Molecular Insight into Ion-Specific Interactions

Vibrational sum frequency study of the carboxylic acid moiety

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Molecular insight into ion specific interaction – Vibrational sum frequency study of the carboxylic acid moiety
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« Au milieu de l’hiver, j’apprenais enfin qu’il y avait en moi un été invincible »

Albert Camus
Abstract

Ion specific effects at charged interfaces find numerous applications in colloidal sciences and play a vital role in many biological processes. Despite having been studied for over a century, starting with the work of F. Hofmeister in the 1880s, a comprehensive molecular understanding remains elusive. It is currently believed that specific molecular interactions between the ions and the various chemical functional groups, including the disruption of the interfacial water structure, are the key underlying steps. The research presented in this doctoral thesis focuses on the carboxylic acid moiety which is one of the chemical functionalities most frequently encountered at biological interfaces. Vibrational sum frequency spectroscopy (VSFS), a non-linear optical technique with an exquisite surface specificity that probes molecules with a preferred orientation at the interface, was used to investigate the molecular interactions between the carboxylic acid moiety of a fatty acid Langmuir monolayer with monovalent (Li⁺, Na⁺, K⁺, Cs⁺), divalent (Ca²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Co²⁺), and trivalent (Y³⁺, La³⁺) cations. The studies also focused on understanding the remarkable effects that some selected negatively charged co-ions (Cl⁻, Br⁻, I⁻, SCN⁻) can have in the cation – carboxylate interactions. Particular attention was drawn to the special role of the hydronium (H₃O⁺) counter-ion, including the identification of resolved spectral features linked to a specific structural configuration adopted at the negatively charged interface. VSFS allowed, among others, to quantify the surface charge, determine the type of cation binding with the carboxylate headgroup, and monitor structural changes in the interfacial water molecules depending on the identity of the ion, concentration, and solution pH. Overall, the results shine new light onto the fundamental understanding of the electrical double layer (EDL) at charged interfaces.

In accordance to the predictions of the classical Poisson Boltzmann (PB) theory, it was found that at sufficiently low monovalent salt concentrations (< 50 mM), the charging behaviour of the fatty acid monolayer was independent of the cation or anion identity. However, by further increasing the amount of salt in solution, it was demonstrated that the ion-specific size of the hydrated cation played a determining factor in the properties of the EDL, marking the departure from the PB formalism. Specific interactions between the alkali metal cations and the charged and uncharged forms of the carboxylic acid, lead to additional ion-specific effects: Li⁺ ions interacted more favourably with the charged carboxylate group, while larger and more polarizable (softer) cations such as Cs⁺ showed relatively stronger adsorption to the uncharged moiety. This effect produced a reversal of the cation affinities depending on the degree of deprotonation of the monolayer.
In contrast, divalent and trivalent cations were shown to experience much stronger ion-specific interactions with the carboxylic acid moiety, which could be observed from sub-micromolar concentrations, and varied depending on the ion charge and intrinsic electronic properties. In particular, the trivalent cations examined tended to associate through molecular coordination interactions. The different types of binding could be distinguished by examining the vibrational signatures of the carboxylate headgroup: from electrostatically driven (solvent-shared or contact ion pairing) to more covalent binding complexes, varying with the nature of the ion and experimental conditions (pH, concentration). The binding of trivalent ions led to a reversion of the surface charge, from negative to positive, which in turn caused the adsorption of the OH– co-ion. One implication is that the electric field associated with the charged surface influences the ion speciation in the interfacial region.

An unexpected finding was the striking effect that the nature of the negatively charged co-ions (i.e. Cl–, Br–, I–, SCN–) could have on the interactions of the sodium cations with the carboxylic acid monolayer at molar concentrations. In the presence of the softer I– and SCN– co-ions, the monolayer was substantially more deprotonated when compared to NaCl. The results indicate that the physical-chemical properties of the molecular layers in close proximity to the interface reflects a subtle balance of molecular and electrostatic competitive interactions which involves both the counter- and co-ions.

Finally, throughout this thesis, an effort is made to highlight the importance of the ever-present hydronium cation, which competes with all the other cations examined to pair with the carboxylic acid moiety. In this regard, one of the key novelties of this research, was the identification of resolved spectral features linked to a specific configuration adopted by the hydrated proton at the charged surface. This finding offers a new molecular perspective for tracking and understanding the behaviour of hydrated protons at interfaces.

**Keywords:** specific ion effects, carboxylic acid, fatty acid Langmuir monolayer, vibrational sum frequency spectroscopy, VSFS, electrical double layer.
Sammanfattning

Det finns många tillämpningar för specifika joneffekter vid laddade gränsytor inom kolloidala vetenskaper och dessa effekter spelar en viktig roll i många biologiska processer. Trots att man har studerat detta i över ett sekel, med början med F. Hofmeisters arbete på 1880-talet, är en fullständig molekylär förståelse svår att nå. Det anses för närvarande att de viktigaste underliggande stegen är de specifika molekylära interaktionerna mellan jonerna och de olika kemiska funktionella grupperna och även förändringar av vattensstrukturen vid gränsytan. Forskningen som presenteras i denna doktorsavhandling fokuserar på karboxylsyradelen, som är en av de kemiska funktionaliteter som oftast uppträder vid biologiska gränsytor. Vibrationsumfrekvensspektroskopi (VSFS) är en icke-linjär optisk teknik med en extrem ytspecificitet och detekterar molekyler med en föredragen orientering vid gränsytan. Tekniken användes här för att undersöka molekylära interaktioner mellan karboxylsyragruppen i ett Langmuir-monoskikt av en fettsyra med envärda (Li⁺, Na⁺, K⁺, Cs⁺), tvåvärda (Ca²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Co²⁺) och trevärda (Y³⁺, La³⁺) katjoner. Studierna fokuserade också på att förstå de anmärkningsvärda effekterna som vissa utvalda negativt laddade medjoner (Cl⁻, Br⁻, I⁻, SCN⁻) kan ha i interaktionerna mellan katjoner och karboxylater. Särskild uppmärksamhet riktades mot den speciella hydroniummotjonen (H₃O⁺) och identifieringen av upplösta spektrala band som är kopplade till en specifik strukturell konfiguration som obervationerades vid den negativt laddade gränsytan. VSFS möjliggjorde bland annat en kvantifiering av ytladdningen, en bestämning av typen av katjonbindning med karboxylathuvudgruppen och VSFS kunde även användas för att observera strukturella förändringar av vattenmolekylerna på gränsytan, beroende på identiteten av jonen, koncentrationen och pH i lösningen. Sammantaget sprider resulterna nytt ljus över den grundläggande förståelsen av det elektriska dubbellagret (EDL) vid laddade gränsytor.

I överensstämmelse med den klassiska Poisson-Boltzmann (PB) -teorin konstaterades det att vid tillräckligt låga saltkoncentrationer (<50 mM) av envärda joner var laddningsbeteendet för fettsyramonolagret oberoende av katjonens eller anjonens identitet. Genom att ytterligare öka mängden salt i lösningen visade det sig emellertid att den jonspecifika storleken av den hydruiserade katjonen spelade en avgörande roll för egenskaperna av EDL, vilket innebar en avvikelse från PB-formalismen. Specifika interaktioner mellan alkalimetalldjonerna och de laddade eller oladdade formerna av karboxylsyran ledde till ytterligare jonspecifika effekter: Li⁺ interagerade mer gynnsamt med den laddade karboxylatgruppen, medan större och mer polariserbara (mjukare) katjoner såsom Cs⁺ uppvisade relativt starkare adsorption till den
oladdade karboxylsyradelen. Denna effekt resulterade i en omvändning av katjonaffiniteterna beroende på graden av deprotonering av monoskiktet.

Däremot visade det sig att tvåvärda och trevärda katjoner skapade avsevärt starkare jonspecifika interaktioner med karboxylsyragruppen, vilket kunde observeras vid sub- mikromolär koncentrationer och varierade beroende på jonladdningen och de elektroniska egenskaperna. I synnerhet tenderade de trevärda katjonerna som undersöktes att associeras genom molekylära koordinationsinteraktioner. De olika bindningstyperna kunde särskiljas genom att undersöka vibrationsstrukturen för karboxylat-huvudgruppen: från elektrostatiskt driven (joner separerade av lösningsmedel eller i direkt kontakt) till mer kovalenta bindningskomplex, där bindningstypen varierademed jonens natur och experimentella förhållanden (pH, koncentration). Bindningen av trevärda joner ledde till en omvändning av ytladdningen, från negativ till positiv, vilket i sin tur resulterade i adsorption av medjonen OH⁻. En följd av detta är att det elektriska fältet som är associerat med den laddade ytan påverkar vilka joner som finns på gränsytan.

En oväntad observation var den påtagliga effekten som de negativt laddade medjonerna (d.v.s. Cl⁻, Br⁻, I⁻, SCN⁻) kan ha på interaktionerna mellan natriumkatjonerna och monoskiktet av karboxylsyran vid molkoncentrationer. I närvaro av de mjukare medjonerna I⁻ och SCN⁻ deprotonerades monoskiktet väsentligt mer än i närvaron av NaCl. Resultaten visar att de fysikalisk-kemiska egenskaperna hos molekylskikten i närheten av gränsytan återspeglar en fin balans mellan molekylära och elektrostatiska konkurrerande interaktioner som involverar både med- och motjoner.

Slutligen har det genom hela denna avhandling strävats efter att belysa vikten av den ständig närvarande hydroniumkatjonen, som tävlar med alla andra katjoner om att paras ihop med karboxylsyradelen. Relaterat till detta var ett av det viktigaste nya resultatet i denna forskning identifieringen av upplösta spektralstrukturer kopplade till en specifik konfiguration av den hydratiserade protonen vid den laddade ytan. Denna observation bidrar med ett nytt perspektiv för att detektera och förstå beteendet hos hydratiserade protoner vid gränsytor.

**Nyckelord:** specifika joneffekter, karboxylsyra, fettsyra, Langmuir-monoskikt av fettsyror , Vibrationssumfrekvenspektroskopi, VSFS, elektriska dubbellagret.
List of articles

The doctoral thesis is based on the following articles:

Article I. Molecular insight into carboxylic acid - alkali metal cations interactions: reversed affinities and ion-pair formation revealed by non-linear optics and simulations
Adrien P. A. Sthoer, Jana Hladílková, Mikael Lund, Eric C. Tyrode

Article II. Interaction of Na⁺ cations with a highly charged fatty acid Langmuir monolayer: molecular description of the phase transition
Adrien P. A. Sthoer, Eric C. Tyrode
J. Phys. Chem. C, 2019, DOI: 10.1021/acs.jpcc.9b06435

Article III. Anion specific effects at negatively charged interfaces: the influence of Cl⁻, Br⁻, I⁻ and SCN⁻ on the interactions of Na⁺ with the carboxylic acid moiety
Adrien P. A. Sthoer, Eric C. Tyrode
Manuscript

Article IV. La³⁺ and Y³⁺ interactions with the carboxylic acid moiety at the liquid/vapor interface: identification of binding complexes, charge reversal, and detection limits
Adrien P. A. Sthoer, Helen M. Adams, Sanghamitra Sengupta, Robert W. Corkery, Heather C. Allen, Eric C. Tyrode
Manuscript

Article V. Vibrational spectral signature of Eigen-like hydrated protons at negatively charged surfaces
Eric C. Tyrode, Sanghamitra Sengupta, Adrien P. A. Sthoer
Manuscript
The author’s contribution to the appended papers:

**Article I.** Part of planning. Performed all the VSFS experiments. Major contributions in the data evaluation and interpretation excluding the MD simulations. Part of writing.

**Article II.** Part of planning. Performed all the experimental work. Major contributions in the data evaluation calculation, and writing.

**Article III.** Part of planning. Performed all the experimental work. Major contributions in the data evaluation calculation, and writing.

**Article IV.** Major part of planning. Shared contribution in the experimental work. Major part of data evaluation and writing.

**Article V.** Part of planning. Shared experimental work and evaluation. Minor part of writing.

Research project linked to the doctoral thesis:

**Interactions of divalent cations with the carboxylate moiety: a vibrational sum frequency study**, Mikael Scott, *MSc thesis*, KTH Royal Institute of Technology, Stockholm, Sweden, 2018. *(Available online)*

Designed the research project, supervision and review.
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A. Introduction

Water is considered as the source of life on Earth. To be precise, one should rather mention aqueous electrolytes, as no organism would survive without its little grain of salt. In a larger aspect, an aqueous electrolyte refers to minerals, acids, proteins and any molecule that dissociates into a positively (cation) and a negatively (anion) charged particle when in contact with water, giving the ability to conduct electrical information. Electrolytes are encountered everywhere, as for example, the concentration of electrolytes in the human body is ~150 mM, and four times higher in sea water. Ion-specific interactions define ion behaviours beyond the limitation of classical electrolyte theories, when direct molecular interactions equal or dominate a purely electrostatic description. The term has been largely popularized thanks to the pharmacologist F. Hofmeister, who made in 1887 a systematic investigation on the effect of salts on protein folding properties, although J.L.M. Poiseuille, physicist and medical doctor, was probably the first to report on ion-specific interactions with his work on the viscosity of aqueous salt solutions in 1847. Since then it has been demonstrated that ion-specific effects play a key role in a wide range of physical phenomena, from the structure and reactivity of interfaces to complex biological functions. If a comprehensive overarching molecular explanation remains elusive, the topic has recently regained a new interest thanks to the development of experimental techniques and the emergence of new theories.

Understanding, copying, and engineering are the key steps towards innovations. Understanding specific effects of electrolytes near macromolecules or at charged interfaces is believed to be as important in the physical-chemical sciences as Mendel’s work was for genetics. The phenomena have plentiful technological implications, specifically of relevance for many of the UN global goals for sustainable development. Findings of this research could find an audience in thematic as wide as for instance cleaning water sources and contaminated soil from metallic contamination (Goal 6), understanding biological processes and effects of drugs for the cure of human diseases and/or threats on biodiversity and human health (Goal 3 & 15), chemical reaction in aerosols and their effect on global warming (Goal 13), human health (Goal 3) and/or atmospheric corrosion (Goal 9), development of hybrids nanotechnologies that ally microelectronics and biological systems (Goal 7 & 9). The list is non-exhaustive. For example, monovalent alkali metal salts are
particularly abundant in biology as well as in industry.\textsuperscript{22} Na\textsuperscript{+} or K\textsuperscript{+} composes the background electrolyte in sea water and living cell activities, where their concentration regulation through the sodium-potassium pump channels has a key role as membrane potential determining agents in nerve cells communication.\textsuperscript{23} The importance of Li\textsuperscript{+} is constantly increasing for industrial applications in energy storage,\textsuperscript{24} but the alkali metal cation is also used for medicinal treatment of bipolar disorder.\textsuperscript{25} On another hand polyvalent metals cations due to their variety of coordination modes are key reactant for biomimetic synthesis of inorganic-organic hybrid materials,\textsuperscript{26-27} and trace metal divalent and trivalent cations in concentration ranges as low as sub-micromolar can be responsible for driving the interactions in aerosols or biological organisms.\textsuperscript{4, 28-31} Ni\textsuperscript{2+} and Mn\textsuperscript{2+} were shown to be a cause of toxicity at minute concentrations, due to strong preferential interactions with proteins, and an accumulation mechanism in certain organs, such as in the liver or in the brain cells for Mn\textsuperscript{2+}.\textsuperscript{25, 32} At comparable concentrations, the adsorption of trivalent ions to proteins induces a charge reversion of the surface that drastically affects the reactivity and the stability of macromolecules towards coagulation.\textsuperscript{33-35} Similarly, the pH determining ions, namely hydronium and hydroxide ions, play a significant role in numerous chemical processes, particularly if implying acid/base reactions or in the oxidation of polyvalent ions. The enrichment of those ions at hydrophobic interfaces such as the air/water interface, was argued for decades, until recent spectroscopic investigations.\textsuperscript{36-37} The high rate of protons transport via a H-bond hopping mechanism is held responsible for the high surface conductivity of biological membranes, which gives the proton a major acting part in bioenergetics.\textsuperscript{36}

The carboxylic acid and the phosphate moieties, two common functional groups carried by lipids which are the building block of organic matter and are also widely used in hybrid nanotechnologies,\textsuperscript{38} are particularly known to attract positively charged ions. The proton concentration gradient they create at cells membranes is considered to be a driving force for membrane transport and the synthesis of the adenosine triphosphate (ATP) transporter, the energy carrier of the cells. However, a molecular description of such interactions and the concurrent effect of the hydroxide co-ions, remain under question.\textsuperscript{36} Interestingly, and despite being ignored for a long time, it has been recently highlighted that even the nature of the co-ions affects the adsorption or repulsion of its counter-ion at interfaces.\textsuperscript{39-40} In a counter-intuitive way, halide co-ions (F\textsuperscript{-}, Cl\textsuperscript{-}, Br\textsuperscript{-}, I\textsuperscript{-}) were shown to affect the interactions of the sodium cations with oppositely charged functional groups,\textsuperscript{41-43} when reaching a critical
concentration where ion/co-ions interactions cannot be neglected. These observations echo in a more complex molecular picture the early phenomenological description of ion-specific effects by Hofmeister,\textsuperscript{1,44} where anions and cations were considered together as a pair and not independently.

It has long been believed that ion-specific interactions originate from long-range ordering forces on the aqueous matrix. Instead, direct ion-surface interactions together with the associated and concurrent disruption of the interfacial water structure, are currently considered to be the underlying causes of ion-specific phenomena.\textsuperscript{4,9} Having as a starting point the statistical treatment of the Poisson Boltzmann formalism that describes the behaviour of ions at charged interfaces purely based on electrostatic considerations, the more recent theories extend this description by accounting for intrinsic properties of ions, such as their size, hydration forces, polarizability and/or dispersion forces.\textsuperscript{45-50} The final aim is to gain a full molecular perspective that includes ion-ion, ion-solvent, and ion-solvent-ion interactions. Similarly, a molecular description of the pH determining ions, the hydronium and hydroxide ions, has yet to be uncovered, and their role in the interfacial electrolyte organization remains underestimated. To challenge and extend these theories, direct experimental observations of the molecular structure from ions and water in the interfacial region are required.

In this thesis, vibrational sum frequency spectroscopy (VSFS), a non-linear optical technique with an exquisite surface specificity is used to gain a molecular perspective into ionic specific interactions with the carboxylic acid moiety and its negatively charged carboxylate base. The carboxylic acid moiety is one of the reactive sites of amino acids, the building blocks of proteins, and is commonly present on bio-membranes. It is an important functional group due to its prevalence in biological and atmospheric chemistry systems.\textsuperscript{51-55} The use of long chain fatty acids, which self-organize in monomolecular Langmuir monolayers, provides a convenient model to investigate ion-specific interactions at negatively charged interfaces.\textsuperscript{51} Combined with VSFS, it allows to determine the charging of the monolayer, and gain information on the electrical double layer (EDL) properties, including the variation of the electrical surface potential, the formation of contact ion pairs and molecular complexes, the competition with the hydronium counter-ions, and the presence of the anionic co-ions. Together they provide new experimental insights that can be used as a benchmark to validate and extend current theories on ion-specific interactions.
B. Theoretical Background

1. Fatty acid Langmuir monolayers

   a. The fatty acids

An amphiphile is an organic compound that possesses both hydrophilic and hydrophobic (lipophilic) properties, as for example lipids, metallic soaps, blockcopolymers and surfactants in general. Changing the size and/or the nature of either the hydrophilic or hydrophobic components allows tuning the physicochemical properties of the amphiphile.\textsuperscript{56-57} A primary classification of the surface active amphiphiles is made on the basis of the charge of the hydrophilic headgroup, distinguishing anionic, nonionic, cationic, and zwitterionic amphiphiles, the later carrying both a positive and a negative charge. Among the negatively charged surfactants, we find the family of fatty acids that consists of an aliphatic alkyl chain attached to a carboxylic acid headgroup.

The fatty acids are abundantly present in nature. They are an important component in a multitude of biological tissues,\textsuperscript{53, 58-59} and are widely used by animals as a dietary fuel.\textsuperscript{60} Their properties, like for example solubility and packing configurations when confined, largely depend on the balance between hydrophobic and hydrophilic interactions. For instance, the type and length of the aliphatic chain (saturated / unsaturated, branched, or single alkyl chain) affect the hydrophobic interaction,\textsuperscript{61-62} while the reactivity of the carboxylic acid headgroup (i.e.; deprotonation, polymerization, or ion binding) controls the hydrophilicity, meaning the affinity towards the subphase.\textsuperscript{63-64} Single chain saturated fatty acids ($\text{C}_n\text{H}_{2n}\text{O}_2$) similar to that depicted in Figure B.1.1, are rather simple compounds, and essentially insoluble in water for $n > 16$. Deposited at the air/water interface, they offer a straightforward proxy for mimicking ionisable biological interfaces that expose carboxylic acid groups to solution.\textsuperscript{51, 65}
b. Surface tension & monomolecular films

Surface tension.

The surface tension, $\gamma$, reflects the difference in interaction energy when a molecule is dragged from the bulk to the surface, where it loses a number of neighbouring molecules. It can be written as the change in free energy per unit area, or a force acting perpendicular to the surface normalized per unit length, as shown in equation (1).\(^65\text{-}66\)

$$\gamma = \left( \frac{\partial G}{\partial A} \right)_{T,P} = \frac{F}{2l} \quad (1)$$

The absorption of molecules or ions to a surface causes variations of the surface tension.\(^56\text{-}57, 67\text{-}68\) The adsorption process is favoured if the sum of the free energy of interaction is lowered, i.e. if the surface tension decreases. Particularly for an insoluble amphiphile adsorbed on the air/water interface, the hydrophilic/hydrophobic duality decreases the tension of the air/water interface:

$$\gamma_{\text{air/amphiphile}} + \gamma_{\text{water/amphiphile}} < \gamma_{\text{air/water}} \quad (2)$$

The difference in surface tension upon adsorption compared with that of the neat interface defines the surface pressure $\Pi = \gamma_0 - \gamma$. The neat air/water interface has a surface tension $\gamma_0$ equal to ~72.8 mN/m at 20°C. The Wilhelmy plate method, as depicted in Figure B.1.2 allows measuring the surface tension by determining the force experienced by the fully wetted plate (paper) when partly immersed in the solution.

\[\text{Figure B.1.2. Schematic of a Wilhelmy plate used for measuring the surface tension.}\]
The surface tension is calculated using equation (3), where \( t \) and \( w \) are the thickness and width of the plate taken parallel to the water surface, \( \Delta \rho \) is the density difference between the air and the liquid, \( g \) the gravitational constant and \( l \) the immersion depth of the plate. The Wilhelmy method requires to keep the plate completely wetted so that the contact angle \( \theta \) is zero. The pressure is calculated from equation (4) assuming that the thickness is negligible compared with the width.

\[
\gamma = \frac{F - t.w.\Delta \rho .g.l}{2(t + w) \cos \theta} \quad \text{(3)}
\]

\[
\Pi = -\frac{1}{2} \Delta \rho .g.\Delta l.w \quad \text{(4)}
\]

**Langmuir monolayer.**

The monomolecular film formed by the deposition of an insoluble amphiphile at the air/water surface is called a Langmuir monolayer. Agnes Pockel, considered the pioneer in the fundamental research of molecular thin films, was the first to systematically study the relationship between surface tension on an oil contaminated air/water interface with molecular surface confinement.\(^{69}\) However, it was the work of Irvin Langmuir and Katherine Blodgett, decades later that gave birth to the Langmuir Blodgett through technique, for studying the chemistry and physics of monolayers and multilayers.\(^{70-72}\) The schematic of the apparatus is shown in Figure B.1.3, and a detailed description is given in the experimental part (section C.4). Briefly, it consists of a Teflon trough containing the liquid subphase, where the insoluble amphiphile is deposited at the interface using a highly volatile solvent. Once the solvent evaporates the monolayer can be compressed and the surface pressure recorded.

![Figure B.1.3. Illustration of a Langmuir-Blodgett trough. The deposited fatty acid molecules form a monolayer at the air/water interface.](image-url)
c. 2D isotherms and phase transitions

The Langmuir through records the change of surface pressure as a function of the area per molecule \( \Pi = f(A) \), which can be interpreted as a 2D phase diagram in analogy to the P-V / P-T phase diagrams for bulk materials. The isotherms give a direct insight into the thermodynamic properties of the monolayer. Similarly to bulk studies, the three main phases in 2D phase diagrams are the gaseous (G), the expanded (E), and condensed phases (C). These are classified depending on the freedom of movement of the amphiphile and the rheological properties of the monolayer. However, as discussed in a later section, there are many additional subdivisions. In the gaseous phase, the amphiphiles present little to no interactions between each other \( \Pi \approx 0 \text{ mN/m} \) with a quasi-null surface viscosity. Their movements at the surface are dictated by thermal motion. In the expanded state the amphiphiles start interacting with each other, but the hydrophobic parts are still randomly orientated, yet sensitive to temperature variations. Finally, in the condensed phase, the amphiphiles approach the closest packing configuration, showing a high surface viscosity. An intermediate state (I) is sometimes identified between the expanded and condensed states as a complex phase transition.

The particular case of the Langmuir isotherm of single-chain saturated fatty acids has been extensively studied in the last decades, by combining various techniques such as surface potentiometry \( \Delta V - A \), surface rheology, Brewster angle microscopy (BAM), polarized fluorescence microscopy (PFM), IR spectroscopy (FT-IR), electron microscopy (TEM), X-Ray diffraction, molecular dynamic simulation and more recently VSFS. A detailed 2D-phase diagram has been proposed, mostly originating from the structural packing configuration.

Figure B.1.4 shows the Langmuir isotherm of the eicosanoic acid (commonly named arachidic acid, C20) on a pure water, and NaOH pH 12 subphase at ambient conditions of pressure and temperature (1bar, 22°C). The compression isotherm of arachidic acid on a pure water subphase shows a coexistence region between a gaseous (G) and a condensed phase (C) from the largest area per molecule measured down to \( \sim 23 \text{ Å}^2 \). This is followed by a pure condensed phase. However, in the isotherm, at least two distinct condensed phases can be identified from the different slopes observed in the \( \Pi - A \) curve. The two condensed phases are expected to adopt a hexagonal packing configuration, yet differ in the tilt of the alkyl chain between a tilted condensed (TC, lower) and untilted condensed (UC, stiffer slope) phase. X-Ray diffraction and VSFS experiments
both concluded in an angle of $\sim 30^\circ$ with regards to the water surface for a fatty acid monolayer in the TC phase. The monolayer in the UC phase ($\sim 18 \, \text{Å}^2$) has a higher compressibility. With even further compression, the monolayer undergoes collapse at a critical surface pressure $\Pi_c \geq 50 \, \text{mN/m}$, transforming into a more stable 3D phase.

The features observed in Langmuir isotherms result from a subtle balance between cohesive and repulsive forces that respectively promote or oppose the condensation of the monolayer. Cohesive forces include short-range interactions such as the Van der Waals forces between the alkyl chains, intermolecular bridging of the headgroup through hydrogen bonding, or polymeric complexation with counter-ions. On the other hand, repulsive forces include among others steric hindrances, electrostatic repulsion for charged amphiphiles, surface diffusion (Marangoni effect), or thermal motions. For example, in Figure B.1.4 increasing the pH of the aqueous subphase forces the deprotonation of the fatty acid, introducing a new repulsive electrostatic force between the molecules. It results in changes in the organization of the monolayer and the appearance of a plateau of phase transition. The

**Figure B.1.4.** Langmuir isotherms of arachidic acid (C$_{20}$) on a pure water subphase (blue) and NaOH pH 12 (orange). The isotherms show distinct phases corresponding to the organisation of the fatty acid molecules at the air/water interface.
temperature has been shown to have a similar effect on the monolayer.\textsuperscript{81, 85} At large areas per molecule the repulsion forces dominate, creating a liquid expanded phase (LE), followed upon compression by a TC-LE coexistence region at a higher surface pressure (plateau). The nature of the phase transition had been argued for decades, primarily because of the non-horizontality of the plateau, which contravenes Gibbs rules for a first-order phase transition. However, more recent Brewster angle spectroscopy observations, have shown that condensed domains in the TC phase are found in equilibrium surrounded by a LE phase, which confirms the first-order nature of the transition.\textsuperscript{80} Based on an earlier observation of Langmuir that suggested a 2D aggregation process for the TC-LE region,\textsuperscript{96} Vollhardt et Fainerman proposed an equation of state ($\Pi-A$) derived from a generalization of the Volmer’s equation and a 2D micelle’s like aggregation model, that predicted the apparent non-ideality.\textsuperscript{81, 97} However, the model failed to transpose unambiguously from one system to another because it remains difficult to quantify independently, the hydrophobic or hydrophilic interactions and their cohesive or repulsive effects.\textsuperscript{98-103} Still, due to experimental limitations, the key role played by the headgroup during the phase transition had been investigated only to a limited degree, especially regarding the charging of the monolayer, the counter-ion complexation, or the packing morphologies.\textsuperscript{26, 82, 104-105} Hence, the role of the headgroups in the packing configuration of the amphiphile remained unclear before the studies presented in this thesis.

Langmuir monolayers of arachidic acid have been extensively characterized in the literature, which is an advantage when defining the model system for studying the charging behaviour and ion-specific interactions. Moreover, the Langmuir trough technique allows to precisely control the area per molecule, thus the surface density, a valuable input when interpreting and quantifying the molecular information obtained from VSFS.
2. The charged interface

In certain conditions of pH, temperature, and electrolyte concentration, the fatty acid monolayer in contact with the aqueous subphase can ionize. The charging process results from the dissociation of the carboxylic acid moiety into a negatively charged carboxylate and a free hydronium cation, as shown in equation (5).

\[ RCOOH \leftrightarrow RCOO^- + H^+ \]  

The charging of the interface, together with the rearrangement of the counter-ions (i.e.; opposite charge, attracted to the interface) and the co-ions (i.e.; same charge, repelled from the interface) creates an electrical potential that decays from the interface.

\[ \text{Electrical potential} \]

\[ \psi \]

\[ \psi_d \]

\[ \psi_0 \]

\[ \kappa^- \]

\[ Z \]

\[ E_z \]

\[ \text{IHP} \]

\[ \text{OHP} \]

\[ \text{Figure B.2.1. Schematic representation of the electrical double layer at charge Langmuir monolayer in contact with an electrolyte solution, and the expected variation of the electrical potential.} \]

The formation of the oppositely charged ion cloud in response to the charging of the interface is described by the Electrical Double Layer model (EDL). The ion condensation assures the local electroneutrality at the expense of a substantial entropic cost, subjecting the ion to a statistical equilibrium of attractive and repulsive interactions. The terminologies have slightly varied from the original two plates capacitor model presented by Helmholtz, or the statistical Gouy-Chapman model, to a mixed multi-layered model. A generally accepted representation of the EDL is depicted in Figure B.2.1. The system is composed of
a fixed layer of adsorbed ions and a second, more mobile that is experiencing both thermal fluctuations and coulombic interactions. The latter is called the diffuse layer, as the ions are free to diffuse from the surface. The fix ionic layer in the near vicinity of the interface is usually referred to as the Stern layer \((0 < z < d)\). The counter-ions are immobilized at the surface, attracted by the interfacial electric field, but constrained to a certain distance by their own finite size. The Stern layer can be further divided into two layers, namely the inner Helmholtz plane (IHP) and the outer Helmholtz plane (OHP). The OHP is located at the center of the ionic layer interacting with the surface through their hydration shell. On the other hand, the IHP corresponds to the distance of bare ions interacting with the surface after removing their hydration shell. The ions are either electrostatically or covalently bound. Thus the thickness of the Stern layer is usually in the order of the ionic radius (several Å).

Understanding the physico-chemical properties of the EDL is of enormous significance, as the concept can be translated into many biological and/or colloidal mechanisms, such as membrane-membrane interactions in biological cells, proteins-electrolyte interactions or the quantification of the colloidal stability with the DLVO (Derjaguin-Landau-Verwey-Overbeek) theory.\(^{109}\)

\[ a. \text{ The Poisson Boltzmann model} \]

The Poisson-Boltzmann model (PB) for describing a charged interface, proposed independently by Gouy\(^{106}\) and Chapman\(^{107}\), describes the repartition of ions near the charge interface by taking into account the electrostatic potential and the entropic contribution promoting the formation of the diffuse layer. In a classical approach of the PB model the ions are treated as point-like charges in a mean-field potential approximation. The distribution of ions \(C^i\) along the \(z\) axis normal to the planar surface is given by a statistical Boltzmann distribution by equation (6):

\[
C^i(z) = C^i_\infty \exp \left( -\frac{ze\psi(z)}{kT} \right)
\]  

(6)

where \(k\) is the Boltzmann constant, \(T\) the absolute temperature, \(e\) the elementary charge, \(z_i\) the charge of the considered \(i\)-ion, \(C^i_\infty\) its concentration in the bulk, and \(\psi(z)\) the electric potential at a position \(z\). In dielectric media, the variation of charge density is related to the electrical potential through the Poisson equation. The combination of the ion distribution
function with the Poisson equation leads to a second-order differential equation, the Poisson-Boltzmann equation:

$$\nabla^2 \psi(z) = -\frac{e}{\varepsilon \varepsilon_0} \sum_i z_i C_i^i \exp\left(-\frac{z_i e \psi(z)}{kT}\right)$$  \hspace{1cm} (7)

**Debye-Hückel approximation, the linearized PB.**

In the limits of low surface potentials, also known as the Debye-Hückel approximation, when the electrical interaction becomes negligible compared to the thermal energy, i.e. $\psi \ll kT$, the Poisson Boltzmann equation (7) simplifies considerably. The virial expansion to the first order in $\psi$ leads to the linearized Poisson-Boltzmann equation:110

$$\nabla^2 \psi(z) = \left(\sum_i \frac{C_i^i z_i^2 e^2}{\varepsilon \varepsilon_0 kT}\right) \psi(z) = \kappa^2 \psi(z)$$  \hspace{1cm} (8)

The general solution of the linearized PB equation is:

$$\psi(z) = \psi_0 \exp(-\kappa z)$$  \hspace{1cm} (9)

where the electrical potential decays exponentially. The value $\kappa$ is known as the Debye-Hückel parameter and its reverse $\kappa^{-1}$, as the Debye screening length. The Debye length is one of the most important concepts in the colloidal chemistry of charged particles and sets the length scale of the range of electrostatic interactions. In the Debye-Hückel approximation, $\kappa^{-1}$ corresponds to the distance at which the potential has dropped to 1/e of its value at the surface, $\psi_0$. The Debye length is also referred to as the double layer thickness and commonly used to discuss the extent of screening of the surface charge. However, the electrical potential propagates much further away and can affect the motion of ions and dipole up to $\sim 4 \kappa^{-1}$ away from the surface. The Debye length can be calculated with the general expression (10) and depends on both the concentration and the valencies of the ions. The higher the concentration of electrolyte in the subphase, the shorter the Debye length.

$$\kappa^{-1} = \left(\sum_i \frac{C_i e^2 z_i^2}{\varepsilon \varepsilon_0 kT}\right)^{-1/2}$$  \hspace{1cm} (10)

The Debye-Hückel approximation is strictly valid only when $z_i$, i.e. $\psi \ll kT$, which corresponds to a surface potential $\psi_0 < 25$ mV at ambient temperatures. These conditions
are exceeded in many practical situations, and the electrical potential cannot be considered
to decay exponentially. For such cases the full solution of the PB equation is necessary.

The Gouy-Chapman equation.
The PB equation for a planar charged interface in contact to an electrolyte solution, can be
readily solved analytically, as first proposed by Gouy\textsuperscript{106} and Chapman\textsuperscript{107}, for a symmetrical
1:1 electrolyte. The solution links the surface potential ($\psi_0$) to the surface density of charge $\sigma$, is known as the Gouy-Chapman (GC) equation:

$$\psi_0 = \frac{2kT}{e} \text{arcsinh} \left( \frac{\sigma}{\sqrt{C_\infty 8\epsilon \epsilon_0 kT}} \right)$$ (11)

This correlation was further extended by Grahame, for divalent and asymmetric electrolytes:\textsuperscript{108}

$$\frac{\sigma^2}{2\epsilon \epsilon_0 kT} = \sum_i C_i - \sum_i C_i^i$$ (12)

$$\frac{\sigma^2}{2\epsilon \epsilon_0 kT} = \sum_i C_i^i \left[ \exp \left( \frac{-z_i \psi_0}{kT} \right) - 1 \right]$$ (13)

where $C_i^i$ is the concentration of the i-species at the surface expressed by equation (6).

Generalization of the Gouy-Chapman equation.
For z:z symmetrical electrolytes one obtains a general expression by simply dividing
equation (11) with the valence z:

$$\psi_0^z = \frac{2kT}{ze} \text{arcsinh} \left( \frac{\sigma}{\sqrt{C_\infty 8\epsilon \epsilon_0 kT}} \right) = \frac{\psi_0^{1z}}{z}$$ (14)

Now considering asymmetrical salts, where the valency of the anion is equal to 1 and the
cation valencies are given by $z > 1$, like for example MnCl$_2$ or YCl$_3$, the Grahame equation
gives:

$$C_0^+ = C_\infty^+ \exp \left( -\frac{ze\psi_0}{kT} \right)$$

$$C_0^- = C_\infty^- \exp \left( \frac{e\psi_0}{kT} \right) = zC_\infty^+ \exp \left( \frac{e\psi_0}{kT} \right)$$
\[
\frac{\sigma^2}{2\varepsilon\varepsilon_0 kT} = C^+ \left\{ \exp\left( -\frac{z e \varphi_0}{kT} \right) + z \exp\left( \frac{e \varphi_0}{kT} \right) - (z+1) \right\}
\]  \hspace{1cm} (15)

By factorizing the general expression under bracket by that for monovalent salts \((z = 1)\) given in equation (16), we obtain a generalized relationship between the surface charge and the surface electrostatic potential as shown in equation (17).

\[
\left( \exp\left( \frac{e \varphi_0}{kT} \right) - 2 + \exp\left( -\frac{e \varphi_0}{kT} \right) \right)
\]  \hspace{1cm} (16)

\[
\sigma = \sqrt{8\varepsilon\varepsilon_0 kT \sinh\left( \frac{e \varphi_0}{2kT} \right)} \left\{ C^+ + C^2+ \left[ 2 + \exp\left( -\frac{e \varphi_0}{kT} \right) \right] \right\}^{\frac{1}{2}}
\]  \hspace{1cm} (17)

The expression remains valid even for mixtures of salts with different valencies. Contrary to the Gouy-Chapman equation for 1:1 electrolyte, there is no simple way to isolate an expression for the surface potential. However, the equation can be solved numerically.

**Limitations.**

Although the PB theory has been a valuable breakthrough for the description of charged interfaces, there are important shortcomings due to the many assumptions associated with its derivation. First, the Boltzmann distribution of charge neglects all kind of ion-ion and ion-solvent interactions at the molecular level. In general, the predictions from the theory show good agreement with experimental results in dilute electrolyte solutions and low surface potentials; however, they can fail even qualitatively for highly charged surfaces and/or high electrolyte concentrations (>50 mM for monovalent salt). The divergence increases with the valence of the salt. Additionally, the mean-field potential approximation considers the electrolyte as a continuum and the distribution of charges is seen in a homogenous force field. This assumption disregards the discreteness of the system and neglect short-range molecular specific interactions. Moreover, the discontinuity of the dielectric constant at the interface adds a repulsive force, known as the image force that the model ignores. On the other hand,
treating ions as point-like charges neglects steric repulsive forces originating from their finite size, which could lead to an unphysical accumulation of counter-ions near the interface.

b. Steric saturation of counter-ions in the EDL

To account for some of the limitations described above, several modifications to the PB theory have been proposed in the literature. Here are presented the most relevant alternatives for the work carried out in this thesis.

**The Gouy-Chapman-Stern model (GCS).**

The analytical description of the EDL was extended by Stern, who assumed a capacitor model in the first layer of co-ions followed by a classical PB model of the Gouy Chapman type at $z = d$, the edge of the Stern layer. While Helmholtz assumed that the transiently bound co-ions could totally cancel the surface charge, Stern proposed that a partial charge could be found beyond the edge of the bound ionic layer. The model considers the Stern layer as a parallel plate capacitor. In an aqueous electrolyte solution, the difference of potential between the two charging plates is given by:

$$\frac{\Delta \psi}{\text{thickness}} = \sigma \left( \frac{\psi_d - \psi_s}{d_{\text{Stern}}} \right)$$

with $\psi_d$ the electrical potential at the edge of the Stern layer (outer Helmholtz plate), and $d_{\text{Stern}}$ the distance from the surface. The Stern and diffuse layers can then be considered as two capacitors in series to obtain a more complete picture of the EDL. Brown et al. recently proposed an experimental approach to determine the Stern layer thickness, from the experimentally measured surface potential and zeta potential, of silica nanoparticles in a monovalent salt solution. The $d_{\text{Stern}}$ thicknesses calculated were in the range of the hydrated counter-ions’ diameters (~6-8 Å). Nonetheless, the approach required several assumptions and neglected, for instance, the finite ion size, as well as the known dependence of the dielectric permittivity with ion concentration.

**The composite diffuse-layer model.**

Explicitly accounting for the finite size, ensures that the ion density at the surface does not exceed the physically limiting values. A dimensional parameter linked to the ion size would account for the hard-core steric repulsion between the charged particles. Two different
approaches, one proposed by Orland et al.,\textsuperscript{117-118} and the other by Iglic et al.,\textsuperscript{119-120} define an excluded ion volume from the mean volume fraction of ions in the bulk solution:

\[ \nu = 2a^3 C_\infty \]  

(19)

where \( a \) is a phenomenological parameter linked to the effective ion size. The composite diffuse-layer model adopts the Poisson-Boltzmann formalism, with the condition that the maximal concentration of counter-ion at the surface is given by \( 1/a^3 \). This leads to the formation of a compact condensed layer of counter-ions, a concept that shares some similarities with the Stern model. Nonetheless, the thickness of the compact condensed layer naturally varies depending on the ion concentration and surface charge, and starts forming when the surface potential exceeds a critical value, \( \psi_c \) as defined by equation (20), when adopting a capacitor model for the condensed layer.\textsuperscript{111}

\[ |\psi_c| = \frac{kT}{e} \ln \left( \frac{2}{\nu} \right) \]  

(20)

**The modified Poisson-Boltzmann (MPB).**

The formalism has been completed in a continuum approach that cancels the “edge effect” (the discontinuity of the electrostatic potential at Stern or compact condensed layer thickness), which is referred here as the modified Poisson-Boltzmann model (MPB).\textsuperscript{111, 117-118} The model assumes a modified Boltzmann distribution with a steric correction:

\[ C_{(z)} = \frac{C_\infty \exp \left( \frac{-e\psi_{(z)}}{kT} \right)}{1 + 2\nu \sinh^2 \left( \frac{e\psi_{(z)}}{2kT} \right)} \]  

(21)

The resulting modified Poisson-Boltzmann equation for a \( z:z \) electrolyte becomes:

\[ \nabla^2 \psi_{(z)} = \frac{zeC_\infty}{\varepsilon \varepsilon_0} \frac{2 \sinh \left( \frac{ze\psi_{(z)}}{kT} \right)}{1 + 2\nu \sinh^2 \left( \frac{ze\psi_{(z)}}{kT} \right)} \]  

(22)

Similarly to the Gouy-Chapman equation, analytical solutions are available for the MPB model in the case of a planar charged interface.\textsuperscript{111}
\[
\sigma^2 = \frac{4z^2\varepsilon_0kTC_e}{\nu} \ln \left[ 1 + 2\nu \sinh^2 \left( \frac{ze\nu_0}{2kT} \right) \right] 
\]  \hspace{1cm} (23)

Or alternatively when solving for the surface potential:

\[
\left( \psi_0 \right)_{mPB} = \frac{2kT}{ze} \sinh^{-1} \left( \frac{1}{2\nu} \left( \exp \left( \frac{\nu_0^2}{4z^2\varepsilon_0kTC_e} \right) - 1 \right) \right) 
\]  \hspace{1cm} (24)

The model naturally converges to the Gouy-Chapman predictions at low salt concentrations, and/or low surface charge. The steric effect becomes apparent at high salt concentrations and/or highly charged surfaces. Figure B.2.2 shows the counter-ion density profiles calculated with the PB and MPB models (effective ion size \( a = 8\AA \)), for a highly charged surface that mimics the behaviour of a fatty acid monolayer in contact with an aqueous subphase at pH 12. The MPB model predicts a saturation of the counter-ion concentration at \(~3.3\) M, while the PB model predicts surface concentrations that are order of magnitude above the solubility limit of the salt. The thickness of the saturated compact layer is not equal to the dimensional fitting parameter \( a \), and depending on conditions, it can be composed of several layers of counter-ions. The thickness of the layer depends on the ion size and the surface charge, as shown in equation (25):

\[
l^* \approx \frac{a^3\sigma}{ze} 
\]  \hspace{1cm} (25)

Although the MPB model described accounts for the finite size of the ions, it still has several limitations. First, it assumes that the dielectric constant is independent of the ion concentration, and neglects direct ion-ion and ion-surface specific interactions. However, at high salt concentrations as the steric effect becomes predominant, most solvent molecules will be participating in hydration shells of the counter-ions. Thus ion-solvent, ion-solvent-ion, and solvent-solvent interactions should become more relevant. In this regard, Yaakov, Andelman and Podgornik obtained similar ion concentration profiles to those presented in Figure B.2.2 by explicitly considering the solvent polarizability and the local variation of the dielectric constant. Further details are given in section B.3.b.
Figure B.2.2. Calculated ion density profiles of Na\(^+\) counter ions near a Langmuir monolayer of arachidic acid compressed at 20Å\(^2\), on an aqueous subphase NaOH pH 12.0. The MPB model predicts the saturation of the ion concentration close to the surface. The Figure is reproduced from paper II.

c. Counter-ion condensation

Direct molecular interactions of the counter-ion with the carboxylate can be included in the analytical formalism.

Dissociation of the carboxylic acid.

Let’s consider the dissociation of the carboxylic acid group of the fatty acid monolayer in the presence of a symmetrical monovalent salt (1:1). According to the interfacial chemical equilibria presented in equation (5), the dissociation constant can be expressed as:

\[
K_a = \frac{[H^+]_0 [RCOO^-]_0}{[RCOOH]_0} = \frac{\alpha}{1 - \alpha} [H^+]_0
\]

(26)

where the indices “0” correspond to surface concentrations, and \(\alpha\) the degree of dissociation. Assuming a Boltzmann distribution of charge, the surface concentration of hydronium ions can be expressed as a function of the bulk concentration and the surface potential, giving:

\[
K_a = \frac{\alpha}{1 - \alpha} [H^+]_\infty e^{-\alpha \psi_0 / kT}
\]

(27)

\[
K_a = \frac{\alpha}{1 - \alpha} [H^+]_\infty e^{-\alpha \psi_0 / kT} \Rightarrow \ln \left( \frac{1 - \alpha}{\alpha} \right) = \ln [H^+]_\infty - \ln K_a - \frac{e\psi_0}{kT}
\]

(28)
Solving for the surface potential in equation (28):

\[
\psi_0 = \frac{kT}{e} \left[ \ln \left( H^+ \right)_0 - \ln K_a - \ln \left( \frac{1 - \alpha}{\alpha} \right) \right]
\]

The surface potential is related to the surface charge density by the Gouy-Chapman equation (equation (11)). The surface charge density of the monolayer is calculated from the degree of deprotonation \( \alpha \), and the density of fatty acid molecules at the interface (obtained from the Langmuir trough). Combining those two relations with equation (29) allows to express the degree of dissociation of the monolayer as a function of the bulk concentration of electrolyte and hydronium ions, resulting in:

\[
\sigma = \frac{\alpha e}{A_d}
\]

\[
\sqrt{C_e} = \frac{\alpha e}{A_d \sqrt{8 \varepsilon_0 kT}} \left\{ \sinh \left( \frac{1}{2} \left( \ln \left( H^+ \right)_0 - \ln K_a - \ln \left( \frac{\alpha}{1 - \alpha} \right) \right) \right) \right\}^{-1}
\]

This derivation had been previously considered for explaining the apparent shift of the acidity of the carboxylic acid moiety at the interface.\(^{64, 122}\) It has been recently experimentally confirmed by measuring the monolayer deprotonation with VSFS, that while the intrinsic acidity constant of the carboxylic acid remains similar to that observed for shorter chain carboxylic acids in the bulk (pKa ~5), at the surface, the monolayer is 50% deprotonated at much higher pH. The measured apparent pKa was ~10.8 for a compact monolayer (20 Å²).\(^{123}\)

The shift of acidity is explained by the reorganization of the counter-ion when charging the monolayer. The negative charge attracts positively charged ions at the surface, locally increasing the concentration of hydronium ions.

**Accounting for the steric size.**

The finite size of the counter-ion, can be explicitly considered using the expressions for the surface potential of the MPB model described above (equation (24)), and following a similar derivation as for equation (31):

\[
\sqrt{C_e} = \sqrt{e^{\frac{e^3 \alpha \varepsilon_0}{kT}} \left( \frac{4 \alpha^3}{d^3} \right) \left\{ \sinh \left( \frac{1}{2} \left( \ln \left( H^+ \right)_0 - \ln K_a - \ln \left( \frac{\alpha}{1 - \alpha} \right) \right) \right) \right\}^{-1}}
\]

The expression above, however, neglects the finite size of the hydronium ion.
Metallic cation condensation.

If other cations with a strong affinity to the carboxylic moiety are present in solution, the model can be extended to account for the competitive binding with the hydronium ions.\(^{64, 82, 86, 124}\)

\[
RCOO^- + M^+ \leftrightarrow RCOO-M \tag{33}
\]

\[
K_M = \frac{[RCOO-M]_0}{[M^+]_0[RCOO^-]_0} \tag{34}
\]

where \(K_M\) is the binding constant of the monovalent cation \(M^+\) with the carboxylate. The degree of dissociation is then calculated including the proportion of bound carboxylate:

\[
\alpha = \frac{[RCOO^-]}{[RCOO^-] + [RCOO-M] + [RCOOH]} \tag{35}
\]

\[
\alpha = \frac{1}{1 + \left(K_M [M^+]_\infty + \frac{[H^+]_\infty}{K_a}\right) e^{-\psi_0/kT}} \tag{36}
\]

The chemical equilibria can be introduced either in the classical or modified PB models. For simplicity, the PB is used. Equation (29) becomes:

\[
\psi_0 = \frac{kT}{e} \left[ \ln \left( \frac{[H^+]_\infty}{K_a} + K_M [M^+]_\infty \right) - \ln \left( \frac{1 - \alpha}{\alpha} \right) \right] \tag{37}
\]

Finally, assuming \([H^+]_\infty \ll [M^+]_\infty \approx C_\infty\) it is obtained:

\[
\sqrt{C_\infty} = \frac{\alpha e}{A_M \sqrt{8\pi\varepsilon_0 kT}} \left[ \sinh \left( \frac{1}{2} \ln \left( \frac{[H^+]_\infty}{K_a} + K_M [M^+]_\infty \right) - \ln \left( \frac{1 - \alpha}{\alpha} \right) \right) \right]^{1/2} \tag{38}
\]

The degree of deprotonation and bound carboxylate can be calculated as:

\[
D_H = \frac{1 + K_M [M^+]_0}{1 + K_M [M^+]_0 + \frac{[H^+]_0}{K_a}} \tag{39}
\]

\[
D_M = \frac{K_M [M^+]_0}{1 + K_M [M^+]_0 + \frac{[H^+]_0}{K_a}} \tag{40}
\]
Although this approach appears useful for determining the binding constants of cations with the carboxylic acid at the interface, it appears that the binding is systematically overestimated at high concentrations, and underestimated at low concentrations.82

3. Ion specific interactions

In the ideal electrolyte solution, inter-ion interactions are considered to be negligible and ions behave like in an infinitely dilute solution. Deviations from ideality are well described by the Debye-Hückel theory that predicts variations of the activity coefficients of the species by considering inter-ionic electrostatic interactions.125 However, at high salt concentrations (~0.1M, i.e., comparable to the biophysical concentrations of monovalent chloride salts), water can no longer be regarded as a continuum. Instead, water molecules in the hydration shells are shared, and the solvent behaves as a connected matrix.5, 126 This is particularly true for concentrations above 1 M, where due to the compact packing of the hydrated ions, no “free water molecules” remain available. Such high concentrations can be easily reached upon formation of an electrical double layer in close proximity to a charged interface. Consequently, ion-ion, ion-solvent, and solvent-solvent interactions cannot be neglected, and ions should be considered together with their close molecular environment. This introduces ion-specific interactions, for which the behaviour of the ion depends on its nature and cannot be simply predicted by classical theories of electrolyte, i.e solely by its charge.4, 127-129

Ion specific effects are present in numerous physicochemical processes, influencing among others, the viscosity, surface tension, forces between charged surfaces, and colloidal stability (DLVO theory).4 They also play a determining role in an extensive range of biological phenomena, including ion-membrane interactions, membrane permeability (ion-channel), regulation of the osmotic pressure in cells, and protein folding.4 For many decades ion-specific effects were thought to originate from long-range interactions in the solvent, as an “ordering” effect on the water matrix. However, it is currently acknowledged that the underlying mechanism of action involves direct molecular interactions between the ions and the chemical functional groups of interest, as well as those water molecules in close proximity within the first hydration shells.4, 9, 45, 48, 130-131
Salt specific interactions have been extensively reported since the early work of F. Hofmeister, a pharmacologist of the University of Prague, who published a series of papers between 1887 and 1898, entitled “Zur Lehre von der Wirkung der Saltz”. Hofmeister systematically classified the ability of different salts to dissolve or precipitate proteins in aqueous solution. In his original formulation, Hofmeister interpreted his results as a “salt specific effect”, a global effect that included both the counter and co-ions in solution. Even if an empirical ranking for the different ions emerged from his work, Hofmeister also reported discrepancies in the ordering of salts depending on the nature of the studied protein. The extended Hofmeister sequence is presented below (Figure B.3.1):

![Hofmeister Series](image)

**Figure B.3.1.** A typical arrangement of ions in the anionic and cationic Hofmeister series

The ions were originally classified as “salting out” or “salting in” depending on their ability to precipitate proteins from solution. Later, they were also described in terms of their ability to “structure water” as kosmotropes (water structure maker) or chaotropes (water structure breaker). Even if both terminologies are still used today, the salts are no longer characterized with regards to their long-range impact on water. Instead, one should precise that the “salting out” salts correspond to strongly hydrated anions and weakly hydrated cations, whereas the “salting in” salts are the opposite, weakly hydrated anions, and strongly hydrated cations.

**Alteration of the Hofmeister series.**

The Hofmeister series is a phenomenological observation rather than a rigorous prediction of the salt behaviour, as many exceptions have been reported. For instance, the ordering of the ions regarding their “salting effect” depends on the functional group present on the studied protein, the proportion of its hydrophobic backbone and also, on the specific anion – cation combination. Similarly, at solid / liquid interfaces, experiments have shown that the
ordering of ion adsorption affinities, could vary depending on the nature of the counter-ions, surface chemistry, and solution conditions. In reality, the ion specificities result from a complex interplay of multiple factors that include ion (and solvent) polarizability, ion size, the surface charge, and the hydrophilicity / hydrophobicity of the surface.

For the specific case of fatty acid Langmuir monolayers, Schwierz, Horinek and Netz recently proposed a “Hofmeister phase diagram” based on MD simulations. With respect to the Hofmeister series arrangement, the diagram shows that the ordering of ion interactions with the carboxylic acid moiety, could be either direct, reversed, or in between, depending on the ion concentration and pH of the subphase. They suggested that the reversion of the Hofmeister series is “rather a rule than an exception”. However, no direct molecular experimental confirmation was available until Paper I in this thesis was published.

b. Theoretical approaches

Several different models have been proposed to explain the Hofmeister effect, and in particular, predict the reversion or alteration of the ionic affinities. Aiming to describe the specificity of the interactions, they all include one or several corrections to account for intrinsic properties of the system, such as the ion size, the heterogeneity and discreetness of the media, ion-ion and ion-solvent correlations, and/or ion and solvent polarizabilities. The most relevant models are reviewed in what follows.

The law of matching water affinity.

One of the most popular theories is the empirical concept of matching water affinity proposed by K. Collins, also known as Collin’s law. Collins recognized that the electrostatic forces of ions on the surrounding water molecules are negligible beyond the first hydration shells. The dominant interactions are those of chemical nature between the close hydration water molecules and the electrons of the outer orbitals of the ions. This means that ion specific interactions originate from the properties of the first hydration layers, i.e. ion-water interactions. Accordingly, the empirical law proposed by Collins explained the Hofmeister series in terms of hydration and dehydration forces, which can be qualitatively correlated with the ion size and surface charge density. In this regard, kosmotrope ions, which are relatively smaller with a higher surface charge density are examples of strongly hydrated ions. The comparatively larger chaotrope ions are instead weakly hydrated ions. As a consequence of their ability to form inner–sphere ion pairs, two oppositely charged ions with
a similar Gibbs free energy of hydration will preferably associate: kosmotrope-kosmotrope and chaotrope-chaotrope interactions are favoured. A concept later referred to as “like seeks likes” by W. Kunz, where soft and hard ions will interact favourably with ion of the same nature, mostly depending on the size, geometry, and polarizability. The concept can be empirically extended to any functional group by considering their hydration energy, such as hydrophobic group or uncharged polar group, provided a useful qualitative tool for predicting ion specific interactions. For example, the carboxylic acid group is considered as kosmotropic, while the fatty acid alkyl chain would be chaotropic.

**Solvent polarizability.**

Ben-Yaakov, Andelman and Podgornik proposed a formalism to modify the mean field approach, in order to account for local heterogeneity of the system in regards to ion-solvent interactions and solvent polarity, through local variations of the dielectric constant of the media. The dielectric constant of an electrolyte has been shown to be systematically lower than that for pure water. The reasons are two fold; first, the ion is intrinsically less polarizable than the solvent, and second, water molecules in the hydration shell of the ion are strongly oriented by the local electrostatic field, which substantially reduces their response to an external electric field. The model proposed a decrement of the dielectric response of the media as a function of the electrolyte concentration, which is implemented in a classical PB approach. It somehow indirectly accounts for the finite size of the ion by introducing a similar limitation to the ion concentration at the surface, and leads to similar descriptions as the MPB model at interfaces.

**Ion polarizability: the Levin’s theory.**

Levin and co-workers adopted a different formalism by focusing on the ion polarizability. The model, known as Levin’s theory, expresses the ion solvation potential in terms of purely geometrical considerations combined with charge relocalisation at the surface of the ionic sphere. The theory considers four contributions: the electrostatic self-energy (relocalisation of the charge), the cavitation energy (hydrophobic solvent interaction), the image potential, and the hard sphere repulsion (exclusion principle). In agreement with Collin’s law, the ion size and the charge of the ion are important factors, but also its close environment and the properties of the solvent. In this regard, “big” “softer” ions, can delocalize their charge more effectively to interact with hydrophobic, uncharged particles, or ions of the same nature through hydrophobic interactions. The model successfully predicted the adsorption of anions
to the air/water surface. The ion polarizability is proportional to the ion volume, which scales similarly to hydrophobic solvation contribution, thus dispersion forces.

**Dispersion forces: Ninham’s theory.**

Ninham, Boström, and co-workers suggested that a complete prediction of the specific ionic interactions could only be achieved by considering an interplay of hydration, electrostatic, and short-range dispersive forces. The dispersion forces refer to quantum forces, which includes all non-electrostatic ion-ion and ion-solvent interactions, and depends on the ion concentration, polarizability, and electron affinity of the ion. The model requires to include an additional potential to the PB formalism, obtained from the extension of the Lifshitz theory: 

$$
\mu_{\text{disp}}(z) = h(z). B / z^3,
$$

with B being the dispersion coefficient, accounting for the ionic polarizability, and h(z) a size constrained distribution function. The dispersion forces as presented by Ninham allows the possibility for charged species to adsorb to hydrophobic, neutral hydrophilic surfaces, or even negative species to a negatively charged surface. The theory was shown to be capable of predicting the reversion of ion affinities in proteins, and accurately account for ion-specific effects at pH electrode surfaces. The theory has been recently extended to account for cooperative adsorption, and chemisorption onto protein structures. However, it remains difficult to generalize to other extended interfaces, as the non-electrostatic parameters included in the interaction potentials are not readily available. In general, the theory provides consistent results for highly concentrated electrolyte solutions, and surfaces with a low surface charge. For higher surface charge densities however, it is argued that steric effects and the dielectric response of the solvent are the predominant factors.

**Molecular dynamics simulations.**

All the aforementioned models propose different ways to introduce an additional potential to the PB formalism, with the aim of modifying, restraining, or refining the Boltzmann distribution of charge. Hence molecular dynamics simulations appear, up to now, as the best unifying approach to account for the complex interplay of the different mechanisms. The recent developments in simulation techniques have shone new light on our understanding of ion-specific effects, and help regain research interest in the field. The complementary of molecular dynamics simulations on the atomic scale, with coarse-grained models where the solvent is introduced implicitly in the parametrization, represents a useful tool to compare experimental results and gain more depth in the molecular understanding of ion-specific effects.
at interfaces. However, discussions are ongoing on the optimization of the parametrization of the studied systems, such as how to properly account for the polarizability of the ions and solvent, as well as the systematic overestimation of ion-ion interactions.40, 154-156

c. The classification of binding

In extensions of the Poisson Boltzmann theory that account for ion association, no distinction is made between the different types of binding. The only assumption made is that the surface charge is fully compensated by the adsorbing ions. Moreover, in most of the models mentioned above, an emphasis is made to describe the forces that govern the ionic interactions (i.e.; dispersion forces, electrostatic forces, hydration forces), but few report on the spatial configuration of ion association and the possibility of covalent binding through electron cloud sharing. The type and configuration of binding can be an important source of ion-specific interactions, especially for polyatomic ions such as the carboxylate moiety, or for polyvalent ions where the electronic structure of the outer orbital shell of the ions is more complex. A description of the main types of ion associations of interest for this thesis is presented below.

**Ion pairing.**

At high electrolyte concentrations, ions of opposite charge are separated from each other by a limited amount of water molecules, to the extent that they can share their hydration shells. Such type of ionic association can be observed even in dilute solutions, leading to deviations from dilute electrolyte theories and the appearance of ion-specific effects. This ionic association is referred to as ion pairing. Despite being observed in different systems for decades, Marcus and Hefter first proposed in a review in 2006,48 a generalized definition to distinguish ion pairing from the more accepted notion of covalent binding. Ion pairing results from electrostatic long-range attraction, and/or short-range dipole-dipole interactions. In contrast to covalent binding, ion pairing is a non-directional association, meaning that there is no specific coordination for monoatomic ions regardless of the charge.

Different types of ion pairing have been proposed to form depending on the inter-ionic distance and solvent sharing. The different types are depicted in Figure B.3.2, and include: a solvent separated ion pair, where the hydration of each ion remains intact (distant of two solvent molecules, 2SIP); a solvent shared ion pair, when only a single solvent layer separates the ions (SIP); and a contact ion pair (CIP), when the ions associate without any solvent molecules in between. Ion pairs have been recently experimentally characterized,
for instance, by comparing the diffusion times of the single ions with the paired species, or the constrained dynamics of water molecules in their close proximity.\textsuperscript{5,10} The propensity of the ions to form ion pairs depend on their nature and the interaction with the solvent (ion-ion, ion-solvent, and ion-solvent-ion interactions). Thus the ion pair formation is highly ion-specific and strongly depends on the size and charge of the ions, placing the concept in perspective with Collin’s law.

\textbf{Coordinative binding.}

The concept of ion complexation is more widely accepted. It describes short-range directional interactions, through a donor-acceptor mechanism of the outer electron orbital shell.\textsuperscript{157} The coordinative binding of ions strongly depends on their electronic structure, thus on the nature of the ion, giving to the ionic association a high ionic specificity. Figure B.3.3 depicts a generally accepted schematic of possible configurations that can be adopted for ionic coordinative association between a carboxylate anion and a metallic cation.\textsuperscript{158} The metal-carboxylate complexes are commonly sorted into four classes, respectively unidentate (I), chelating bidentate (II), bridging bidentate (III) and a polymeric form (IV) involving several carboxylate groups. The coordination modes (III) and (IV) can further be divided in subcategories depending on relative arrangement of the oxygen-ion bond (anti/syn) and the coordination of the metallic ions. An additional structure is often mentioned in the case of hydrated carboxylate group, namely the pseudo-bridging or H-bonded unidentate, corresponding to bridging bidentate where one metallic ion is replaced by a water molecule bonded with the other metallic atom. Attempts have been made in order relate the vibrational

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure_B.3.2.png}
\caption{Schematic representation of the three types of Ion Pairing, according to Marcus classification.}
\end{figure}
signature of the carboxylate group with its binding configuration in solid,\textsuperscript{158-159} liquid,\textsuperscript{160} and gaseous phase,\textsuperscript{161} or at interfaces.\textsuperscript{162} If no systematic rule has emerged, it remains a good comparative indication as discussed in Paper IV. All the type of binding can be described thermodynamically with a constant of association, referred as $K_M$ in section B.2.c. The electronic structure of the metallic ions, the steric confinement of the carboxylate, the binding mechanism, all introduce sources of ion specificity for the interaction of ions with the fatty acid Langmuir monolayer.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{coordination_modes.png}
\caption{Schematic representation of the coordination modes of the carboxylate group with metallic cations, reproduced from Paper IV.}
\end{figure}
4. Vibrational Sum Frequency Spectroscopy, VSFS

This chapter presents an introduction to the theory and applications of vibrational spectroscopy, with a specific emphasis on VSFS and other recent developments explored in the frame of this research.

a. Linear and non-linear optics

In the absence of absorbance, when light interacts with matter, it typically induces a polarization that acts as a source for reemitting radiation. Depending on the intensity of the electromagnetic radiation, the induced response can be linear or non-linear with the field.\(^{163-165}\)

In linear optics, the electric dipole moment can be expressed in terms of the static dipole moment \(\mu_0\), the molecular polarizability \(\alpha\), and the excitation field \(E\):

\[
\alpha = \mu_0 + \alpha E \tag{41}
\]

In dielectric media, it is more convenient to use the macroscopic polarization \(P\), which results from averaging the electric dipole moment per unit volume. \(P\) can be expressed as the sum of the static polarization \(P^{(0)}\) and a term accounting for the induced dipole moment, which is directly related to the polarizability:

\[
P = P^{(0)} + \varepsilon_0 \chi^{(1)} E \tag{42}
\]

where \(\chi^{(1)}\) is the linear susceptibility, a first order tensor, which depends on the number of molecules per unit volume and the molecular polarizability \(\alpha\), averaged over all the molecular orientations in the material. In an isotropic medium, the refractive index is given by the square root of the relative permittivity of the medium, \(\varepsilon_r\), which is linked to the linear susceptibility by:

\[
n = \sqrt{\varepsilon_r} = \sqrt{1 - \chi^{(1)}} \tag{43}
\]

The real part of the refractive index \(n\) characterizes the speed of the light in the dielectric media, while the imaginary part relates to absorption. When the intensity of the electric field becomes comparable to that felt by electrons in a molecule, the polarization response no longer depends linearly with the field, and higher-order terms need to be included. The macroscopic polarization is then expressed as:\(^{163,166}\)
\begin{equation}
P = P^{(0)} + P^{(1)} + P^{(2)} + P^{(3)} + \ldots \tag{44}
\end{equation}

\begin{equation}
P = P^{(0)} + \varepsilon_0 \chi^{(1)} E + \varepsilon_0 \chi^{(2)} : EE + \varepsilon_0 \chi^{(3)} : EEE + \ldots \tag{45}
\end{equation}

where the second and third order terms depend on the non-linear susceptibility $\chi^{(2)}$ and $\chi^{(3)}$, which are tensors of rank 3, and rank 4, respectively. The term depending on $\chi^{(2)}$ in equation (45) is responsible for the second order non-linear phenomena behind several surface specific spectroscopies, which include second harmonic generation (SHG), sum frequency generation (SFG), and difference frequency generation (DFG).

\textbf{b. Vibrational spectroscopy}

The interactions of light with matter offer a wealth of information regarding, among others, the identity, composition, and electronic structure of the material. Depending on the frequency of the electromagnetic radiation used (e.g.; microwave, infrared, visible, UV, or X-Ray), different type of information can be obtained from the system. Vibrational spectroscopy targets molecular vibrations that are typically probed, either directly by absorption of infrared radiation (IR), or indirectly, through the inelastic scattering of higher energy photons (Raman).

To a first approximation, the molecular vibration of a diatomic molecule can effectively be modeled as harmonic oscillators, where atoms are represented as spheres of known mass and the covalent bond as mass-less spring. The natural frequency of the vibration $\nu$, is then dependent on the spring constant $F$, linked to the covalent bond strength, and the reduced mass $m^*$, of the two atoms involved in the vibration, as shown in equation (46).

\begin{equation}
\nu = \frac{1}{2\pi} \sqrt{\frac{F}{m^*}} \tag{46}
\end{equation}

The frequency of vibration depends on the strength of the covalent bond, which can in turn be affected by the chemical environment and the geometrical configuration of the molecule. For instance, close interactions of ionic species with a charged molecular group, will have an effect on the vibrational frequency. On another hand, isotopic substitution also causes a shift in the frequency of vibration by changes in the reduced mass. For example, replacing the hydrogen atoms with deuterium causes a $\sim 1.36$ redshift of the vibrational frequency, an
effect that is commonly used to displace the spectral signatures from alkyl chains (CH to CD’s), as well as those involving water molecules (H₂O to D₂O).

In polyatomic molecules, more complex vibrations are expected. The number of fundamental modes of vibration is dictated by the total number of atoms N in the molecule, with 3N-6 for non-linear molecules, and 3N-5 for those displaying a Cₙ axis of symmetry. The number of effective vibrational modes available can nonetheless be reduced depending on the symmetry properties of the molecule or functional group under investigation. Molecular symmetry can also be used to predict if a vibrational mode will be either IR, Raman, or SF active (both IR and Raman active).¹⁵⁷

**IR induced dipole moment.**

A permanent dipole moment in a molecule results from the inhomogeneous distribution of partial charges, linked to differences in the atoms electronegativity and molecular geometry. When the frequency of an incoming electromagnetic wave matches one of the natural frequencies of vibrations of the molecule, it induces a spatial variation in the charge distribution, which changes the permanent dipole moment and brings the molecule to an excited energy level. The infrared light is absorbed and later dissipated as thermal energy. For a vibrational mode to be IR active, the dipole molecule must change during the vibration:

$$\left( \frac{\partial \mu}{\partial Q} \right)_0 \neq 0$$  (47)

where Q is the normal coordinate axis.

**Raman scattering.**

Molecular vibrations can also be probed with Raman scattering, which uses as excitation source electromagnetic radiation, typically in the visible frequency range, that does match any of the absorption frequencies of the molecule under study. When light interacts with the molecule it induces a short-lived dipole moment by distorting its electron clouds. In this virtual state, the molecule acts as a source of radiation, reemitting light with the same frequency as the excitation source before decaying back to its original state. In this case, the emitted light is referred to as elastic or Rayleigh scattering. However, a very small fraction of the molecules in the virtual state, do not decay back to their original energy levels and scatter light at slightly different frequencies. Two options are possible, either decay to an
energy level higher than the original (Stokes Raman scattering), or lower (Anti-Stokes Raman scattering) as depicted in Figure B.4.1. The differences in the frequencies between the excitation source and the Raman scattered photons are related to the molecular vibrational energy levels. According to equation (41) the induced dipole can be expressed in term of the polarizability $\alpha$, a second rank tensor, and the applied electric field $E$, as follow:

$$\mathbf{\mu}_{\text{ind}} = \alpha \mathbf{E}$$  \hspace{1cm} (48)

To be Raman active, a disturbance of the polarizability in time and space is required, giving the following selection rule:

$$\left( \frac{\partial \alpha}{\partial Q} \right)_{0} \neq 0$$  \hspace{1cm} (49)

In order for a vibrational mode to be SF active, a molecule needs to fulfil both the IR and Raman selection rules.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{raman_scattering_diagram.png}
\caption{Illustration of the Raman scattering process. Elastic Rayleigh scattering involves excitation to a virtual level and a decay back to the original vibrational energy. In Raman scattering the photon either loses (Stokes) or gains (Anti-stokes) energy in the process.}
\end{figure}
c. Vibrational Sum Frequency Spectroscopy

Vibrational sum frequency spectroscopy (VSFS) is a second-order non-linear optical technique with an intrinsic surface specificity and sensitivity. The theoretical foundations were established in the 1960’s by the team of the 1981 Nobel Physics laureate N. Bloembergen.\textsuperscript{168-169} However, it was not experimentally demonstrated in condensed phases until the late 1980’s, following the pioneering work of Shen and co-workers.\textsuperscript{170-171} The strength of VSFS lies in its intrinsic surface specificity and selectivity. VSFS can be used to identify molecules with a preferred orientation at the interface, and under certain conditions, also its composition.

The theory of VSFS has been extensively described in the literature.\textsuperscript{166, 171-172} Briefly, two pulsed laser beams, one fixed in the visible frequency range ($\omega_{\text{vis}}$), and the other tuneable in the infrared ($\omega_{\text{IR}}$), are overlapped in time and space at the interface of interest as depicted in Figure B.4.2. In the non-centrosymmetric environment of an interface, a second-order non-linear optical response generates a third laser beam (SF) carrying the sum of the frequencies of the two incident beams ($\omega_{\text{SF}}$). The SF signal is resonantly enhanced when the incoming IR

![Figure B.4.2](image)
frequency matches the vibrational modes of the molecules with a preferred orientation present at the interface.

In the co-propagating geometry presented in Figure B.4.2, the incident beams and the SF beam propagate in the same plane of incidence (XZ-plane), which is perpendicular to the surface plane (XY-plane) with respect to the laboratory coordinate system (X,Y,Z). The SF angle is given by the conservation of momentum in the X direction with:

\[
n_{SF} \omega_{SF} \sin \theta_{SF} = n_{vis} \omega_{vis} \sin \theta_{vis} + n_{IR} \omega_{IR} \sin \theta_{IR}
\]  

(50)

Referring to equation (45) the polarisation vector for sum frequency generation is:

\[
\mathbf{p}^{(2)}_{SF} = \varepsilon_0 \chi^{(2)} : \mathbf{E}_{vis} \mathbf{E}_{IR}
\]  

(51)

If we explicitly consider the two electric fields \( \mathbf{E}_{vis} = E_{vis}(r) \cos(\omega_{vis}t) \) and \( \mathbf{E}_{IR} = E_{IR}(r) \cos(\omega_{IR}t) \), equation (51) can be written as:

\[
P^{(2)} = \varepsilon_0 \chi^{(2)} \left[ \left( E_{vis}^2 + E_{IR}^2 \right) + \left( E_{vis}^2 \cos(2\omega_{vis}t) + E_{IR}^2 \cos(2\omega_{IR}t) \right) \right. \\
\left. + \frac{1}{2} E_{vis} E_{IR} \cos((\omega_{vis} - \omega_{IR})t) + \frac{1}{2} E_{vis} E_{IR} \cos((\omega_{vis} + \omega_{IR})t) \right] 
\]  

(52)

Therefore the intensity of the Sum Frequency beam (last term in the above equation) is proportional to the square of the modulus of the nonlinear susceptibility tensor \( \chi^{(2)} \) and the intensity of the visible and IR beam,

\[
I_{SF}(\omega) \propto |\chi^{(2)}|^2 I_{vis}(\omega_{vis}) I_{IR}(\omega_{IR})
\]  

(53)

The second order non-linear susceptibility links the macroscopic observable and the molecular response to the electromagnetic perturbation. It can be separated into two components, a non-resonant contribution \( \chi^{(2)}_{NR} \) and a resonant contribution \( \chi^{(2)}_{R,n} \).

\[
\chi^{(2)} = \chi^{(2)}_{NR} + \sum_n \chi^{(2)}_{R,n}
\]  

(54)

The non-resonant contribution, \( \chi^{(2)}_{NR} \) arises from different second-order optical phenomena, such as multipole interactions and is, as its name suggest, largely frequency independent. The
resonant non-linear second-order susceptibility $\chi_{R,n}^{(2)}$ carries the molecular information of interest, and is enhanced when the frequency of the IR beam matches a vibrational resonant frequency of an interfacial molecule. $\chi_{R,n}^{(2)}$ is proportional to the number density of molecules at the surface $N$, and the orientationally averaged molecular hyperpolarizability $\beta_n^{(2)}$, with $\varepsilon_0$ the permittivity of vacuum.

$$\chi_{R,n}^{(2)} = \frac{N}{\varepsilon_0} \langle \beta_n^{(2)} \rangle \tag{55}$$

The hyperpolarizability $\beta^{(2)}$ contains the information about the resonance selection rules in the molecular frame and can be expressed in the local frame of the molecule (a, b, c) as a product of the Raman polarizability tensor and the IR transition dipole moment:

$$\beta_{abc}^{(2)} = \frac{\alpha_{ab} \mu_c}{\omega_n - \omega_{IR} - i\Gamma_n} \tag{56}$$

where $\alpha_{ab}$ is the Raman tensor element, $\mu_c$ the IR transition dipole moment, $\omega_{IR}$ is the IR frequency, $\omega_n$ the frequency of the $n$th vibrational mode and $\Gamma_n$ the damping constant, also defined as the reciprocal of the lifetime of the excited state (homogenous broadening).

Equation (56) shows how the SF signal is selectively enhanced when the IR frequency ($\omega_{IR}$) approaches a vibrational frequency mode $\omega_n$. The detected SF intensity depends then in the convolution of several factors, the square number of contributing molecular oscillators ($N^2$), their average orientation ($\langle \rangle$), and the IR and Raman tensor elements.

Symmetry considerations.

The non-linear second-order susceptibility $\chi^{(2)}$ is a third-ranked tensor, composed of 27 elements $\chi_{ijk}^{(2)}$, where i, j and k could be the 3 directions in the laboratory frame X, Y, or Z, taking Z as the normal to surface plane, and Y the perpendicular to the plane of incidence as depicted in Figure B.3.2. According to Neumann’s principle\textsuperscript{173} in the electric dipole approximation, the second-order susceptibility is zero in centrosymmetric media.

$$\chi_{ijk}^{(2)} = \chi_{i-j-k}^{(2)} = -\chi_{jik}^{(2)} = 0 \tag{57}$$

Consequently, the SF signal is only generated when the inversion symmetry of the medium is broken. This situation occurs typically at interfaces, but not in bulk isotropic media. This
is the property that makes VSFS an \textit{intrinsically surface-specific} technique. The number of non-zero $\chi^{(2)}_{ijk}$ elements can be further reduced upon consideration of the interfacial symmetry. For the azimuthally isotropic air/liquid interface with $C_\infty$ planar symmetry, only seven components remain: $\chi^{(2)}_{xzz} = \chi^{(2)}_{yyz}, \chi^{(2)}_{xxz} = \chi^{(2)}_{yzy}, \chi^{(2)}_{zxx} = \chi^{(2)}_{zyz}, \text{and } \chi^{(2)}_{zzz}$ where the indices refers to the SF, visible, and IR, respectively. The polarization of the beams can be either parallel (P) or perpendicular (S, from german \textit{senkrecht}) relatively to the plane of incidence, both orthogonal to the propagation vector as shown in Figure B.3.2. By selecting different polarization combinations of the SF, visible, and IR beams specific elements of the susceptibility tensor can be probed. Table B.4.1 summarizes the different polarization combinations that give rise a non-zero SF signal for surfaces with $C_\infty$ symmetry. Far from electronic transitions, the Kleinman symmetry ensures the symmetry of the Raman tensor. Thus the two first indices of the $\chi^{(2)}$-elements are interchangeable, giving an additional simplification SPS = PSS. Consequently, three polarization combinations suffice to describe our studied system.$^{166}$

<table>
<thead>
<tr>
<th>$\chi^{(2)}$ element</th>
<th>Polarization combination (SF, vis, IR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi^{(2)}<em>{zzz}, \chi^{(2)}</em>{zzz}, \chi^{(2)}<em>{xzz}, \chi^{(2)}</em>{xxz}$</td>
<td>PPP</td>
</tr>
<tr>
<td>$\chi^{(2)}_{yzy}$</td>
<td>SSP</td>
</tr>
<tr>
<td>$\chi^{(2)}_{zyz}$</td>
<td>SPS</td>
</tr>
<tr>
<td>$\chi^{(2)}_{zyy}$</td>
<td>PSS</td>
</tr>
</tbody>
</table>

**Local field correction.**

The complete expression of the intensity of the SF beam is given by:$^{174}$

$$I_{sf}(\omega) = \frac{8\pi^3}{e^3} n_1 (\omega_{sf}) n_1 (\omega_{vv}) n_1 (\omega_{ir}) \left| \chi_{eff}^{(2)} \right|^2 I_{vv}(\omega_{vv}) I_{ir}(\omega_{ir})$$

where $\chi_{eff}^{(2)}$ is the effective susceptibility, $n_i(\omega_n)$ the refractive index of the media $i$ at frequency $\omega_n$, and $\theta_n$ the angle to the surface normal of the beam $n$. The effective
susceptibility $\chi_{eff}^{(2)}$ is linked to $\chi^{(2)}$ through the unit polarization vector $\hat{e}(\omega_n)$ and tensor $L^\prime$.\(^{90}\)

$$\chi_{eff}^{(2)} = \left[\hat{e}(\omega_{SF}) \cdot L^\prime(\omega_{SF})\right] \cdot \chi^{(2)} \cdot \left[L^\prime(\omega_{vis}) \cdot \hat{e}(\omega_{vis})\right] \cdot \left[L^\prime(\omega_{IR}) \cdot \hat{e}(\omega_{IR})\right]$$ (59)

In a co-propagating configuration, the unit polarization vector can be decomposed in the laboratory frame using the rotational matrix $R_y$ about the y axis for the incident beams, and its transpose for the SF beam:

$$\hat{e}_n = \begin{pmatrix} \cos \theta_n \\ 1 \\ \pm \sin \theta_n \end{pmatrix}$$ (60)

As mentioned in the previous section, only three polarization combinations give an independent overview of the microscopic structures. Expanding equation (59) for the three polarization SSP, SPS and PPP yields:

$$\chi_{eff, SSP}^{(2)} = L^\prime_{yy}(\omega_{SF}) L^\prime_{yy}(\omega_{vis}) L^\prime_{zz}(\omega_{IR}) \sin(\theta_{IR}) \chi^{(2)}_{yzy}$$ (61)

$$\chi_{eff, SPS}^{(2)} = L^\prime_{yy}(\omega_{SF}) L^\prime_{zz}(\omega_{vis}) L^\prime_{yy}(\omega_{IR}) \sin(\theta_{vis}) \chi^{(2)}_{yzy}$$ (62)

$$\chi_{eff, PPP}^{(2)} = -L^\prime_{xx}(\omega_{SF}) L^\prime_{xx}(\omega_{vis}) L^\prime_{zz}(\omega_{IR}) \cos(\theta_{SF}) \cos(\theta_{vis}) \sin(\theta_{IR}) \chi^{(2)}_{xxz}$$

$$-L^\prime_{xx}(\omega_{SF}) L^\prime_{zz}(\omega_{vis}) L^\prime_{xx}(\omega_{IR}) \cos(\theta_{SF}) \sin(\theta_{vis}) \cos(\theta_{IR}) \chi^{(2)}_{xxz}$$

$$+L^\prime_{xx}(\omega_{SF}) L^\prime_{zz}(\omega_{vis}) L^\prime_{xx}(\omega_{IR}) \sin(\theta_{SF}) \cos(\theta_{vis}) \cos(\theta_{IR}) \chi^{(2)}_{xxz}$$

$$+L^\prime_{zz}(\omega_{SF}) L^\prime_{zz}(\omega_{vis}) L^\prime_{zz}(\omega_{IR}) \sin(\theta_{SF}) \sin(\theta_{vis}) \sin(\theta_{IR}) \chi^{(2)}_{zzz}$$ (63)

The far-field and the local field are linked by the tensor $L^\prime(\omega)$ which is the product of the macroscopic Fresnel factor $L(\omega)$ and the microscopic Fresnel factor $l(\omega)$. $L_{xx}, L_{yy}$ and $L_{zz}$ are the diagonal coefficients of the macroscopic Fresnel factor associated with the frequency of the corresponding beam. They can be expressed with the refractive index of the media and the respective refractive angle $\theta_n$ obtained from the Snell-Descartes law. The Fresnel factor

37
depends on the geometry, and the nature of the interface, as well as the frequencies of the beams.\textsuperscript{175} Their values have been derived and presented in what follows:\textsuperscript{90, 175-176}

\begin{align}
L_{xx}(\omega_n) &= \frac{2n_1(\omega_n) \cos(\theta_n)}{n_1(\omega_n)\cos(\theta_n) + n_2(\omega_n)\cos(\theta_n)} \quad (64) \\
L_{yy}(\omega_n) &= \frac{2n_1(\omega_n) \cos(\theta_n)}{n_1(\omega_n)\cos(\theta_n) + n_2(\omega_n)\cos(\theta_n)} \quad (65) \\
L_{zz}(\omega_n) &= \frac{2n_2(\omega_n) \cos(\theta_n)}{n_1(\omega_n)\cos(\theta_n) + n_2(\omega_n)\cos(\theta_n)} \quad (66)
\end{align}

The correction for the local field factors $l_{nn}$ appears in the expression of the non-linear susceptibility $\chi^{(2)}$. Shen and Heinz proposed a simplified model to take into account the local field in the expression of the macroscopic Fresnel factor.\textsuperscript{177-178} The system exposes a three layers configuration where the bulk medium 1 and 2 are separated by an ultrathin polarization layer characterized by its “refractive index” $n'$, defined as the ratio of $l_{xx} = l_{yy}$ over $l_{zz}$. The model suggests to account for the local field by correcting the expression (63) of the macroscopic Fresnel factor $L_{zz}$ by multiplying with the ratio:

$$\left(\frac{n_2}{n'}\right)^2$$

A common approach considers $n'$ as equal to one of the bulk refractive indexes, either $n_1$ or $n_2$, or, in the case of an organic monolayer, as the bulk refractive index of the material at the interface. Alternatively, theoretical and experimental models have been suggested other approaches to estimate the $n'$ value.\textsuperscript{90} The situation becomes even more complex when considering that the refractive index of the medium 2, and specifically at a charged interface, is varying at high salt concentrations. Properly accounting for the microscopic local field factors remains a subject of debate. The formalism adopted in this thesis follows the derivations and Fresnel factor corrections proposed by Liljeblad et al.\textsuperscript{175}

**Modeling the SF signal.**

The emission of the SF light from an interface is a coherent process, and information is carried both in the phase in the magnitude of the signal. To extract quantitative information from the recorded SF spectra, the fitting procedure needs to account for interference, both
constructive and destructive, between adjacent modes as well as the non-resonant background. In this study the SF signal is modelled using Voigt-like profiles of the form presented in equation (68),

\[
I_{SF} \propto A_{NR}^{(2)} + \sum_{n} \int \frac{-A_n e^{-\left((\omega_\text{IR} - \omega_n)^2/2\sigma_n^2\right)}}{\sqrt{2\pi\sigma_n^2 \left(\omega_\text{IR} - \omega_n^I + i\Gamma_n^I\right)}} d\omega_n^I \tag{68}
\]

where, \(A_{NR}\) is the non-resonant contribution to the SF signal, \(A_n\) the amplitude or oscillator strength of the \(n^{th}\) resonant mode, \(\omega_\text{IR}\) the infrared frequency, \(\omega_n\) the peak position, and \(\Gamma_n\) and \(\sigma_n\), to the Lorentzian and Gaussian line widths, respectively. For dielectric media, the NR contribution is taken as a real constant as we are far from electronic transitions. Alternatively, a simpler fitting function is sometimes used, consisting of only Lorentzian line shapes based on the formalism of equations (53) to (56). This method has the advantage of presenting fewer fitting parameters and thus reducing the number of assumptions. However, the convolution of Lorentzian and Gaussian line shapes account for both homogeneous (Lorentzian line shape) and inhomogeneous broadening (Gaussian line shape). The fitted amplitude is directly proportional to the number of oscillators and opposite signs may reveal contrasting net polar orientations.

d. Orientation analysis

As mentioned in equation (55) each \(\chi^{(2)}\) tensor elements are related to the hyperpolarizability \(\beta^{(2)}\) specific to a given vibrational mode, and thus a certain chemical group. Probing the interface with different polarization combinations gives a direct insight into the interfacial orientation of the molecules. Three different coordinate systems need to be considered: the laboratory frame \((X,Y,Z)\), the surface frame \((x,y,z)\), and the molecular frame \((a,b,c)\). The two first coordinate systems coincide for an isotropic planar surface. The hyperpolarizability is expressed in the molecular frame, while the observables are detected in the laboratory frame. Thus the orientation analysis consists in determining the elements needed to relate the molecular to the laboratory frame, in terms of the azimuthal, tilt, and twist angles \((\psi,0,\phi)\) as shown in Figure B.4.3. With a given experimental geometry, and knowing or assuming the molecular symmetry properties of the vibrational mode under consideration, the intensity of the SF signal is calculated for the three polarization combinations and quantitatively
compared with the fitted amplitudes from the VSF spectra. A detailed protocol has been given by Wang et al. considering different chemical groups and geometries.\textsuperscript{172}

Numerous investigations have used an orientation analysis procedure to determine the average orientation of the alkyl chain of fatty acid Langmuir monolayers at the air/water interface,\textsuperscript{89, 91, 95} as well as in closely related systems giving information on the packing of the chain,\textsuperscript{62, 180} and the orientation of the carboxylic acid headgroup.\textsuperscript{181-183} The following section focuses on the method applied in paper I for the particular case of the stretching mode of the carbonyl bond.\textsuperscript{184}

**Protocol**

**Local symmetry group.** The first step is to consider the symmetry properties of the functional group under investigation to determine the corresponding hyperpolarizability tensor elements in the molecular frame (a,b,c). For the fatty acid monolayer three groups are mostly considered: the terminal methyl group (-CH\textsubscript{3}), which when freely rotating can be regarded as having C\textsubscript{3v} symmetry, the methylene group (-CH\textsubscript{2}) with C\textsubscript{2v} symmetry, and the hydroxyl (-OH) as well as the carbonyl (C=O) groups with C\textsubscript{∞v} symmetry, when assuming that the OH and/or carbonyl groups are energetically decoupled from the rest of the molecule. For a C\textsubscript{∞v} group, there are only three non-zero hyperpolarizability tensor elements of which two are independent: $\beta_{abc} = \beta_{bbc} = R \beta_{ccc}$, where $R$ is the ratio between the two independent hyperpolarizabilities.

**Raman depolarization ratio.** The ratio between the two independent hyperpolarizability tensor elements in a C\textsubscript{∞v} vibrational mode can be determined from the Raman depolarization ratio.
ratio, $\rho$. The procedure can also be followed for symmetric $C_{3v}$ modes, which have the same non-zero independent elements. The Raman depolarization ratio is calculated from the Raman intensity ratio of the vibrational mode of interest collected at a polarization perpendicular and parallel to that of the incident radiation: $\rho = I_\perp / I_\parallel$. This ratio can then be expressed in terms of $R$ as follows:

$$\rho = \frac{3}{4} \left[ 1 + \frac{5}{4} \left( \frac{2R+1}{R-1} \right)^2 \right]^{-1} \quad (69)$$

since $R$ is also equivalent to the ratio of the independent diagonal elements in the Raman tensor:

$$R = \beta_{\alpha\alpha} / \beta_{\varepsilon\varepsilon} = \alpha_{\alpha\alpha} / \alpha_{\varepsilon\varepsilon}$$

**Euler angle transformation.** The conversion between the molecular system and the surface or laboratory coordinate system is performed by Euler angle transformations as shown in Figure B.4.3. The expression for the Euler transformation matrix is given in Table B.4.2 for a Cartesian system as a function of the azimuthal, tilt, and twist angles ($\psi, \theta, \phi$).\(^{172, 185}\) For the special case of a Langmuir monolayer at the air/liquid interface we consider an azimuthal isotropic interface. The Euler angles $\phi$ and $\psi$ can thus be spatially integrated. The remaining elements in the final expression of $\chi^{(2)}_{ijk}$ are only dependent on $\theta$, the angle between the molecular $c$ axis and the surface normal ($z$).

**Table B.4.2.** Elements of the Euler rotation matrix as presented by Wang et al.\(^{172}\)

<table>
<thead>
<tr>
<th></th>
<th>$m_{x,a}$</th>
<th>$m_{x,b}$</th>
<th>$m_{x,c}$</th>
<th>$m_{y,a}$</th>
<th>$m_{y,b}$</th>
<th>$m_{y,c}$</th>
<th>$m_{z,a}$</th>
<th>$m_{z,b}$</th>
<th>$m_{z,c}$</th>
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<tbody>
<tr>
<td>$m_{x,a}$</td>
<td>$\cos \psi \cos \phi - \cos \theta \sin \phi \sin \psi$</td>
<td>$-\sin \psi \cos \phi - \cos \theta \sin \phi \cos \psi$</td>
<td>$\sin \theta \cos \phi$</td>
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<td>$-\sin \theta \cos \phi$</td>
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<tr>
<td>$m_{x,b}$</td>
<td>$-\sin \psi \cos \phi - \cos \theta \sin \phi \cos \psi$</td>
<td>$\cos \psi \cos \phi - \cos \theta \sin \phi \sin \psi$</td>
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<td>$\sin \theta \cos \phi$</td>
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<tr>
<td>$m_{y,a}$</td>
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<td>$-\cos \psi \sin \phi$</td>
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<tr>
<td>$m_{y,b}$</td>
<td>$-\cos \psi \sin \phi$</td>
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<td>$\sin \theta \cos \phi$</td>
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</table>

**Expressing the susceptibility elements.** The $\chi^{(2)}$-elements are related to the averaged orientation of the non-zero hyperpolarizability tensor elements as described in equation (55).
For the stretching mode of the carbonyl (C=O), the final expressions for the second-order susceptibility elements in the laboratory frame are:

\[ \chi_{yzz} = \frac{N}{\varepsilon_0} \left\langle \beta_{yzz} \right\rangle - \frac{1}{2} \frac{N}{\varepsilon_0} \beta_{ecc} \left[ (R+1)\left\langle \cos \theta \right\rangle + (R-1)\left\langle \cos^3 \theta \right\rangle \right] \]  
(70)

\[ \chi_{yzy} = \frac{N}{\varepsilon_0} \left\langle \beta_{yzy} \right\rangle - \frac{1}{2} \frac{N}{\varepsilon_0} \beta_{ecc} \left[ (1-R)\left\langle \cos \theta \right\rangle - \left\langle \cos^3 \theta \right\rangle \right] \]

\[ \chi_{zzz} = \frac{N}{\varepsilon_0} \left\langle \beta_{zzz} \right\rangle - \frac{N}{\varepsilon_0} \beta_{ecc} \left[ R\left\langle \cos \theta \right\rangle + (1-R)\left\langle \cos^3 \theta \right\rangle \right] \]

where \( N \) refers to the number of molecules, \( \theta \) the tilt angle, \( R \) the hyperpolarizability ratio and \( \left\langle \ldots \right\rangle \) the averaging function over all angles.

**Angle distribution function.** The averaging of the tilt angle requires a choice of distribution function. A \( \delta \)-function is commonly used meaning that all molecules at the surface tilt with exactly the same angle. However, a more realistic alternative is a Gaussian distribution function, as shown in the equation bellow,

\[ \left\langle f(\theta) \right\rangle = \frac{1}{\sigma \sqrt{2\pi}} \int_{-\infty}^{\infty} f(\theta) \exp \left( -\frac{(\theta - \theta_0)^2}{2\sigma^2} \right) \sin \theta d\theta \]  
(71)

where \( \theta_0 \) is the mean orientation angle, \( \sigma^2 \) the variance, and \( f(\theta) \) the function to average.

**SF amplitude calculation.** Once the Fresnel factors are calculated, using equations (64) to (66), the effective second-order susceptibility from can be expressed as a function of the tilt angle \( \theta \), using equations (61) to (63) and (70). In this thesis, the refractive indexes of the bulk incident phase (\( n_i \), air) are trivially set to 1, while for the bulk liquid phase (\( n_2 \)) those from pure water at the corresponding frequencies are used. The refractive indexes for the interfacial thin polarization sheet are arbitrarily set to 1.25. The effective second-order susceptibility elements are directly related to the intensities \( I_{SSP} \), \( I_{SPS} \), and \( I_{PPP} \) measured in experiments. Unknown parameters that are redundant in each equation are trivially set to 1 for comparison, but the ratios of the different combinations are usually discussed (SSP/SPS, SPS/PPP, and SSP/PPP). The amplitude ratios determined from the experimental spectra are then compared, and the tilt angle identified at the intersection with the computed curves, as shown in Figure B.4.4 for the carbonyl stretching mode in an arachidic acid monolayer.
As detailed previously, in the dipole approximation VSFS only detects molecules with a preferred orientation (non-centrosymmetric media), meaning that the technique requires no background subtraction when probing interfacial molecules. However, at a charged aqueous interface, the static electric field generated at the surface propagates through the immediate bulk solution forming an electrical double layer (EDL), that perturbs or partially orient water dipoles. The reorientation results in a break of the centrosymmetry of the bulk solution in the diffuse double layer, and consequently leads to an additional non-linear optical response. Eisenthal and co-workers were the first to account for this response by establishing a linear relationship with the surface potential in second harmonic generation studies. In the original derivation, the SH (or SF) response was divided into two elements, the first includes contributions from molecules in the direct interfacial region \( \chi^{(2)} \), while the second arises from contributions further into the bulk in the diffuse layer, \( \chi^{(3)} \). The later represents all phenomena induced by interactions with the propagating electric field \( E_{dc}(z) \), such as the field-induced oriented water dipoles or bulk dipoles interactions.

**e. Probing the surface potential: the \( \chi^{(3)} \) model**

As detailed previously, in the dipole approximation VSFS only detects molecules with a preferred orientation (non-centrosymmetric media), meaning that the technique requires no background subtraction when probing interfacial molecules. However, at a charged aqueous interface, the static electric field generated at the surface propagates through the immediate bulk solution forming an electrical double layer (EDL), that perturbs or partially orient water dipoles. The reorientation results in a break of the centrosymmetry of the bulk solution in the diffuse double layer, and consequently leads to an additional non-linear optical response. Eisenthal and co-workers were the first to account for this response by establishing a linear relationship with the surface potential in second harmonic generation studies. In the original derivation, the SH (or SF) response was divided into two elements, the first includes contributions from molecules in the direct interfacial region \( \chi^{(2)} \), while the second arises from contributions further into the bulk in the diffuse layer, \( \chi^{(3)} \). The later represents all phenomena induced by interactions with the propagating electric field \( E_{dc}(z) \), such as the field-induced oriented water dipoles or bulk dipoles interactions.
From equation (45), the SF response can be expressed as a sum of a second and third-order NL phenomena:

$$P_{SF}^{(2)} = P_{s}^{(2)} + P^{(3)}$$ (72)

$$P_{SF}^{(2)} = \varepsilon_0 \chi_0^{(2)} : E_{vis} E_{IR} + \varepsilon_0 \chi_0^{(3)} : E_{vis} E_{IR} E_{DC} (z)$$ (73)

Projecting on the z-axis, the third-order polarizability can be written as:188-191

$$P^{(3)} = \varepsilon_0 \chi_0^{(3)} E_{vis} E_{IR} \left( \psi_0 + i \Delta k_z \int_0^z \psi(z) e^{i \Delta k_z z} dz \right)$$ (74)

Similarly, from the extension of equation (53), the intensity of the SF signal ($I_{SF}$) is proportional to:

$$I_{SF} \propto I_{vis} I_{IR} \left| \chi_0^{(2)} + \chi_0^{(3)} \int_0^z E_{DC} (z) e^{i \Delta k_z z} dz \right|^2$$ (75)

where $I_{vis}$ and $I_{IR}$ are the visible and IR beam intensities, respectively, and $\Delta k_z$ is a phase factor accounting for the limited coherence length of the SF process.

**Coherence length.**

Depending on how rapidly the electric field decays from the surface, the probing depth can significantly vary. For the lowest ionic strengths, this depth could be of several microns. However, since SF generation is a coherent process involving three different beams, the SF signal generated will only add constructively within a limited probing depth, defined by the coherence length of the non-linear optical process, $\pi \Delta k_z^{-1}$. This length depends on the geometry of the experimental setup, which for the case of a co-propagating reflexion geometry is given by:191

$$\Delta k_z^{-1} = \left| k_{(e_0,z)} + k_{(e_0,\perp z)} - k_{(e_0,\perp z)} \right|^{-1}$$ (76)

$$k_{(e_0,z)} = \left( \frac{2 \pi n z}{\lambda_1} \right) \cos \theta_i$$ (77)

where $\lambda_1$ is the wavelength in vacuum, and $k_{(e_0,\perp)}$ the wave vector. If the surface electric field propagates further that the coherent length, destructive interference will reduce the global SF intensity, and the $\chi_0^{(3)}$ contribution will no longer be proportional to the surface potential.
**Interference factor.**

In the EDL formalism, the interfacial electric field interacts with water dipoles in the diffuse double layer, thus the maximum probing depth should approximately correspond to the length of the diffuse double layer. In the frame of the Debye-Hückel approximation, valid for surface potentials below ~25 mV, the propagating electric field is expected to exponential decay from the surface. Equation (75) then becomes:

\[ I_{SF} \propto I_{vis} I_{th} \left| \chi_s^{(2)} + \chi^{(3)} \psi_0 \left( \frac{\kappa}{\kappa - i\Delta k_z} \right)^2 \right. \]  

(78)

Where \( \kappa^{-1} \) refer to the Debye screening length, and \( \frac{\kappa}{\kappa - i\Delta k_z} \) the interference factor. In the geometry of our experiments \( \Delta k_z^{-1} \approx 51 \) nm. The Debye length depends on the concentration of electrolyte and the valence of the salts. If \( \kappa^{-1} \ll \Delta k_z^{-1} \), typically for concentrations above 1 mM, the interference factor is \( \approx 1 \). Consequently the \( I_{SF} \) is proportional to the surface potential, while for \( \kappa^{-1} \gg \Delta k_z^{-1} \) signal from within the diffuse double layer \( \chi^{(3)} \) will totally cancel due to destructive interference. However, for surface potential above ~25 mV, which is typically the case at low ionic strengths in most charged surfaces, \( \psi_{(z)} \) does no longer decay exponentially and equation (78) is no longer valid. A general expression of the third order contribution has been recently presented by considering the full analytical solution to the Poisson-Boltzmann equation and where the interference factor is expressed as a series expansion.191
C. Experimental section

This chapter presents the materials, chemicals, and instruments used in the frame of this thesis. Particular attention is drawn on the cleaning and purification procedures that involved a significant time of the project. The two main techniques used to carry out the experiments, vibrational Sum Frequency spectroscopy, and the Langmuir-Blodgett (LB) trough, are thoroughly described.

1. Materials

   a. Fatty acid

In this thesis, the focus has been on the saturated eicosanoic acid (C\textsubscript{20}H\textsubscript{40}O\textsubscript{2}), also known as arachidic acid and depicted in Figure C.1. With a twenty carbon long alkyl chain, the compound is essentially insoluble in water, making it a perfect candidate to form monomolecular Langmuir monolayers at the air/water interface. The saturated alkyl chain allows forming a highly ordered and densely packed monolayer. The perprotonated eicosanoic acid, CH\textsubscript{3}(CH\textsubscript{2})\textsubscript{18}COOH (≥99%, CAS 506-30-9) and the corresponding deuterated compound, eicosanoic-d\textsubscript{39} acid CD\textsubscript{3}(CD\textsubscript{2})\textsubscript{18}COOH (97%, CAS 39756-32-6) were obtained from Sigma-Aldrich and used as received.

![Molecular structure of the eicosanoic acid, C\textsubscript{20}H\textsubscript{40}O\textsubscript{2}.](image)

Figure C.1. Molecular structure of the eicosanoic acid, C\textsubscript{20}H\textsubscript{40}O\textsubscript{2}.

In order to widen the perspectives and conclusions of this work other noteworthy amphiphile monolayers were investigated, which included a fatty alcohol, a fatty sulphate, and a fatty amine. Changing the nature of the hydrophilic headgroup allowed exploring the effect of the headgroup charge at the interface, which ranged from neutral in the case of the fatty alcohol, to positively charged for the fatty amine, and finally negatively charged over the full pH range in fatty sulphate case. Nonetheless, only limited results from these studies are presented in this thesis, which mainly focused on the fatty acid Langmuir monolayers, exposing the carboxylic acid moiety to solution.
b. Salts & speciation

The purity grade and suppliers of the salts investigated in this work are presented in Table C.1.1. The list can be divided in 5 main categories depending on the nature of the salt: alkali metal hydroxides (1:1), alkali metal chlorides (1:1), sodium thiocyanate and halides (1:1), and divalent (1:2) and trivalent (1:3) transition metal and rare earth chloride salts. All were supplied with the highest purity grade available on the market. The importance of the purity of the salts is discussed in detail in the next section.

The speciation of the salts, particularly those involving divalent and trivalent ions can show a complicated behaviour in solution, as some can oxidize or form hydroxide complexes depending on the concentration and pH. The speciation of the salts was first evaluated using the software HYDRA and MEDUSA, which allows building speciation diagrams showing the concertation of the species of interest as a function of pH, as well as predominance and Pourbaix diagrams (E-pH diagram). Based on the SOLGASWATER\textsuperscript{192} and HALTAFALL\textsuperscript{193} algorithms, the software authored by Ignasi Puigdomenech can be downloaded free of charge from the author’s webpage. HYDRA contains a database of chemical equilibria constant at 25°C. The package only requires as input the selection of the ionic and/or complex species, their concentrations and the range of pH of the study. The amount of carbonate can be specified either with the atmospheric partial pressure of CO\textsubscript{2} (3.5·10\textsuperscript{-4} atm) or in terms of the adsorbed concentration of carbonate ions. In this studies, the concentration of carbonate species (CO\textsubscript{3}\textsuperscript{2-}) was set to 1 \(\mu\)M in order to match the usual amounts found in biological systems and experimental conditions.\textsuperscript{194-195} Figure C.2 shows an example of the speciation diagrams for 1 \(\mu\)M (left) and 1 mM (right) YCl\textsubscript{3} at room temperature. The diagram for 1 \(\mu\)M YCl\textsubscript{3} is plotted for 10\textsuperscript{-6} M (bold line) and 10\textsuperscript{-4} M (dot lines) total concentration of dissolved CO\textsubscript{2}, while the diagram for 1 mM YCl\textsubscript{3} is plotted for 10\textsuperscript{-6} M dissolved CO\textsubscript{2}. The diagrams show the concentration of each species in logarithmic scale as a function of pH. Further examples can be seen for the speciation diagram in terms of fractions of the total amount for each species on Figure C.5, and the Pourbaix diagram on Figure C.6.
Figure C.2. Speciation diagrams of 1 μM (left) and 1 mM (right) of YCl₃ salt at room temperature. The left diagram is plotted with 10⁻⁶M (bold line) and 10⁻⁴M (dot lines) total concentration of dissolved CO₂. The diagrams were obtained from HYDRA and MEDUSA software.

2. Cleaning procedures

The fatty acid monolayers were found to be highly sensitive to the presence of organic and/or ionic impurities. Thereby, a number of different precautions were taken when preparing the experiments, which involved several steps for cleaning or purifying, both materials in contact with the solutions, as well as the as received salts.

a. Glassware cleaning procedure

The glassware was cleaned following a 3 steps sonication procedure: first in ethanol (95%) for 10 min, followed by 5% Deconex solution (commercial alkaline agent, Borer Chemie) for 20 min, and finally in ultrapure MilliQ water, obtained from an Integral 15 Millipore system featuring a constant resistivity (18.2 MΩ.cm) and low total organic content (< 3 ppb). Between each step, the glassware was rinsed at least ten times with ultrapure water. The volumetric flasks required additional precautions as the neck also had to be carefully cleaned. This was accomplished by immersing the flasks upside down inside beakers containing the same cleaning solutions and repeating each sonication step. Once finalized, the glassware surfaces expected to be in contact with the salt solutions were kept immersed in ultrapure water and sealed until further use. Plastic materials, such as the Teflon beaker or syringe tubes, are cleaned in the same way. However, the Deconex step is skipped for the rubber materials, as this material is highly porous and soaps could be absorbed.
<table>
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<th>Salts</th>
<th>CAS Nr.</th>
<th>Purity</th>
<th>Grade</th>
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<tr>
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<td>EDTA</td>
<td>60-00-4</td>
<td>99.995 %</td>
<td>Sigma-Aldrich</td>
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**Table C.1.1.** List of the salt investigated in this thesis, including specifications of the purity grade and the supplier.
b. Salt purification: Removal of organic contaminants

It was found that many of the as received salts contained significant amounts of surface-active organic contaminants that could influence the properties of the Langmuir monolayers. This became evident by measuring the surface tension of the pure salt solutions. It is thought that organic contaminants are introduced into the salts during the various purification steps performed by the supplier to achieve the trace metals purity grade specification. In order to remove the organic impurities, the salts were either baked at high temperatures or alternatively subjected to a multi-step aspiration procedure, similar to that proposed by Lunkenheimer for purifying surfactant solution.68, 196-197 The latter option was used for the temperature-sensitive salts that could not sustain the heat treatment. The two purification procedures are described in detail below.

**Baking procedure.**

The salt is poured inside a dry ceramic crucible that had been previously cleaned following the procedure described above for glassware. The crucible is then covered by a loosely fit ceramic lid and placed in an oven at 500°C for at least one hour. The salt is then slowly cooled down to ~120°C before being removed from inside the oven. This procedure is used for the alkali metal chloride salts (NaCl, LiCl, KCl, CsCl) and sodium halides, yet cannot be applied for hydrated salts, as well as those that can easily oxidize.

**Multi-step aspiration procedure.**

This procedure was used for removing surface-active organic contaminants from solutions containing divalent ions or NaSCN. It is worth noting that when heated, the thiocyanate salt can oxide and produce the extremely poisonous hydrogen cyanide gas. The reaction does not happen spontaneously at room temperature.198-200 Salt solutions were cleaned using either the automated Lukenheimer high-performance purification apparatus,196-197 or an equivalent manual procedure. In this method, surface-active contaminants are removed by repeatedly aspirating the surface of highly concentrated salt solutions. In practical terms, the solution is first poured into a thoroughly cleaned glass container and gently stirred with a magnetic stirrer for a given amount of time (5 to 10 minutes). Stirring accelerates the adsorption of surface active contaminants expected at trace level concentrations in the bulk, allowing equilibrium conditions to be reached at a faster rate. The surface of the solution is then aspirated using a Teflon or stainless steel pipette, removing a volume that is typically in the
order of 0.2 ml or less. This procedure is repeated several times (2 to 200 times) until the contaminants are depleted from the bulk solution, as evidenced by a constancy in the surface tension (see Figure C.3). During the whole procedure, emphasis is made to minimise contact with the external atmosphere. Surface tension was measured using the Wilhelmy plate method, which records the change in weight experienced by a platinum plate when brought into contact with an electrolyte solution. An example from two different CaCl₂ salts is shown in Figure C.2. The surface tension of most salt solutions above ~10 mM is expected to be higher than that of pure water (72.8 mN/m at 20°C), with the actual value depending on the concentration and nature of the salts. The surface tension of the “as received” 99.5% CaCl₂ 500 mM solution (Figure C.2 left), is 67 mN/m at time zero and decreases further with time, clearly indicating the presence of surface active impurities. After three aspiration steps, the surface tension increases to 73 mN/m and become stable with time, showing that most of the contaminants have been effectively removed. For the case of the anhydrous 99.99% CaCl₂ (Figure C.2 right), the surface tension of the solution from step zero is stable and already above that of pure water with no further improvement observed upon aspiration of the surface. This implies that the “as received” anhydrous sample was essentially free from surface-active organic contaminants and that no aspiration steps were required. It is worth noting that all anhydrous samples purchased showed no significant amounts of surface-active contaminants, probably reflecting the fact that when manufactured, the samples are typically heated up to high temperatures to remove water, a process that most certainly also eliminates organic compounds.

Figure C.3. Cleaning procedure of the salts. Surface tension measurements of the hydrated CaCl₂ (99.5% trace metal analysis), and the anhydrous CaCl₂ (99.99% trace metal analysis). Data reproduced from Mikael Scott’s Masterthesis.203
3. Solution preparation

Stock solutions are prepared by weighing in the balance the correct mass with at least three significant units directly in cleaned and N₂ dried volumetric flasks. Solutions with lower concentrations are typically prepared by serial dilution starting from the stock. The volume is extracted with a glass Pasteur pipettes, and diluted in ultrapure MilliQ water. Depending on the salt used, additional precautions are taken as described in the following subsections.

a. Removal of trace metal ions

Trace metal polyvalent ions at sub-micromolar concentrations can have a large influence on the properties of the fatty acid monolayers. Even salt purchased with a 99.999% trace metals basis grade meets will contain 10 ppm of ionic impurities, which correspond to ~10 μM when preparing 1 M solutions of the given salt. For salts with 99.98% trace-metals purity, the concentration of unwanted ionic species will accordingly increase to ~200 μM. The following metal cations are typically listed as potential ionic impurities in the salts included in table C.1: Na⁺, K⁺, Rb⁺, Ca²⁺, Mg²⁺, Sr²⁺, Zn²⁺, Ni²⁺, Cu²⁺, Fe²⁺, Al³⁺, Fe³⁺, As³⁺ (…) Of particular concern are the divalent and trivalent ions which can strongly interact with the carboxylate headgroup and compete for adsorption sites on the fatty acid monolayer. The effect of trace amounts of polyvalent ions was observed in solutions containing monovalent salts, such as alkali metal chloride and alkali metal hydroxide salts, as well as divalent salts with relatively low trace metals purity, such as 99.5% calcium dichloride for concentrations starting from 100 mM.

Ethylenediaminetetraacetic acid (EDTA)

![Molecular structure and acidity diagram of the EDTA. The diagram was obtained from the HYDRA and MEDUSA software.](image)

**Figure C.4.** Molecular structure and acidity diagram of the EDTA. The diagram was obtained from the HYDRA and MEDUSA software.
In order to remove traces of polyvalent impurities, ethylenediaminetetraacetic acid (EDTA) in a concentration ranging between 0.5 and 20 μM was added to solution. EDTA is a chelating agent exposing four carboxylic acid groups, thus it shares the same ionic affinity as the fatty acid monolayer. The stability constants of EDTA – metal complexes have been tabulated by Anderegg et al. for a large number of metal cations obtained from numerous studies. The affinity for the divalent and trivalent ions are typically 10 to 20 order of magnitude higher than for the monovalent salts, which makes EDTA very effective in capturing trace amounts of polyvalent cations even in the presence of a larger amount of monovalent cations in solution. Figure C.4 shows the molecular structure of the EDTA and the speciation diagram obtained from the HYDRA and MEDUSA software.

An illustration of the effect is shown in Figure C.5. The VSF spectra of dAA monolayers on what appear to be identical subphases (i.e. 1 M NaCl), show large variations in the carboxylate and carbonyl stretching features, as well as a complex time-dependent behaviour (spectra in grey). Solutions were prepared using NaCl with the highest purity grade available in the market (99.999% trace metals), from either different suppliers, different batches of the same supplier, or even the same batches. It is worth noting that no significant changes could be detected in the surface pressure vs molecular area isotherms. The addition of 0.5 μM (red) EDTA to the subphase was sufficient to remove all inconsistencies and time-dependent features, ensuring the repeatability of the experiments.

Iron (II) is taken here as an example to illustrate the effect of EDTA. Iron is a common component present as a trace metal impurity in many salts, and in principle, due to corrosion, also found as an airborne contaminant in dust. The binding constant of Fe²⁺ with the carboxylate group is at least 10 order of magnitude higher than the one of the Na⁺ cations. The speciation diagram of 1 μM iron (II) is shown in Figure C.5.b in the presence of 1 M NaCl. The fraction shows the relative proportion of iron species in solution. Approximately 40 % of iron (II) remains in the form of the free metallic ion until pH 10, and forms solid Fe(OH)₂(s) that precipitates out from solution at higher pH. The graph in Figure C.5.c shows the effect of the EDTA when introduced in the same proportion. From pH 4, most of the iron in the solution forms a stable complex with EDTA, and the amount of free ions will decrease by two orders of magnitude in the biologically relevant pH range that extended between pH 4 and 8. Noteworthy, since EDTA releases hydronium ions when
dissolved in water, it will lower the pH when the concentration exceeds 1 μM. Thus the pH should be carefully controlled.

EDTA has long been used in the Langmuir monolayers community in order to remove trace metal divalent and trivalent ions from solution.\textsuperscript{80, 206-207} It has a relatively high solubility in water (1.37 mM at 20°C) and shows no surface enrichment, as proven by surface tension and VSFS measurements. See the appendix of paper I for details.\textsuperscript{184}

**Figure C.5.** a) VSF spectra collected in the SSP polarization combination in the carboxylate-carbonyl stretching region (1200 – 1900 cm\(^{-1}\)) of dAA Langmuir monolayers on a 1 M NaCl (99.999%, trace metal basis) salt solutions. b) & c) HYDRA and MEDUSA speciation diagrams of iron (II) 1 μM in NaCl 1 M, as a function of solution pH in the presence or absence of EDTA (1 μM).
b. pH adjustment

The pH of the solutions was adjusted using NaOH or HCl (36.5%). The pH was measured using two different electrodes connected to a Mettler Toledo pH meter FiveEasy™ FE20.

- Electrode 1: pH electrode LE409 from Mettler Toledo, porous pin geometry
- Electrode 2: pH electrode pHC2701-6, high KCl flow, annular geometry, purchased from Radiometer Analytica

The first electrode, less sensitive, was used for the preparation of high alkaline solutions. The second, having a faster equilibration rate, was suitable for measuring the pH of low ionic strength solutions. The two electrodes were calibrated using a set of two of the three AVS TITRINORM uncoloured buffers (4.00, 7.00 and 10.00) purchased from VWR.

Ionic specific effect at the pH electrode.

In certain occasions, the two electrodes failed to provide accurate pH values for solutions with high salt concentrations, or even just μM concentrations of specific divalent and trivalent cations. This effect is mostly due to ionic specific interactions between the negatively charged silica surface of the electrode and the cation and/or anions of the electrolyte. The effect was most noticeable for highly polarizable anions such as SCN⁻ and I⁻, as well as metallic cations with a high affinity to the silica surface that can compete with K⁺ for adsorption sites on the electrode’s surface. The contamination of the electrode with such electrolytes can also decrease the electrolyte flow in the salt bridge junction. The electrodes were thus ineffective for determining the pH of concentrated NaI and NaSCN solutions, as well as those containing NiCl₂, MnCl₂, YCl₃, and LaCl₃. For such cases, the pH was adjusted prior to adding the salt crystals. After dissolution the pH was verified with a series of different pH indicator papers (MColorpHast™ pH 0 – 14, 4.0 – 7.0, 6.5 – 10 and 11.0 – 13.0 and Whatman® Panpeha™ pH 0 – 14 from Merck).

Further experimental precautions for controlling the pH control.

Once divalent or trivalent ions adsorbed to the electrode surface, to avoid cross-contamination and repeatability of the results, the electrode surface was regenerated following the procedure suggested by the supplier. Briefly, the electrode was soaked in a slightly acidic solution (pH ~4) overnight, and then copiously rinsed in water, the KCl solution inside the electrode was replaced.
The adsorption of the CO$_2$ from the ambient air causes the ionic strength to increase and acidifies the solution with a significant change of pH over time that could affect the fatty acid Langmuir monolayer.$^{65, 80, 195}$ However, in the conditions of our experiments the variation of pH was measured to be stable within the time of acquisition ($\pm 0.1$ for 1h30). It is worth noting that the sample position of the VSF spectrometer is purged with filtered air free from CO$_2$.

c. Oxidizing species

Some of the ions studied in this research project are involved in Red-Ox reactions, and can spontaneously oxide or reduce in contact with air and/or water. Figure C.6 shows the Pourbaix diagram of Iodide and Manganese calculated with the HYDRA and MEDUSA software, using Na$^+$ and Cl$^-$ as counter-ions respectively. A Pourbaix diagram, or potential/pH diagram ($E_{\text{SHE}}$-pH, voltage potential with respect to the standard hydrogen electrode) gives the predominant species of an electrochemical system as a function of the pH of the solution. The green lines mark the stability region for water. The lines in the diagram show the equilibrium conditions calculated from the Nernst equation (vertical transitions) and/or complexation equilibrium (horizontal transitions). However, no information can be drawn concerning the kinetics of the reactions.

\[ 
\begin{align*} 
E_{\text{SHE}} / V & \quad \text{pH} \\
\text{a) NaI} & \quad \text{b) MnCl}_2 
\end{align*} 
\]

**Figure C.6.** Pourbaix diagram of NaI *(left)* and MnCl$_2$ *(right)* at respectively 1 M, and 10 mM electrolyte concentrations. The diagrams have been plotted using the HYDRA and MEDUSA software, with a concentration of absorbed CO$_2$ equal to $10^{-6}$ M and at 25°C.

In the whole pH range examined, the oxidized forms of iodide, mainly I$_5^-$, I$_2$, and IO$_3^-$, are stable in the stability range of water (green dashed lines on the Pourbaix diagram).
The oxidation reaction of iodide with dioxygen is promoted in acidic media, following the chemical reaction: \( \text{O}_2(\text{aq}) + 4\text{H}^+ + 6\text{I}^- \rightarrow 2\text{H}_2\text{O} + 2\text{I}_3^- \).\textsuperscript{22,208-210} This reaction is spontaneous but slow in ambient conditions. The kinetic of oxidation depends on the concentration of anions, the pH, the amount of dissolved \( \text{O}_2 \), and is accelerated when exposed to UV radiation. The presence of triiodide gives a pale yellowish tint to the solution and also increases the pH by consuming \( \text{H}^+ \) ions. In order to avoid the oxidation of iodide, 10 to 50 mM of sodium thiosulphate, a strong reducing agent, was added to the solution. The chemistry follows the reaction of the well-known iodometry titration. However, it was confirmed that at neutral and high pH the presence or absence of the reducing agent had no significant effect on the VSF spectra of dAA (Paper III). Regarding manganese, the Pourbaix diagram in Figure C.6 shows that many different Mn oxides are stable in aqueous solution. It is experimentally observed that the oxidation of Mn(II) to Mn(III) occurs spontaneously for 100 mM MnCl\(_2\), when increasing the pH to 8 upon addition of NaOH, giving a yellow/brownish tint to the solution. In order to verify that only Mn\(^{2+}\) ions in their free form were present in solution in the experimental conditions used in the VSF studies, UV-Vis adsorption experiments were performed for different MnCl\(_2\) concentrations in ultrapure water at pH ~5.8, in the presence and absence of sodium thiosulphate in solution.

\textit{d. Hygroscopic salts}

As many of the salts used in this thesis are highly hygroscopic, such as anhydrous CaCl\(_2\), MnCl\(_2\), NiCl\(_2\), and some additional ultradry salts, they were manipulated and weighted in a chamber (110x75x60 cm\(^3\)) purged with dry air with a relative humidity lower than 1\% (HMT333, VAISALA, Helsinki, Finland).

\textit{e. Potential adsorption of cations to the glass vessels}

Trivalent cations can adsorb to the glass surfaces of the volumetric flasks, potentially depleting the concentration of ions in solution, particularly at submicromolar concentrations. To discard this possibility, additional experiments were performed using volumetric flasks made out of polymethylpentene (PMP) and polypropylene (PP). However, no differences were observed in the VSF spectra of dAA when compared with solutions made in glass volumetric flask. Consequently, it was concluded that there is no significant depletion of La\(^{3+}\) and Y\(^{3+}\) ions from the bulk solution due to adsorption to the glass surfaces.
4. Langmuir-Blodgett (LB) trough

Description.
The Langmuir-Blodgett (LB) trough is a technique used for studying insoluble monomolecular monolayers at the air/water interface. The operating principles of the technique are based on the pioneering work of Agnes Pockels who studied the surface tension of film using an apparatus known as the slide trough. The instrument was later improved by Irving Langmuir in his work on floating monolayer, before being extended by Katharine Blodgett to allow for the sequential deposition of monolayers on solid substrates.

The LB trough is composed of three main parts: the trough, which is made out of Teflon, the barriers, which are typically made of the hydrophilic polymer Delrin (polyacetal) and a balance to measure the surface tension, using the Wilhelmy plate method. To ensure that the plate is fully wetted by the solution (i.e. contact angle of zero degrees), the plates used were made out of filter paper. The temperature in the trough was accurately controlled using a circulating fluid connected to a cooling/heating system (F12-MA, JULABO, Germany). If not stated otherwise, the temperature of the trough was set to 22 ±0.2 °C.

Measurements were carried out using two different troughs:

- A large KSV NIMA 5000 trough (KSV NIMA Instruments, Helsinki, Finland), which was 150 mm wide, and 519 mm long, giving a useful total surface area of ~77,850 mm² (after subtracting the barriers). Thanks to the larger surface areas, more accurate Langmuir compression isotherms could be recorded.

- A small Minimicro trough from KSV NIMA, which was 50 mm wide and 195 mm long, giving a total area of ~8,300 mm². The trough was small enough to be placed in the VSF setup, and had a circular glass window in its center to let trough the transmitted visible and IR beams, and reduce the scattering.

Cleaning procedure.
As mentioned above, the properties of the Langmuir monolayers were highly sensitive to the presence of surface active contaminants. Therefore, the trough and accessories had to be carefully cleaned before each measurement. The procedure is described in detail in what follows.
The large trough being less mobile was cleaned in situ by rinsing it several times in pure water and fully aspirating the liquid starting from the top layer. The trough was gently wiped with an optical cleaning tissue first soaked with chloroform, and then in absolute ethanol (99.5%). Finally, the trough was rinsed two additional times in ultrapure MilliQ water.

The mini trough could be more carefully cleaned. It was first copiously rinsed by placing it under warm tap water, followed by absolute ethanol (99.5%). The mini trough was delicately wiped with optical cleaning tissue soaked in chloroform and/or absolute ethanol. Care was taken to avoid wiping with chloroform the section surrounding the glass window, as it can weaken the Epoxy resin glue holding the window in place. Finally, the trough was rinsed with ultrapure water and dried with filtered N₂. The same procedure was used for the Delrin barriers, only that chloroform was not used as it can dissolve the polyacetal. The trough is held on a Standa (Lithuania) remotely controlled motorized stage, on which is attached a

Figure C.8. Photograph of KSV Minimicro 1S trough. The trough is contained in a Plexiglas box to limit contamination of the subphase by the airborne particles during the experiment. The grey holder controls the position of the barriers, and the black part above the trough is a precision balance holding the Wilhelmy plate. The trough is held on a Standa motorized stage.
The Plexiglas box shown in Figure C.8. The holders and the surrounding elements of the VSF sample position are systematically dusted before each experiment and wiped with a paper soaked in ethanol. Once cleaned, the trough is kept filled with ultrapure water. Further, before pouring the solution to be investigated, the trough was rinsed three times with ultrapure water, and the top layer of the aqueous subphase aspirated before depositing the monolayer.

Surprisingly, it was found that polyvalent ions could absorb to the Teflon trough. This phenomenon was sometimes clearly visible as it decreased the contact angle of the water on the Teflon trough, increasing its wettability. After the experiments with polyvalent salts, the trough was soaked for at least one hour in a saturated solution of EDTA, copiously rinsed, and then left filled with ultrapure water overnight.

**Deposition of the monolayer**

The solution of fatty acid is prepared by dissolving ~10 mg of the compound in ~1 mL of chloroform. The concentration is accurately determined using a balance, given the density of chloroform. Ten to twenty microliters of the solution were spread on the surface of the trough with a glass syringe. A waiting time of 10 min was observed in order to let the solvent fully evaporate before compressing the monolayer by moving the Delrin barriers at a rate that ranged between 5 and 10 mm/min until reaching the desired surface pressure or area per molecule.

**5. Spectrometers**

The Femtosecond VSF and Raman spectrometers described in this section were already operational by the time the research carried out in this thesis was started. The instruments are home-built at KTH and display a high degree of automatization. They are described in detailed elsewhere and only the key features are presented below.

1. *Femtosecond VSF Spectrometer*

   The most prominent part of the thesis work has been carried out with the Femtosecond VSF spectrometer shown in Figure C.9.

   The laser source consists of a Ti:sapphire broadband oscillator (Quantronix, USA) generating 90 fs / 1 kHz / 805 nm pulses that are subsequently amplified in an Integra-HE amplifier (Amplitude, France), with a total output power of 6 W. A tuneable IR pulse (1.14 – 20 μm) is produced by a traveling optical wave parametric amplifier (HE-TOPAS-C, Light
Conversion, Lithuania), pumped by ~4.5 W of the output power of the amplifier. The remaining power is converted to a bandwidth-tunable picosecond pulse in a home-built beam shaper. The 805 nm “visible” and tunable IR pulses are directed to the sample position in a co-propagating geometry. The angles of incidence are set to 70° for the visible and 55° for the IR beam. The SF signal is collected with a combined spectrometer and EM-CCD camera (Shamrock SR202i-B, Andor Ireland, and Newton, Andor, Ireland). The set-up allows measurements in broad spectral regions (1000 cm⁻¹ to 4000 cm⁻¹) at different polarization combinations with a typical spectral resolution of 3 cm⁻¹, that can be reduced to <1 cm⁻¹ at the expense of the output visible power. The visible power at the sample position was varied between typically ~20 to 50 mW, depending on the system studied. Unless otherwise stated the power of the IR beam at the sample position was set to 4 mW by a combination of polarizers. The visible and IR beams are focused at the sample with a typical beam size (short access of the ellipse) of respectively, ~300 μm and ~150 μm (~5 mJ/cm²). The position of the through at the focal point is adjusted remotely by a motorized stage, with a 1/8 step resolution of 0.625 μm (8MVT70-13 – Motorized stage, Standa).

The VSF lab is equipped with a temperature-controlled air system that ensures a rate of organic compound inferior to 10,000 particles count per cubic feet, corresponding to the cleanroom classification US FED STD 209E. The humidity in the lab never exceeded 35% RH, and the temperature is fixed to 21 ±1°C all year long.

**Surface heating.**

When investigating Langmuir monolayers showing a phase transition plateau (Paper II), heating of the sample surface by the IR beam caused a convection flow that displaced the most ordered phase out from the area examined. Similar heating issues have been previously reported for fatty alcohols and phospholipid monolayers.²¹²-²¹⁴ To avoid this effect an optical chopper system was placed in the IR path (MC2000B with a MC1F2P10 blade with only one opening, turning at 200Hz, Thorlabs), in order to decrease the number of consecutive IR pulses hitting the same position, and simultaneously reducing the IR power to ~0.2 mW (i.e. 5% of the power without the chopper). The drop in the signal to noise ratio was compensated by increasing the time of acquisition.
Normalization.

A 100 nm thick gold-coated silica plate is used to normalize the SF signal with the gold non-resonant background. The side wings of the Gaussian-shaped broadband gold spectra are truncated at approximately 10% of its maximum value in order to reduce noise during the normalization routine. This is described in detail elsewhere. The spectrometer is calibrated each day prior to experiments, using as reference the adsorption bands of a thin polystyrene (PS) film placed in the IR beam path. An example of this procedure is shown in Figure C.10 for the spectral region corresponding to the carbonyl and carboxylate stretching bands. The VSF spectrum of gold with the PS film included in the IR path (red spectrum) is compared with the IR transmission spectrum of the same film. The SF spectra are calibrated by blue-shifting the X-axis with a linear correction factor using as reference the fundamental and overtone absorption bands from the aromatic PS ring.
Figure C.10. Example of the wavelength calibration procedure. VSF spectrum of a gold sample with a PS film introduced in the IR path (red), compared with the transmission FTIR spectrum of the same PS film (grey). In this example the SF spectrum needs to be blue-shifted with the linear function \( f(\nu) = 1.021 \cdot \nu + 81.3 \) (cm\(^{-1}\)). The calibration function is defined before each experiment. The same procedure is carried out for the higher frequency region with the CH vibration modes.

b. Raman spectrometer

The Raman spectrometer has been used for the determination of the depolarization ratio of the carbonyl band in paper I. The spectrometer consists of 532 nm CW light source (Laser Quantum, UK) of ~2 mm in diameter. The beam is focused at the sample position with a Gradium lens (focal length 80 mm). The sample is sealed in a transparent glass tube of 4 mm in diameter. An ultra-long working distance objective (M-Plan Apo 50X, Na 0.55, Mitutoyo, Japan) is used to collect the scattered Raman light in a 90° scattering configuration. The light is detected using the same combination of spectrometer and camera as for the VSFS set-up.
D. Summary of research and perspectives

This chapter summarises the key results of the papers included in the thesis. The results are also placed in the perspective of manuscripts under preparation and future research initiatives as an extension to this work.

1. Description of the charging interface
   
   a. Spectral description of the studied system

   Langmuir monolayers of arachidic acid provide numerous advantages for the investigation of ion-specific interactions at a charged interface and their direct molecular interactions with the carboxylic acid moiety. First of all, the surface pressure isotherms offer a macroscopic description of the monolayer and a precise account of the average molecular density. Additionally, with an apparent pKa of ~10.8, the monolayer is ionizable at convenient and biologically compatible experimental conditions. The system has been readily characterised using various experimental approaches, offering valuable structural information. However, due to its intrinsic surface specificity, VSFS offers unique possibilities, especially if the carboxylic acid headgroup and its molecular environment want to be precisely targeted. It is not more than ten years ago that H. Allen and co-workers used VSFS spectroscopy to investigate the ion-specific interactions of K⁺, Na⁺, Ca²⁺, and Mg²⁺ with the carboxylic acid group, studies that triggered a renewed interest on the carboxylate headgroup and its specific vibrational signature at the interface. Nevertheless, the influence of organic and ionic impurities in the interpretation of the results had been largely neglected, and only a few systematic studies of the carboxylic acid headgroup at interfaces have become available since then. Recently, Tyrode & Corkery presented a full perspective of the behaviour of the fatty acid interface in contact with low monovalent salt concentrations (<50 mM), by simultaneously considering the headgroup, the alkyl chain, and the interfacial water response. The results showed good agreement with classical EDL theories (i.e. Gouy-Chapman) with no dependence on the identity of the monovalent cations and anions in solution.

The research presented in this thesis aims to provide a comprehensive overview of the molecular structure and behaviour of monovalent, divalent, and trivalent ions when
interacting with a charging carboxylic acid monolayer, at conditions beyond the limitations of classical EDL theories, when ion-specific interactions are the governing driving forces.

**VSFS characterisation of the arachidic acid Langmuir monolayer.**

Most of the research presented in this thesis, with the exception of paper II, focuses on the study of AA and dAA Langmuir monolayers compressed at 20 mN/m (20Å²), in the tilted condensed phase (TC). Figure D.1 shows the VSF spectrum of the deuterated fatty acid (dAA) monolayer collected under the SSP polarization combination. The spectrum is composed of four main regions of interest, labelled as A, B, C, and D. Region A, extending between 1250 cm⁻¹ and 1800 cm⁻¹, comprises the vibrational features of the carboxylic headgroup, including the single C-O stretch ~1300 cm⁻¹, the carbonyl stretch ~1720 cm⁻¹, the symmetric (1400-1500 cm⁻¹) and asymmetric (1500-1600 cm⁻¹) carboxylate stretching modes, and the OH bending modes from water that overlap with the carbonyl stretch at ~1660 cm⁻¹. Region B corresponds to the CD symmetric and asymmetric stretches of the deuterated alkyl chain, which have been described in detail in the literature.¹²³,¹⁸⁰ Region C refers to the so-called “proton-continuum” range that actually extends between the fundamental OH bending and OH stretching modes of water, and corresponds to the O-H absorption bands of hydronium and hydroxide ions.³⁶⁻³⁷ Finally, region D features the OH stretching modes of interfacial water molecules. Regions A, B, and D are extensively described in Papers I, II, III and IV, while the less explored region C is the main focus of Paper V.

**Figure D.1.** VSF spectra of dAA on 1 μM NaCl, collected in an extended spectral region 1100 - 4000 cm⁻¹ under SSP polarization combination. The areas highlighted in the spectra correspond to the frequency ranges of prevalence for the research presented in the thesis, respectively A for the fatty acid headgroup, B for the alkyl chain, C for the proton continuum, and D for the fundamental OH stretches of water.
Perprotonated and deuterated arachidic acids were used depending on the region studied. Since the CH₂ / CH₃ bending and wagging modes from the AA monolayer, overlap with the carboxylate features, the perprotonated amphiphile was only used when studying the OH regions (C & D). In the case of deuterated AA, the CD₂ /CD₃ bending and wagging modes are shifted <1200 cm⁻¹, leaving the fundamental vibrational modes of the carboxylic acid headgroup free from overlapping interferences.

The symmetric and asymmetric methyl stretching modes are useful indicators for tracking the conformational order and packing of the alkyl chain. They were used as references for spectral normalization in order to extract comparative quantitative information, and for confirmation of the 2D phase of the Langmuir monolayer. Paper II reports on the structural description of the alkyl chain configuration during the 2D phase transition of arachidic acid Langmuir monolayer on high pH NaOH solutions (see section B.1.c). The conformational order of the deuterated monolayer could be tracked by following the amplitude of the asymmetric methyl and methylene stretching modes, A(CD₃) / A(CD₂). Our results show the co-existence of the LE and TC phases on the plateau of phase transition, with the conformational order being in agreement with the SF intensities calculated from the lever rule, as expected from the first-order nature of the phase transition determined from BAM images. Besides, the spectra in the plateau region could be reconstructed from the individual SF spectra collected in the pure LE (~4 mN/m) and TC (~20 mN/m) regions, which confirmed the presence of two distinct phases in equilibrium.

**b. Vibrational signature of the headgroup: quantification of the deprotonation**

**Free or hydrated carboxylate: surface charge density.**

Upon addition of salt and/or increase of the subphase pH, the monolayer starts to deprotonate. The deprotonation is revealed in region A of the VSF spectra by a decrease of the characteristic features of the carboxylic acid group, namely the single C-O (~1300 cm⁻¹) and carbonyl (~1720 cm⁻¹) stretches, and the appearance of the symmetric (~1408 cm⁻¹) and antisymmetric (~1535 cm⁻¹) stretching modes of the carboxylate moiety, seen predominantly in the SSP and SPS polarizations, respectively. The vibrational signature of the carboxylate at 1408 cm⁻¹ is readily assigned to the hydrated carboxylate species at the interface. Investigations on the pH dependence of the monolayer deprotonation showed that the
The carboxylate group remains anchored at the surface with no detectable changes in its orientation when changing the packing density. Given that the surface density of the fatty acid monolayer is known from the trough measurements, the cross-section of the hydrated carboxylate SF signal, as well as the degree of deprotonation of the monolayer can be determined. From the percentage of free carboxylate species, the surface charge density of the monolayer can be readily measured. This methodology was employed to experimentally determine the thermodynamic (~5.0) and surface apparent pKa (~10.8) of the fatty acid in agreement with predictions from the Gouy-Chapman model for low salt concentrations.\textsuperscript{123}

Paper I reports on the charging of the monolayer in contact with concentrated monovalent salt solutions (up to 1M). As shown for NaCl salt in Figure D.2, increasing the salt concentration promotes the deprotonation of the monolayer. Starting from 100 mM, the surface charge deviated from the Gouy-Chapman (GC) prediction. Agreements were found instead with a modified theoretical model (MPB model) that accounts for the finite size of the ion through a dimensional parameter “a”. This parameter was found to be similar to the reported hydrated Na\textsuperscript+ ion diameter (a \approx 8 Å). The results highlight the importance of steric repulsion when predicting ion-specific interactions.

\textbf{Figure D.2.} (a) VSF spectra of dAA collected under SSP polarization combination in the carboxylate / carbonyl region, for varying NaCl concentrations, at pH 6.0 and temperature 22°C. Upon addition of salt, the symmetric stretching mode of the carboxylate increases, while the carbonyl band decreases. (b) Deprotonation isotherm as a function of NaCl concentration compared with the predictions from the Gouy-Chapman (GC) and modified Poisson Boltzmann (MPB) models, using a fitting parameter a = 8Å.
Carbonyl: percentage of deprotonation.

Following a similar methodology, one should be able to confirm the proportion of the remaining uncharged carboxylic acid molecules by considering the intensities of the carbonyl (C=O) band (the single C-O is unsuitable because of its significantly weaker cross-section). This method was employed in Paper II and Paper IV, where we could respectively determine the amount of reprotonation of the monolayer upon compression at high pH, and calculate the binding constant of trivalent ions to the surface. However, it is important to stress the experimental limitations of this approach. First of all, the carbonyl stretch has a weaker SF response than the symmetric carboxylate stretch, and also partially overlaps with the OH bending modes of interfacial water molecules. Both of these factors lead to higher experimental errors. Additionally, as shown in Paper I, the orientation of the C=O was seen to vary depending on the degree of deprotonation of the monolayer, with the tilt angle shifting, relative to the surface normal, from ~75° to ~60° upon addition of salt. All-atom MD simulations using two alternative force fields qualitatively confirmed this change in orientation upon charging. This change in orientation of the carbonyl bond is experimentally visualized by contrasting trends in the SF response in the SSP and SPS polarization combinations. Consequently, to estimate the proportion of uncharged species at the surface, an average of the variation in intensities in the two polarization combinations is necessary.

CIP formation: partial screening of the charge.

Paper II reports on the changes experienced by a fully deprotonated dAA monolayer upon compression in a Langmuir trough at pH 12.0. Increasing the pH of the subphase above pH 11.0 (when the monolayer is more than 50% deprotonated), a plateau of phase transition appears on the surface pressure vs molecular area isotherms (Π-A) (see section B.1.c). The Π-A isotherms display a rather complex behaviour depending on experimental conditions, such as pH, ionic strength, and temperature. This phase transition in fatty acid monolayers had been the subject of numerous previous studies. However, none had provided direct molecular information on the carboxylic acid headgroup, and the interpretation of the changes occurring during the phase transition remained speculative at best.

By tracking the conformational order of the alkyl chain during the phase transition, the presence of TC and LE phases confirmed the first-order nature of the transition. Focusing on the vibrational signature of the carboxylate moiety during the phase transition, we observed a blue shift of 9 cm⁻¹ for the symmetric vibrational mode as shown in Figure D.3.a.
The higher frequency peak at 1417 cm\(^{-1}\) was assigned to the formation of a contact ion pair (CIP) between the carboxylate and the Na\(^+\) counter ion. Under the pressure of lateral electrostatic forces, the dehydration of the carboxylate moiety from solvent separated / solvent shared configuration to CIP formation (see section B.3.c), allows a more efficient screening of the charge.

The relative proportions of the carboxylate and reprotonated species were quantified as a function of monolayer compression (Figure D.3.b). From the carbonyl and hydrated carboxylate cross-sections, we could estimate the cross-section of the COO\(^-\)Na\(^+\) being within ±15 % equivalent to that of the hydrated species. The monolayer is fully charged in the LE phase up to the transition plateau region at an area per molecule of ~60 Å\(^2\), equivalent to a charge density of ~0.26 C/m\(^2\). Further compression of the monolayer triggers the reprotonation of the carboxylate group and the formation of counter ion pairing, COO\(^-\)Na\(^+\). At the end of the transition in the pure TC phase, the three headgroup species are found roughly in a 2:3:1 proportion for COOH, COO\(^-\)Na\(^+\), and the hydrated COO\(^-\), respectively.

\[ \nu(COO^{-}Na^{+}) \]
\[ \nu(C=O) \]

**Figure D.3.** (a) VSF spectra of dAA on NaOH pH 12.0 subphase in the TC phase at 20 mN/m. The inset shows an enlargement of the carboxylate symmetric stretching mode before and after the phase transition plateau, where a ~10 cm\(^{-1}\) blue shift is clearly resolved. Figure (b) reports the experimental value of deprotonation of the fatty acid monolayer and the relative proportion of each carboxylate species upon compression of the monolayer when varying the area per molecule, showing up to ~33±8% of reprotonation of the monolayer in the condensed phase at pH 12.0.
A fully deprotonated monolayer compressed at 20 Å² would result in a surface charge of ~0.8 C/m². At pH 12.0, calculations from the PB model predict the complete deprotonation of the monolayer (Paper II), while, according to our calculation, those including a binding constant for Na⁺ counter ions lead a fully bound monolayer. On the other hand, the MPB model shown in Figure D.3.b by a dotted line predicts a partial reprotonation, with ~40% of the monolayer remaining charged. One of the implications of the results presented in Paper II is that the COO⁻Na⁺ CIP must partially contribute to the total monolayer charge. This statement is in agreement with the electrostatic nature of the ion pairing classification proposed by Marcus.48

c. Water Response: determination of the surface potential

Targeting the OH stretching region D, provides information on the water molecules in close proximity to the fatty acid headgroup (χ⁵(2)), as well as those further away in the diffuse layer, perturbed by the interfacial electric field associated with the charged surface (χ⁵(3)).187, 189-191, 224 The SF water response to the charging of the monolayer has been investigated in Paper I and Paper II in order to gain information on the interfacial electric field and the surface potential. The χ³(5) contribution in the OH signal is in principle proportional to the surface potential. However, at low ionic strengths, when the decaying electrostatic potential perturbs water molecules far beyond the non-linear coherence length of the SF process $\kappa^{-1} \geq \Delta k_z^{-1}$, destructive interference takes place, decreasing the SF OH response (see Section B.4.e). Changes in the OH amplitude when increasing the NaCl concentration in solution have been shown to be consistent with the predictions of the χ³(5) model at low ionic strengths (Figure D.4.a).122 The OH signal reaches a maximum for a concentration of ~1 mM NaCl, when $4\kappa^{-1} \approx \Delta k_z^{-1}$. At high ionic strengths, however, when the probing depth is shorter than the non-linear coherence length, the χ³(5) contribution is expected to be proportional to the surface potential, which in turn can be independently estimated from the experimentally determine surface charge using the MPB model, as seen in Figure D.2.b. For concentrations > 10 mM, discrepancies appear that could originate from the fact that at high salt concentrations, the water molecules probed include those in the more centrosymmetric environment hydrating the cations in the condensed layer, which do not effectively contribute to the measured SF signal. To test this hypothesis, further calculations were made by introducing in the χ³(5) model, the MPB potential at the edge of the saturated compact layer l⁺,
instead of that from the surface. The predictions from this revised “condensed diffuse layer” (CDL) model provide a much better fit to the experimental results as shown in Figure D.4.a. Nonetheless, further experiments would be needed to better decouple the surface and DL contributions, either by variation of the non-linear coherence length as presented recently by D. Hore and E. Tyrode, or using phase sensitive measurements.

**Figure D.4.** VSF OH stretch amplitude as a function of (a) the bulk NaCl concentration at pH ~6, and (b) area per molecule (i.e. surface charge density) at constant ionic strength and NaOH pH 12.0

(a) The data are compared with theoretical \( \chi^{(3)} \) models with values of surface potential calculated from the MPB and CDL model. Contributions from both water molecules in the immediate surface \( \chi_s^{(2)} = -0.12(1 - \alpha) \) and diffuse double layer \( \chi^{(3)} \) are accounted for, with the former depending on the monolayer charge. (b) The experimental data are directly compared with the surface potential calculated from the PB model and MPB model. The dimensional fitting parameter is set to \( a = 8 \text{ Å} \) in the MPB calculations.
In Paper II, we measured the SF water response upon variation of the surface charge density, when compressing the monolayer. At pH 12.0, corresponding to 10 mM of NaOH, the $\chi^{(3)}$ contribution does not experience interference, and is expected to be proportional to the surface potential. The measured SF water response shows that the surface potential decreases more rapidly (i.e. becomes more negative) than what the PB model predicts, yet it is in agreement with the trend of the MPB prediction (Figure D.4.b). Deviations between the two models appear from a surface charge of $\sim$0.1 C/m$^2$, equivalent to that observed at pH $\sim$6 when increasing the salt concentration (i.e. 50 mM NaCl). Interestingly, it is the significant drop in surface potential that explains the hydronium condensation that leads to the headgroup reprotonation. The more negative surface potential causes a significant increase in the surface concentration of H$^+$, which despite having a bulk concentration 10 orders of magnitude lower than Na$^+$ ions, still preferentially interacts with the carboxylate. The experimental deduced surface charge and surface potential variations are well in agreement with the predictions of the MPB model that accounts for steric effects due to the finite size of Na$^+$. However, the theory still neglects other physical phenomena that could have an influence, such as the non-uniform dielectric constant, dispersion interactions, and ion-ion or ion-solvent interactions. Our experimental results could be used as a benchmark to test potential extensions to the theory.

2. Specific molecular interactions

a. Salt specific interactions

Cation specific effect.

The charging behaviour of the monolayer was observed to depend on the identity of the monovalent counter-ion. Paper I reports on specific interactions of LiCl, NaCl, and CsCl salts upon increasing concentrations up to 1M, as well as changing the solution pH. The cations show contrasting interaction preferences for either the uncharged carboxylic acid or the charged carboxylate, following the order: Cs$^+$ > Na$^+$ > Li$^+$, and Li$^+$ > Na$^+$ > Cs$^+$ respectively. The formation of a contact ion pair was even observed for Li$^+$ at pH 9.0, when the monolayer deprotonation exceeded 40%. Coarse-grained and all-atom MD simulations supported the interpretation of having relatively higher concentrations of cesium ions near the mostly uncharged monolayer, with lithium ions having a higher preference for the
charged surface exposing more carboxylate groups. Figure D.5 reports on the percentage of deprotonation of the monolayer from Paper I and III, where the swap of ionic affinities becomes evident. The effective “ion size” introduced by the MPB model, though important, was not sufficient to explain the observed behaviour, as other ion-specific mechanisms come into play.

Although alterations of the Hofmeister series had been widely observed close to proteins and macromolecules exposing both hydrophilic carboxylic acid functional groups and hydrophobic backbone patches, systematic alterations on surfaces exposing just carboxylic acid groups had only been theoretically predicted by Schwierz and co-workers using MD-simulation. Paper I and the methodology of charge quantification in Paper II, brings experimental confirmation for the proposed “Hofmeister phase diagram” of surface charge density vs pH or concentration. The experimental results unveil the significance of close molecular interactions between the ions and the interface for understanding ion specificity.

![Figure D.5](image)

**Figure D.5.** Degree of deprotonation of the fatty acid monolayer as a function pH for a series of electrolyte solutions at a fixed concentration of 1M.

The dependence of the counter-ion identity has also been observed in extensions of the research presented in Paper II for hydroxide electrolytes at pH 12.0 (Figure D.6). The increase in surface pressure of the plateau of phase transition with the identity of the cation, reveals a stronger repulsion between the charged fatty acid molecules. This is linked to the inability of Cs⁺ to form contact ion pairs with the carboxylate and reduce the surface charge. On the contrary, Li⁺ covalently binds to the carboxylate at pH 12.
The VSF spectra in the OH region confirm this interpretation, with CsOH salt leading to the highest signal (i.e. higher surface charge), as no differences in hydronium condensation were noticed between Na\(^+\) and Cs\(^+\) ions. However, further investigations are required to systematically quantify this behaviour, as presented previously for NaOH (Paper II).

**Figure D.6.** (a) Langmuir isotherms of AA on CsOH, NaOH and LiOH electrolytes at pH 12.0. (b) VSF spectra collected in the OH stretching region under the SSP polarization for AA monolayers, compressed at 20 mN/m as indicated in (a).

### Specific co-ion effect.

Surprisingly and interestingly, at molar salt concentrations, the nature of the negatively charged co-ions was observed to affect the interactions of the Na\(^+\) counter-ion with the carboxylic acid moiety (Paper III). The deprotonation of the fatty acid monolayer follows the series NaI > NaSCN > NaBr > NaCl, which remains similar when increasing the pH from 6.0 to 9.0 (values reported in Figure D.5). In particular, iodide co-ions were found to drastically promote the deprotonation of the monolayer up to ~75 % at pH 6.0. Bromide behaved similar to chloride, while thiocyanate anions showed an intermediate behaviour both at low and high pH. The relative proportions of the hydrated carboxylate and CIP configurations were quantified for each electrolyte, showing good agreement with the charge dependence of the CIP occurrence as presented in Paper II.

Additionally, since thiocyanate is a molecular ion it can be detected by VSFS, provided it is present at the interface and has a preferred orientation. This is exemplified in Figure D.7.a, for a fatty amine monolayer on a 1 M NaSCN subphase. SCN\(^-\) anions are attracted to the
positively charged surface, and the CN triple bond stretch from the thiocyanate ion can be readily detected at \( \sim 2060 \text{ cm}^{-1} \). On the other hand, for the negatively charged fatty acid monolayer, the nitrile stretching vibrational features at low and high pH (Figure D.7.b for pH 6) suggests a relative depletion of the anion from the interface, or at least no special interaction with the carboxylic acid moiety. Yet, the presence of thiocyanate anions could be detected. Besides, the interaction of the Na\(^+\) counter-ion with the carboxylic group is found inversely proportional to the affinity of the sodium for its co-ion as predicted by Collin’s law of matching water affinity, which points out specific molecular interaction between the sodium and its co-ion near the interface, maybe through their interactions with the water molecules or at the edge of the counter-ion condensed layer. The results of paper III highlight the importance of systematically considering the co-ion when discussing about ion specific interactions.

![Figure D.7](image.png)

**Figure D.7.** VSF spectra collected under SSP, SPS and PPP polarization combinations in the nitrile (SC≡N) stretching region, showing (a) the adsorption of thiocyanate ions as counter-ion at a positively charged fatty amine monolayer, and (b) its depletion as co-ions from the negatively charged fatty acid monolayer.
b. Detection of hydronium counter-ion: rising the role of the proton in ion-specific interactions

The results presented in sections D.1.b and c emphasize the importance of hydrogen ions in the charging mechanism of the monolayer. We have seen that hydronium ions compete with the Na⁺ for binding with the carboxylate group, despite being present at orders of magnitude lower concentrations in the bulk solution. From the research carried out in this thesis, only La³⁺ and Y³⁺ trivalent ions showed a significantly higher affinity for the carboxylic acid group.

The proton, H⁺, is never found in its bare form in aqueous solution but instead solvated by water molecules, which makes it, in principle, targetable by VSFS. The exact number of molecules participating in this solvation shell is, however, debatable. Attempts to describe the molecular structure of the hydrated proton have generally revolved around two structural motifs, the Eigen²²⁶ and Zundel.²²⁷ In the former, the proton is localized in a single central water (H₃O⁺) that is surrounded by three additional strongly hydrogen-bonded molecules, forming the H₃O⁺(H₂O)₃ complex. In the latter, the proton is instead equally shared between two water molecules H₅O²⁺. However, despite recent experimental and theoretical advances, a molecular description of the hydrated proton in solution remains elusive.³⁶ One of the main reasons is that hydrated protons in bulk give rise to an extremely diffuse vibrational signature that makes it difficult to assign to specific conformers. The situation can be different at surfaces. Previous VSF studies had mainly focused on the influence of H⁺ on the OH water features, while the “proton continuum” region, characteristic of hydrated protons, (Region C in Figure D.1) was only targeted in a recent VSF investigation of the air/water interface of acidic solutions, where no specific hydronium enrichment was observed down to pH 2.³⁷ This is not the case at negatively charged interfaces, as hydronium ions are enriched and could give rise to a response in the SF spectra, provided they have a preferred orientation. Paper V shows that a resolved spectral feature from the hydrated proton can be detected at the surface of a partly charged dAA monolayer. Figure D.8.a shows the isotopic identification of the OH stretch from the hydrated proton at a dAA monolayer on 1 μM NaCl, corresponding to an optimum compromise between surface charge and surface potential. The peak centred at ~2540 cm⁻¹ was assigned to the antisymmetric OH stretching of the H₃O⁺ core in an Eigen-like proton (H₅O³⁺). The assignment was reached after comparison with previous IR studies on ultra-cold gas-phase clusters.²²⁸-²³⁰ The signal was shown to be much
more pronounced in the SPS polarization combination, an observation that was consistent with the assignment as an antisymmetric OH stretch of the C$_{3v}$ Eigen core. The proton ion has been identified at various experimental conditions, for different salt or by changing surfactant, with a negatively charged sulphate moiety for example. In this regard, Figure D.8.b shows preliminary data of the hydronium identification at the fatty acid monolayer when varying the nature of the co-ions in the experimental conditions of Paper III. The identification of the proton molecule as presented in Paper V, open new and exciting molecular perspectives for understanding the behaviour of hydrated protons at interfaces, including its implication in ion specific interactions.

**Figure D.8.** (a) VSF spectra of dAA Langmuir monolayer collected under SPS polarization for identification of the OH stretching vibration of the Eigen-like proton by isotopic substitution. (b) Effect of the negative co-ion (1M) on the proton stretching modes at the fatty acid monolayer (preliminary data).

c. **Direct molecular interaction: the carboxylate binding pattern, detection and identification**

The fatty acid monolayer has been shown to be extremely sensitive to the binding of polyvalent metal ions (Paper I). The propensity for polyvalent ions to interact with the carboxylic acid is reinforced at flat charged interfaces, which display a surface electric field that facilitates the interaction. Even when using ultrapure monovalent or divalent salts (>99.999%, trace metal basis), sub-ppm trace metal impurities are sufficient to cause remarkable inconsistencies in the vibrational features of the carboxylate group. The presence of such interactions is sometimes revealed by multiple vibrational contributions in the symmetric carboxylate region or time-
dependent variations in the peak intensities. Extra precautions need to be taken in order to remove trace polyvalent ions from solutions, such as the use of a soluble chelating agent (see Paper I and experimental section C.3.a). The results presented in Paper I, cast doubts on the interpretation of a large body of studies on extended charged interfaces using medium to high monovalent (and divalent) salt concentrations.162, 190, 219

To improve our understanding of the interaction mechanism between trivalent ions and the carboxylate headgroup, systematic studies using trivalent chloride salts YCl₃, and LaCl₃, (Paper IV) were performed. As part of this research project, additional studies were carried out on the divalent chloride salts CaCl₂, MnCl₂ and NiCl₂. They are described in detail in M. Scott’s Master thesis project entitled “Interactions of divalent cations with the carboxylate moiety: a vibrational sum frequency study”.203 The master thesis is available online, and part of the results will be shortly discussed here when describing Paper IV.

**Detection of polyvalent ions.**

The onset of monolayer deprotonation in the presence of the divalent ions Ni²⁺, Mn²⁺, and Ca²⁺ could be detected from concentrations starting from 1 to 10 μM. On the other hand, for trivalent ions the onset could be detected from concentrations below 300 nM (Figure D.9). The detection limit of trivalent ions could even be improved to concentration <100 nM by the addition of a monovalent background electrolyte or by targeting their effect on the OH stretching intensities. Another striking observation is the evolution of the deprotonation with increasing salt concentrations. While Y³⁺ and La³⁺ cause full deprotonation of the monolayer at ~5 μM, a significant deprotonation of the monolayer could only be detected at concentrations > ~1 mM for Mn²⁺, and Ni²⁺. Below this concentration, the deprotonation induced by the divalent ions were according to the predictions of the GC model.

Multiple frequencies were identified for the symmetric vibrational mode of the carboxylate moiety. We have previously reported on the free hydrated carboxylate (~1408 cm⁻¹) and the CIP configuration (~1417 cm⁻¹) for monovalent ions, resulting from ionic interactions. At low concentration, the deprotonation of the monolayer with divalent ions shows similar frequencies. Trivalent ions, however, show a significant blue shift of the carboxylate symmetric stretching mode (~1458 cm⁻¹ for Yttrium) from the onset of deprotonation. At millimolar concentrations, all polyvalent salts were showing a significant blue shift of the carboxylate vibrational signature, which has been reported as characteristic of covalent bonding and complex formation.158, 160
Figure D.9. VSF spectra of dAA Langmuir monolayer on (a) MnCl$_2$ electrolyte and (b) YCl$_3$ for various concentrations, pH 5.8 and temperature 22 °C. The detection limit recorded for YCl$_3$ and LaCl$_3$ is two orders of magnitude lower than for the tested divalent cations (CaCl$_2$, NiCl$_2$, and MnCl$_2$), revealing interactions between the trivalent ions and the fatty acid moiety from concentrations as low as 300 nM.

**Overcharging effect.**

Specific interactions of the trivalent ions with the carboxylic acid were shown to cause an overcompensation of the surface charge for concentrations > ~10 μM (Paper IV). The fatty acid monolayer then effectively show a positive charge to solution. The reversion of charge is further supported by the detection of the hydroxide co-ions adsorbing to the interface. This emphasizes the importance of hydrogen ions in local variations of ionic speciation at a charged interface. No changes in the carboxylate vibrational pattern were observed when the charge reversion intensifies with increases amounts of YCl$_3$ in solution, which indicates that the phenomena stems from molecular interactions between the bound cations and the underlying electrolyte. It is worth noting that up to 100 mM, no charge reversion was observed with the divalent ions investigated.

**Identification.**

Spectral signatures of Y$^{3+}$ and La$^{3+}$ trivalent ion binding were established through the carboxylate vibrational modes. As seen on Figure D.10.a, three distinct vibrational modes are unambiguously identified for the symmetric and asymmetric stretching modes, corresponding to three different carboxylate species. Attempts have been made to correlate
the vibrational pattern of the carboxylate to its coordinative mode (see section B.3.c).\textsuperscript{158, 160-161} If no strict rule has emerged,\textsuperscript{220, 231} and although the binding configuration must be strongly affected by the restriction of the chelation due to the interfacial geometry, the literature in the solid and liquid phase remains a valuable comparison tool. Comparison with lamellar lanthanum metallic soap allowed us to conclude that the spectral features were linked to both ionic and covalent binding type interactions, with the latter composed, in principle, of a mixture of bridging and chelating bidentate configurations. The quantification of each contributions were reported for yttrium and lanthanum as a function of the salt concentration (Figure D.10.b). The concentration dependences indicate an ion specificity in their interactions with the interface. The quantification and identification of the interfacial binding pattern could have numerous implications, such as the understanding and predication of the arrangement of complexed ions in organo-metallic hybrid microelectronics,\textsuperscript{26} or the vibrational characterization of metal organic frameworks (MOF’s),\textsuperscript{27} where lanthanum and yttrium are commonly employed.

**Figure D.10.** (a) VSF spectra of dAA on LaCl\textsubscript{3} electrolyte at various concentrations, under three polarization combinations SSP (orange), SPS (blue), and PPP (black). The spectra show three clearly distinguishable symmetric and antisymmetric vibrational modes. (b) VSF amplitude of the carboxylate symmetric contributions as a function of the salt concentration. The data reported in Paper IV for YCl\textsubscript{3} electrolytes show similar carboxylate contributions, yet with variations in the relative proportions, suggesting a different molecular binding pattern with the carboxylate and specific molecular interactions between the two salts.
The high frequency features remain somewhat challenging to assign without theoretical and simulations support. The quite simple lithium cation for instance, Li\(^+\) (1s\(^2\) 2s\(^0\)), interacts with the carboxylate moiety in many ways, as revealed by the numerous identified contributions of the carboxylate vibrational features (Figure D.11). Extension of the research on Paper II with high pH LiOH solution has revealed multiple interactions of the lithium at 1408 cm\(^{-1}\), 1418 cm\(^{-1}\), 1430 cm\(^{-1}\) and 1438 cm\(^{-1}\), which are not caused by metallic impurities, but rather depends on experimental conditions, such as the ionic strength, the pH and the surface density of charge. The first two frequencies are attributed to electrostatic interactions, but the two last would rather indicate a complexation structure. The fact that lithium monovalent ion interacts with the carboxylate group in a “trivalent” fashion may explain its strong biological specificity.

**Figure D.11.** VSF spectra of the carboxylate moiety of a dAA Langmuir monolayer on LiOH electrolyte in the condensed phase (20 mN/m, 20 Å\(^2\)) at pH 10, 11 and 12, and during the phase transition (~1 mN/m, 40 Å\(^2\)). Lithium cation, Li\(^+\) (1s\(^2\) 2s\(^0\)), interacts with the carboxylate moiety in many ways, as revealed by the numerous identified contributions of the carboxylate vibrational features.
Concluding remarks

The results presented in this thesis have demonstrated the unique capabilities of vibrational sum frequency spectroscopy for investigating ion-specific interactions at the charged carboxylic acid interface. VSFS allowed, among others, to quantify the surface charge, determine the type of cation binding with the carboxylate headgroup (ionic, CIP, complexation), and monitor structural changes in the interfacial water molecules depending on the identity of the ion, concentration, and solution pH. Investigations on the charging process of the fatty acid Langmuir monolayer has shown that from monovalent salt concentrations > 50 mM, and significant surface charge > -0.1 C/m², the ion-specific size of the hydrated counter-ion becomes a determining factor in the properties of the EDL. Additional ion-specificity arises from the molecular affinities of the cations with the charged and uncharged carboxylic acid moiety, as well as with its co-ions. Those properties intrinsically depend on the nature of the ions. Those findings indicate that the physical-chemical properties of the interface depend on a subtle balance of electrostatic and molecular cooperative interactions, that could be elucidated by carefully considering other physical parameters. Overall, the results shine new light onto the fundamental understanding of the electrical double layer (EDL) at charged interfaces.

From the vibrational pattern of the carboxylate moiety, different types of binding were distinguished, from electrostatically driven (solvent-shared or contact ion pairing) to more covalent binding complexes. The quantification of each contribution revealed a strong ion-specificity, not only depending on the charge, but also the size and the electronic structures of the ions, which affect their coordination in the confined environment of the interface. Divalent and trivalent ions notably displayed much stronger affinities with the carboxylic acid, with interactions detected from nanomolar concentrations. The present work proposes a modus operandi for studying ionic interactions with the carboxylic acid group, as well as other charged interfaces with varying functional groups. We hope that it could lead to further understanding of ion reactivity at biological interfaces, and in particular, the development of more efficient routines for separating environmentally toxic heavy metals.

Finally, the hydronium ion was shown to have a determining role in the physical-chemical properties of the charging interface. The identification of resolved spectral features linked to a specific hydrated proton species opens up a whole new horizon of possibilities for extending our comprehension of ion-specific interactions and charged interfaces in general.
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