The influence of crude oil acids on natural inhibition of hydrate plugs

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Preface

This dissertation is submitted for the degree of Philosophiae Doctor at the University of Bergen. The thesis consists of six parts: four journal papers, one conference paper and a research report. In addition, an introduction to the work and a summary of methods and main results are given as the first part of the thesis. The main part of the work has been performed at the Department of Chemistry, University of Bergen in the period 2005-2009. My PhD project has been part of the HYPERION project (HYdrates in PEtroleum productION - Assessment of Plug Risk), which is a KMB project funded by the Research Council of Norway and StatoilHydro ASA. The HYPERION project is an interdisciplinary collaboration between several fields of research at the University of Bergen and StatoilHydro ASA, and has been combining physical chemistry, petroleum- and organic chemistry, physics and industrial aspects (see Figure 1).

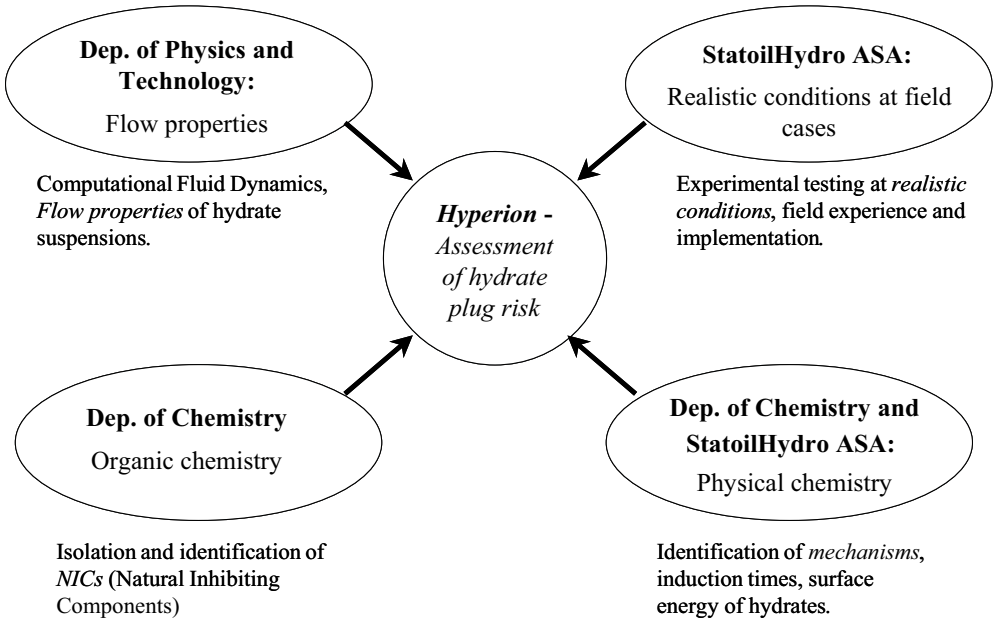

Figure 1: Roles of the partners in the HYPERION project.

The aim of the HYPERION project has been to obtain knowledge about inherent mitigation effects of crude oils on gas hydrate formation, morphology and growth and to predict the risk of hydrate plugging in a given oil, gas, water system. Hydrate plugging tendency is observed to be very different from one crude oil to another. My contribution to this collaboration has been in the fields of organic- and petroleum chemistry, with focus on how crude oil composition influences hydrate plugging tendency. Hydrate mitigating properties has been attributed to chemical components naturally present in some oils, the acidic fraction being of main importance in this context. The aim of this work has been to study petroleum acids, with focus on acid extraction- and analytical characterisation methods, and on investigation mitigating effects of the acids on hydrate plugging.

In May 2008 I had a short, but very valuable stay at the research group of Prof. Dr. Brian Horsfield and PD Dr. Heinz Wilkes, at Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences (GFZ). During this stay Dr. Stefanie Pötz introduced me to, and taught me about electrospray ionisation-mass spectrometry (ESI-MS). This stay has resulted in a continued and fruitful collaboration between our research groups.

Acknowledgements

I would like to express my profound gratitude to my supervisors Tanja Barth, Sylvi Høiland and Per Fotland for your guidance, helpfulness, inspiration and encouragement throughout my PhD project. Your support, as well as your care for my well-being, have been invaluable to me.

The Research Council of Norway and StatoilHydro ASA are thanked for funding the project. It has been very educational and interesting for me to be part of the HYPERION project, and I want to thank all the participants for the collaboration. I also want to express my thankfulness to Stefanie Pötz, Heinz Wilkes and Brian Horsfield for kindly welcoming me to a stay at GFZ.

I wish to thank all my fellow students, colleagues and friends. A special thank goes to Espen N. Vaular and Monica Jordheim who have been my companions throughout all my years of studies, and to Anna E. Borgund who I have been working closely together with, and who has been an invaluable discussion partner and friend. You have all been making my years at University of Bergen such a memorable and unforgettable time. Many people at the Department of Chemistry have lent a helping hand to my project one way or another, something I am truly grateful for. I especially want to thank Terje Lygre for assistance with instruments. Siv Elin and Torunn; I could not ask for any better and more faithful friends than you.

My family has been essential to me during these years. I especially want to thank my parents for all support and care. My dear Steve has been an endless source of encouragement; thank you for being my rock in tough times, for constantly having faith in me and for your love. Finally to my two children Kjetil and Christian; you are always there for me, and I am so proud of you. You both mean the world to me.

Abstract

Gas hydrates can form in petroleum production systems of natural gas, water and crude oil. In some systems the hydrates agglomerate rapidly into large plugs that cause hazardous blockages of e.g. transport pipelines. The dangers associated with hydrate plugs are severe, and hydrate prevention strategies represents huge economical costs for the operators. However, in some systems plugging is never observed. Instead, the hydrates form as tiny crystals that are easily transported within the fluid flow as suspensions. Crude oils that possess low hydrate plugging tendency are believed to contain natural inhibiting components (NICs). One possible mechanism is that the NICs are anti-agglomerants, i.e. surfactant molecules capable of adsorbing to the hydrate surface. The layer of adsorbed surfactants creates an "oil-wet" hydrate surface that makes the particles less prone to agglomeration and plugging. Knowledge about how to isolate these specific compounds from the crude oils, and to elucidate their chemical structures, can provide a valuable tool to hydrate plug assessment- and control. These compounds could potentially be developed into future environmentally friendly low dosage hydrate inhibitors. The origin of these crude oil compounds is unknown. The process of biodegradation seems to be of importance, as most non-plugging oils are biodegraded whereas plugging oils are typically non-biodegraded. However, there are exceptions to these general categories, and this raises the question whether different biodegradation processes influence the plugging potential of oils differently.

This thesis addresses the challenge of isolation and identification of natural inhibiting compounds in crude oils, and also includes testing their hydrate wettability effects. One class of crude oil components that has previously been recognised to display hydrate anti-agglomerating effects is the polar acidic fraction. In the work of this thesis the attention has been directed to this fraction.

A central part of the work has been the development of HPLC- and SPE methods for fractionation of petroleum acids. The HPLC separation gives useful infor-

mation about the distribution of acidic compounds in different oils, and a strong negative correlation between the relative amount of phenolic compounds in the acid extracts and hydrate wettability has been found. Acid extracts and SPE fractions isolated from low plugging potential oils impose changes in the wettability of Freon hydrates. One of the SPE fractions displays particularly high effect at low concentration. This distinctly hydrate surface active fraction contains predominantly weakly polar compounds. FTIR analysis indicates that these molecules contain ester functionalities that are not found in a corresponding fraction of a high plugging tendency oil. On the contrary, the profile of the high plugging tendency oil indicates a larger content of phenolic compounds, which is in accordance with the observed negative correlation between phenols and wettability.

Anaerobic- and aerobic laboratory biodegradation experiments produce acids of different chemical composition. Anaerobically produced acids display higher effectiveness at the oil/hydrate interface than those from aerobic biodegradation. The chemical composition of these acid extracts resembles the trends found for fractions from low- and high plugging potential oils studied in other works; the most hydrate surface active acids hold a larger relative proportion of weakly polar compounds and lower content of phenolic compounds.

The exact chemical structures of the acid fractions isolated in this work remain to be elucidated. The final work of chemical analysis is currently being performed by Dr. Stefanie Pötz at GFZ. Preliminary results from ESI-MS analysis provide very promising data, and reveal clear structural differences in acid compositions between low- and high plugging tendency oils.

List of Publications

Papers

- Paper I: Normal phase high performance liquid chromatography for fractionation of organic acid mixtures extracted from crude oils, A.E. Borgund, K. Erstad and T. Barth, Journal of Chromatography A 1149 (2): 189-196, 2007.

- Paper II: Fractionation of Crude Oil Acids by HPLC and Characterization of Their Properties and Effects on Gas Hydrate Surfaces, A.E. Borgund, K. Erstad and T. Barth, Energy & Fuels 21 (5): 2816-2826, 2007.

- Paper III: Influence of Petroleum Acids on Gas Hydrate Wettability, K. Erstad, S. Høiland, P. Fotland and T. Barth, Accepted for publication in Energy & Fuels, in Press. Available online, DOI: 10.1021/ef8009603. Publication Date (Web): February 27, 2009.

- Paper IV: Isolation and Molecular Identification of Hydrate Surface Active Components in Petroleum Acid Fractions, K. Erstad, S. Høiland, T. Barth and P. Fotland, Conference Proceedings of the 6th International Conference on Gas Hydrates, 6.-10. July 2008.

- Paper V: Changes in crude oil composition during laboratory biodegradation; acids and interfacial properties, K. Erstad, I.V. Hvidsten, K.M. Askvik and T. Barth, Submitted to Energy & Fuels, January 2009.

Research report

- Mass spectrometric studies of crude oil acid extracts and their solid phase extraction (SPE) subfractions, K. Erstad, S. Pötz, Research report, December 2008.

Abbreviations

AA	Anti-Agglomerant
API	American Petroleum Institute
ASTM	American Society for Testing and Materials
ATR	Attenuated Total Reflection
DCM	Dichloromethane
EA	Elemental Analysis
EI	Electron Impact
ESI-MS	Electrospray Ionisation-Mass Spectrometry
ELSD	Evaporative Light Scattering Detector
FID	Flame Ionisation Detector
FT-ICR	Fourier Transform Ion Cyclotron Resonance
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography
IFT	Interfacial Tension (mN m^{-1})
KI	Kinetic Inhibitor
KMB	Kompetanseprosjekter Med Brukermedvirkning
LC	Liquid Chromatography
LDHI	Low Dosage Hydrate Inhibitor
MEG	Monoethylene glycol
MS	Mass Spectrometry
NIC	Natural Inhibiting Component
NIGOGA	Norwegian Industry Guide to Organic Geochemical Analyses
NSO	Nitrogen, Sulphur, Oxygen
SARA	Saturated, Aromatics, Resins and Asphaltenes
SEC	Size Exclusion Chromatography
SPE	Solid Phase Extraction
TAN	Total Acid Number (mg KOH per g oil)
TCD	Thermo Conductivity Detector
THF	Tetrahydrofuran
TIC	Total Ion Chromatogram
TLC-FID	Thin Layer Chromatography-Flame Ionisation Detection
UCM	Unresolved Complex Mixture
UV/VIS	Ultraviolet/Visible
WOGC	Whole Oil Gas Chromatography

Contents

Chapter 1

Introduction

In petroleum production the formation of gas hydrate plugs in pipelines and platform equipment is a major challenge and concern, and represents a major problem in terms of economical loss for oil companies and safety dangers for the personnel that handle them. Gas hydrates are ice-like, crystalline solids, consisting of guest molecules trapped inside cavities in a framework of hydrogen bonded water molecules [1]. Presently, the oil- and gas industry is moving the subsea exploration and production towards increasing water depths, where the temperature and pressure conditions are well within the range for hydrate formation. As the phase behaviour of hydrates is well mapped and understood, hydrate phase diagrams can be constructed from commercial software, based on the composition of water, gas and oil phases in each particular system. As of today most operators are dealing with the hydrate problem using conservative and expensive strategies, assuming that plugging will always occur if the T-P conditions for hydrate formation are present. To avoid the formation of hydrates, the transport conditions are designed in such ways that the well-stream is always outside the hydrate stable area of the pressure, temperature phase diagram. Preventing hydrate block-

age is achieved mostly by adding thermodynamic inhibitors (methanol or glycols) or by insulation. Thermodynamic inhibitors are effective in preventing hydrate formation by shifting the hydrate phase equilibrium boundary towards lower temperatures and higher pressures conditions. However, high concentrations of these chemicals are required, which impose negative consequences for the project economics as well as unfavourable impact on the environment. An alternative prevention method is inhibition by use of low dosage hydrate inhibitors (LDHIs) [2]. As of today the use LDHIs is prohibited in the Norwegian sector due to toxic effects. Details on gas hydrates and inhibitors are discussed further in Chapter 3.

During the latest years, the presence of certain natural inhibiting components in the crude oils that possess a mitigating effect on the plugging tendency of the hydrates, has been reported in several works [3–7]. These components cause the hydrates to form as colloid suspensions rather than plugs. The hydrate suspensions are easily transportable as slurries, though the transportability is somewhat limited by viscosity of the slurry at high particle loadings. The active components are believed to be natural anti-agglomerants (whether such compounds also may have the effect of kinetic inhibitors has not been much explored). As of today the specific chemical structures of the natural inhibiting components are unknown, and the exact mechanisms that take place are not fully understood. AAs molecules are believed to interact with the hydrate surface by adsorption mechanisms; the polar end of the molecule being attracted to the hydrate surface and with the other, hydrophobic end interacting with the bulk hydrocarbon oil phase. Several works suggest that such compounds are located in the polar resin- and asphaltene fractions [6, 8] or in the acidic crude oil fractions [9–11]. To obtain detailed knowledge of these compounds is of great interest, as these

may have applications as environmentally friendly low dosage hydrate inhibitors that may reduce the use of thermodynamical inhibitors, or maybe even replace them. Hence, suitable methods for extraction and characterisation of the natural inhibiting components need to be developed. This represent a considerable challenge, as the concentration of these components in crude oils is anticipated to be small, and the crude oil is an extremely complex matrix with regards to chemical composition.

Sloan [12] points to the fact that there is a paradigm shift with regard to hydrates in petroleum industry. The shift is from awareness and control of the problem to a calculated risk of plugging the line, based on detailed knowledge. If more knowledge on these natural hydrate inhibitors of crude oils can be obtained, it may open new fields for production and help making operations of current field simpler, safer and with less chemicals.

In the works of this thesis the main focus has been on isolation and characterisation of petroleum acids as hydrate anti-agglomerants, although it must be emphasised that AA effects from other crude oil components than acids cannot be excluded.

Chapter 2

Crude Oil

2.1 Elemental composition

Crude oil is a complex mixture of hydrocarbons, but also containing minor amounts of organic compounds with the heteroatoms nitrogen, sulphur and oxygen. Crude oils also contain trace amounts of metallic constituents (Ni, V, Cu, Fe). The elemental composition, based on available data from petroleum sources from all over the world, is given in Table 2.1 [13].

Table 2.1: Elemental composition of petroleum. From Speight [13]

Element	%
Carbon	83.0-87.0
Hydrogen	10.0-14.0
Nitrogen	0.1-2.0
Oxygen	0.05-1.5
Sulphur	0.05-6.0
Metals (Ni and V)	$<$ 1000 ppm

2.2 Classifications of petroleum constituents

The number of chemical compounds in crude oils is immense, causing a great complexity in composition [14]. Due to the extreme complexity a complete molecular identification of every single component in petroleum is impossible, and one has to settle for simplifications. This is achieved by separation of the crude oils into fractions, or classes, of compounds of similar properties by means of chemical or physical methods [13]. There are numerous ways to classify petroleum fractions and many different separation schemes are used for fractionation of petroleum into various types of subclasses. One common way of classifying petroleum is to name the pure hydrocarbon compound groups as *paraffins* (saturated hydrocarbons, straight chained or branched), *naphthenes* (cyclic saturated compounds with one or several rings that may have paraffinic side chains, and are often also termed "alicyclics") and *aromatics* (hydrocarbons with one or several aromatic nuclei that also can be bonded to naphthene rings and/or paraffinic side chains). *Olefins* (unsaturated compounds; alkenes) are rarely reported. The fractions that contain heteroatom compounds are often referred to as *resins* and *asphaltenes*. Resins and asphaltenes are often termed "polar compounds", or "NSO" compounds. Such fractions can be abbreviated as "SARA" fractions; Saturate, Aromatic, Resin and Asphaltene [15]. The compound groups are often subdivided into further subclasses, like for example the aromatics into *mono-, di-, tri-* and *tetraaromatics* [14], or the resins into acids, bases and neutrals [16, 17].

Sometimes petroleum- or petroleum fractions are described as "light" or "heavy", based on distillation properties or API gravity [13, 14]. Again, the definition of these terms may not be very restricted. In general, light oils are enriched in paraffinic compounds and have a higher proportion of lower-boiling components. The so-called "light hydrocarbons" are the range of

compound between C_1-C_9 [18]. Light oils have low viscosity, high API gravity and low heteroatom content. Heavy oils are depleted in light hydrocarbons, enriched in asphaltenes, have low API gravity, high viscosity, high content of sulphur and acidic compounds. These oils are often severely biodegraded. The process of biodegradation is described in the next section (Section 2.3)

2.3 Biodegradation

Petroleum biodegradation is the alteration of crude oil caused by living organisms [19]. For a long time it was assumed that hydrocarbon degradation only was possible in the presence of oxygen, the processes being carried out by aerobic bacteria (oxygen electron acceptors) [19–21]. However, it has been recognised that anaerobic bacteria (bacteria that utilise other electron acceptors than oxygen, e.g. sulphates and nitrates) also are capable of hydrocarbon degradation in subsurface petroleum reservoirs [22, 23]. Some authors suggest that the latter described process may be predominant in deep subsurface petroleum biodegradation [23–25]. There has also been reported about certain facultative microorganisms that possess the ability to exist under both aerobic and anaerobic conditions [26–28]. The biodegradation processes are controlled by conditions that support microbial life and suit the specific bacteria, important factors being reservoir temperature, water pH, salinity- and nutrient concentrations and the accessibility to electron acceptors and hydrocarbons [29,30]. Biodegradation has a negative impact on the oil quality and renders both the oil recovery and refining process difficult, as the molecular changes that takes place in the oil as a result of biodegradation make the oil increasingly "heavier" (as discussed in Section 2.2).

The process is generally considered to be selective and quasi-stepwise,

based on the view that microorganisms prefer certain constituents of the crude oil above others. Although the rates at which the different petroleum hydrocarbons are degraded are not fully understood and the systems are very complex [30], the removal of the hydrocarbons has generally been found, very briefly stated, to happen in the following succession: n-alkanes, isoprenoids and other branched alkanes, cyclic alkanes and aromatic hydrocarbons. However, this is also dependent of the type of microorganism, as individual organisms can be very specific for the hydrocarbon substrates they can metabolise [31, 32]. Several schemes for qualitative assessment of biodegradation levels are reported in the literature, two of the most well-known being the Peters and Moldowan (PM) scale [33] that emphasises moderate to severe biodegradation levels, and a modified scheme by Wenger et al. [32] that expands the assessment of initial/moderate stages of biodegradation. In a recent paper by Elias et al. [34] an improved method of biodegradation assessment is proposed. This approach allows for a quantification of the extent of depletion of individual hydrocarbons within series of related oils.

Figure 2.1 illustrates chromatographic profiles of a non-biodegraded oil compared to a moderately biodegraded oil. The chromatogram of the non-biodegraded oil has a typically "n-paraffin envelope", where almost all the n-alkanes are intact and dominating the chromatogram. In the chromatogram showing the biodegraded oil, the n-alkanes are almost completely consumed, but some branched- and cyclic alkanes and aromatic hydrocarbons have not been utilised by the bacteria at this stage of biodegradation.

Not only hydrocarbons are susceptible to biodegradation. Although less documented, the polar (NSO) compounds in petroleum are also affected by microbial processes, including petroleum acids. Several authors report that carboxylic acids are generated during both aerobic and anaerobic biodegrada-

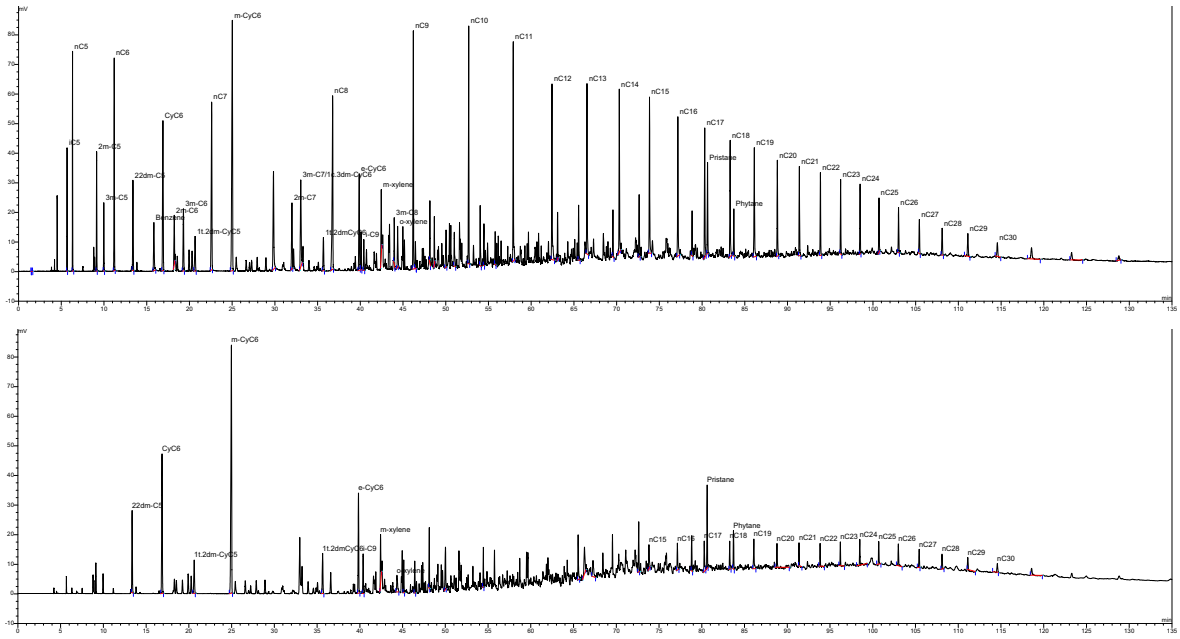

Figure 2.1: Illustration of changes in gas chromatographic profiles as a result of biodegradation. Upper: a typically non-biodegraded crude oil. Lower: a crude oil that has undergone moderate biodegradation (from crude oils belonging to the HYPERION project, University of Bergen 2007).

tion [35–41]. However, during the same processes acids can also be removed, utilised as substrates for growth by the bacteria [35,38]. Studies on biodegradation of naphthenic acids in the laboratory support this [42–45]. Hence, these polar acidic compounds are not as bioresistant as once believed. Acidic constituents of petroleum and interfacial properties are described in more detail in the next sections (Sections 2.4-2.5).

2.4 Acidic constituents of petroleum

The presence of acidic compounds in petroleum was reported for the first time by Hell and Medinger [46], as early as in 1874. By 1930, after extensive research in order to determine the structures of acids in petroleum, it was stated that phenols and carboxylic acids had been found in crude oils. The

acids were found to be both aliphatic and alicyclic (Lochte [47] and references therein). The origins of the petroleum acids are still a subject of research. In the early stage it was debated whether the acids were artefacts formed during various refining processes. However, it was shown that only a small quantity of acids were produced during these processes [48]. As of today it is generally assumed that acids may have been incorporated into the oil from three different sources; 1. Acidic compounds found in source rocks, derived from the original organic matter that created the crude oil (plants and animals) [49–51]. 2. Neo-formed acids during biodegradation [35–41, 52] (although the high acid concentration in biodegraded oils is believed to be related principally to the removal of non-acidic compounds, leading to a relative increase of the acid concentration levels). 3. Acids that are derived from the bacteria themselves, e.g. from cell walls that the organisms leave behind when their life cycle is completed. Indications of such types of species are reported by Tomczyk et al. [53]. In their work species containing more than just carboxylic acid functionalities are identified, e.g. sulphur- and nitrogen functionalities. The latter may originate from amino acid in the cell walls. An additional possible source, although not much explored in this context, is the presence of so-called *biosurfactants*. The bacteria that cause biodegradation, must exist in the water phase, and they need to reduce the interfacial tension between oil and water in order to access the carbon source (oil). This is done by producing a biosurfactant [30, 54]. In general, biosurfactants are complex molecules, but the chemical composition of many biosurfactants classes includes acidic functionalities (fatty acids and amino acids moieties) [55, 56].

Despite the reports of presence of compounds in petroleum acidic fractions that do not solely contain the carboxylic acid functional group, most of the

research on petroleum acids comprises the so-called naphthenic acids. The term "naphthenic acids" is commonly used to describe an isomeric mixture of carboxylic acids containing one or several saturated alicyclic rings [57, 58]. However, in petroleum terminology it has become customary to use this term to describe the whole range of organic acids found within crude oils. Naphthenic acids are generally described on the formula $C_nH_{2n+Z}O_2$, where n is the number of carbons, Z is the "hydrogen deficiency" index. Z is either zero (for simple fatty acids with one carbon-oxygen double bond) or a negative even integer (acids with additional rings/double bonds) that specify homologous series [59–61].

Some general, simplified structures of typical acidic compounds found in petroleum are given in Figure 2.2 (modified from Qian et al. [60], Clemente et al. [58] and Laredo et al. [62]). Recently, some new and very interesting high molecular weight acid structures have been discovered [63, 64] and described [65–67]; the so-called "ARN" acids. These are a mixture of C_{80} tetraprotic acids with 4-8 alicyclic rings, found in crude oils of North Sea UK, Norwegian Sea and West African oilfields. An example of an ARN acid structure is given in Figure 2.3. The origin of these acids is still not clarified, but one possibility is that they may be biosurfactants produced by oil degrading bacteria. The unusually high interfacial activity these acids display, compared to monoprotic acids, supports this idea [65]. Interfacial properties of oil constituents are discussed further in the next section (Section 2.5).

Acidic crude oils are generally considered as problematic from an oil quality point of view. The acids cause corrosion problems in the refinery processes and due to their toxicity, they also represent a pollution source in refinery wastewaters [42].

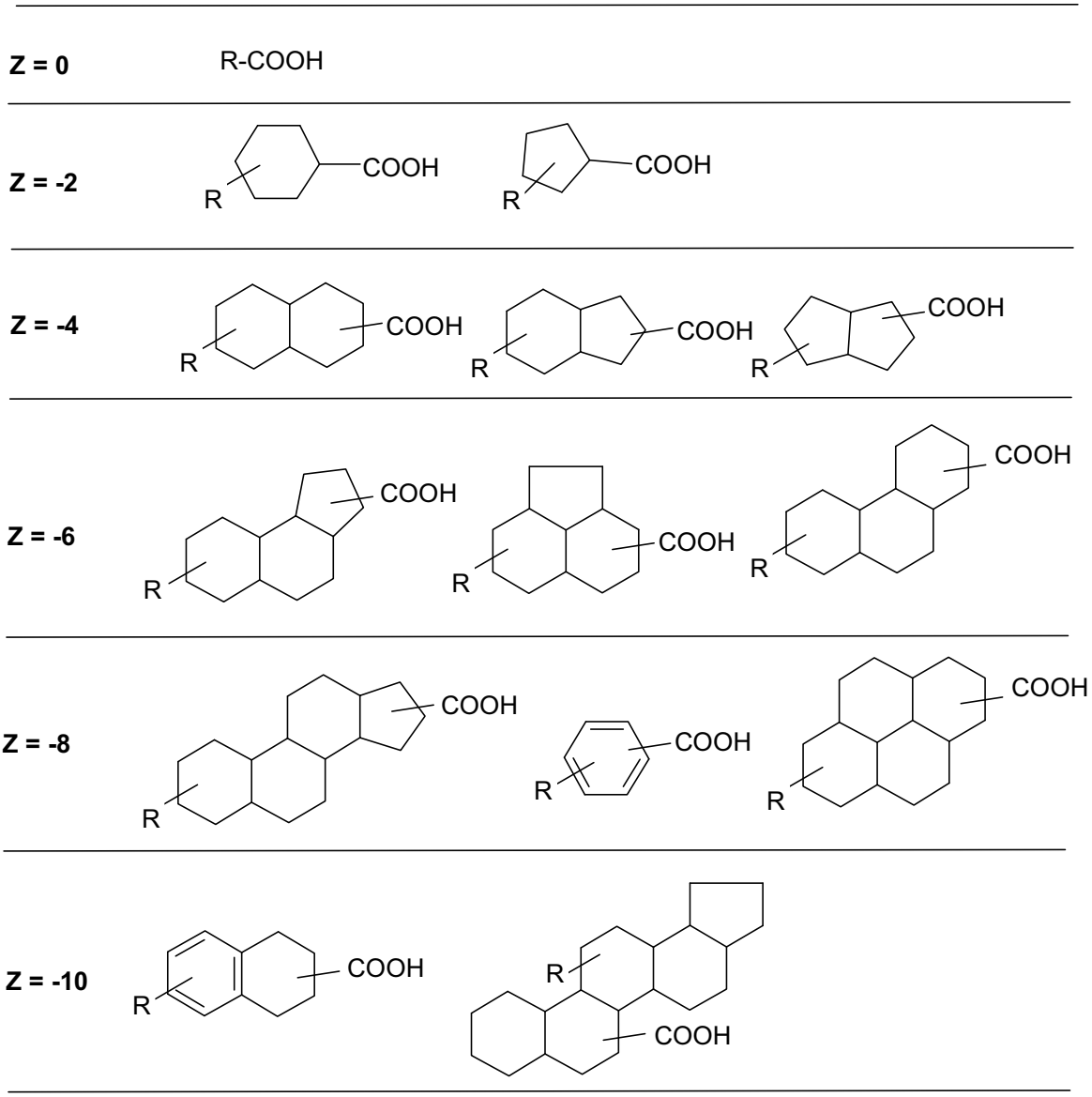

Figure 2.2: Some examples of suggested structures of naphthenic acid types (in Z families 0 to -10) reported in the literature [58, 60, 62]. Note that the COOH group may also be a part of aliphatic chains.

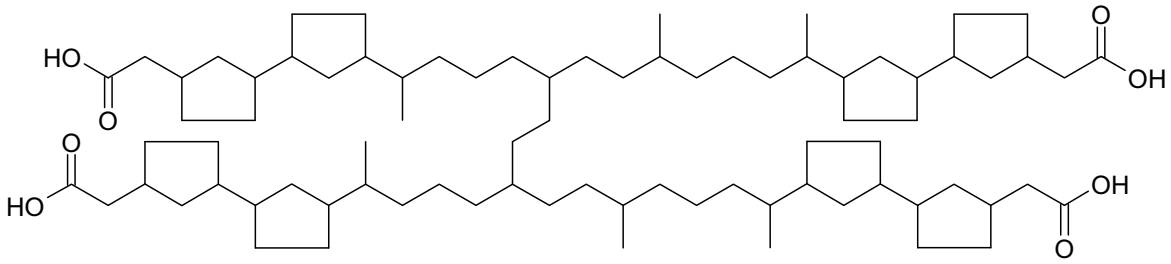

Figure 2.3: Example of a proposed structure of an 8 ring C_{80} tetraprotic ARN acid (Smith et al. [66]).

2.5 Interfacial properties

Crude oil components that are surface active (at the boundary between liquid/solid or liquid/gas) and interfacial active (in the interface between two liquids) span over a large range of chemical structures and molecular weights. Asphaltenes and resins, including naphthenic acids, are examples of petroleum constituents that display such properties [17, 68–71], thus acting as natural surfactants. Surfactant molecules are amphiphilic, meaning that they have both hydrophilic and hydrophobic parts in the molecules, and for this reason adsorb strongly at interfaces [72]. Oil/water interfacial active compounds typically reduce the interfacial tension between the two phases and enhance stabilisation of water-in-oil (w/o) emulsions. The formation of stable w/o emulsions is generally undesirable and causes serious challenges in petroleum production in terms of separation and refining processes [73, 74]. As mentioned in the previous section (Section 2.4) the newly discovered "ARN" types of acids have shown particularly strong interfacial properties.

Surface active compounds in crude oils are also important for reservoir production challenges [75–77]. Although the processes involved are complex and still not well understood, polar compounds present in crude oils are generally assumed to be involved in adsorption interactions or deposition

mechanisms that take place at the crude oil/brine/rock interface. These surface active components may contribute to wettability alteration of the rock from initial water-wet, to less water-wet or oil-wet reservoirs.

It should be mentioned in this context that indigenous components found in some crude oils display surface activity in the interface between crude oil and gas hydrates [3–7]. These natural surfactants display preventive properties on the formation of gas hydrate plugs, and are believed to be anti-agglomerants. Anti-agglomerants and gas hydrates are described in more details in the next chapter (Chapter 3).

Chapter 3

Gas Hydrates

3.1 Background

Generally, Joseph Priestley is believed to be the first discoverer of hydrates in 1778, as a result of laboratory curiosity. However, there exist no records that confirm the experiments of Priestley [1]. The first documentation on gas hydrates is generally credited Sir Humphrey Davy, with his paper published in 1811 [78]. In 1810 Davy observed in his experiments that chlorine gas dissolved in water froze more readily than pure water. What he had discovered was chlorine hydrates. Over a long period after this discovery the interest in hydrates was purely academic: the early works (1810-1934) were focused on mapping all types of compounds that, when mixed with water, transformed into solid hydrates. In 1934, the first hydrate plug in a gas transport line was reported by Hammersmith [79], and gas hydrates as a challenge to the industry became a major focus in hydrate research. Since this discovery the field of gas hydrate research has become large. Only for the five last decades it has been recognised that gas hydrates naturally occur in deep seas and permafrost, and that these may represent a potential source of energy for the

future. However, they are also being considered a potential source to future climate changes and to geohazards [1].

3.2 Molecular structures

Hydrate clathrates are crystalline inclusion compounds. They consist of small guest molecules trapped inside cavities in a framework formed of hydrogen bonded water molecules. They look very much like snow or ice (Figure 3.1), but can exist at temperatures far above the freezing point of water. Dependent on the size of the trapped molecules, the common types of structures are: cubic structure I, cubic structure II and hexagonal structure H, as shown in Figure 3.2 (illustration from Sloan [80]). Figure 3.3 shows a segment of cubic structure I in more details, illustrated with methane as a guest molecule.

Figure 3.1: Decomposing propane hydrates (photo by Kristin Erstad, Department of Chemistry, University of Bergen, 2006).

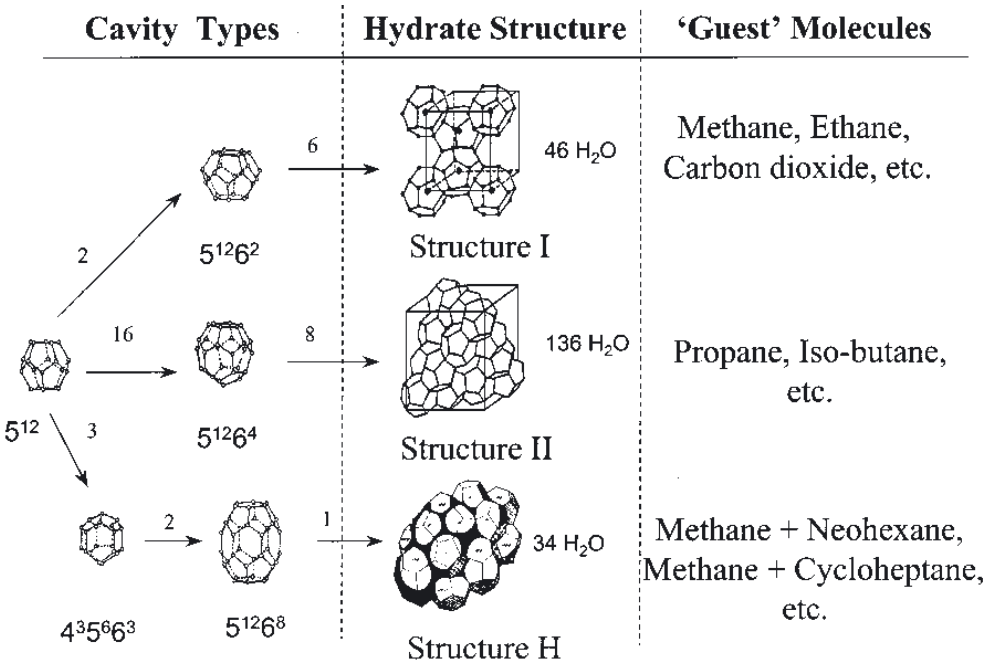

Figure 3.2: Figure taken from Sloan [80]: Three common hydrate unit crystal structures. Nomenclature: $5^{12}6^4$ indicates a water cage composed of 12 pentagonal and 4 hexagonal faces. Along the lines are indicated the numbers of cage types. Example: the Structure I unit crystal is composed of 2 5^{12} cages, 6 $5^{12}6^2$ cages, and 46 water molecules.

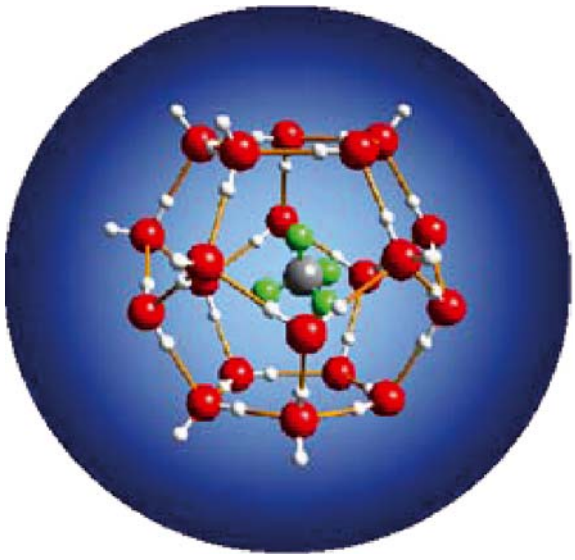

Figure 3.3: Simplified illustration of framework of cubic structure I hydrate, with an encapsulated methane guest molecule. The figure is adapted from University of Göttingen, GZG.

3.3 Phase equilibriums of gas hydrates

The formation of hydrates is restricted to confined temperature- and pressure ranges. The T-P area of where hydrate formation is possible is determined by "hydrate phase diagrams". The positions of the phase boundaries in such diagrams are dependent on the total composition of the system. I.e. the nature of the hydrate forming component (i.e. type of gaseous guest molecule, single- or multiple component gas), the composition of the water phase (pure water or water with dissolved salts/inhibitors) and if an additional fluid is present (e.g. a crude oil); the composition of the specific fluid. Based on this information, theoretical hydrate phase diagrams can be constructed with commercial hydrate simulator software. The accuracy of such prediction tools is evaluated by comparing the theoretical data with experimental data [81]. An example of a hydrate phase diagram for a system consisting of pure water and a single hydrate former (propane) is shown in Figure 3.4.

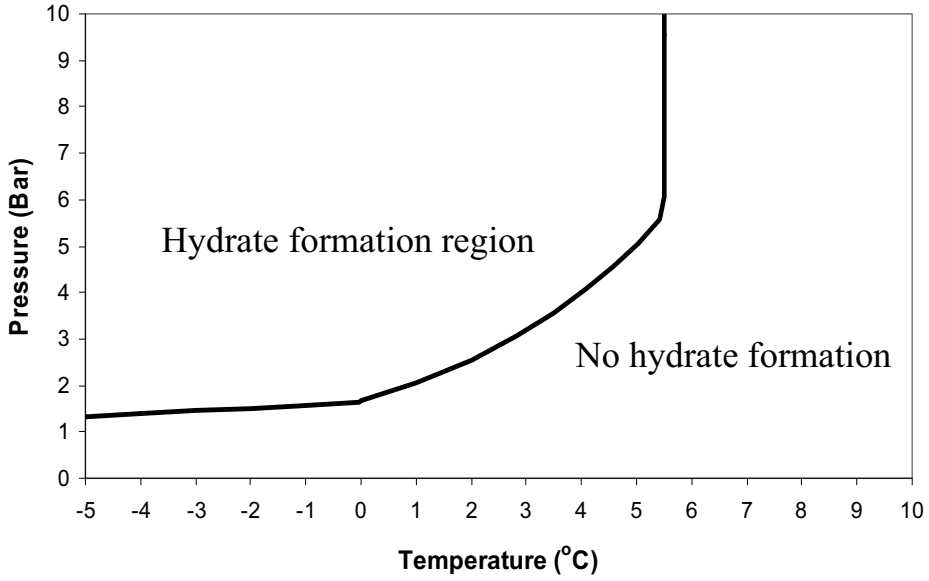

Figure 3.4: Simple schematic overview of gas hydrate behaviour in a system of pure water and propane. The phase diagram is calculated by means of the software PVTsim from Calsep.

The thick line illustrates the hydrate phase boundary . To the left of this boundary P-T conditions that favor hydrate formation are present, whereas in the region to the left hydrate formation is not possible in this system.

3.4 Gas hydrates in an industrial perspective

In petroleum production, the formation of hydrates is observed in pipelines and production facilities [1]. Petroleum associated natural gas consists mainly of methane, ethane and propane. Hence, the presence of propane facilitate the formation of structure II hydrates in multiphase transport pipelines [82] (see also Figure 3.2), although a possible coexistence of structure I and II has been predicted [83]. The oil- and gas industry is moving the production into deeper waters and at longer distances from the shore. This involves transport of unprocessed gas and oil over long distances, where the fluids are gradually cooled to sea floor temperature. Here, at high pressure- and low temperature, the conditions for hydrate formation are ideal, and the risk of hydrate plug formation is impending [82]. The risk of blockage of oil- and gas pipelines due to formation of hydrate plugs is a severe problem, and represents a negative impact of the project economy as well as safety hazard for the production personnel. Removal of plugs by depressurisation can cause the plug to become a hazardous projectile that can cause severe damage on equipment, or even loss of life [84]. Figure 3.5 shows a propane hydrate plug being dissociated by depressurisation during laboratory experiments, the pressure being released very carefully from both side of the plug. This is considered the safest method of hydrate plug remediation in pipelines [82].

Figure 3.5: Dissociation of propane hydrate plug by two-side depressurisation in the laboratory (photo by Kristin Erstad, Department of Chemistry, University of Bergen, 2006).

3.5 Chemical inhibitors

3.5.1 Thermodynamic inhibitors

To avoid hydrate plugging scenarios described in the previous section, the oil companies undertake hydrate prevention strategies. Traditionally these strategies are conservative and expensive, assuming that plugging will always occur. In practical applications the strategy means that operators design the transport conditions such that the well-stream is never allowed to reach the hydrate stable area of the pressure, temperature phase diagram. A major tool for manipulating the phase diagram is thermodynamic inhibitors (typically methanol and monoethylene glycols (MEG)) [1]. As shown in Figure 3.6 the addition of methanol shifts the phase boundary to the left, towards higher pressures and lower temperatures. Hence, their performances are very similar to those of anti-freezing agents. This prevention strategy is expensive, because large amounts are required; 10-50 % of the water phase [85].

In addition, this type of treatment involves recovery of the alcohols from the processes. Hence, if the field is small or the transport length is too long, then the hydrate strategy may be a project stopper. As many of the larger fields are in their tail production phase, large volumes of water and gas must be handled. During shut down and restart of these fields huge amounts of methanol and MEG are normally used.

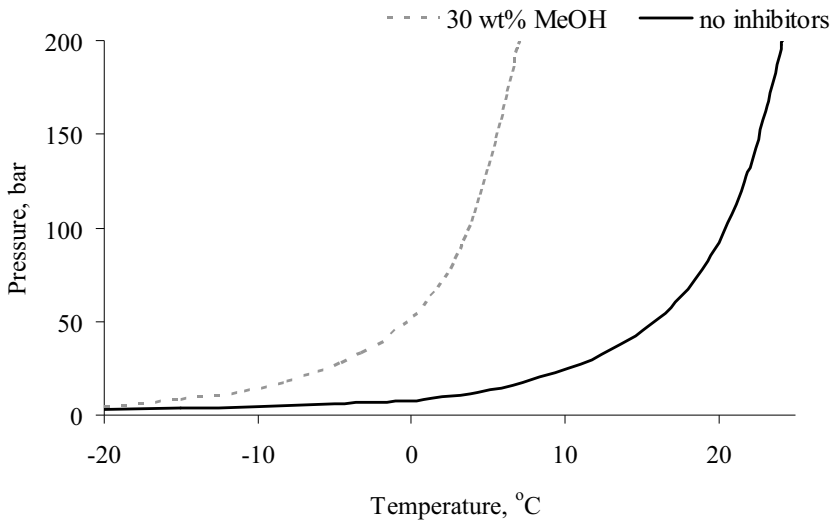

Figure 3.6: Shift of hydrate equilibrium line towards lower temperature and pressure by addition of 30 % methanol (grey stippled line) compared to a system with no inhibition (black line). The hydrate former is a natural gas consisting of 90.4 % methane, 5.2 % ethane, 2.1 % propane and trace amounts of N_2, CO_2, *iso*-butane, *n*-butane, *iso*-pentane, *n*-pentane and C6). The phase diagrams are calculated by means of the software PVTsim from Calsep.

3.5.2 Low dosage hydrate inhibitors

The cost considerations associated with thermodynamic inhibitors has motivated the search for alternative hydrate strategies. The development of so-called "Low dosage hydrate inhibitors" (LDHIs) as replacement for alcohols has been a subject of research for many decades. A comprehensive review of

the literature within this research field is given by Kelland [2]. Presently two main types of LDHIs exist; kinetic inhibitors (KIs) and anti-agglomerants (AAs). Neither of these alters the thermodynamic equilibrium of hydrates, but their surface properties affect the hydrate kinetics and agglomeration, respectively. LDHIs are effective at low concentrations (typically 0.5-3 wt %) [86]. Their performances are also dependent on the extent of subcooling in the system, i.e. the difference between the hydrate equilibrium temperature and the operating temperature at a given pressure [2].

KIs work by delaying the initial hydrate nucleation, i.e. increasing the induction time of hydrate formation. Also, kinetic inhibitors adsorb onto growing hydrate crystals at the hydrate/water interface, preventing small hydrate crystals to grow into larger crystals, thus slowing the rate of growth and prolonging the period of time before catastrophic growth occurs [1]. This delay in hydrate growth means that one may operate within the hydrate stable area of the phase diagram for a given amount of time without the appearance of hydrates. KIs are typically water-soluble polymeric compounds [87–89].

The second group of LDHIs, the AAs, are inhibitors that prevent agglomeration but not formation of hydrates [1]. The key to the AA effectiveness is their structures and surfactant properties. AA surfactants are thought to work by containing polar head groups that can interact with the lattice of hydrate water molecules, and a hydrophobic tail group that attracts the hydrocarbon phase [90, 91]. Such a mechanism influences the hydrate wettability, rendering the hydrate surface "oil-wet". Oil-wet particles tend to stabilise w/o emulsions [7, 9]. The AA surfactants are believed to stabilise the water phase as small droplets dispersed in the hydrocarbon phase as a w/o emulsion, and the hydrates form within these small droplets [1]. Hence, AAs cause the hydrates to form dispersions or suspensions that are kept in

the oil phase and easily transported as slurries in production lines. An additional proposed mechanism is that AAs disperse the hydrate particles by preventing them sterically from contact and adhering together [92].

LDHIs have a huge application potential to replace the thermodynamic inhibitors (methanol and glycols). As of today the way of how LDHIs work at a molecular level is not yet fully understood or documented, even though they have now been applicated in the field [93]. However, the use of LDHIs is restricted on the Norwegian continental shelf due to their toxicity. The main concern is their low biodegradability. The work of developing new and more environmentally friendly LDHIs is currently ongoing [85, 89, 94].

3.6 Natural inhibitors

In multiphase transport systems where crude oils are present, plugging is never observed in some cases, even though the system may be located well within the stable P-T area of hydrate formation. The natural ability of some crude oils to inhibit hydrate agglomeration has been reported by several authors [3–8]. This phenomenon is attributed to crude oil composition and the presence of indigenous hydrate mitigating components. Some authors name these compounds "natural inhibiting components" (NICs). These are believed to be natural anti-agglomerant surfactants with affinity for the hydrate surface, adsorbing to the hydrate surface by hydrogen bonding interactions (as discussed in the previous section). This idea is illustrated in Figure 3.7, where the NICs create "oil-wet" hydrate surface, the particles being surrounded by a layer of NICs. This mechanism prevents agglomeration of hydrate particles that otherwise may lead to building up of hydrate plugs. The generation of oil-wet hydrates correlates with low hydrate plugging ten-

dency, as a result of a change in surface energy of petroleum hydrates. [7,95]. It has been reported that the effectiveness of the natural anti-agglomerants is strongly dependent of salinity and pH [96].

Although the field of research is in its early stage and published material is sparse, several authors emphasise that acid fractions from some crude oils impose anti-agglomerating effects on hydrates and preventing plugging [9,11,91,97]. The work presented in this thesis is solely based on isolation and characterisation of petroleum acids in this context, although it is important to have in mind that other classes of petroleum constituents also may be natural inhibitors.

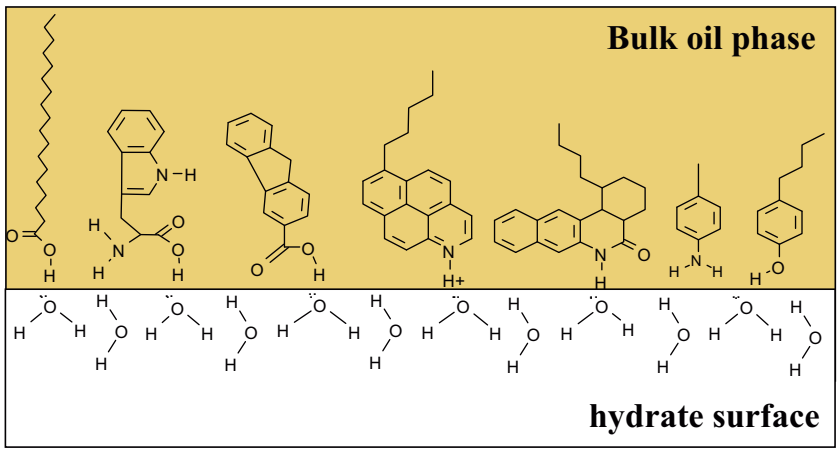

Figure 3.7: Illustration of how interactions in the crude oil-hydrate interface may take place. Polar, natural inhibiting components in the oil adsorb to the hydrate surface, making it oil-wet (adapted by courtesy of Sylvi Høiland).

Chapter 4

Methods for Characterisation of Crude Oil and Crude Oil Fractions

4.1 Determination of acid content

Quantification of the acid content in petroleum and petroleum fractions is mostly done either directly, by non-aqueous titration methods, or by various acid extraction methods. The titration methods are destructive, i.e. they provide a quantitative measure of the of total acid content, but after the measurement is done the samples can not be taken to further analysis. These methods do not provide detailed compositional information about compound groups present in the sample. Using acid extractions methods, however, acids are physically isolated from the bulk matrix. The isolates can be quantified and also be taken further for characterisation with other analytical methods.

4.1.1 Acid extraction

A wide range of separation schemes for isolating acidic compounds has been reported in the literature. Typical separation schemes involve extensive multistep separations that require pre-separation of the resin fraction prior to the acid extraction [59, 62]. Other methods extract the acids directly from the crude oil matrices. Liquid-liquid extraction techniques are widely used. Here, alkaline base solutions are used to remove the acids based on partition principles [39,48,53,68,98,99]. Another common direct method is solid-liquid extraction. Such methods often make use of ion-exchange resins [100–102]. The solid phase (ion exchange resin) typically consists of quaternary ammonium amines with bonded anions that are exchanged with the anions of the analyte [103]. Also, liquid chromatographic techniques based on adsorption/polarity of compounds have been been reported [104, 105].

In the works presented in this thesis two different methods have been applied; liquid-liquid extraction, described by Costantinides and Arich [48] and others [40,71] (Paper I and II), and ion-exchange methodology described by Mediaas et al. [102] (Paper I-V and Research report). This ion-exchange method uses a sugar-based resin and has proved to have both high effectiveness and selectivity.

4.1.2 TAN - Total Acid Number

TAN is a non-aqueous potentiometric titration procedure commonly used in the oil industry for measuring acidity of crude oils [37, 62, 101, 106]. TAN is defined as the amount (mg) of potassium hydroxide (KOH) necessary to titrate 1 g sample to a well-defined inflection point. Procedures have been standardised by the American Society for Testing and Materials (ASTM).

ASTM664-89 [107] is a method where the titration is performed with a solution of KOH in 2-propanol. This method has recently been modified by Barth and Strand in order to achieve an improved detection of the weaker acids in crude oils [108]. Although TAN is the standardised method in oil industry, other non-aqueous potentiometric titration procedures are reported for determination of acidic and basic content in petroleum [109, 110].

4.2 Chromatographic methods

Generally, *chromatography* is a technique in which the components of a mixture are separated based on the rates at which they are carried through a stationary phase by a gaseous or liquid mobile phase [111]. There exist a multitude of chromatographic methods. Some of these are described in the following sections (Sections 4.2.1 - 4.2.5).

4.2.1 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a chromatographic method where compounds are separated based on their affinity to, and partition between, a liquid mobile phase and a stationary phase that consist of a finely divided material. Based on the polarities of the mobile and stationary phases, the chromatographic separation can be divided into two types. In *normal-phase chromatography* the stationary column is packed with a polar material. The polarity of the material can be varied, and is normally chosen based on an assessment of the polarity of the sample. Typically functional groups used are amino, cyano or diol [103]. The mobile phase in such systems is usually a non-polar solvent (if a single single solvent is used). In normal-phase chromatography the least polar analyte elutes first. In *reversed-phase*

chromatography a non-polar stationary phase and a polar mobile phase are utilised. The stationary phase is typically a silica based material modified with a hydrocarbon group, the most common one being C18. In reversed-phase chromatography the least polar compounds are eluted last.

The solvent mobile phases are chosen based on achieving the most effective separation for the particular system in question. *Isocratic elution* means that the mobile phase consists of one single solvent, as described above. However, *gradient elutions* are more common. Here, two or more solvents are varied throughout the run, to achieve a better separation.

There exist no universal detectors for HPLC, but a range of detector types are available, each having specific performances. Thus, it is important to choose a detection system that has specific properties that suits for the nature of the sample one wants to study, and be aware of the limitations the detector represents. Some common used HPLC detectors are based on UV/VIS absorbance, refractive index, fluorescence, electrochemistry and light scattering principles. The output of the detection is a *chromatogram*, which is a plot of peaks giving an indication of concentration of sample components as a function of their elution time through the chromatographic column.

Various HPLC analytical procedures are used to separate crude oils and crude oil fractions into various component groups [13, 112–114]. HPLC procedures developed specifically for naphthenic acids [58] and acidic petroleum fractions [66, 67, 104, 115] are reported in the literature.

In the works of this thesis (Papers I, II, IV and V) HPLC in normal-phase has been used to study our petroleum acid extract samples. A cyano column of intermediate polarity, and a three component mobile phase gradient consisting of hexane, dichloromethane and methanol have been applied.

Two types of detectors have been used; the UV detector is selective towards, and suitable for, compounds that contain unsaturated organic functional groups that can absorb light in the ultraviolet or visible region, so-called *chromophores*. Thus, this detector has a limited applicability for the constituents of a sample that possess an aliphatic nature. Hence, a complementary detector has also been used. The evaporative light scattering detector (ELSD) can be applied to most compounds with molecular weights from about 200 g/mol [103]. Thus, if the sample contains molecules of low molecular weights and low boiling points they will not be detected if they evaporate together with the eluent solvents.

4.2.2 Solid Phase Extraction

Solid phase extraction (SPE) is a chromatographic technique that has many benefits: it is rapid, inexpensive and reproducible, and requires low sample- and solvent amounts [116]. The technique is based on partition principles, where the analytes have stronger affinity for, and adsorb to, a solid phase rather than the sample matrix. After adsorption the compounds can be removed from the adsorbent material and collected sequentially, using solvent eluents that have affinity for the different compounds that are retained on the solid phase. The most common application of SPE is to use cartridges packed with a suitable solid phase (these are generally similar to the HPLC columns described in Section 4.2.1). This technique has been used for petroleum samples, e.g. for separation of aliphatic and aromatic hydrocarbons [117], and also for analysis of naphthenic acids [99, 100]. In this work a SPE technique for rapid, preparative subfractionation of crude oil acid extracts is developed (Paper I). The technique has further been applied in the works of Paper III-IV and the Research report.

4.2.3 Gas Chromatography

In gas chromatographic techniques the components in a sample are separated based on their volatility, and on their affinity to a stationary phase and a mobile, gaseous phase. The instrumental set up is typically as shown in Figure 4.1. The most common stationary phases used for complex mixtures like petroleum are capillary columns [103]. These consist of a non-volatile liquid that covers the inside walls of the column. The mobile phase is a chemical inert gas, typically helium. An injection system is designed to introduce the sample into the gas stream and to the column. By programming the temperature oven to increase step-wise or continuously, samples with a broad boiling range achieve increased chromatographic separation on the column, and they reach the detector at different retention time. The detector most commonly used for organic compounds in GC analysis is the flame ionisation detector (FID). The principle behind this type of detection is that the electrical conductivity of a gas is proportional with the concentration of charged particles, i.e. ions, in the gas. And the number of ions produced is related to the concentration of sample material that is combusted in the flame.

A "Whole Oil Gas Chromatography" (WOGC) technique for assessment of the extent of biodegradation in crude oil samples has been applied in Paper V. "The Norwegian Industry Guide to Organic Geochemical Analyses" (NIGOGA) [118] is produced in order to achieve consistency in the analytical laboratory procedures of Norwegian oil companies and the Norwegian Petroleum Directorate. The instructions given in this guide are used for identification of compounds, and for assessment of the quality of the data. This includes using a thoroughly characterised standard oil (the Norwegian Geochemical Standard oil, NSO-1) for assignment of peaks.

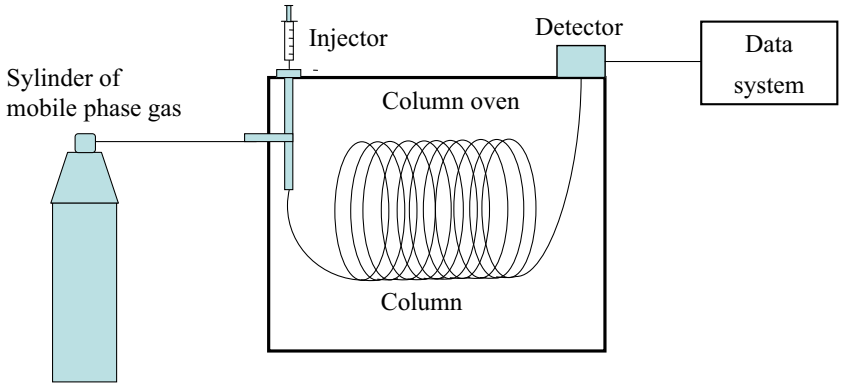

Figure 4.1: Schematic illustration of components in a gas chromatographic system.

4.2.4 Gel Permeation Chromatography

Gel Permeation Chromatography (GPC), sometimes called Size Exclusion Chromatography (SEC), is a technique that separates molecules according to size and shape [119]. In the chromatographic system a column packed with a porous material, having a given pore size, is used as the stationary phase. Traditional stationary phases in GPC consist of polysaccharides or polystyrenes. The sample molecules are separated due to their exchange between a liquid mobile phase and the stagnant liquid inside the pores in the packing material, by diffusion. Dependent on the pore size, the largest molecules will retain the least time in the column as they will be excluded from the pores, and elute first. The smallest molecules will spend longer time in the pores and elute last. In GPC, the relationship between retention time and molecular weights is calibrated with standard compounds of known molecular weights. When selecting the mobile phase it is of major importance to choose a solvent of sufficient "solvent power" for the specific sample in question. The phenomenon of molecular aggregation/polymerisation, especially for the asphaltene fraction is a well-known challenge in these types of analysis, and can lead to incorrect M_W range determinations [120]. Al-

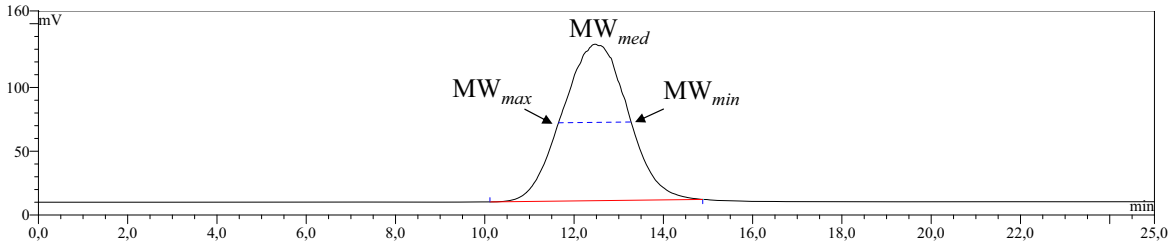

Figure 4.2: Example of a GPC chromatogram.

though GPC is a commonly applied technique for petroleum samples, it is not an exact method. It has several disadvantages; the lack of realistic standards similar to petroleum constituents, adsorption to the gel material on the column, and influence of molecule geometry being common factors that can obscure the analysis [13, 40].

In this thesis (Paper II, III and IV) GPC has been applied to the samples, using the same instrumental setup as for HPLC. The stationary phase column consists of polystyrene/divinylbenzene. An isocratic (one-solvent system) mobile phase elution with tetrahydrofuran (THF) is used, and the ELSD for detection. The output is a chromatogram like shown in Figure 4.2. The retention time that represent the maximum height of the peak is used to calculate the medium molecular weight of the sample (MW_{med}), which is assessed as an "average" molecular weight of all the molecules that constitute the sample. The retention times at the width at half the peak height can be used as an evaluation of maximum molecular weights (left side, MW_{max}) and minimum molecular weights (right side, MW_{min}) respectively. Having in mind the limitations of the method, only approximate molecular weight ranges can be achieved. However, modern high resolution mass spectrometric techniques are now emerging, providing molecular weight measurements with high degree of accuracy (see Section 4.3.3).

4.2.5 Thin Layer Chromatography - Flame Ionisation Detection

Thin Layer Chromatography - Flame Ionisation Detection (TLC-FID) is a method used for determination of the distribution of saturated (aliphatic) hydrocarbons, aromatic hydrocarbons, resin- and asphaltene fractions in petroleum and solvent extracts from rocks [38, 77, 118, 121, 122]. This is a rapid way of performing SARA analysis. The technique is often also titled with the trade name "Iatroscan", which is a special instrument equipped with a FID. The stationary phase in this system consists of special designed thin layer rods coated with silica or aluminium oxide (Chromarod®). The samples are applied near the end of the rods, and the rods are placed in special development tanks, saturated with the respective eluent solvents of increasing polarity. The different fractions in the sample are separated as they migrate upwards on the rods to different distances, depending on which solvent system they elute with. The rods are then scanned in the Iatroscan instrument, by leading them through the hydrogen flame of the FID.

This technique has been applied to three oil samples in Paper V, where further details can be found.

4.3 Spectroscopic methods

Spectroscopy involves absorption of, and resolution of, different types of electromagnetic radiation into their wavelengths to produce a *spectrum*. Spectroscopic techniques based on UV/visible and infrared radiation are described in the following sections (Sections 4.3.1 and 4.3.2). As new techniques have evolved the concept of spectroscopy does not any longer apply solely to techniques were electromagnetic radiation is involved, but has been extended

to involve e.g. mass- and electron spectroscopy [111]. Mass spectrometry (MS) (described in Section 4.3.3) differs from other organic spectral analysis by being determined by chemical reactivity rather than electromagnetic radiation. However, it is customary to consider MS along with the common spectroscopic methods. Spectroscopic methods are very central tools in general organic chemistry. During the last decades, application of spectroscopic studies in the evaluation of petroleum and petroleum products have become of increased importance. Many analytical spectroscopic methods have now been integrated and standardised by the American Society for Testing and Materials (ASTM) [13].

4.3.1 Ultraviolet/Visible Spectroscopy

The ultraviolet- and visible (UV/VIS) spectra of organic compounds are based on transitions between electronic energy levels when the radiation is absorbed by a molecule. The UV region comprises wavelengths between approximately 200-400 nm, while the visible region is between approximately 400-800 nm. The degree of absorption intensities follows the Lambert-Beer‘s laws [123]. The technique is applicable to analytes containing *chromophores*, i.e. compounds that contain electrons involved in double- and triple bonds or heteroatoms. Thus, pure aliphatic compounds are difficult to study with this technique.

UV/VIS absorbance spectra are typically recorded from dilute solutions in a spectrophotometer, where a beam of light is passed through the sample solution. The solution is commonly placed in silica- or quartz cuvettes that have a certain thickness. Several works cited in the literature have utilised UV/VIS spectroscopic techniques to study aromatic and heterocyclic constituents of petroleum, e.g. the highly condensed asphalthenes [124–126] and

polar fractions [127]. As discussed in Section 4.2.1, detection systems based on UV absorbance are also often used in HPLC, providing spectroscopic information of the compounds that constitutes the peaks in a chromatogram. In this thesis an UV detector coupled to the HPLC system has been applied in the works presented in Paper I, II and V. In Paper IV a spectrophotometer instrument and a 1×1 cm quartz cuvette have been used.

4.3.2 Fourier Transform Infrared Spectroscopy

Infrared spectroscopic methods yield information on functional groups present in the sample molecules, e.g. C-H, C=C, C=O, O-H functions, and so on. When the molecules are irradiated by infrared light, an alteration in their vibrational energy state occurs when the radiation is absorbed. The absorption is registered by a *spectrometer* instrument, and this is the foundation of the *infrared spectrum* of absorption bands. Fourier Transform Infrared Spectroscopy (FTIR) spectrometers provide rapid analyses with very high sensitivity. Here, the signals are transformed by a mathematic operation (Fourier Transformation) into a plot of absorbance versus wavenumber. FTIR is widely used for characterisation of petroleum fractions [38], including acidic fractions [17, 40, 53, 98]. FTIR has been a central method in the work of this thesis, and has been applied for characterisation of acid extracts in Paper I, II, IV and V. A spectrometer with an Attenuated Total Reflection (ATR) measuring cell has been used. This technique involves sending the infrared radiation into a diamond crystal that is in contact with the sample. The radiation is reflected in the diamond, and a wave is sent into the sample where it is attenuated in the region of the spectra where the sample absorbs energy. This causes an alteration in the radiation emitted from the diamond crystal, which is detected and gives the basis of the FTIR spectra for the

sample.

4.3.3 Mass Spectrometry

Mass spectrometry (MS) is an unique, analytical method that provides a means of studying samples on a molecular level. Generally, the technique involves ionisation of the sample molecules in an *ionisation source*. Depending of the type of ionisation source applied, the charged molecules can further break up into fragment ions, where the fragmentation pattern is unique for the molecule in question. This fragmentation pattern is used for identification of the molecule. The ions are separated in a *mass analyser* prior to detection. The output of the detector is a *mass spectrum*, which is a plot of the mass/charge ratios (m/z) versus detector response.

Often MS is combined with pre-separation using chromatographic methods, like liquid chromatography - mass spectrometry (LC-MS) and gas chromatography - mass spectrometry (GC-MS). In these hyphenated techniques the compounds are introduced into the mass spectrometer one by one, after the chromatographic separation. Unfortunately when analysing complex mixtures, especially heavy petroleum and heteroatomic petroleum fractions such as naphthenic acids, several disadvantages with these techniques exist [59, 61, 128–130]. Due to their high molecular weights and non-volatile nature such samples often fail to be analysed by GC. Derivatisation methods are applied to increase the volatility, but these methods often involve several work-up steps and consequently loss of sample. Both the ionisation source and mass analysers used in conventional GC-MS may often not be powerful enough to produce nor detect sample ions. In addition, the high level of complexity of the samples combined with poor resolving power of the instrumentation often produce a hump of unresolved peaks (UCM) in the

chromatograms [37, 53, 59]. However, the latest years, new and powerful MS techniques are developed, and are now on the verge of solving many of these problems.

Electrospray ionisation (ESI) in negative mode generates a negative charged molecular ion $(M\text{-}H)^-$, and produce minimal fragmentation. Non-fragmenting mass spectrometric techniques provides a wealth of information on practically all the components in a fraction, and molecular formulas can be assigned and concentrations determined [14]. ESI is highly suitable for ionisation of acidic species [57, 131, 132]. ESI can be applied directly to the sample matrix without pre-chromatographic isolation, due to its high selectivity for polar heteratomic compounds and the suppression of the bulk hydrocarbon mixture [35, 130]. However, pre-separation is still highly favourable, and will increase the resolution even further (personal communication, Dr. Stefanie Pötz). The extremely complex nature of petroleum requires a mass analyser with ultra-high resolution capacity [133]. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), is now emerging as the superior tool for such challenges in terms of accuracy and resolution, providing resolution 10-100 times higher than any other analysers [134].

Mass spectrometric techniques have been applied in the work of this thesis (Research Report) to study the molecular composition of acid extracts and their subfractions (SPE fractions). A GC-MS with Electron Impact (EI) ionisation technique and a quadropol mass analyser has been applied for a quick screening analysis without sample derivatisation, with the purpose of detecting possible ester compounds. The same samples are also analysed with ESI-MS (Electrospray Ionisation-Mass Spectrometry) by Dr. Stefanie Pötz at Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences. This work is still in progress, but some preliminary results are presented in

the Research Report. At this centre a MS with a double focusing magnetic sector field mass analyser and an ESI source is available.

4.4 Physical properties

Some methods for assessing physical properties of crude oils and crude oil fractions are presented in the following sections (Sections 4.4.1 - 4.4.3).

4.4.1 Elemental Analysis

Elemental analysis gives the percentages of carbon, hydrogen, nitrogen, oxygen and sulphur for organic compounds. Such types of analyses are widely used to perform an evaluation of the nature of petroleum samples [13,98,135]. Assessment of the overall character of petroleum samples are often given as ratios. H/C gives a measure of the aromaticity, N/C, O/C and S/C provide assessments of heteroatom concentrations. In Paper IV a combustion analyser has been used to determine the content of C, H, N and S in acid extracts and SPE fractions. The content of O is calculated by difference. The basic principle of quantitative CHNS-O analysis is high temperature combustion. The gaseous combustion products are transferred by a helium carrier gas into a copper tube where they are purified and separated into their various components in specific adsorption traps, then sequentially analysed with a thermo conductivity detector (TCD).

4.4.2 Density

Density is defined as the mass of a unit volume of material at a specified temperature, and is a common parameter for assessment of the quality of a crude oil. [13, 14]. Biodegradation processes (described in Section 2.3) are

known to increase the densities of crude oils [40]. Density may be measured by means of several techniques. In the work of this thesis, two different methods have been applied. A glass *pycnometer* is a rapid and convenient way of measuring the density of a liquid. The device is a volumetric glass vessel equipped with a thermometer. The vessel is filled up with the liquid at a chosen temperature and weighted. The density is then determined from the volume and weight of the liquid (Paper III). The second means of measuring density (Paper III and V) has been by use of a *densitometer* instrument. In this technique the liquid is filled in a special designed tube that is subjected to harmonious oscillations by an electromagnet. The density can be calculated from the period of the oscillation, which is dependent on the density of the fluid [136].

4.4.3 Interfacial Tension

Interfacial tension (IFT) is a measure of the force acting at a boundary between two immiscible liquid phases (or *surface tension* if the boundary is between liquid/solid or liquid/gas). There exist numerous techniques to measure interfacial- and surface tension properties [137, 138], and they are readily used for assessment of petroleum samples [13]. We have applied a classical drop-volume method for IFT measurements (Paper III and V), described by Harkins and Brown [139] and Adamson [137]. The technique is based on measuring the maximum volume of a pendant drop formed at the tip of a capillary in the bulk of another liquid (Figure 4.3). The interfacial tension is measured from the following equation (Equation 4.1).

$$\gamma = \frac{V \cdot \Delta\rho \cdot g}{2\pi \cdot r \cdot F} \tag{4.1}$$

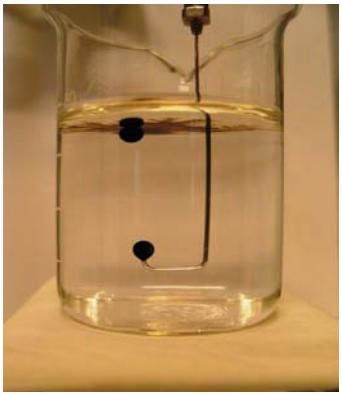

Figure 4.3: Liquid oil droplet formed in the bulk of liquid water, at the tip of an inverted needle.

where γ is the interfacial tension (mN/m), V is the volume of one droplet (m^3), $\Delta\rho$ = density difference between the two liquids (kg/m^3), g is the gravity constant (m/s^2), r is the radius of the inverted needle (m) and F is a dimensionless, empirical correction coefficient.

4.5 Hydrate wetting properties tests

Wettability is another term, or measure, of the energies associated with surface tension. The surface energy of petroleum hydrates is believed to be closely related to the plugging tendency, as discussed in Section 3.6. As mentioned in Section (4.4.3), a range of techniques are available for measuring surface- and interfacial energies. On the other hand, general and convenient methods for measuring the wettability/surface energies of *hydrate surfaces* have hitherto not been available. However, Høiland et al. [7] have developed a method where the wettability of Freon hydrates can be assessed quantitatively from their emulsion behaviour. This method has been applied in the work of Paper III, and is described in the next section (Section 4.5.1). Freon hydrates are convenient to work with in the laboratory as they form at atmospheric pressure, and requires no pressurised or advanced instrumenta-

tion. In Paper V the hydrate wettability, or the hydrate plugging tendency, has been assessed using a high pressure sapphire cell, see Section 4.5.2. Here a synthetic natural gas mixture is used as the hydrate former. This set-up resembles more realistic hydrate formation conditions.

4.5.1 Inversion points in crude oil/brine emulsions

The basic approach to this method is that solid particles that are present in an emulsion of a water/oil system, at the interface between the two phases, may obtain various wettability states depending on the oil/water/solid interfacial tensions of the system. The classifications are labelled oil-wet, water-wet or intermediate wet, depending on which of the fluids that are most likely to wet the particles. This is dependent on the chemical composition of the crude oil, i.e. whether there are components present in the crude oil that possess affinity for the solid surface [7]. According to the theory of colloid particle stabilised emulsions, the wettability state of the solid particle will determine whether it stabilises an water-in-oil emulsion (oil-wet solid surface) or an oil-in-water emulsion (water-wet solid particle) [140, 141]. The inversion point of such particle stabilised emulsion systems can be determined by a step-wise alteration the volume ratio of the two fluids [142].

Figure 4.4 is a simplified illustration of the method, in this case for system of *oil-wet* Freon particles. The upper system is the initial system, without particles. This is the reference system, measured for each specific oil sample. This is to take into account each individual oils original chemical composition, which will give the initial state of the particular oil sample in question. At low volume fractions of water, the system is an *oil-continuous* emulsion. When step-wise increasing the volume fraction of water, at some point the emulsion system will no longer be able to maintain the water droplets dispersed. At

this stage the system ceases to be oil-continuous, a water phase becomes visible and the viscosity of the system alters abruptly. This stage represents the *point of phase inversion*, φ^{inv}. As can be seen in the example given in Figure 4.4, for the initial system φ^{inv} has a value of 0.45. Increasing the volume fraction of water even further, the system will eventually transform into a completely *water-continuous* emulsion system. The lower system is the same oil/water system, but in which hydrate particles are present. In this particular case the hydrate particles are oil-wet, from adsorbing components present in the oil. As can be seen, in such a system the emulsion can hold the water droplets dispersed to a higher volume fraction of water before the oil-continuous emulsion breaks. And the point of phase inversion shifts to 0.65. This is due to the stabilising effect on the water-in-oil emulsion the oil-wet hydrate particles impose (in a system of water-wet particles the effect would be the opposite, the point of phase inversion would be *lowered*). This can also be understood from the illustrations of the curvatures of the interfaces packed with oil-wet particles: the situation to the left, where the emulsion is oil-continuous, is more favourable for a system containing oil-wet particles than the water-continuous situation to the right. Figure 4.5 shows examples of the appearance of a water-in-oil emulsion and an oil-in water emulsion.

4.5.2 Plugging tendency tests

Instrumental set-ups for assessment of hydrate plugging tendency, using different types of reactors and flow loops- or simulators, are commonly described in the literature [4, 6, 8, 11, 90, 96, 143, 144]. The plugging tendency tests of crude oil samples described in Paper V have been performed in a high-pressure cell rig set-up. Specifications about this set-up are reported in the literature [3, 95]. A simplified illustration of the instrumentation is

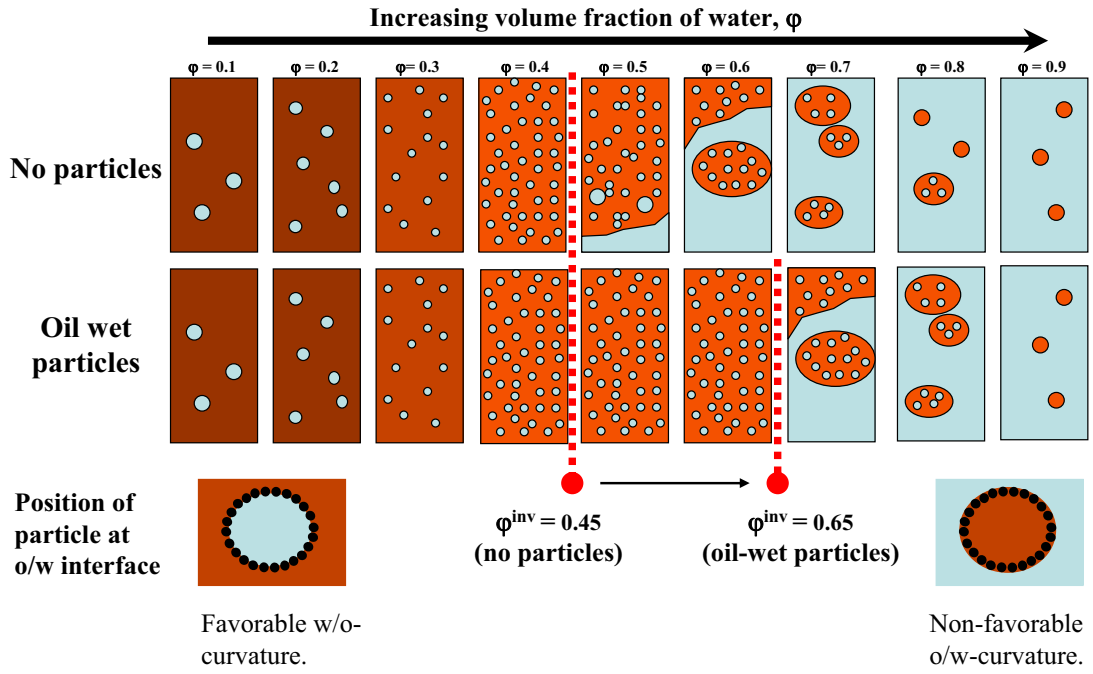

Figure 4.4: Phase inversion in crude oil/brine emulsions containing oil-wet hydrate particles (adapted by courtesy of Sylvi Høiland).

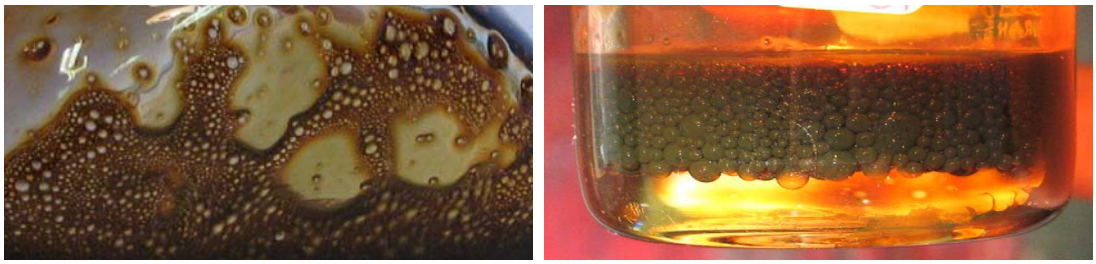

Figure 4.5: Oil-continuous (water-in-oil) emulsion (left) and water-continuous (oil-in-water) emulsion (right) (by courtesy of Sylvi Høiland).

shown in Figure 4.6. The core of the system is a transparent sapphire cell, which is placed inside a climate chamber where the temperature can be controlled. The instrumentation is equipped with a pump system that supplies the fluids (oil sample, aqueous phase and a synthetic natural gas mixture) into the cell where the fluids are mixed. The system is then programmed to a cooling- and heating cycle that provides the hydrate formation- and decomposition. During the experiments the development of temperature, pressure,

volume and cell torque is monitored. In addition, still pictures and videos are recorded, allowing for a visual inspection of morphologies of the hydrates formed. The wettability, or plugging tendency, is assessed in a qualitative manner from the visual images. The cell torque data provides some support in this evaluation, as the torque gives an impression of the viscosity of the fluids inside the cell.

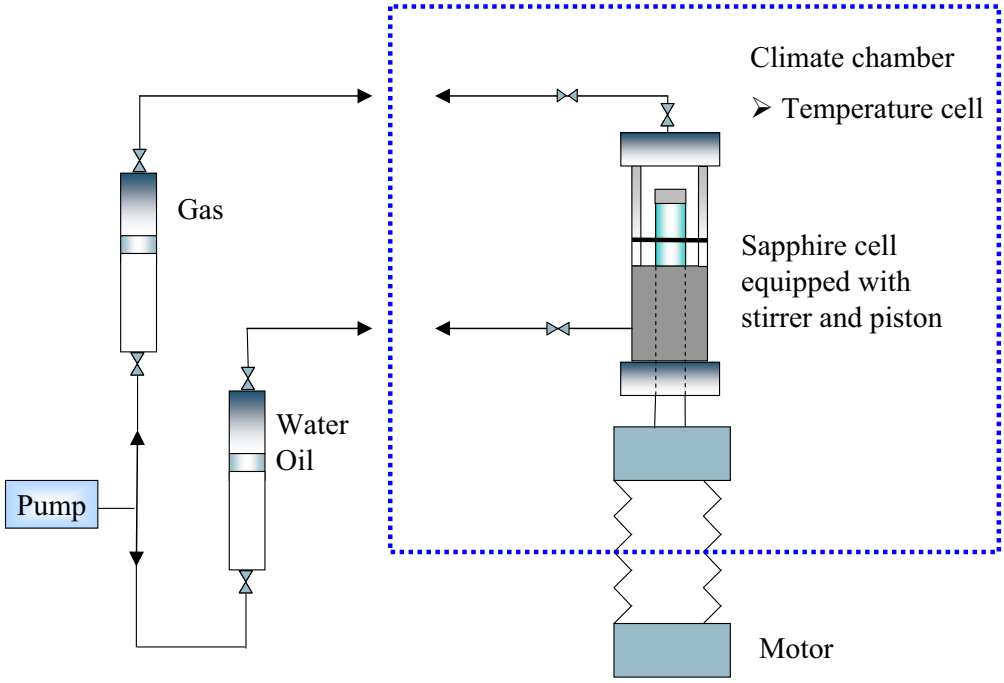

Figure 4.6: Simplified illustration of the high-pressure cell rig, for assessment of hydrate plugging tendency (adapted by courtesy of Kjell Magne Askvik).

Chapter 5

Main Results

5.1 Introduction and outline

In this chapter the main results from the Papers I-V, and the Research report are presented. The chapter is structured roughly by dividing the observations into three sections. The first part emphasises the development of methods for isolating natural inhibiting components from crude oils. This includes testing acid extraction methods that are reported in the literature, and finding the most effective and best suited method for our samples. Moreover; methods for separation of acid extracts further into subfractions are developed. The yields and composition of the isolated extracts and subfractions are presented.

In the second part the results from investigating the separate effects of these acid extracts and their subfractions on hydrate wettability are given. Finally, the results from the analytical characterisation of these samples are presented in the third main part.

The acids have been extracted from a range of crude oils that differ much in chemical composition, physical properties and hydrate plugging tendency.

Table 5.1: Some crude oil characteristics[a] and assignment to the works they have been used in.

Crude oil label	Biodeg. level[b]	Wettability $\Delta\varphi^{*}$[c]	Plugging tendency[d]	In paper/R.r.[e]
B1c	2	-0.32	plug	I, II, IV, R.r.
B2b	6	0.26	dispersion	I, II, III, IV, R.r.
B2c	5	int. wet.[f]	n.m.	I, II
B4a	8	0.35	dispersion	I, II, III, IV, R.r.
B4c	2	0.38	dispersion	I, II, III, IV, R.r.
B4d	n.m.	n.m.	n.m.	I, II
B7a[g]	n.m.	n.m.	plug	V
B7b[g]	n.m.	n.m.	plug	V
S3b	0	-0.06	plug	I, II, IV, R.r.
S7b	0	(0.00) 0.21, 0.27	plug	I, II, III
S9	n.m.	n.m.	plug	V

[a] The data are taken from Paper I-V and and Høiland et al. [7]. Numbers given in brackets indicate that values changed over time (see Paper III). The newest measurements are presented outside the brackets.
[b] Peters and Moldowan scale [33].
[c] Wettability index, see Høiland et al. [7] and Paper III.
[d] From combined field experience and tests performed in high pressure sapphire cells at StatoilHydro ASA R&D centre.
[e] R.r. Research report
[f] Int.wet. This oil contain large amounts of water, and has been evaluated to be of intermediate wettability.
[g] Biodegraded in the laboratory.
n.m. not measured.

A general overview of the crudes, with assignments to which work they have been used for, is given in Table 5.1.

The crude oils originate from the Norwegian continental shelf, and are mostly supplied by StatoilHydro ASA (the S9 oil is provided by Prof. Terje Torsvik, Centre for Integrated Petroleum Research). Some of the properties listed in Table 5.1 are already existing data. Biodegradation levels are assessed on the Peters and Moldowan scale (see [33] and [40]) and wettability values are determined by Høiland et al. [7]. The samples are denoted with the capital letters "B" for biodegraded samples, and "S" for sweet, non-

biodegraded crude oils. These are followed by a number that indicates the fields they are sampled from. The last letter (in lowercase) denotes wells or different batches within one field.

5.2 Fractionation of petroleum acid extracts - method development

The natural hydrate anti-agglomerants present in crude oils are believed to be constituents of the acid fraction. Although the petroleum acid fraction comprises a very small part of the crude oils, it still represents a considerably complex matrix. Hence, in the search for these components, methods that are able to resolve this matrix into subfractions are required, in order to obtain more detailed analyses. The developments of high performance liquid chromatography (HPLC)- and solid phase extraction (SPE) fractionation methods for characterisation of petroleum acids (Paper I) have been essential works in this thesis. These methods have provided a foundation for all the other works in this thesis.

5.2.1 HPLC methods

A cyano column with intermediate polarity (between silica and C18) has been found most suitable for HPLC analysis of the petroleum acid samples (Paper I). An eluent gradient of hexane, dichloromethane and methanol is applied, which ensures elution of acid compounds having a wide range of polarities and functionalities. This type of column material, combined with the chosen eluent program, provides a separation of the acid extracts into four well-defined groups of subfractions with similar chemical features: weak

acids with no acidic protons, saturated carboxylic acids, phenolic compounds and polyfunctional compounds. The responses from two different types of detection give slightly different profiles, see Figure 5.1. The ELSD detects most compounds apart from low-boiling compounds that evaporate together with the solvent. Hence, e.g. certain phenolic compounds will fail to be detected in this system. For this reason an UV detector has been applied for comparison, as this detector provides a sufficient response also for such compound types. The assignments of chromatographic peaks to the given compound classes are done my means of a set of standard compounds. Even after fractionation the subfractions are still too complex for identification of individual compounds, but the method provides a mean of comparison of acid extract from crude oils that possess different properties with regards to e.g. biodegradation and hydrate plugging tendency.

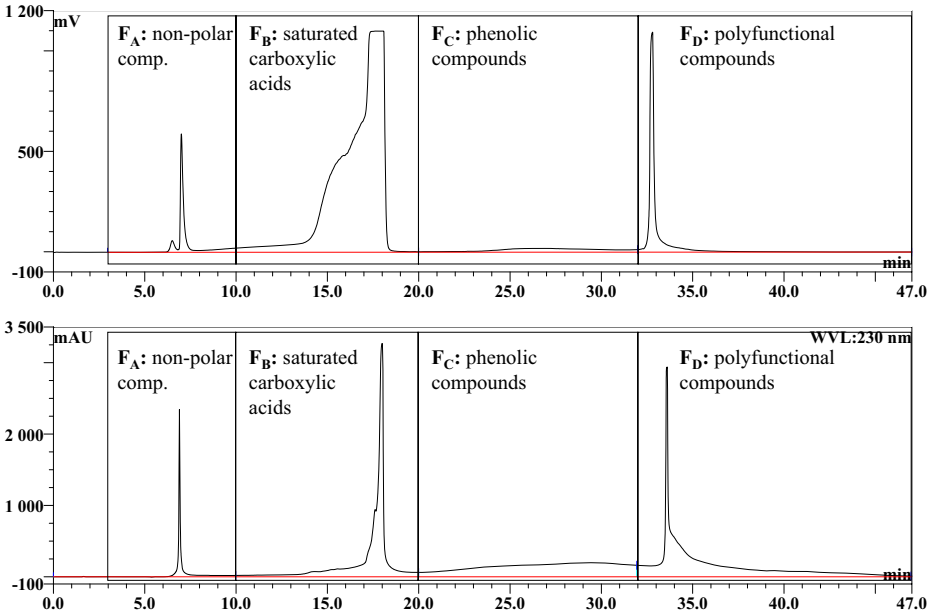

Figure 5.1: Chromatographic profiles of B4c acid extract (from ion exchange method) using an analytical HPLC column and two different detectors. Upper: ELS detector (Figure 2, Paper I). Lower: UV detection at 230 nm (Figure 4, Paper I).

This method has also been developed in a semi-preparative scale, which allows for a physical separation and collection of each subfraction. Thus, sufficient amounts of material for further analysis by complementary methods are obtained. The semi-preparative HPLC method has a slightly better resolution of the carboxylic acid fraction (the peak F_B fraction in Figure 5.1 is separated into two fractions: F_{B1} and F_{B2}).

5.2.2 SPE fractionation

Even though the semi-preparative HPLC method allows for collection of larger fractions, there is still need for a more simple, rapid and upscaled preparative method for separation of even larger acid amounts. A separation corresponding with HPLC fractionation methods is attempted by using cyano SPE cartridges. Even after optimisation of the eluent composition, the SPE columns cannot reproduce the HPLC fractions exactly. This is reflected in limited capacity to resolve the components in the sample. Also, a very strongly polar eluent (containing formic acid) is needed to remove the most polar compounds from the column adsorbent. However, the four fractions obtained with this method, SPE_A-SPE_D, are suitable for testing physio-chemical properties of the acids.

5.2.3 Comparison of selectivity and effectiveness of acid extraction methods

In the two first papers of this thesis (Paper I and II) two methods of extracting acids from crude oil have been applied: an ion exchange method [102] and a liquid-liquid extraction method [40, 48, 71]. The yields of extractions are compared and information about the distribution of acid compound classes

are obtained by means of applying the HPLC methods described in Section 5.2.1. Figure 5.2 shows extraction yields and relative distributions of acid compound classes determined from HPLC using ELSD, and the same acid profiles when quantified with UV detector. Overall, the ion exchange method is generally much more efficient than the liquid-liquid method. The oil B4a has been extracted by both methods, and the amount of material in the ion exchange extract is threefold the amount extracted by liquid-liquid extraction. The liquid-liquid extract of B4a contains a larger relative amount of phenols and non-polar compounds, and a smaller amount of carboxylic acids compared to the corresponding ion-exchange extract. The two biodegraded oils B1c and B2b contain highest amounts of acids, while the two non-biodegraded oils S3b and S7b contain the least (ion-exchange method). As can be seen the ELS detector gives strongest response to saturated carboxylic acids. The response to carboxylic acids is much weaker with UV detection. The UV detector, however, gives a much stronger response to both phenolic compounds and polyfunctional compounds. As for ELSD, the UV detection also indicates that liquid-liquid extraction method yields acid extracts that have a relatively larger amount of non-polar compounds, lesser amounts of carboxylic acids and larger amounts of phenolic compounds compared to ion-exchange extraction.

5.2.4 Distribution of acid compounds in HPLC and SPE subfractions

The main observation from Figure 5.2 (Section 5.2.3) is that B1c, B2b, B4a and B4c ion exchange extracts have very similar distribution of acid compounds, measured by ELSD. B2c is very different from these. The two acid extracts from non-biodegraded oils S3b and S7b are not similar to each other,

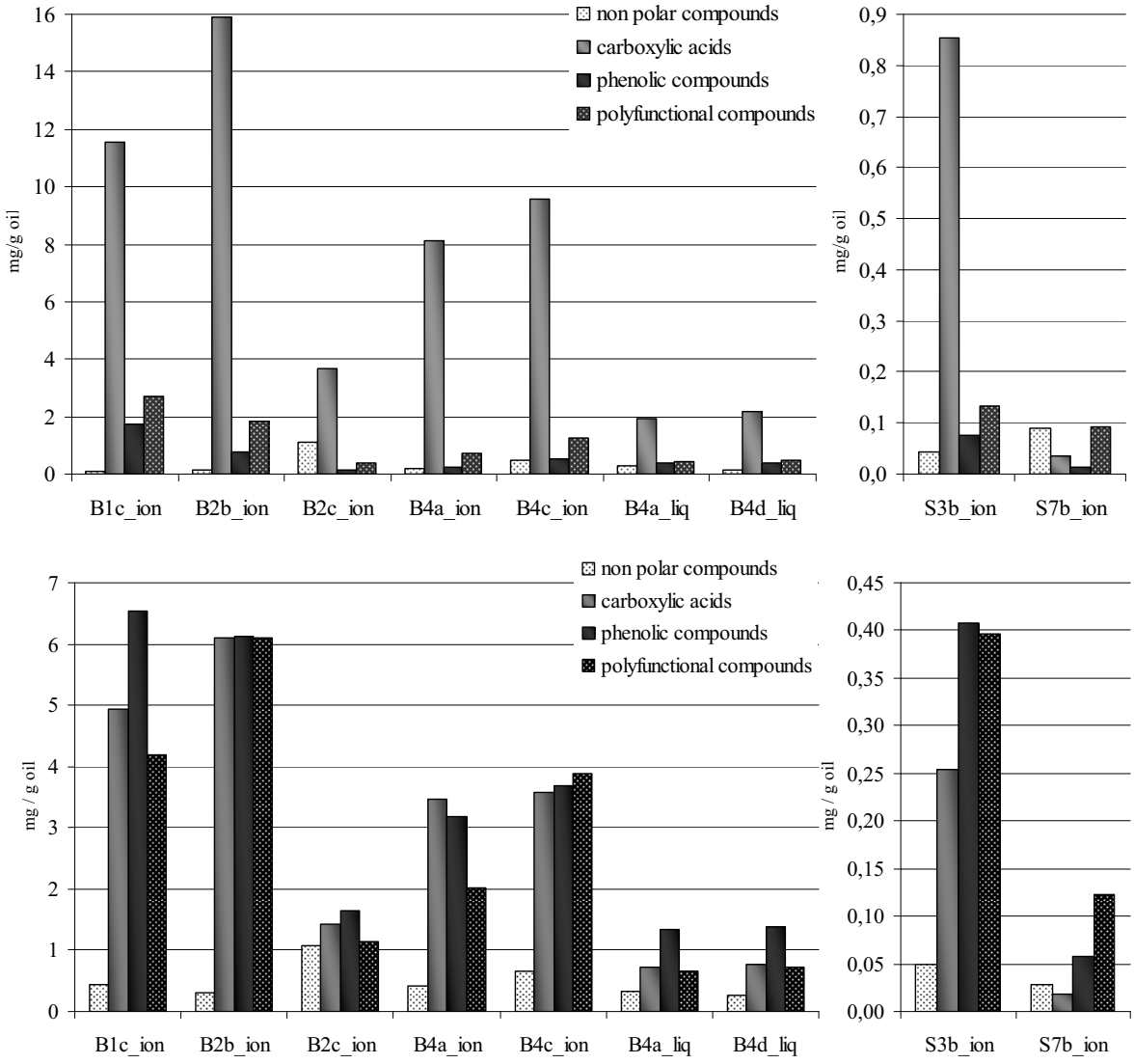

Figure 5.2: Comparison of acid extraction yields, distribution of acid compound classes for a set of different crude oils. The oils marked with "ion" have been extracted by the ion exchange method, and the oils marked with "liq" have been extracted by the liquid-liquid extraction. Upper: ELS detector (Figure 5, Paper II). Lower: UV detection at 230 nm (Figure 6, Paper II).

and the S7b acids has a distribution which is very different from all the other samples. UV detection gives larger differences in acid profiles within the same sample set of crude oils.

In Paper V, acids are extracted (ion exchange method) from a non-biodegraded oil, S9, and the same oil after it has been undergoing separate anaerobic- and aerobic biodegradation in the laboratory for 10 months. The biodegradation leads to an increase in acid content, from 0.74 mg/g oil in the original oil S9 to 1.87 mg/g in the anaerobically biodegraded oil B7a and to 2.25 mg/g oil in the aerobically biodegraded oil B7b. Hence, the laboratory biodegradation during this time scale has not increased the amount of acids to the same extent as in the naturally, reservoir biodegraded oils studied in Paper II (shown in Figure 5.2). However, even though the duration of biodegradation is negligible, compared to a geological time scale, the composition of acid extracts change significantly, shown in Figure 5.3. The anaerobic biodegradation has increased the relative amounts of weakly polar compounds and decreased the relative amount of phenolic compounds (B7a oil). The most interesting feature about the acid extracts from aerobic biodegradation (B7b) is the relative decrease in weakly polar compounds and the increase of polyfunctional compounds.

In Paper IV acids have been extracted from a set of five oils: B4a, B4c, B2b, B1c and S3b (ion exchange method). The extraction yields are very similar to those presented in Paper II, and demonstrate the reproducibility of the ion exchange extraction method. Four of these acid extracts are further separated into four SPE fractions: SPE_A-SPE_D. Figure 5.4 shows the relative amount of material that elute in each fraction. Generally for all oils, the major part of the acids elute in the SPE_A fraction. Smaller but significant

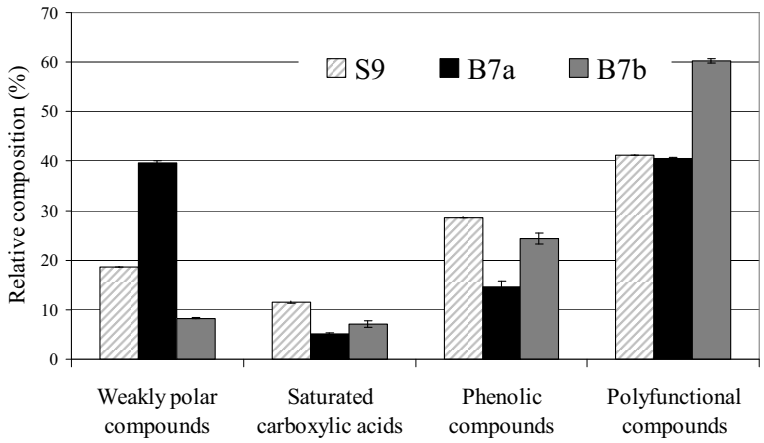

Figure 5.3: Distribution of acid compound classes in a non-biodegraded oil S9, and after laboratory biodegradation for 10 months: B7a anaerobically biodegraded and B7b aerobically biodegraded. Detection at 230 nm using an UV detector. (Figure 6, Paper V).

parts elute in the SPE_B and SPE_C fractions, while the SPE_D fraction contain only a very small fraction of the total acid extract. The acid extract from the high plugging potential B1c oil contains a larger relative amount of material in the SPE_C fraction and lower relative amount of SPE_A compared to the low plugging potential oils B4a, B4c and B2b. Subsequently, the composition of the acid extracts and each of their SPE fractions are studied by HPLC with ELS detection. The whole acid extracts and the SPE_A-SPE_C fractions have a relative distribution pattern that is specific for the nature of each type of fraction and shows fairly similar distributions, and major variations between oils are not observed (see details in Paper IV, Figure 5). However, in the most polar SPE_D fraction there are distinguishable differences in profiles between B1c and the other oils B4a, B4c and B2b, see Figure 5.5. The SPE_D fraction of B1c is enriched in polyfunctional compounds and depleted in weakly polar compounds relatively to B4a, B4c and B2b.

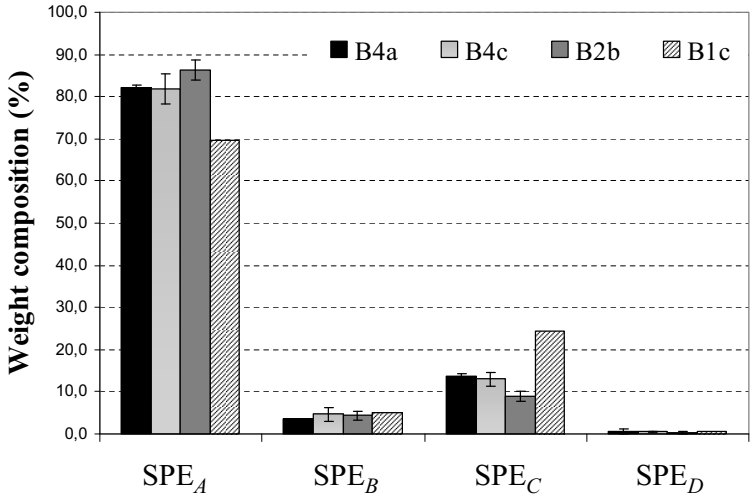

Figure 5.4: Relative amounts of organic material found in the SPE fractions of different oils (Figure 3, Paper IV).

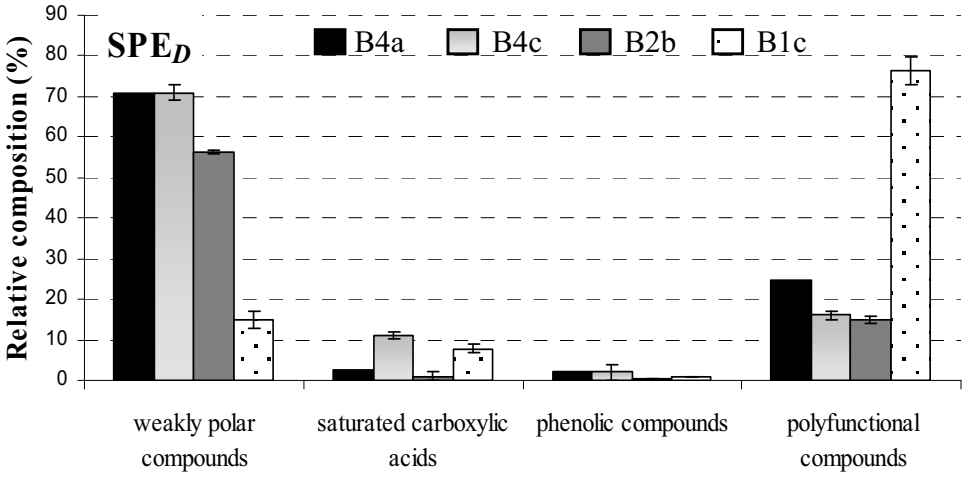

Figure 5.5: Relative amounts of compound classes present in the SPE$_D$ fractions of different oils, based on HPLC using ELS detection (modified from Figure 5, Paper IV).

5.3 Petroleum acids - wettability and interfacial properties

In the search to find the specific structures that possess mitigation properties on hydrate plug formation, the acid extracts have been divided into subfractions as described in the previous section (Section 5.2). The content of acids and distribution of acid compound classes in different types of crude oils have been shown. Different types of oils are observed to have variable amounts of acids and the acids differ very much in chemical composition. In this section, the separate effects of acids and acid subfractions on hydrate wettability are shown. Three different approaches have been applied, as presented in the following sections:

5.3.1 Correlations between HPLC fractions and wettability

In Paper II a study of relationships between the amounts of organic material found in the different HPLC fractions and general crude oil properties are performed. Though the data set is not large, a very interesting finding is that there exist a strong negative correlation between the relative amount of material found in HPLC fraction F_C (phenolic compounds) and wettability index of hydrates formed in the crude oil (R = -0.94), see Figure 5.6. This observation suggests that a large amount of phenolic compounds will reduce the values of wettability. Low wettability values correlate with water-wet hydrate particles and higher degree of hydrate agglomeration.

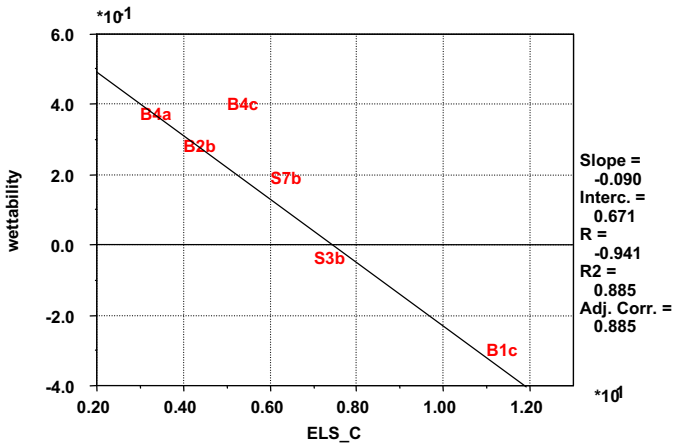

Figure 5.6: Correlation between the wettability and the relative amount of material in the F_C fraction using the ELS detector (Figure 11, Paper II).

5.3.2 Effects of acid extracts and SPE fractions on freon hydrate wettability

The influence of whole acid extracts and their SPE fractions on hydrate wettability has been examined in Paper III, for three oils that possess low plugging tendency (B4a, B4c and B2b). The tests show that all the whole acid extracts are able to alter the wettability state of Freon hydrates towards a more oil-wet state. Some very interesting effects are observed when the SPE fractions of the respective acid extracts are tested separately. Some of the SPE fractions show equal or even larger effects than the whole acid extracts they are derived from. The most striking observation is the particularly high effect found for the SPE_D fractions from all three oils, at very low concentration. This is illustrated in Figure 5.7, where the effect of each fraction is related to the number of moles of additives (see more details in Paper III). In the paper it is discussed whether the high effectiveness is caused by particular active compounds being present in these fractions and/or that a change in acid monomer-multimer equilibrium may influence the degree of activity of such compounds when they are isolated from the main acid matrix.

Interestingly, when measuring the effects of interfacial tension (IFT) between water and oil (acid extract and SPE fractions of B4a), no significant lowering of IFT is observed. These results suggest that the components in these fractions are not active at the oil/water interface, but adsorb preferentially at the hydrate-oil interface.

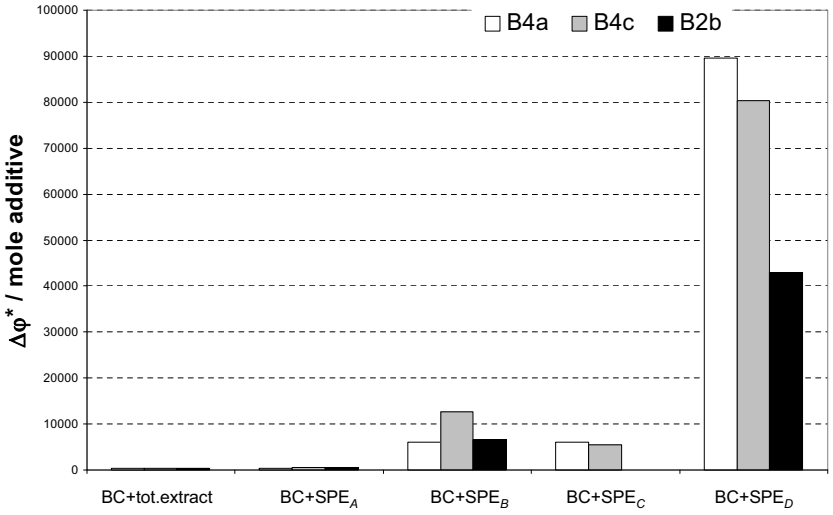

Figure 5.7: Effect of acids and SPE fractions on the wettability index ($\Delta\varphi^*$) relative to the number of moles added. Data from the SPE$_C$ fraction from B2b is missing due to loss of sample (Figure 7, Paper III).

5.3.3 Wettability effects of acids produced during anaerobic and aerobic biodegradation

The acid yields and composition of acid extracts isolated from a crude oil before and after it has been subjected to anaerobic and aerobic laboratory biodegradation was discussed in Section 5.2.4 (this work is presented in Paper V). The interfacial tension (IFT) between oil and water are measured for all three oils. Only for the aerobic biodegraded oil (B7b) a decrease in IFT is observed (from 23 mN/m in the original oil S9 to 17 nN/m in the B7b oil). This may be attributed to production of biosurfactants and can possible be

related to the relative increase in polyfunctional compounds found in the acid extract that was discussed in Section 5.2.4 and shown in Figure 5.3.

The amounts of acids extracted from these oils are too small to perform further subfractionations. However, the hydrate plugging mitigation properties of each of the three whole acid extracts are investigated at realistic hydrate formation conditions, e.g. conditions that resemble pipeline oil transport systems (Paper V). None of the acids extract are able to form systems of completely dispersed hydrate particles; plugging occurs in all the systems. This is attributed to the quantities and types of acids produced during 10 months of biodegradation not being sufficient to make a significant impact on the hydrate morphology. However, plugging seems less aggressive and takes place at a slower rate in the systems where the acid concentration has been increased. This is interpreted as the hydrates in these systems are more oil-wet, and residing to a larger extent in the oil phase, and having less access to water for growth and building up of plugs. This is also an indication of kinetic hydrate inhibiting effects of the acids. Kinetic effects can be related to wettability and morphology of hydrates when formed [95]. The largest effect is observed of acids produced during anaerobic biodegradation. In Figure 5.8 this is illustrated from still pictures recorded from the onset of hydrate formation (point A) until the end of the hydrate formation and growth period (point B). S9 is the original non-biodegraded oil. The S9 + B7a acids is the same oil modified with acids produced during anaerobic biodegradation. Volume versus temperature-, and torque versus time development data from the experiments support these findings.

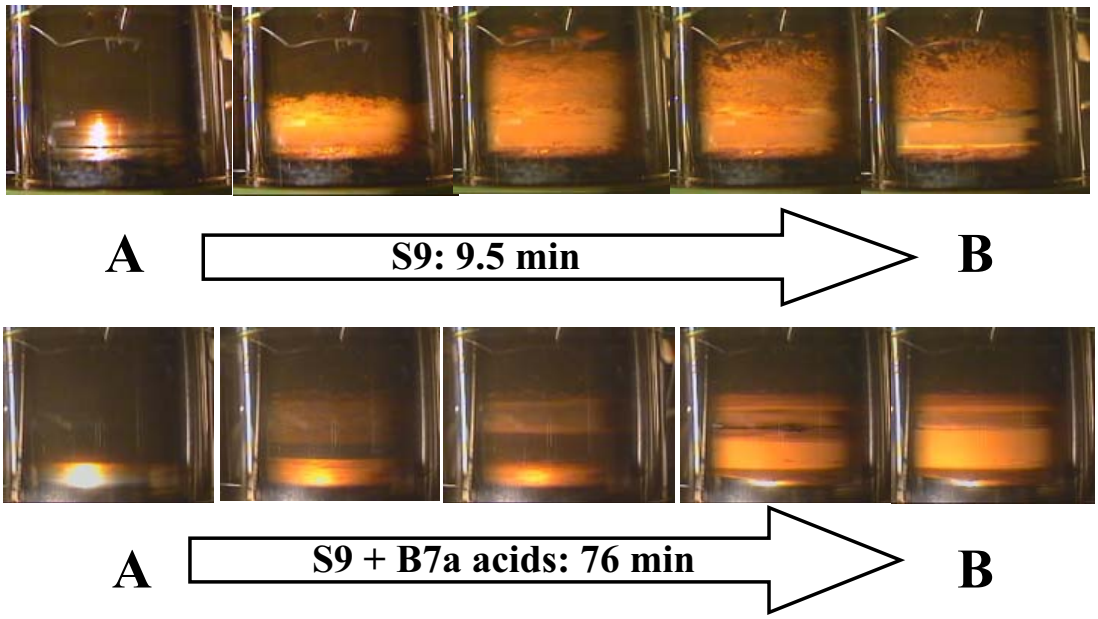

Figure 5.8: Still pictures from plugging tendency tests during the stage of hydrate formation and growth between point A (onset hydrate formation) and point B (end of hydrate formation- and growth). Upper: original non-biodegraded crude oil S9. Lower: S9 modified with acids produced during anaerobic biodegradation (modified from Figure 12, Paper V).

5.4 Chemical characterisation of petroleum acids and subfractions

5.4.1 Composition of acid fractions from preparative HPLC

In Paper II preparative HPLC fractions from three oils (B1c, B4a and B4c) have been studied by means of Fourier Transform Infrared spectroscopy (FTIR), and molecular weights are determined by means of gel permeation chromatography (GPC). In general, the FTIR spectra show very little variation between oils of high and low plugging tendencies. However, each type of subfraction possesses specific compositions, shown in different types of bands indicating functional groups, and with different degree of intensity

that is uniform within each type of fraction.

GPC of two whole acid extracts (B4c and S3b) gives average molecular range of approximately 500 g/mol. Studying the molecular weights of each preparative fraction reveals a larger range of molecular weights, that is more or less uniform within each fraction (from roughly 450 g/mol in the F_{B2} fractions, while the F_C fractions all have molecular weights larger than 800 g/mol).

In section 5.3.1, it has been shown that there exist a strong negative correlation between the HPLC fraction F_C (phenolic compounds) and wettability. As discussed, FTIR and GPC can not reveal clear compositional distinctions. Hence, a closer examination of this particular fraction is done by means of extracting UV spectra from the F_C area of the chromatograms. Compositional differences within the phenolic components are indicated, shown in Figure 5.9. The F_C fractions from the two high plugging potential oils B1c and S3b have a higher secondary absorbance in the region above 250 nm, and may contain components that are not present in the low-plugging tendency oils B2b and B4a.

5.4.2 Composition of acid fractions from SPE

In Section 5.3.2 the effects of acid extracts and SPE fractions on Freon hydrate wettability have been demonstrated. A chemical, analytical characterisation study of these samples has been performed (Paper IV). The SPE_D fractions are particularly addressed. As discussed in a previous section (Figure 5.7, Section 5.3.2), this specific fraction stands out from the other SPE fractions by displaying a very high degree of hydrate surface activity for three oils of low plugging tendency: B4a, B4c and B2b. In addition, the same three SPE_D fractions have shown to have very different distribution of acid com-

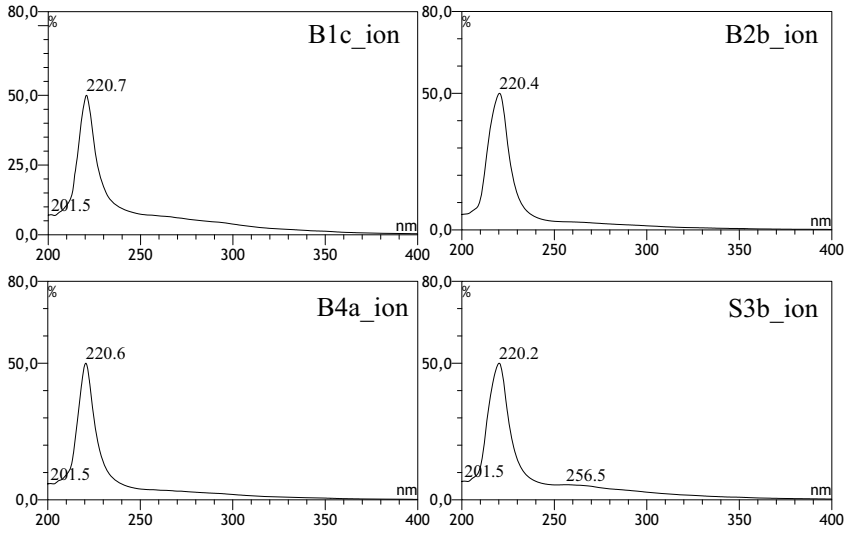

Figure 5.9: UV spectra of fraction F_C from some acid extracts (Figure 7, Paper II).

pound classes from that of a high plugging tendency oil, B1c (Figure 5.5, Section 5.2.4).

In the work of Paper IV an important observation is made from the FTIR analyses of the SPE_D fractions. In each of the FTIR spectra from low plugging tendency oils B4a, B4c and B2b a band of very strong intensity at wave number 1735 cm^{-1} is found. This band is attributed to the carbonyl group (C=O) from compounds with ester functionalities in the molecule. In Figure 5.10 the spectrum from B4a is compared to a high plugging potential oil, B1c. Interestingly, in the SPE_D fraction of B1c the same band is not found. A band of much lower intensity is located at lower frequency (1710 cm^{-1}). This band is assigned to C=O from carboxylic acids that absorb typically in this area of the spectrum. The spectrum of B1c also has a more "phenolic" character, indicated by a broad O-H stretch band in the region of approximately 3600-3200 cm^{-1}, which is stronger in B1c than in B4a. As discussed in Section 5.3.1 larger amounts of phenolic compounds are connected to higher risk of agglomeration and plugging. Similar differences in FTIR

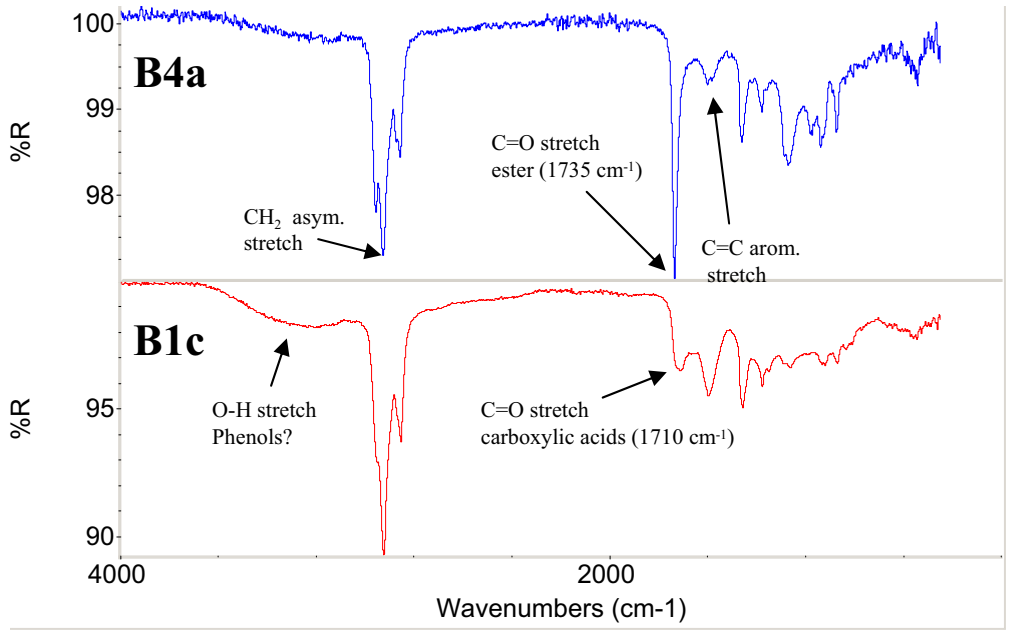

Figure 5.10: Comparison of FTIR spectra from SPE_D fractions of B4a (upper), and B1c (lower) (modified from Figure 6, Paper IV).

profiles between polar fractions of high- and low plugging tendency oils have been reported by Borgund [10]. In that work, hydrate adsorbing components were extracted from crude oils using Freon hydrates as an extraction phase.

The indication of ester compounds in the SPE_D fractions of B4a, B4c and B2b may be attributed to the large amount of weakly polar compounds detected in HPLC analysis (as shown in Figure 5.5). However, the actual presence of such structures has not been confirmed. In the discussion of Paper III it is debated whether these components may be plain ester structures, part of more complex structures or even if these signals found in the FTIR spectra originate from the monomeric form of the carboxylic acids. When resolving the acid matrix into subfractions the monomer-multimer equilibrium of the acids may change. A higher hydrate surface activity would be expected if the acids exist in their monomeric forms, and this may be one possible explanation to the high degree of hydrate surface affinity these SPE fractions

display.

Some ester structures are found in these samples by performing a GC-MS screening analysis (see the Research Report). The findings imply that these ester compounds are most abundant in low plugging tendency oils, which supports the findings from FTIR and HPLC. However, the total ion chromatograms (TIC) are dominated by UCMs, with very few resolved peaks. More detailed and accurate information on the chemical structures that constitute the SPE fractions is needed. The work of examining these samples with electrospray ionisation mass spectrometry (ESI-MS) is in progress, performed by Dr. Stefanie Pötz at the Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences (GFZ). Some preliminary results, from SPE_A fractions only, are presented in the Research Report. A very interesting observation is that all of the SPE_A fractions contain compounds that hold two oxygen, exclusively. This observation is in accordance with the HPLC analysis of these fractions (Paper IV), that shows that the SPE_A fractions consist almost solely of saturated carboxylic acids (shown in Figure 5, Paper IV).

However, the number of oxygen determined for compounds in the SPE_A fractions shows inconsistency with the average formulas estimated in Paper IV. These are obtained by means of combining GPC and elemental analysis, giving oxygen from roughly 3-7 for the SPE_A fractions. The GPC methodology and elemental analysis are both associated with significant uncertainties. Hence, these results serve to demonstrate the advantages of using ESI-MS for accurate molecular formula assignments.

Figure 5.11 shows how different types of acid species (holding two oxygen) are distributed in SPE_A fractions of B1c, B2b and B4a. The "O_2" notation used is described in the Research Report, Section 3.2.4. A general explana-

tion is that the more negative value of "O_2", the more hydrogen deficient are the acids. Generally, in all three SPE_A fractions the -4O_2 compounds are most abundant. These are most likely two-ring alicyclic compounds. However, significant differences in distributions of O_2 compounds between the high plugging tendency crude oil B1c and the two low plugging tendency oils B2b and B4a are observed; interestingly there are almost no pure fatty acids (0O_2 compounds) in the SPE_A fraction of B1c, while B2b and B4a hold significant amounts of these. Also, the second most abundant species in B1c are the -6O_2 compounds, while for B2b and B4a the compounds with second largest intensities are the -2O_2 compounds. This indicates that B1c contains a larger proportion of hydrogen deficient species.

B4a is the most depleted in terms of highly hydrogen deficient compounds of these three samples (no compounds $>$ -20O_2 are detected).

5.4.3 Composition of acid extracts produced during laboratory biodegradation

The wetting properties and HPLC distributions of the three acid extracts studied in Paper V (S9, B7a and B7b) have been discussed in previous sections. It has been shown that the B7a acid extract, isolated from an anaerobically biodegraded oil, differs from the other extracts by imposing stronger hydrate anti-agglomerating properties compared to the S9 and B7b acids (discussed in Section 5.3.3). Also, it has been shown that this extract contains a larger relative amount of weakly polar compounds and a lower relative amount of phenols than the S9 and B7b acid extracts (Figure 5.3, Section 5.2.4). UV spectra has been extracted from the phenolic compound area of the chromatogram for the three acid extracts, shown in Figure 5.12 (only B7a and B7b shown, the spectrum of S9 is very similar to B7b). The

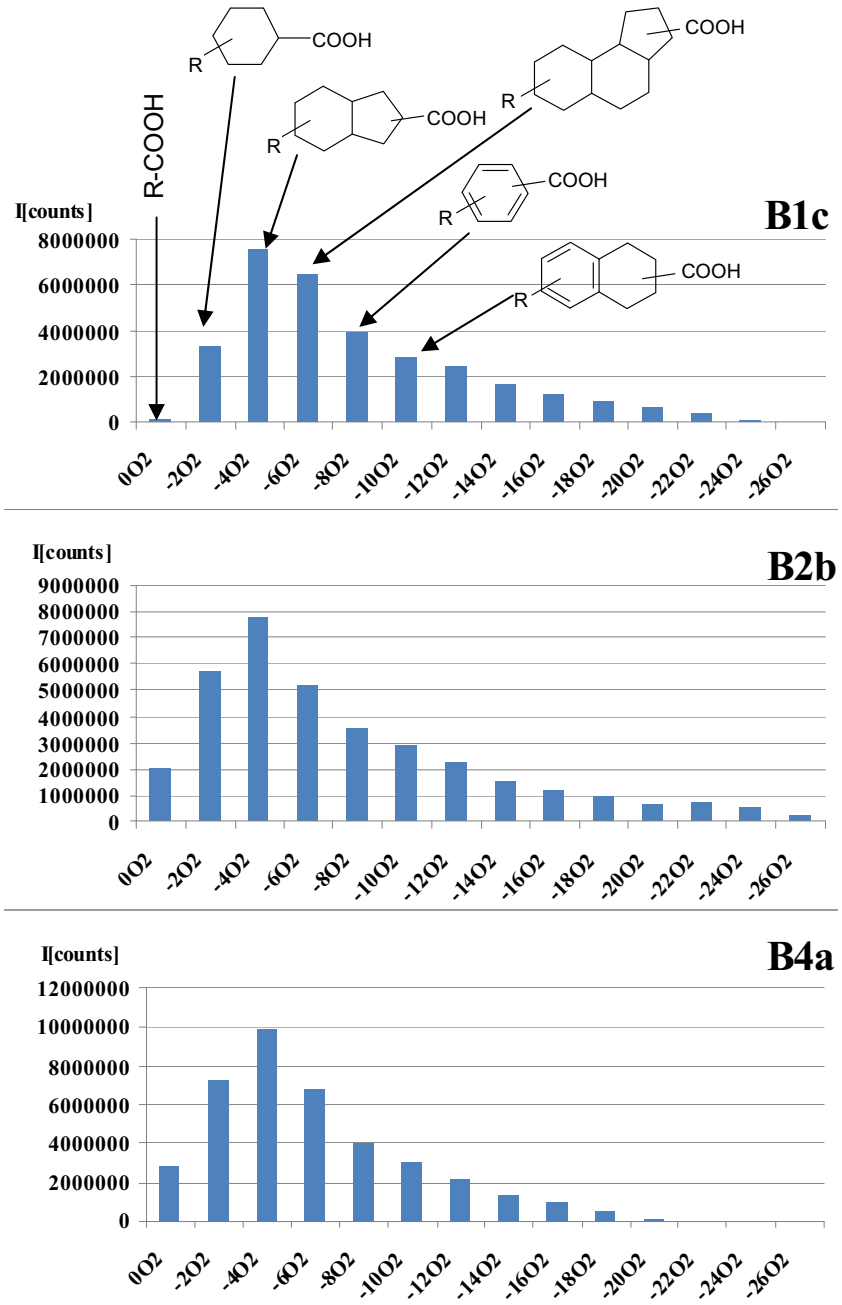

Figure 5.11: Sum of intensities of O_2 species in the SPE_A fractions of B1c (upper), B2b (middle) and B4a (bottom) acid extracts, shown with examples of representative acid structures for some of the O_2 classes (modified from Figure 6, Research Report).

B7b phenolic fraction resembles very much a standard UV spectra of phenol [145], with maximum absorbance at 218 nm and a secondary absorption shoulder around 272 nm. As seen in Figure 5.12 the secondary absorbance for B7a is very weak compared to B7b. The observations are also supported from the recorded FTIR spectra of the acid extracts: S9 and B7b have more intense signals from phenolic compounds than B7a (see Figure 8, Paper V). Interestingly, the differences in UV profiles of B7a and B7b in the spectra shown in Figure 5.12 are very similar to those of non-plugging and plugging oils previously shown in Figure 5.9. Again, we relate these findings to the observations from Paper II: there are strong indications that larger amounts of phenolic compounds present in the acid extracts may impose higher degree of water-wetting effects on the hydrate surface, and hence higher risk of agglomerating and hydrate plugging.

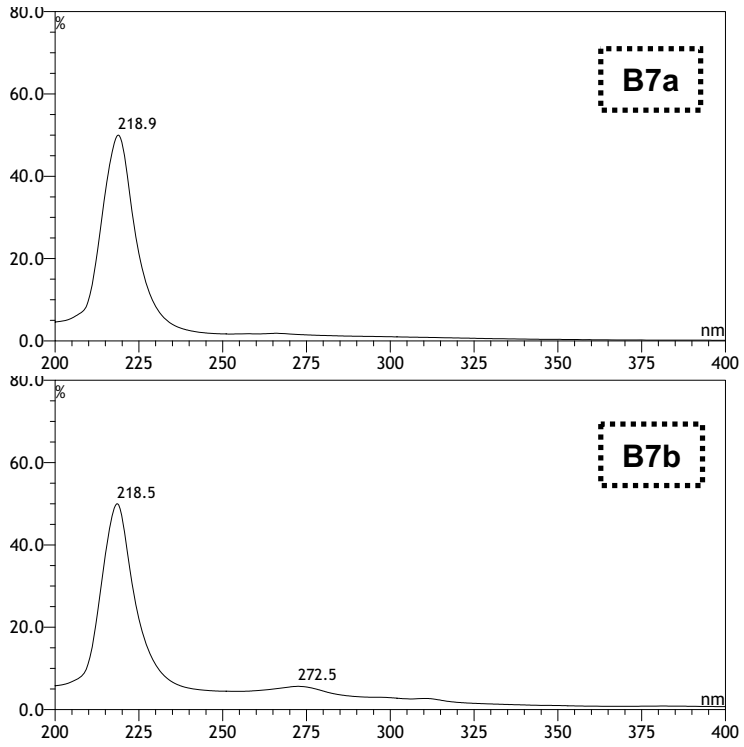

Figure 5.12: UV spectra of the phenolic fractions from B7a and B7b. (Figure 7, Paper V).

Chapter 6

Summary and Suggestions to Further Work

In the work of this thesis the task of identifying the natural inhibiting components (NICs) has been approached by extracting and analysing acidic fractions of crude oils. The identification of the specific structures of these compounds still remains, but subfractions that possess mitigating effects on the plugging tendency of the hydrates have been located and isolated. A general impression of the most hydrate surface active subfractions of low plugging tendency oils suggests that these are dominated by weakly polar compounds, and there are strong indications that these contain certain types of organic functional groups (C=O ester). However, the approach is still not a straightforward one. Several new aspects have arisen during the work of this thesis.

The nature of the systems seems to be very complex. Even after subfractionation the acid matrix still comprise thousands of different species, and the mechanisms that influence hydrate surface wettability remain to be clarified. The effectiveness of the NICs may be dependent on the position of monomer-multimer equilibriums, which again may be influenced by the

"chemical environment" in which the NICs exist. Interactions with the bulk crude oil matrix (acting as a solvent for the NICs) may be a determining factor, as well as the influence of other classes of components that comprise the acid fractions. The latter is strongly supported by a trend observed in several works of this thesis: higher amounts of phenolic compounds within the acid fractions seem to possess a counteracting effect on the hydrate plugging mitigation effect.

An additional factor that must be considered in this matter is the total concentration of acid species in the crude oils. This seems to be related to the amounts of acids produced during the biodegradation processes, but cannot be explained solely by this. Here, more knowledge is required before any conclusions can be drawn. However, results from work of this thesis indicate that anaerobic and aerobic biodegradation processes produce acid fractions of widely different composition. The acids produced during anaerobic biodegradation impose larger hydrate inhibition effects than the aerobically produced acids, and also larger effects than the acids that are originally present in the oil, i.e. acids that are incorporated into the oil from other sources than biodegradation. This may also be related to specific types of biosurfactants that are excreted from organisms capable of anaerobic degradation of petroleum. The biodegradation history of the majority of the crude oil sample set studied in this thesis is still unexplored in this context.

Based on the up to present obtained knowledge, future work should emphasise the structure elucidation of the types of compounds that comprise the very effective SPE_D fractions isolated in this work. This work is being continued by Dr. Stefanie Pötz at the Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences (GFZ). We hope that eventually, this work will provide detailed information on exact types, molecular sizes and

concentration levels of the key compounds that is being searched for. If this can be achieved it will strongly facilitate a selection of constricted, targeted model compounds which can be tested further for wettability- and plugging tendency properties. Possibly, these compounds can serve as strong candidates as low dosage hydrate inhibitors (LDHIs). Detailed structures of these compounds can also be integrated in theoretical modelling of such systems with regards to details on surface energies, e.g. by using a computational molecular modelling approach.

Bibliography

[1] E.D. Sloan, Jr. *Clathrate hydrates of natural gases. Second Edition, Revised and Expanded.* Marcel Dekker, Inc., New York, 1998.

[2] M.A. Kelland. History of the Development of Low Dosage Hydrate Inhibitors. *Energy & Fuels*, 20(3):825–847, 2006.

[3] F.H. Fadnes. Natural hydrate inhibiting components in crude oils. *Fluid Phase Equilibria*, 117(1-2):186–192, 1996.

[4] C. Gaillard, J.P. Monfort, and J.L. Peytavy. Investigation of Methane Hydrate Formation in a Recirculating Flow Loop: Modeling of the Kinetics and Tests of Efficiency of Chemical Additives on Hydrate Inhibition. *Oil & Gas Science and Technology - Reveue De L Institut Francais Du Petrole*, 54(3):365–374, 1999.

[5] L. Bergfløtdt. *Influence of crude oil based surface active components and synthetic surfactants on gas hydrate behaviour.* Department of Chemistry, University of Bergen, 2001. Dr.Scient. Thesis.

[6] E.M. Leporcher, J.L. Peytavy, Y. Mollier, J. Sjöblom, and C. Labes-Carrier. Multiphase transportation: hydrate plugging prevention through crude oil natural surfactants. *Society of Petroleum Engineers*, paper 49172, 1998.

[7] S. Høiland, K.M. Askvik, P. Fotland, E. Alagic, T. Barth, and F. Fadnes. Wettability of Freon hydrates in crude oil/brine emulsions. *Journal of Colloid and Interface Science*, 287(1):217–225, 2005.

[8] A. Sinquin, X. Bredzinsky, and V. Beunat. Kinetic of Hydrates Formation: Influence of Crude Oils. *Society of Petroleum Engineers*, paper 71543, 2001.

[9] S. Høiland, A.E. Borgund, T. Barth, P. Fotland, and K.M. Askvik. Wettability of Freon Hydrates in Crude oil/Brine Emulsions: the Effect

of Chemical Additives. *Proceedings of the Fifth International Conference on Gas Hydrates*, Trondheim, Norway, June 2005.

[10] A.E. Borgund. *Crude oil components with affinity for gas hydrates in petroleum production.* Department of Chemistry, University of Bergen, 2007. PhD Thesis.

[11] P.V. Hemmingsen, X.Y. Li, J.L. Peytavy, and J. Sjöblom. Hydrate Plugging Potential of Original and Modified Crude Oils. *Journal of Dispersion Science and Technology*, 28(3):371–382, 2007.

[12] E.D. Sloan. A changing hydrate paradigm-from apprehension to avoidance to risk management. *Fluid Phase Equilibria*, 228-229:67–74, 2005.

[13] J.G. Speight. *The Chemistry and Technology of Petroleum. Third Edition, Revised and Expanded.* Marcel Dekker, Inc., New York, 1999.

[14] K.H. Altgelt and M.M. Boduszynski. *Composition and Analysis of Heavy Petroleum Fractions.* Marcel Dekker, Inc., New York, 1994.

[15] D.M. Jewell, E.W. Albaugh, B.E. Davis, and R.G. Ruberto. Integration of Chromatographic and Spectroscopic Techniques for the Characterization of Residual Oils. *Industrial & Engineering Chemistry Fundamentals*, 13(3):278–282, 1974.

[16] J.B. Green, R.J. Hoff, P.W. Woodward, and L.L. Stevens. Separation of Liquid Fossil-Fuels into Acid, Base and Neutral Concentrates. 1. An Improved Nonaqueous Ion-Exchange Method. *Fuel*, 63(9):1290–1301, 1984.

[17] C. Ovalles, M.D. Garcia, E. Lujano, W. Aular, R. Bermudez, and E. Cotte. Structure/interfacial activity relationships and thermal stability studies of Cerro Negro crude oil and its acid, basic and neutral fractions. *Fuel*, 77(3):121–126, 1998.

[18] F.D. Mango. The light hydrocarbons in petroleum: a critical review. *Organic Geochemistry*, 26(7-8):417–440, 1997.

[19] J. Connan. *Biodegradation of Crude Oils in Reservoirs*, pages 299–335. In: Advances in Petroleum Geochemistry (J. Brooks and D. Welte, eds.). Academic Press, London, 1984.

[20] E.J. McKenna and R.E. Kallio. The Biology of Hydrocarbons. *Annual Review of Microbiology*, 19:183–208, 1965.

[21] D.W. Waples. *Geochemistry in petroleum exploration.* Reidel Publishing Company, Boston, 1985.

[22] A. Vieth and H. Wilkes. Deciphering biodegradation effects on light hydrocarbons in crude oils using their stable carbon isotopic composition: A case study from the Gullfaks oil field, offshore Norway. *Geochimica et Cosmochimica Acta*, 70(3):651–665, 2006.

[23] I.M. Head, D.M. Jones, and S.R. Larter. Biological activity in the deep subsurface and the origin of heavy oil. *Nature*, 426(6964):344–352, 2003.

[24] C.M. Aitken, D.M. Jones, and S.R. Larter. Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. *Nature*, 431(7006):291–294, 2004.

[25] H. Huang, S.R. Larter, B.F.J. Bowler, and T.B.P. Oldenburg. A dynamic biodegradation model suggested by petroleum compositional gradients within reservoir columns from the Liaohe basin, NE China. *Organic Geochemistry*, 35(3):299–316, 2004.

[26] V.G. Grishchenkov, R.T. Townsend, T.J. McDonald, R.L. Autenrieth, J.S. Bonner, and A.M. Boronin. Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. *Process Biochemistry*, 35(9):889–896, 2000.

[27] C.C. Gaylarde, F.M. Bento, and J. Kelley. Microbial contamination of stored hydrocarbon fuels and its control. *Revista de Microbiologia*, 30(1):1–10, 1999.

[28] N.A. Yemashova, V.P. Murygina, D.V. Zhukov, A.A. Zakharyantz, M.A. Gladchenko, V. Appanna, and S.V. Kalyuzhnyi. Biodeterioration of crude oil and oil derived products: a review. *Reviews in Environmental Science and Biotechnology*, 6(4):315–337, 2007.

[29] M. Magot, B. Ollivier, and B.K.C. Patel. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, 77(2):103–116, 2000.

[30] K.E. Peters, C.C. Walters, and J.M. Moldowan. *The Biomarker Guide. Second Edition. Volume 2 Biomarkers and Isotopes in Petroleum Exploration and Earth History.* Cambridge University Press, Cambridge, 2005.

[31] R.C. Prince. *Petroleum and other hydrocarbons, biodegradation of*, pages 2402–2416. In: Encyclopedia of Environmental Microbiology (G. Bitton, ed.). John Wiley & Sons, New York, 2002.

[32] L.M. Wenger, C.L. Davis, and G.H Isaksen. Multiple Controls on Petroleum Biodegradation and Impact on Oil Quality. *Society of Petroleum Engineers*, paper 71450, 2001.

[33] K.E. Peters and J.M. Moldowan. *The Biomarker Guide*. Prentice-Hall, New Jersey, 1993.

[34] R. Elias, A. Vieth, A. Riva, B. Horsfield, and H. Wilkes. Improved assessment of biodegradation extent and prediction of petroleum quality. *Organic Geochemistry*, 38(12):2111–1230, 2007.

[35] S. Kim, L.A. Stanford, R.P. Rodgers, A.G. Marshall, C.C. Walters, K. Qian, L.M. Wenger, and P. Mankiewicz. Microbial alteration of the acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry. *Organic Geochemistry*, 36(8):1117–1134, 2005.

[36] K.A. Thorn and G.R. Aiken. Biodegradation of crude oil into nonvolatile organic acids in a contaminated aquifer near Bemidji, Minnesota. *Organic Geochemistry*, 29(4):909–931, 1998.

[37] W. Meredith, S.-J. Kelland, and D.M. Jones. Influence of biodegradation on crude oil activity and carboxylic acid composition. *Organic Geochemistry*, 31(11):1059–1073, 2000.

[38] J.S. Watson, D.M. Jones, R.P.J. Swannell, and A.C.T. van Duin. Formation of carboxylic acids during aerobic biodegradation of crude oil and evidence of microbial oxidation of hopanes. *Organic Geochemistry*, 33(10):1153–1169, 2002.

[39] A. Fafet, F. Kergall, M. Da Silva, and F. Behar. Characterization of acidic compounds in biodegraded oils. *Organic Geochemistry*, 39(8):1235–1242, 2008.

[40] T. Barth, S. Høiland, P. Fotland, K.M. Askvik, B.S. Pedersen, and A.E. Borgund. Acidic compounds in biodegraded petroleum. *Organic Geochemistry*, 35(11-12):1513–1525, 2004.

[41] H. Wilkes, S. Kühner, C. Bolm, T. Fischer, A. Classen, F. Widdel, and R. Rabus. Formation of *n*-alkane- and cycloalkane-derived organic

acids during anaerobic growth of a denitrifying bacterium with crude oil. *Organic Geochemistry*, 34(9):1313–1323, 2003.

[42] J.S. Clemente and P.M. Fedorak. A review of the occurence, analysis, toxicity, and the biodegradation of naphthenic acids. *Chemosphere*, 60(5):585–600, 2005.

[43] X. Han, A.C. Scott, P.M. Fedorak, M. Bataineh, and J.W. Martin. Influence of Molecular Structure on the Biodegradability of Naphthenic Acids. *Environmental Science & Technology*, 42(4):1290–1295, 2008.

[44] O.V. Biryukova, P.M. Fedorak, and S.A. Quideau. Biodegradation of naphthenic acids by rhizosphere microorganisms. *Chemosphere*, 67(10):2058–2064, 2007.

[45] J.S. Clemente, M.D. MacKinnon, and P.M. Fedorak. Aerobic Biodegradation of Two Commercial Naphthenic Acids Preparations. *Environmental Science & Technology*, 38(4):1009–1016, 2004.

[46] C. Hell and E. Medinger. Ueber das Vorkommen und die Zusammensetzung von Säuren im Rohpetroleum. *Berichte der deutschen chemischen Gesellschaft*, 7(2):1216–1223, 1874.

[47] H.L. Lochte. Petroleum Acids and Bases. *Industrial and Engineering Chemistry*, 44(11):2597–2601, 1952.

[48] G. Costantinides and G. Arich. *Non-hydrocarbon compounds in petroleum*, pages 109–175. In: Fundamental Aspects of Petroleum Geochemistry (B. Nagy and U. Colombo, eds.). Elsevier Publishing Company, London, 1967.

[49] R. Jaffé and P.R. Gardinali. Generation and maturation of carboxylic acids in ancient sediments from the Maracaibo Basin, Venezuela. *Organic Geochemistry*, 16(1-3):211–218, 1990.

[50] A.S. Mackenzie, R.L. Patience, D.A. Yon, and J.R. Maxwell. The effect of maturation on the configurations of acyclic isoprenoid acids in sediments. *Geochimica et Cosmochimica Acta*, 46(5):783–792, 1982.

[51] A.S. Mackenzie, G.A. Wolff, and J.R. Maxwell. *Fatty acids in some biodegraded petroleums. Possible origins and significance*, pages 637–649. In: Advances in Organic Geochemistry (M. Bjorøy et al., eds.). Wiley, Chichester, 1981.

[52] F.H. Behar and Albrecht P. Correlation between carboxylic acids and hydrocarbons in several crude oils. Alteration by biodegradation. *Organic Geochemistry*, 6:597–604, 1984.

[53] N.A. Tomczyk, R.E. Winans, J.H. Shinn, and R.C. Robinson. On the Nature and Origin of Acidic Species in Petroleum. 1. Detailed Acid Type Distribution in a California Crude oil. *Energy & Fuels*, 15(6):1498–1504, 2001.

[54] E. Kowalewski, I. Rueslåtten, K.H. Steen, G. Bødtker, and O. Torsæter. Microbial improved oil recovery - bacterial induced wettability and interfacial tension effects on oil production. *Journal of Petroleum Science and Engineering*, 52(1-4):275–286, 2006.

[55] J.D. Desai and I.M. Banat. Microbial Production of Surfactants and Their Commercial Potential. *Microbiology and Molecular Biology Reviews*, 61(1):47–64, 1997.

[56] S. Lang. Biological amphiphiles (microbial biosurfactants). *Current Opinion in Colloid & Interface Science*, 7(1-2):12–20, 2002.

[57] M.P. Barrow, L.A. McDonnell, X.D. Feng, J. Walker, and P.J. Derrick. Determination of the Nature of Naphthenic Acids Present in Crude Oils Using Nanospray Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: The Continued Battle Against Corrosion. *Analytical Chemistry*, 75(4):860–866, 2003.

[58] J.S. Clemente, T.-W. Yen, and P.M. Fedorak. Development of a High Performance Liquid Chromatography method to monitor the biodegradation of naphthenic aicds. *Journal of Environmental Engineering and Science*, 2(3):177–186, 2003.

[59] C.A. Hughey, R.P. Rodgers, A.G. Marshall, K. Qian, and W.K. Robbins. Identification of acidic NSO compounds in crude oils of different geochemical origins by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. *Organic Geochemistry*, 33(7):743–759, 2002.

[60] K. Qian, W.K. Robbins, C.A. Hughey, H.J. Cooper, R.P. Rodgers, and A.G. Marshall. Resolution and Identification of Elemental Composition for More than 3000 Crude Acids in Heavy Petroleum by Negative-Ion Microelectronspray High-Field Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *Energy & Fuels*, 15(6):1505–1511, 2001.

[61] C.A. Hughey, S.A. Galasso, and J.E. Zumberge. Detailed compositional comparison of acidic NSO compounds in biodegraded reservoir and surface crude oils by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. *Fuel*, 86(5-6):758–768, 2007.

[62] G.C. Laredo, C.R. López, R.E. Álvarez, and J.L. Cano. Naphthenic acids, total acid number and sulfur content profile characterization in Isthmus and Maya crude oil. *Fuel*, 83(11-12):1689–1695, 2004.

[63] T.D. Baugh, N.O. Wolf, H. Mediaas, J.E. Vindstad, and K. Grande. Characterization of a Calcium Naphthenate Deposit - The ARN Acid Discovery. *Abstracts of Papers of the American Chemical Society*, 228:U172–U172, 2004.

[64] T.D. Baugh, K. Grande, H. Mediaas, J.E. Vindstad, and N.O. Wolf. The Discovery of High-Molecular-Weight Naphthenic Acids (ARN Acid) Responsible for Calcium Naphthenate Deposits. *Society of Petroleum Engineers*, paper 93011, 2005.

[65] B.F. Lutnaes, Ø. Brandal, J. Sjöblom, and J. Krane. Archaeal C_{80} isoprenoid tetraacids responsible for naphthenate deposition in crude oil processing. *Organic & Biomolecular Chemistry*, 4(4):616–620, 2006.

[66] B.E. Smith, P.A. Sutton, C.A. Lewis, B. Dunsmore, G. Fowler, J. Krane, B.F. Lutnaes, Ø. Brandal, J. Sjöblom, and S.J. Rowland. Analysis of 'ARN' naphthenic acids by high temperature gas chromatography and high performance liquid chromatography. *Journal of Separation Science*, 30(3):375–380, 2007.

[67] B. Brocart, M. Bourrel, C. Hurtevent, J.L. Volle, and B. Escoffier. ARN-type Naphthenic Acids in Crudes: Analytical Detection and Physical Properties. *Journal of Dispersion Science and Technology*, 28(3):331–337, 2007.

[68] W.K. Seifert and W.G. Howells. Interfacially Active Acids in a California Crude Oil. Isolation of Carboxylic acids and Phenols. *Analytical Chemistry*, 41(4):554–562, 1969.

[69] D. Langevin, S. Poteau, I. Hénaut, and J.F. Argillier. Crude Oil Emulsion Properties and their Application to Heavy Oil Transportation. *Oil & Gas Science and Technology-Revue De L Institut Francais Du Petrole*, 59(5):511–521, 2004.

[70] S. Poteau, J.F. Argillier, D. Langevin, F. Pincet, and E. Perez. Influence of pH on Stability and Dynamic Properties of Asphaltenes and Other Amphiphilic Molecules at the Oil-Water Interface. *Energy & Fuels*, 19(4):1337–1341, 2005.

[71] S. Hoeiland, T. Barth, A.M. Blokhus, and A. Skauge. The effect of crude oil acid fractions on wettability as studied by interfacial tension and contact angels. *Journal of Petroleum Science and Engineering*, 30(2):91–103, 2001.

[72] R.M. Pashley and M.E. Karaman. *Applied Colloid and Surface Chemistry*. John Wiley & Sons Ltd, Chichester, 2004.

[73] J. Sjöblom, N. Aske, I.H. Auflem, Ø. Brandal, T.E. Havre, Ø. Sæther, A. Westvik, E.E. Johnsen, and H. Kallevik. Our current understanding of water-in-crude oil emulsions. Recent characterization techniques and high pressure performance. *Advances in Colloid and Interfacial Science*, 100-102:399–473, 2003.

[74] M.H. Ese and P.K. Kilpatrick. Stabilization of Water-in-Oil Emulsions by Naphthenic Acids and Their Salts: Model Compounds, Role of pH, and Soap: Acid Ratio. *Journal of Dispersion Science and Technology*, 25(3):253–261, 2004.

[75] L. Cuiec. Rock/Crude-Oil Interactions and Wettability: An Attempt To Understand Their Interrelation. *Society of Petroleum Engineers*, paper 13211, 1984.

[76] J.S. Buckley and Y. Liu. Some mechanisms of crude oil/brine/solid interactions. *Journal of Petroleum Science and Engineering*, 20(3-4):155–160, 1998.

[77] B. Bennett, J.O. Buckman, B.F.J. Bowler, and S.R. Larter. Wettability alteration in petroleum systems: the role of polar non-hydrocarbons. *Petroleum Geoscience*, 10(3):271–277, 2004.

[78] H. Davy. The Bakerian Lecture: On Some of the Combinations of Oxymuriatic Gas and Oxygene, and on the Chemical Relations of These Principles, to Inflammable Bodies. *Philosophical Transactions of the Royal Society of London*, 101:1–35, 1811.

[79] E.G. Hammerschmidt. Formation of Gas Hydrates in Natural Gas Transmission Lines. *Industrial & Engineering Chemistry*, 26(8):851–855, 1934.

[80] E.D. Sloan. Clathrate Hydrates: The Other Common Solid Water Phase. *Industrial & Engineering Chemistry Research*, 39(9):3123–3129, 2000.

[81] L.H. Gjertsen and F.H. Fadnes. *Measurements and Predictions of Hydrate Equilibrium Conditions*, pages 722–734. In: Gas Hydrates, Challenges for the Future (volume 912), Annals of the New York Academy of Sciences (G.D Holder and P.R. Bishnoi, eds.). The New York Academy of Sciences, New York, 2000.

[82] S.R. Davies, M.S. Selim, E.D. Sloan, P. Bollavaram, and D.J. Peters. Hydrate Plug Dissociation. *AIChE Journal*, 52(12):4016–4027, 2006.

[83] A.L. Ballard and E.D. Sloan, Jr. Hydrate phase diagrams for methane + ethane + propane mixtures. *Chemical Engineering Science*, 56(24):6883–6895, 2001.

[84] E.D. Sloan, Jr. *Hydrate Engineering Monograph Vol. 21.* First Printing, SPE Henry L. Doherty series (J.B. Bloys, ed.). Society of Petroleum Engineers Inc, Richardson, Texas, 2000.

[85] J.D. Lee, H. Wu, and P. Englezos. Cationic starches as gas hydrate kinetic inhibitors. *Chemical Engineering Science*, 62(23):6548–6555, 2007.

[86] J.D. York and A. Firoozabadi. Comparing Effectiveness of Rhamnolipid Biosurfactant with a Quaternary Ammonium Salt Surfactant for Hydrate Anti-Agglomeration. *Journal of Physical Chemistry B*, 112(3):845–851, 2008.

[87] R.L. Christiansen and E.D. Sloan Jr. *Mechanisms and Kinetics of Hydrate Formation*, pages 283–305. In: Natural Gas Hydrates (volume 715), Annals of the New York Academy of Sciences. The New York Academy of Sciences, New York, 1994.

[88] J.P. Lederhos, J.P. Long, A. Sum, R.L. Christiansen, and E.D. Sloan Jr. Effective Kinetic Inhibitors for Natural Gas Hydrates. *Chemical Engineering Science*, 51(8):1221–1229, 1996.

[89] L. Del Villano, R. Kommedal, and M.A. Kelland. Class of Kinetic Hydrate Inhibitors with Good Biodegradability. *Energy & Fuels*, 22(5):3143–3149, 2008.

[90] Z. Huo, E. Freer, M. Lamar, B. Sannigrahi, D.M. Knauss, and E.D. Sloan Jr. Hydrate plug prevention by anti-agglomeration. *Chemical Engineering Science*, 56(17):4979–4991, 2001.

[91] L. Bergfløtdt, L.H. Gjertsen, J. Sjöblom, H. Kallevik, and G. Øye. Chemical Influence on the Formation, Agglomeration, and Natural Transportability of Gas Hydrates. A Multivariate Component Analysis. *Journal of Dispersion Science and Technology*, 25(3):355–365, 2004.

[92] M.R. Anklam, J.D. York, L. Helmerich, and A. Firoozabadi. Effects of Antiagglomerants on the Interactions between Hydrate Particles. *AIChE Journal*, 54(2):565–574, 2008.

[93] U. Klomp. The World of LDHI: From Conception to Development to Implementation. *Proceedings of the 6th International Conference on Gas Hydrates*, Vancouver, Canada, July 2008.

[94] M. Arjmandi, D. Leinweber, and K. Allan. Development of A New Class of Green Kinetic Hydrate Inhibitors. *19th International Oil Field Chemistry Symposium*, Geilo, Norway, March 2008.

[95] P. Fotland and K.M. Askvik. Some aspects of hydrate formation and wetting. *Journal of Colloid and Interface Science*, 321(1):130–141, 2008.

[96] A. Sinquin, D. Arla, Peytavy J.L. Prioux, C. and, P. Glenat, and C. Dicharry. Gas Hydrate Formation and Transport in an Acidic Crude Oil: Influence of Salt and pH. *Energy & Fuels*, 22(2):721–728, 2008.

[97] P.V. Hemmingsen, K. Sunghwan, H.E. Pettersen, R.P. Rodgers, J. Sjöblom, and A.G Marshall. Structural Characterization and Interfacial Behavior of Acidic Compounds Extracted from a North Sea Oil. *Journal of Dispersion Science and Technology*, 20(5):1980–1987, 2006.

[98] B. Qi, X. Fei, S. Wang, and L. Chen. Study on Distribution and Composition of Carboxylic Acids of Bohai Crude Oil in China. *Petroleum Science and Technology*, 22(11-12):1435–1446, 2004.

[99] M.C.V. de Campos, E.C. Oliveira, P.J.S. Filho, C.M.S. Piatnicki, and E.B. Caramão. Analysis of *tert*-butyldimethylsilyl derivatives in heavy

gas oil from brazilian naphthenic acids by gas chromatography coupled to mass spectrometry with electron impact ionization. *Journal of Chromatography A*, 1105(1-2):95–105, 2006.

[100] D.M. Jones, J.S. Watson, W. Meredith, M. Chen, and B. Bennett. Determination of Naphthenic Acids in Crude Oils Using Nonaqueous Ion Exchange Solid-Phase Extraction. *Analytical Chemistry*, 73(3):703–707, 2001.

[101] J. Saab, I. Mokbel, A.C. Razzouk, N. Ainous, N. Zydowicz, and J. Jose. Quantitative Extraction Procedure of Naphthenic Acids Contained in Crude Oils. Characterization with Different Spectroscopic Methods. *Energy & Fuels*, 19(2):525–531, 2005.

[102] H. Mediaas, K.V. Grande, B.M. Hustad, A. Rasch, H.G. Rueslåtten, and J.E. Vindstad. The Acid-IER Method - a Method for Selective Isolation of Carboxylic Acids from Crude Oils and Other Organic Solvents. *Society of Petroleum Engineers*, paper 80404, 2003.

[103] T. Greibrokk, E. Lundanes, and K.E. Rasmussen. *Kromatografi Separasjon og deteksjon.* Universitetsforlaget, Norway, 1998.

[104] J.B. Green, B.K. Stierwalt, J.S. Thomson, and C.A. Treese. Rapid Isolation of Carboxylic Acids from Petroleum Using High-Performance Liquid Chromatography. *Analytical Chemistry*, 57(12):2207–2211, 1985.

[105] H. Willsch, H. Clegg, B. Horsfield, M. Radke, and H. Wilkes. Liquid Chromatographic Separation of Sediment, Rock, and Coal Extracts and Crude Oil into Compound Classes. *Analytical Chemistry*, 69(20):4203–4209, 1997.

[106] S.T. Dubey and P.H. Doe. Base Number and Wetting Properties of Crude Oils. *SPE Reservoir Engineering (August)*, pages 195–200, 1993.

[107] ASTM664-89. *Standard test method for acidic number of petroleum products by potentiometric titration.* In: Annual Book of ASTM Standards, Section 5, American Society for Testing Materials, Philadelphia, 1989.

[108] T. Barth and M.H. Strand. An Improved Titration Method for Determination Of Acid and Phenol Content in Crude Oils Compared to Extraction and HPLC Characterisation. *23rd International Meeting*

on Organic Geochemistry, Poster P270-WE, Torquay, UK, September 2007.

[109] P.K. Dubey and R.J. Holland. Acid-Base Characteristics of Petroleum Asphaltenes as Studied by Non-Aqueous Potentiometric Titrations. *SPE Reservoir Engineering (August)*, pages 197–201, 1984.

[110] A. Jada and M. Salou. Effects of the asphaltene and resin contents of the bitumens on the water-bitumen interface properties. *Journal of Petroleum Science and Engineering*, 33(1-3):185–193, 2002.

[111] D.A. Skoog, D.M. West, and Holler F.J. *Fundamentals of Analytical Chemistry. 7th Edition.* Saunders College Publishing, USA, 1996.

[112] N. Varotsis, N. Pasadakis, and V. Gaganis. A novel approach for the characterization of aromatics in petroleum fractions using HPLC-UV-DAD and evolving factor analysis. *Fuel*, 77(13):1495–1502, 1998.

[113] N. Pasadakis, V. Gaganis, and N. Varotsis. Accurate determination of aromatic groups in heavy petroleum fractions using HPLC-UV-DAD. *Fuel*, 80(2):147–153, 2001.

[114] M. Li, S.R. Larter, D. Stoddart, and M. Bjorøy. Liquid-Chromatographic Separation Schemes for Pyrrole and Pyridine Nitrogen Aromatic Heterocycle Fractions from Crude Oils Suitable for Rapid Characterization of Geochemical Samples. *Analytical Chemistry*, 64(13):1337–1344, 1992.

[115] J.B. Green. Liquid-Chromatography on Silica Using Mobile Phases Containing Tetraalkylammonium Hydroxides 1. General Separation Selectivity and Behavior of Typical Polar Compounds in Fuels. *Journal of Chromatography*, 358(1):53–75, 1986.

[116] L.A. Berrueta, B. Gallo, and F. Vicente. A Review of Solid Phase Extraction: Basic Principles and New Developments. *Chromatographia*, 40(7-8):474–483, 1995.

[117] B. Bennett and S.R. Larter. Quantitative Separation of Aliphatic and Aromatic Hydrocarbons Using Silver Ion-Silica Solid Phase Extraction. *Analytical Chemistry*, 72(5):1039–1044, 2000.

[118] H.M. Weiss, A. Wilhelms, N. Mills, J. Scotchmer, P.B. Hall, K. Lind, and T. Brekke. *NIGOGA - The Norwegian Industry Guide to Organic Geochemical Analyses [online]. Edition 4.0.* Published by Norsk Hydro,

Statoil, Geolab Nor, SINTEF Petroleum Research and the Norwegian Petroleum Directorate. 102 pp [cited 07.06.2006]. Available from World Wide Web: http://www.npd.no/engelsk/nigoga/default.htm, 2000.

[119] W.W. Yau, J.J. Kirkland, and D.D. Bly. *Modern size-exclusion liquid chromatography: practice of gel permeation and gel filtration chromatography*. John Wiley & Sons, Inc., New York, 1979.

[120] S. Paul-Dauphin, F. Karaca, T.J. Morgan, M. Millan-Agorio, A.A. Herod, and R. Kandiyoti. Probing Size Exclusion Mechanisms of Complex Hydrocarbon Mixtures: The Effect of Altering Eluent Compositions. *Energy & Fuels*, 21(6):3484–3489, 2007.

[121] S. Bharati, R. Patience, N. Mills, and T. Hanesand. A new North Sea oil-based standard for Iatroscan analysis. *Organic Geochemistry*, 26(1-2):49–57, 1997.

[122] C. Jiang, S.R. Larter, K.J. Noke, and L.R. Snowdon. TLC-FID (Iatroscan) analysis of heavy oil and tar sand samples. *Organic Geochemistry*, 39(8):1210–1214, 2008.

[123] D.H. Williams and I. Fleming. *Spectroscopic Methods in Organic Chemistry. Fifth Edition*. The McGraw-Hill Companies, London, 1995.

[124] X. Yang, H. Hamza, and J. Czarnecki. Investigation of Subfractions of Athabasca Asphaltenes and Their Role in Emulsion Stability. *Energy & Fuels*, 18(3):770–777, 2004.

[125] J.A. Östlund, P. Wattana, M. Nydén, and H.S. Fogler. Characterization of fractionated asphaltenes by UV-vis and NMR self-diffusion spectroscopy. *Journal of Colloid and Interface Science*, 271(2):372–380, 2004.

[126] I.N. Evdokimov and A.P. Losev. On the Nature of UV/Vis Absorption Spectra of Asphaltenes. *Petroleum Science and Technology*, 25(1-2):55–66, 2007.

[127] T. Barth, S. Høiland, P. Fotland, K.M. Askvik, R. Myklebust, and K. Erstad. Relationship between the Content of Asphaltenes and Bases in Some Crude Oils. *Energy & Fuels*, 19(4):1624 –1630, 2005.

[128] C.A. Hughey, R.P. Rodgers, A.G. Marshall, C.C. Walters, K. Qian, and P. Mankiewicz. Acidic and neutral polar NSO compounds in Smackover oils of different thermal maturity revealed by electrospray high field

Fourier transform ion cyclotron resonance mass spectrometry. *Organic Geochemistry*, 35(7):863–880, 2004.

[129] M.P. Barrow, J.V. Headley, K.M. Peru, and P.J. Derrick. Fourier transform ion cyclotron resonance mass spectrometry of principal components in oilsands naphthenic acids. *Journal of Chromatography A*, 1058(1-2):51–59, 2004.

[130] C.S. Hsu, G.J. Dechert, W.K. Robbins, and E.K. Fukuda. Naphthenic Acids in Crude Oils Characterized by Mass Spectrometry. *Energy & Fuels*, 14(1):217–223, 2000.

[131] L.A. Stanford, S. Kim, R.P. Rodgers, and A.G. Marshall. Characterization of Compositional Changes in Vacuum Gas Oil Distillation Cuts by Electrospray Ionization Fourier Transform-Ion Cyclotron Resonance (FT-ICR) Mass Spectrometry. *Energy & Fuels*, 20(4):1664–1673, 2006.

[132] J.V. Headley, K.M. Peru, M.P. Barrow, and P.J. Derrick. Characterization of Naphthenic Acids from Athabasca Oil Sands Using Electrospray Ionization: The Significant Influence of Solvents. *Analytical Chemistry*, 79(16):6222–6229, 2007.

[133] A.G. Marshall and R.P. Rodgers. Petroleomics: The Next Grand Challenge for Chemical Analysis. *Accounts of Chemical Research*, 37(1):53–59, 2004.

[134] S.K. Panda, J.T. Andersson, and W. Schrader. Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: a challenge. *Analytical and Bioanalytical Chemistry*, 389(5):1329–1339, 2007.

[135] W.E. Rudzinski, T.M. Aminabhavi, S. Sassman, and L.M. Watkins. Isolation and Characterization of the Saturate and Aromatic Fractions of a Maya Crude Oil. *Energy & Fuels*, 14(4):839–844, 2000.

[136] P. Picker, E. Tremblay, and C. Jolicoeur. A High-Precision Digital Readout Flow Densimeter for Liquids. *Journal of Solution Chemistry*, 3(5):377–384, 1974.

[137] A.W. Adamson. *Physical Chemistry of Surfaces. Fifth Edition.* John Wiley & Sons Inc, New York, 1990.

[138] L.E. Cuiec. *Evaluation of Reservoir Wettability and Its Effect on Oil Recovery*, pages 319–375. In: Interfacial Phenomena in Petroleum Recovery (Volume 36) (N.R. Morrow, ed.), Surfactant Science Series. Marcel Dekker,Inc., New York, 1991.

[139] W.D. Harkins and F.E. Brown. The determination of surface tension (free surface energy), and the weight of falling drops: The surface tension of water and benzene by the capillary height method. *Journal of the American Chemical Society*, 41(4):499–524, 1919.

[140] P. Finkle, H.D. Draper, and J.H. Hildebrand. The theory of emulsification. *Journal of the American Chemical Society*, 45(12):2780–2788, 1923.

[141] B.P. Binks. Particles as surfactants - similarities and differences. *Current Opinion in Colloid & Interfacial Science*, 7(1-2):21–41, 2002.

[142] R. Aveyard, B.P. Binks, and J.H. Clint. Emulsions stabilised solely by colloidal particles. *Advances in Colloid and Interface Science*, 100-102:503–546, 2003.

[143] O. Urdahl, A. Lund, P. Mork, and T.N. Nilsen. Inhibition of Gas Hydrate Formation by Means of Chemical Additives - 1. Development of an Experimental Set-up for Characterization of Gas Hydrate Inhibitor Efficiency with Respect to Flow Properties and Deposition. *Chemical Engineering Science*, 50(5):863–870, 1995.

[144] K.Y. Song, M. Yarrison, and W. Chapman. Experimental low temperature water content in gaseous methane, liquid ethane, and liquid propane in equilibrium with hydrate at cryogenic conditions. *Fluid Phase Equilibria*, 224(2):271–277, 2004.

[145] V. Talrose, A.N. Yermakov, A.A. Usov, A.A. Goncharova, A.N. Leskin, N.A. Messineva, N.V. Trusova, and M.V. Efimkina. *"UV/Visible Spectra"*. In: NIST Chemistry WebBook, NIST Standard Reference Database Number 69 (P.J. Linstrom and W.G. Mallard, eds.), National Institute of Standards and Technology, Gaithersburg MD, 20899 (http://webbook.nist.gov). June 2005.