference is filled in correctly. A corrected reference was received on 25 October 2024.

II. Education and career development:

Stoyan Iliyanov Iliev received his secondary education at the First Language High School, Varna, Bachelor in Chemistry at the Faculty of Chemistry and Pharmacy of Sofia University, Master in Computational Chemistry at the same Faculty of Sofia University and from 2020 to 2023 is a full-time PhD student at the Department of Physical Chemistry. He speaks English and German. He is a member of the Faculty Student Council from 07/2020 to 07/2023. As a lecturer he has taught Physical Chemistry of Pharmacy in the winter semester of the academic year 2021/2022 and has taught an elective course Molecular Modeling of Pharmacophores in the winter and summer semesters for the period 2020-2024. He has participated as a co-author in nine scientific publications in the six-year period from 2018 to

2024, which is an extremely good indicator for a young scientist, and all these nine publications are refereed in international databases. He has participated in seven scientific forums as a presenting author mainly with oral presentations and participated in eleven scientific forums as a co-author in oral and poster presentations. Stoyan Iliev is a member of the scientific team of one national research project under the VIHREN programme.

III. Topic Relevance:

The dissertation of Stoyan Iliev is dedicated to the study of hexadecane as a model system at the molecular level, as the topic is interesting and topical, especially in the fields of materials science, nanotechnology and molecular dynamics. Hexadecane, as a long-chain hydrocarbon, is often used as a model for understanding the behavior of alkanes and their interactions with various surfaces and media. Research on hexadecane allows a deeper understanding of molecular interactions, which is particularly important in the design of lubricants, polymers, and in the study of the molecular behavior of liquids at phase boundaries. In particular, the goal of this dissertation is to gain a deeper understanding of the behavior of hexadecane after freezing. I believe that the topic is of sufficient relevance and interest, which is confirmed by the citations obtained on the publications included in the dissertation with quartile Q1. A total of 6 independent citations were noted on the first one of the three papers.

IV. Dissertation-related scientific metrics:

The results included in the dissertation have been published in three articles in journals (Web of Science and Scopus) with quartile Q1, the candidate is the first author in two of the articles and the second author in the third. The journals are respectively Journal of colloid and interface science, Molecules, Colloids and Surfaces A: Physicochemical and Engineering Aspects, undoubtedly prestigious international journals in the field. There are 6 (six) citations listed on these publications, which for papers published in 2023 and 2024 is indicative of the relevance of the thesis topic.

V. Structure and content of the dissertation:

The dissertation follows the generally accepted rules of structure and it is composed of sections (chapters): introduction, review, computational methods, results and discussion, followed by conclusion, contributions and bibliography consisting of 72 titles. The dissertation contains 114 pages, 62 figures, 49 equations and 17 tables.

In the introduction, the author focuses on the possibilities of controlling the properties of alkane-containing systems and the need to elucidate the thermodynamic and structural characteristics of their supramolecular formulations and the possible phase transitions between them. The peculiarity of alkanes is discussed - their ability to form transitional phases between isotropic liquid and crystalline phases, where the molecules are periodically arranged in the three-dimensional space, but also have a rotational degree of freedom along the carbon chain length, the so-called "rotatory phases", which can be stable or relatively short-lived and are realized under conditions strongly dependent on the chain length and parity of the hydrocarbon. The question (central to the PhD thesis) of investigating and understanding how this type of phase transition forms and how it affects the properties of the system is posed. A hexadecane model system was selected and four tasks were formulated: i) Develop a computational protocol for molecular modeling of the phase transition from isotropic liquid to solid state in hexadecane-containing systems that can be applied to other alkanes. (ii) Elucidation of the mechanism of the observed liquid-to-solid transition by analysis of thermodynamic and structural characteristics of the systems. iii) Developing a methodology to analyse the structure of the resulting solid phases. iv) Determination of the type of solid phases (crystalline or rotatory).

The chapter Review begins with experimental studies of phase transitions in alkane-containing systems and, in particular, with the structure and properties of n-alkanes in the liquid phase. Results from studies of the diffusion coefficient and density of hexadecane at different temperatures and pressures are discussed, as well as with respect to the mutual orientation of hydrocarbons in the liquid phase before a phase transition takes place. Results are shown confirming the property in normal alkanes - a characteristic supercooling effect, which is more pronounced in odd representatives of the homologous order, i.e. with chain extension the supercooling effect weakens. The important part of this chapter starts with the subchapter "Nucleation and crystal growth", in which the review by Mura & Ding "Nucleation of melt: From fundamentals to dispersed systems" published in Advances in Colloid and Interface Science, 2021 plays a central role. The classical theory of nucleation has been used to describe phase transitions in alkanes, as the author rightly notes, the "second" available theory is also mentioned without indicating its (generally) accepted name of so called "two-step" nucleation theory. For the completeness of this literature review, I would have liked to see in the dissertation a little more description of the two-stage theory of nucleation, or at least a citation to some of the seminal works of Peter Vekilov with co-authors Dimo Kashchiev and Oleg Galkin. The review of Mura & Ding, 2021 is used by the author as a basis for the review devoted to homogeneous and heterogeneous nucleation. I cannot spare my critical remarks in this chapter with regard to the use (or translation) of commonly accepted terms in the field: the use of the word "superoversaturation" is incorrect, the correct term being just "supersaturation"; I believe that the expression "decay" with regard to the nucleus is more properly replaced by "decomposition", notwithstanding the proximity of the two words; I strongly disapprove of the use of the term "substrate" with regard to item heterogeneous nucleation. The so-called rotatory phases in alkane systems, an intermediate phase between the isotropic disordered liquid and the ordered crystalline phase, which is characterized by long-range order along all three directions in space, but unlike the crystalline phases in rotatory phases, the molecules have a rotational degree of freedom along the chain, are discussed in detail. The differences between all rotatory phases known to date with respect to four structural parameters are shown and discussed, viz: order parameter, which determines the degree of deformation of the hexagonal lattice; azimuthal angle, which indicates the direction of offset of the nearest neighbors from the long semi-axis of the ellipse; the tilt angle of the molecules with respect to the normal to the layer in which they are located; and the angle, which is related to the tilt direction of the molecules. A method for determining the temperature intervals of existence of rotatory phases in bulk crystallization of alkanes using differential scanning calorimetry with an adiabatic scanning technique described by Thoen & Seynhaeve (Journal of Molecular Crystals and Liquid Crystals, 1985) is discussed, too. The studies of Sirota and Singer are discussed (E. B. Sirota, D. M. Singer, "Phase transitions among the rotator phases of the normal alkanes" J. Chem. Phys. 101 (1994) 10873) with respect to establishing the possible genus of transitions between the various rotator phases. The transitions from R_{II} to R_I, from R_I to R_V, from R_{IV} to R_{III}, and from R_{II} to R_{IV} are discussed and described. The literature review also includes a description of crystal phases in alkane systems - structures with triclinic, monoclinic, orthorhombic or hexagonal symmetry. The question of elucidating at the molecular level the mechanism of the transition from liquid to rotatory phase and also the transitions between the different rotatory phases is raised. The most important theoretical studies of n-alkanes are also presented, focusing on alkanes with chain lengths between 14 and 24 C (carbon) atoms. Studies on the diffusion and heat capacity of systems of normal alkanes and their mixtures with molecular dynamics (MD) simulations in an NPT ensemble with a COMPASS force field with an Andersen thermostat and a Berendsen barostat are described in detail. The results of MD simulations in an ensemble maintaining constant particle number, stress tensor and temperature (N-sigma-T) using a Flexible Williams force field are shown. A detailed report of results on both pure alkanes and mixed alkanes is presented, describing procedures for predicting the solid-liquid transition temperature from the self-diffusion coefficient and heat capacity, and also from the change in the radial distribution function profile of the distances between carbon atoms in the system. A comparison is made of the accuracy of seven different force fields for describing phase transitions in normal alkanes, as well as the ability to predict melting temperatures, densities, and self-diffusion coefficients.

The following Chapter 3 describes the basic principles of the classical molecular dynamics method, which was used to carry out the research in this thesis, as well as some key approaches for the structural analysis subsequently carried out. Both the molecular dynamics method and the integration of the equations of motion are satisfactorily described. The problem of molecular mechanical force fields used in MD simulations is correctly introduced and described, which differ both in the type and complexity of the functions involved and in their range of applicability. The force field chosen for the simulations presented in this thesis is CHARMM36. This force field was chosen after performing test calculations with a set of force fields - the results of these detailed calculations are described in Chapter 4.8 of this dissertation. In the validated CHARMM36 force field the couplings, valence and dihedral angles are fitted to quantum chemical and experimental data. The dispersion parameters are optimized to be able to reproduce a large set of experimentally measured quantities: density, heat of vaporization of liquid, crystal lattice parameters, heat of sublimation and free energy of dissolution. The partial charges of the atoms are derived from the ab initio interaction energies of model molecules with water. This force field is able to reliably melt an ordered alkane-containing system and, upon subsequent cooling, it undergoes a liquid-to-solid transition. Procedures for efficiently calculating potential interactions are also described, as well as algorithms for keeping temperature and pressure constant. Some of the systems investigated in this dissertation involve a phase interface between surfactant compounds and aqueous media. The water simulated in the systems presented in this dissertation is described by the TIP4P model, as this force field most accurately reproduces the thermodynamic characteristics of bulk water. In addition, to achieve efficiency during the simulations, the author applies holonomic constraints on a fraction of the atoms in the system, i.e. constraints that fix the distance between two atoms at a certain length (this is the equilibrium bond length in the force field) thus the oscillation frequency of this type of bond can be neglected. This in turn allows the choice of a larger time step for the integration of the equations of motion. Such holonomic constraints are most often applied to the hydrogen-containing bonds in the system, since they have the highest oscillation frequency.

Chapters 4.3 to 4.7 on model systems also include a set of structural analyses, most of which were developed as part of the thesis.

Chapter 4 "Results and Discussion" forms the core of the thesis. The model building and simulation protocol is presented, and the developed methodology for structural analysis and phase state determination of the model systems is outlined and validated. Conclusions are drawn about the initial preferred phase of hexadecane-containing systems after cooling to freezing. The probable phase transitions for this type of systems from isotropic liquid to triclinic crystal are also presented.

The models investigated in this thesis are composed of bulk hexadecane (denoted bulk HEX in this thesis) or hexadecane on a flat water surface stabilized by two layers of surfactants (denoted HEX/Surf/water). The two types of model systems were investigated in order to characterize the phase transitions occurring in them upon cooling from the liquid state. Four atomistic force fields, CHARMM36, Lipid17, AMBER99 and OPLS-AA, in combination with the TIP4P water model are tested and it is shown that only the CHARMM36 force field fulfils the necessary requirements, viz: (i) a phase transition from a regular lattice to an isotropic liquid must be observed after heating the system to 350 K; (ii) once the systems are in the isotropic liquid state, their bulk density must reproduce or be close to the experimental one; (iii) a freezing process must take place in the systems after cooling them to different temperatures between 273 K and 300 K.

After the model systems were cooled to a temperature at which a phase transition from isotropic liquid to ordered state (278 K) takes place, some key thermodynamic characteristics (enthalpy and density profiles) were investigated and a large set of structural analyses were performed in order to assess the degree of ordering in the systems and to unambiguously determine the type of ordered phase obtained. All simulations were conducted with the GROMACS 2020 software package. The program package VMD 1.9.4 was used to visualize the trajectories.

Methods for the (structural) analysis of the phase state of polycrystallite structures of model phases composed of quasilinear molecules are described in detail, as well as methods for the identification and separation of the various crystallites in the studied systems using the determination of the crystallite plane by conducting principal component analysis and subsequent determination of the inclination of the molecules with respect to this plane. A procedure was carried out to "prepare" the crystallites for 2D Voronoi analysis and, accordingly, to obtain the value of the average area of the molecules. Calculations were performed to determine the orientation of the molecules relative to each other using the moment of inertia tensor for each molecule of the crystallite. With the method used, it is also possible to identify transitions occurring during the simulation, both from liquid to solid state and between different solid state arrangements. An analysis of the physicochemical properties - temperature, enthalpy and density - for the liquid-to-solid phase transition has been carried out, and shown to be consistent with the expectation that the transition would be of the first order - an abrupt change in the enthalpy and density of the system. It is desirable that this part of the thesis has a more in-depth commentary on the observed "two-step" jump in the enthalpy and density change of the system under study - see Figure 30 on page 67 of the thesis.

The structural analysis starts with the determination of the moment at which the freezing of the system begins, and therefore the formation of the crystal nucleus. Both model systems (bulk HEX and HEX/Surf/water) show a polycrystalline structure at the end of the phase transition, with a small fraction of molecules in both systems remaining unordered and trapped between the ordered layers for the duration of the simulations. The radial distribution functions (normalized to the number of molecules and box volume) were calculated and compared with those for reference crystals in the triclinic phase and in the orthorhombic rotatory phase of a bulk hexadecane. The data for the reference crystals were obtained from separate MD simulations of the two regular structures that are known for hexadecane, an ideal triclinic crystal and an R_I rotatory phase that is characterized by a wall-centered orthorhombic lattice. Comparison of the bulk HEX and HEX/Surf/water crystallites with the reference crystallites shows that the MD-generated crystallites are in the $R_I \rightarrow T$ transition. The structural analysis continues with the local arrangement of the alkyl chains in the crystallites, where the angle of inclination of the molecules relative to the plane of the crystallite is examined - this is an important structural parameter to determine whether a crystallite is in a rotatory phase. The evolution of the tilt angle, averaged over all molecules, with respect to the plane of each crystallite as a function of time is shown. The degree of ordering of the crystallites is reflected very sensitively in the standard deviations of this quantity, justifying its use as a quantitative measure of the degree of ordering in the systems studied. The structural analysis continues with an examination of the average distance between first neighbors (Voronoi analysis) and rotational order analysis. The effect of cooling rate on the formation and type of the resulting ordered phase is also investigated. The type of solid phases is identified and possible sequences of interphase transitions in hexadecane crystallization are proposed.

The last subchapter in the thesis is devoted to the study of the effect of curvature on the freezing process of hexadecane. In order to investigate the effect of curvature on the freezing process of hexadecane, a system of hexadecane, surfactant (cetyl ethoxylate) and water was constructed. The organic phase of the system is organized into a spherical droplet with a diameter of 15 nm, where the core is composed of hexadecane and the surfactant is located at the periphery of the droplet. Again, CHARMM36 force field was used to describe the organic fraction and TIP4P was used for water. Two starting systems (final structures) were obtained after heating to 350 K and after cooling to 300 K, respectively. The final structures thus obtained at these two temperatures were used as the starting ones during cooling to 278 K for a simulation time of 500 ns. The main difference between the two initial configurations (from 350 K and from 300 K) is the phase in which the surfactant molecules are located. At 350 K the whole system is in the liquid state, whereas at 300 K the surfactant (cetyl ethoxylate) is already frozen. Also, at 350 K the droplet remains spherical, whereas at 300 K a change in shape has begun, which is characteristic of the formation of rotatory phases.

VI. Questions, recommendations and comments:

The remarks can be divided into two: one concerns only formal things related to formatting and structuring of the dissertation, where a little more attention to detail was needed in terms of how the final, completed research product looks; the second type of remarks can be attributed to the way the dissertation is "narrated/written" and the style of the dissertation, but this is a process that the PhD student, I am convinced, will be able to improve in future work. I hope the author will continue his research and teaching activities in the department in the future.

VII. Abstract evaluation:

As stated in the abstract itself "The numbering of figures and tables in the abstract corresponds to that in the thesis". This is the case, but there is a clerical error in the numbering of two of the (sub)chapters in the abstract. Sub-chapter 4.11. "Structural analysis of solid phases" and subchapter 4.12. "Determination of the type of solid phases obtained at different cooling rates" contain subheadings 3.11.1-4 and 3.12.1-6, while it should be 4.11.1-4 and 4.12.1-6. This, of course, in no way affects the quality of the abstract. The abstract has been prepared as required and reflects all the essential results of the PhD thesis.

VIII. Conclusion:

Taking into account the level and volume of the conducted scientific research, the quality of the dissertation work and the contributions of the PhD student, I confidently give my **positive** assessment and propose to the Honorable Scientific Jury to award the educational and scientific degree **"PhD"** to **Stoyan Iliyanov Iliev** in Professional Field 4.2 "Chemical Sciences" (Theoretical Chemistry / Computational Chemistry).

Reviewer:

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18/11/2024