Crude oil components with affinity for gas hydrates in petroleum production

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Preface

This thesis, submitted for the degree of Philosophiae Doctor at the University of Bergen, consists of five papers and one Research Report as well as a summary of the work. The work has been performed at the Department of Chemistry (2003) and the Centre for Integrated Petroleum Research (CIPR), University of Bergen in the period 2004-2006. From November 2005 until January 2006 I had a short stay at the University of Newcastle upon Tyne, in Newcastle, UK, funded by Marie Curie Fellowship Association. The work at the University of Bergen has been financed by CIPR, and has been performed in close collaboration with the HYPERION project. The HYPERION (HYdrate in PEtRoleum productION - Assessment of Plug Risk) project is interdisciplinary, combining physical chemistry, petroleum chemistry, physics and the industrial aspects in research on crude oil/water/gas interactions that influence hydrate morphology, and it is a KMB-project financed by The Norwegian Research Council and Norsk Hydro ASA.

The work presented in this thesis has consisted of extraction and characterisation of components in crude oil with affinity for hydrate surfaces. Certain crude oil components have proven able to influence gas hydrate behaviour in petroleum systems, and thus relate to whether the plugging tendency is high or low. Prevention of hydrate plugs in oil pipelines is important for the petroleum industry, and today large amounts of methanol or glycol are used to prevent the plugs from forming. A better knowledge on the components present in crude oil that influence anti-agglomeration behaviour, and hence the plugging tendency, can have positive economical and environmental effects. Surface active compounds can be extracted from the bulk petroleum by several methods. Due to the huge complexity of crude oil, the analysis of the extracts is very challenging.

Acknowledgements

I would like to thank my supervisors Tanja Barth, Sylvi Høiland and Per Fotland. You have always been there for me, helping me, encouraging me, and made me believe in myself and in my work. Even in tight schedules you have found time for me. I would also like to thank CIPR for financing my Ph.D. thesis. Furthermore, I would like to thank my supervisors at my short stay in Newcastle, Helen Talbot and Geoff Abbott, who welcomed me into their research group and taught me about LC-MS.

My close cooperation with the HYPERION group and the interdisciplinary approach to solve a problem for the petroleum industry has been very interesting and educational. I would especially like to thank Kristin Erstad, who has been working together with me for large parts of my thesis.

Thanks to my friends and colleagues who made the Department of Chemistry a good working environment. Monica, Kristin and Espen - what would the Ph.D. period have been without you! I would also like to thank the rest of the staff at the Department, especially Terje Lygre who helped with the installation of the HPLC equipment.

My family has always encouraged me to study hard and follow my dreams. Thank you for always believing in me! And Kay, I am really glad I moved to Bergen and started to study here. You have been here by my side from the beginning of my University life and you have always encouraged me. Thank you for always knowing what to say and do to make me feel better on tough days :-)

Abstract

Some crude oils are believed to contain natural inhibiting components that can prevent hydrate plugging of oil pipelines in petroleum production. A method for classification of the oils that form hydrate plugs, as opposed to those that are not problematical, can change the hydrate inhibiting strategies for oil companies, and result in both economical savings and environmental improvements. Furthermore, an identification of natural hydrate plug inhibiting components can eventually give rise to development of more economical and environmentally friendly inhibitors that can be added to crude oil in pipelines.

This thesis addresses the issues of chemical characterisation of crude oil with respect to identification of natural plug inhibiting components. Natural plug inhibiting components are probably hydrate surface active, e.g. an acid fraction has previously been shown to be able to convert a plugging oil into a non-plugging oil when added in a low concentration. In this work, methods for extraction of surface active compounds in crude oil have been established; two methods for acid extraction have been tested (liquid-liquid and ion exchange), and in addition components with affinity for freon hydrate and ice surfaces have been extracted. The extracts have been characterised with chromatographic and spectroscopic methods, e.g. an HPLC method for separation of extracts into four groups have been developed; non-polar compounds, saturated carboxylic acids, phenolic compounds and polyfunctional compounds.

The results show that the ion exchange is more effective than the liquid-liquid method for extraction of acids from crude oil. Freon hydrates are found to extract a specific fraction with polar compounds from crude oil, while ice does not seem to be a good surface for extraction of components.

Compounds which absorb to hydrate surfaces are found to be dominated by saturated carboxylic acids, and to contain lower amounts of phenols and polyfunctional compounds. In general, crude oil extracts primarily contain components of intermediate molecular weights. This means that neither high-molecular compounds such as asphaltenes, nor simple low-molecular petroleum acids and bases are present to a large extent in these extracts. FTIR analysis can to some degree differentiate between freon hydrate extracts from plugging and non-plugging crude oils. GC-MS is not suited for the extracts used in this thesis due to limitations regarding molecular weights and low volatility. LC-MS analysis with Ion Trap MS has been tested at the University of Newcastle, but was not optimal for our samples. The freon hydrate extracts have been analysed for compounds similar to a biosurfactant structure, but no such compounds could be found.

No structural identification of the natural inhibiting components have been obtained. However, new methods for extraction of components with affinity for freon hydrate and ice surfaces have been developed and acid extraction methods from the literature have been tested. In addition, a set of analytical methods that can characterise the fractions have been established. One reason for the difficulty of identifying the natural inhibiting components can be that they are present in very low amounts, but it may also be that the distribution of compounds in the fractions is as important as the presence of specific molecules.

List of Publications

Papers

- Paper I: Acidic compounds in biodegraded petroleum, T. Barth, S. Høiland, P. Fotland, K.M. Askvik, B.S. Pedersen, A.E. Borgund, Organic Geochemistry 35 (2004) 1513-1525.

- Paper II: Wettability of freon hydrates in crude oil/brine emulsions: the effect of chemical additives, S. Høiland, A.E. Borgund, T. Barth, P. Fotland, K.M. Askvik, Proceedings, Volume 4, 1151-1161, 5th International Conference on Gas Hydrates, 13.-16. June 2005.

- Paper III: Molecular analysis of petroleum derived compounds that adsorb onto gas hydrate surfaces, A.E. Borgund, S. Høiland, T. Barth, P. Fotland, K.M Askvik, Submitted to Applied Geochemistry, in October 2006.

- Paper IV: Normal phase High Performance Liquid Chromatography for fractionation of organic acid mixtures extracted from crude oils, A.E. Borgund, K. Erstad, T. Barth, Journal of Chromatography A 1149 (2007) 189-196.

- Paper V: Fractionation of crude oil acids by HPLC and characterisation of their properties and effects on gas hydrate surfaces, A.E. Borgund, K. Erstad, T. Barth, Submitted to Energy and Fuels, in February 2007.

Research report

- Extraction of crude oil components with affinity for ice surfaces, A.E. Borgund, Report to Norsk Hydro Research Centre 2006.

Abbreviations

AA	Anti-Agglomerant
APCI	Atmospheric Pressure Chemical Ionisation
ATR	Attenuated Total Reflection
ELSD	Evaporative Light Scattering Detector
ESI	Electron Spray Ionisation
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
GPC	Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography
KHI	Kinetic Hydrate Inhibitor
IR	Infrared Spectroscopy
LC-MS	Liquid Chromatography Mass Spectrometry
LDHI	Low Dosage Hydrate Inhibitor
MEG	monoethylene glycol
MeOH	methanol
MS	Mass Spectrometry
NIC	Natural Inhibiting Component
NMR	Nuclear Magnetic Resonance
SARA	Saturates, Aromatics, Resins and Asphaltenes
TAN	Total Acid Number
TBN	Total Base Number
UCM	Unresolved Complex Mixture
UVD	UltraViolet Detector

Contents

Chapter 1

Introduction

In production and transportation of petroleum a number of chemical and physical phenomena has to be considered. Changes in pressure and temperature can cause alterations in the equilibrium between water, gas and crude oil. Some of the most common and most problematic alterations are the formation and deposition of hydrates, wax and asphaltenes. These deposits can cause plugging of transportation pipelines or process equipment, and eventually result in large economic losses. The prevention of these problems causes substantial extra costs and unwanted use of chemicals. In some cases, the costs associated with prevention of hydrate problems, typically high costs regarding chemicals and construction of equipment for pumping and regeneration of the chemicals, can be a "show stopper" for oil companies when evaluating new prospects.

Reliable physical models for prediction of hydrate formation are available [1]. However, these models do not describe the morphology of the hydrate particles, i.e. whether the hydrate particles agglomerate and grow into a plug or remain as a dispersion of small hydrate particles in oil. Observations have indicated that some oils contain natural inhibiting components

that prevent hydrate plugging [2–5]. A possible mechanism for formation of a dispersion is adsorption of special compound types onto the hydrate surface, preventing the small hydrate particles from agglomerating into large plugs. Hence, morphology of the hydrates can be influenced by crude oil composition. Acid fractions for instance, have been shown to contain hydrate plug inhibiting components (Paper II).

The negative consequences when a hydrate plug is formed have led the oil companies to always assume plugging, thus large amounts of inhibitors, like methanol or monoethylene glycol, are used. A better understanding of the plugging tendency of different crude oils, and a differentiation of oils with respect to their composition, will lead to lower operation costs for many oil fields compared to how it is managed today.

Detailed knowledge about the natural plug inhibiting components and which physical mechanisms are causing the anti-agglomeration of hydrate systems, can be helpful to understand the plugging tendency of crude oils. Thus, organic chemical analysis of the crude oil is very important, but the complexity of crude oil makes it impossible to perform a complete structural analysis [6]. The natural plug inhibiting components are believed to interact with the hydrate surfaces and have high hydrate surface activity. Extraction of these surface active components from the bulk crude oil is necessary to obtain detailed knowledge about them. Petroleum acids comprise a large part of the surface active components in crude oil, and several authors have extracted acids using different methods, see Section 4.2. In addition to extraction of acids, this thesis also focus on extraction of components with affinity for hydrate surfaces.

Even after fractionation of crude oil, the analysis of the fractions are challenging. Petroleum analysis is often focused on the hydrocarbon fractions,

and the methods for these analyses are not suitable for the identification of compounds in crude oil fractions containing surface active compounds, due to low volatility and polar properties. Thus, new methods with the focus on polar compounds of intermediate molecular weights (appr. 500 g/mole) have to be developed.

Chapter 2

Gas Hydrates

Gas hydrates have been a research subject from the beginning of the 19^{th} century [7, 8]. The problem with hydrates in natural gas pipelines was first reported by Hammerschmidt in 1934 [9], when he discovered that the plug in a pipeline consisted of hydrates, and not ice.

2.1 Composition of gas hydrates

Gas hydrates consist of gas molecules that are trapped in a framework of water molecules, and a simple example is shown in Figure 2.1. Hydrates are similar to ice, but hydrates can be formed at higher temperatures than ice.

The water molecules in the hydrate structure are held together by hydrogen bondings, and guest molecules are situated in cavities. The hydrate structure is dependent of the guest molecule, and three different hydrate structures can be formed, see Figure 2.2. Methane and ethane are small molecules, and these gases form Structure I hydrates. Propane is a larger molecule, and Structure II hydrates are needed to provide cavities of suitable size [1]. Petroleum associated natural gas consists predominantly of methane.

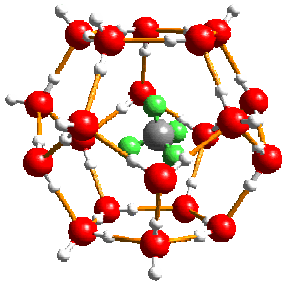

Figure 2.1: Simple illustration of a gas molecule trapped inside a framework of water molecules. ©USGS (U.S. Geological Survey).

Higher weight hydrocarbons, like ethane and propane, are also present in smaller quantities. Non-flammable, non-hydrocarbon components, like carbon dioxide and nitrogen, are often present in trace amounts and are regarded as contaminants [10]. The natural gas contains propane, and Structure II hydrates are formed to make large enough cavities for all the gas components.

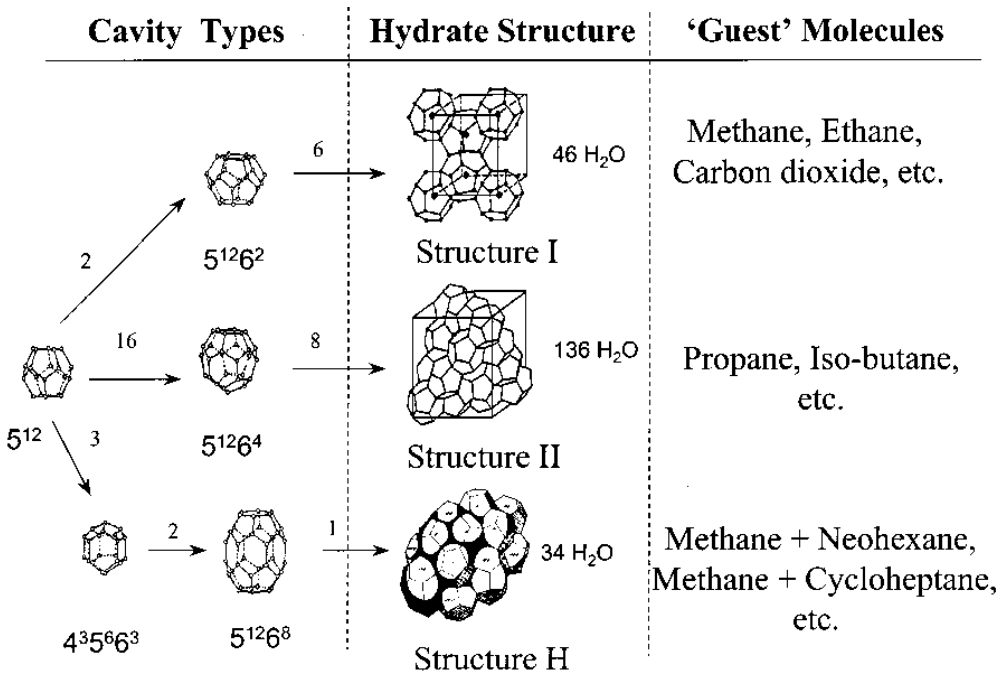

Figure 2.2: Three common hydrate unit crystal structures. Nomenclature: $5^{12}6^4$ indicates a water cage composed of 12 pentagonal and 4 hexagonal faces. Along the lines are indicated the numbers of cage types. Example: the Structure I unit crystal is composed of 2 5^{12} cages, 6 $5^{12}6^2$ cages, and 46 water molecules. The figure is taken from Sloan [11].

2.2 Formation of hydrates

The formation of hydrate crystals can take place when the mixture of water and guest (gas) molecules is within the pressure and temperature region for hydrate formation. Temperatures are typically < 27 °C and pressure typically > 6 bar [11]. Within the pressure and temperature conditions for hydrate formation, it often takes some time for hydrates to form, and this is normally termed the induction period [1]. The hydrate crystals can grow into large clusters of hydrates.

A phase diagram for natural gas is shown in Figure 2.3. This diagram shows that hydrates can be formed in the region to the left of the line in

Figure 2.3, for instance at 5°C and 25 bars. At 15°C and 25 bars there will be no hydrate formation. Different gases give different phase diagrams.

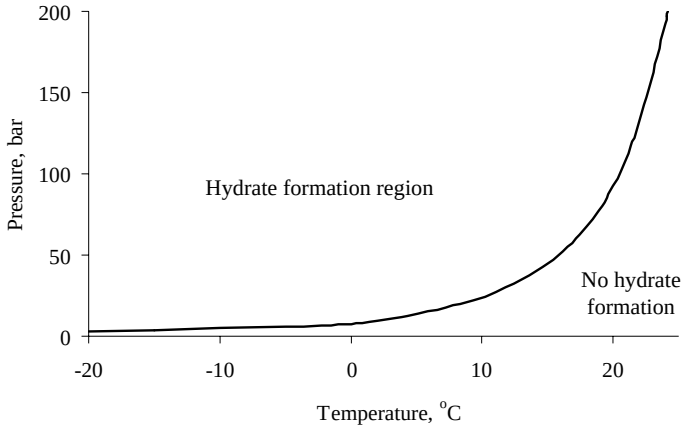

Figure 2.3: Hydrate phase diagram of natural gas (90.4% methane, 5.2 % etane, 2.1 % propane and trace amounts of N_2, CO_2, *iso*-butane, *n*-butane, *iso*-pentane, *n*-pentane and C6) simulated by PVT-sim (from Calsep A/S).

2.3 Industrial aspects: inhibition of hydrates

Formation of a hydrate plug in a petroleum pipeline may create dangerous situations for the oil companies due to pressure build up during plugging and non controllable liberation of gas causing explosion hazard during plug melting. The expenses when removing a hydrate plug is large, including delayed production.

2.3.1 Thermodynamic inhibitors

In order to avoid these problems, the oil companies traditionally design their operating systems to stay outside the hydrate stable region. The hydrate problems are most often avoided by adding methanol (MeOH) or monoethylene glycol (MEG), which are thermodynamic inhibitors. When the ther-

modynamic inhibitors are added to the water phase in the pipelines, the hydrate phase diagram changes. Lower temperatures and higher pressures in the pipelines can be tolerated without moving into the stable hydrate formation area. In Figure 2.4, 30 wt% metanol has been added to the natural gas system (grey stippled line) and this is compared to the gas system with no inhibitor (black line). The phase boundary for hydrate formation is moved to much lower temperatures.

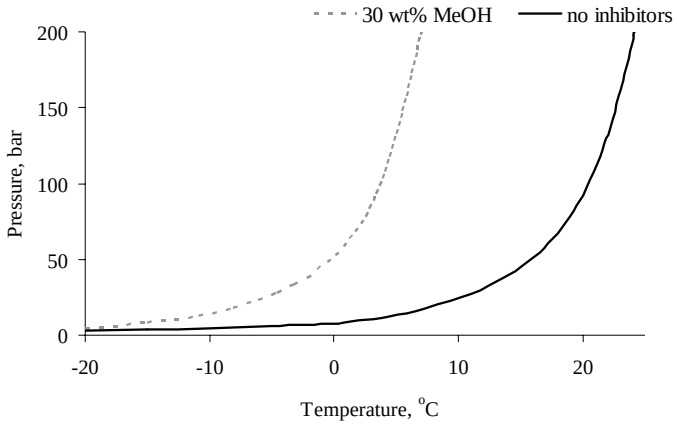

Figure 2.4: Hydrate phase diagram of natural gas system with 30 wt% metanol as inhibitor (grey stippled line) compared to no inhibitors (black line), simulated by PVT-sim (from Calsep A/S).

A production system is often designed to retain sufficient heat to operate outside the hydrate stability region [12]. Inhibitors are needed mostly during "start-up" of cold pipelines that have been shut down either planned or unplanned. The methanol and MEG can be regenerated to use less inhibitor. Still, large amounts are necessary, and storing, transportation and facilities for the regeneration of the inhibitor are needed. Another problem, especially with methanol, is that it can be dissolved in relatively high concentrations in oil before arrival at the refinery, reducing the value of the crude oil [12].

2.3.2 Low dosage inhibitors

Low dosage chemicals can also be used to prevent problems related to hydrate formation in the pipelines. There are two kinds of low dosage hydrate inhibitors (LDHIs); kinetic hydrate inhibitors (KHIs) and anti-agglomerants (AAs).

The KHIs decrease the rate at which hydrates are formed and the growth rate of the hydrates. The hydrate forming fluids can then be transported for a certain period of time before hydrates start to form. The AAs do not prevent the formation of hydrates, but they prevent the agglomeration into large masses by forming a slurry that can be transported. More information about LDHIs can be found in a review article by Kelland [13] and references therein.

KHIs and AAs have different advantages. The AAs are effective at high degrees of sub-cooling, but they cannot be used at high water cuts and they require the presence of a liquid hydrocarbon phase to transport the suspension of the converted hydrate crystals. The KHIs do not need a liquid hydrocarbon phase and can be used at any water cuts, but their effectiveness is limited by sub-cooling [12, 13].

LDHIs are being used in some field applications (e.g [14–16]), but some limitations create problems for the use of LDHIs. The price per volume of the LDHI chemicals is much higher than the thermodynamic inhibitors. However, smaller amounts of LDHIs are needed compared to the thermodynamic inhibitors, so it might be more cost effective to use LDHIs. A main problem is the modification of infrastructure needed for the change from thermodynamic inhibitors to the LDHIs. In addition, since the formation of a hydrate plug will have negative consequences, the oil companies can be reluctant to use LDHIs before their effects has been properly proved [12]. Another im-

portant factor is the toxicity of LDHIs, and a lot of the chemicals used for inhibition are restricted due to their negative environmental effects [17].

2.3.3 Natural inhibitors

Some crude oils have shown to be unproblematic even when operated within thermodynamic conditions for stable hydrate formation without using inhibitors. Several authors have indicated that the plugging tendency of crude oils is dependent on the presence or absence of natural inhibiting components (NICs) [2–5]. A possible mechanism could be the adsorption of special compound types onto the hydrate surface, preventing the small hydrate particles from agglomerating into large plugs, and thus work as a kind of natural AA mechanism.

The natural inhibiting components that are present in some oils are most likely surface active compounds. Examples of surface active compounds in crude oil are asphaltenes, resins, acids and bases. The composition of crude oil will be discussed in Chapter 3.

2.4 Naturally occurring gas hydrates

Gas hydrates are not only a focus in the oil industry, but also attract much interest because they occur naturally in many environments, like in the ocean floor and in the permafrost [1]. Usually methane comprise most (>99%) of the hydrocarbon gas mixtures, and thus Structure I hydrates are most likely to be formed. The gas hydrates buried below the ocean floor are mostly of microbial origin (CO_2 from organic matter is reduced to methane), and the continental gas hydrates often contain a mixture of microbial and migrated thermal methane (thermal decomposition of organic matter), see

review article by Kvenvolden [18] and references therein. Large amounts of methane are present in the gas hydrates, and if obtained, this methane can be used as energy resource. The amounts of methane contained in the naural gas hydrates have been estimated by several authors, and the amounts vary over a wide range [1]. Some authors estimated the amount of carbon in methane hydrates to approximately 2.0×10^{16} m^3 [19, 20]. If correct, this amount would be twice as large as the carbon present in all known fossil fuels [19]. However, the recovery of gas from the hydrate reservoirs is very uncertain, because they are very dispersed and the solid form makes them difficult to recover [1].

2.5 Models for gas hydrates

In this thesis, the experimental work has not involved the natural gas hydrates, due to the need for using expensive equipment that tolerate the temperature and pressure conditions associated with natural gas hydrates. Models for natural gas that are easier and safer to work with in the laboratory, like freon hydrates and ice, have been used.

2.5.1 Freon hydrates as models for natural gas hydrates

Freon (CCl_3F, R-11) forms Structure II hydrates below 8.5°C at 1 bar [21], and is thus suitable for laboratory experiments without pressurised equipment. A procedure for making of the freon hydrates and extracting components with affinity for the hydrate surface is described in Paper III.

2.5.2 Ice as a model for natural gas hydrates

Ice has also been tested as a model for natural gas hydrates. The ice crystals are made as small as possible to get a large surface area for extraction of active material. Crude oil components are allowed to adsorb onto the ice surface by various extraction methods. The procedures for ice extraction are described in the Research Report which is included in this thesis.

Chapter 3

Crude oil

Crude oil is formed from inclusion of biomass of mostly aquatic plants and animals in the sediments. The organic material is altered and decomposed in several steps, and this process results in a complex mixture containing a large variety of compounds and molecular species [10, 22, 23].

3.1 Composition of crude oil

The composition of petroleum can vary depending on many factors, like the location and the age of the field. Crude oil mainly consists of the elements carbon and hydrogen (from organic material). In addition small amounts of nitrogen, oxygen, sulfur and metals can be found [10].

3.1.1 Hydrocarbons in crude oil

Hydrocarbons are compounds consisting of only carbon and hydrogen. In petroleum we can find saturated hydrocarbons with straight or branched chains (paraffins), saturated hydrocarbons containing one or more cyclic structures (naphthenes) and hydrocarbons containing one or more aromatic

nuclei (aromatics) [10]. Examples of different hydrocarbons are shown in Figure 3.1.

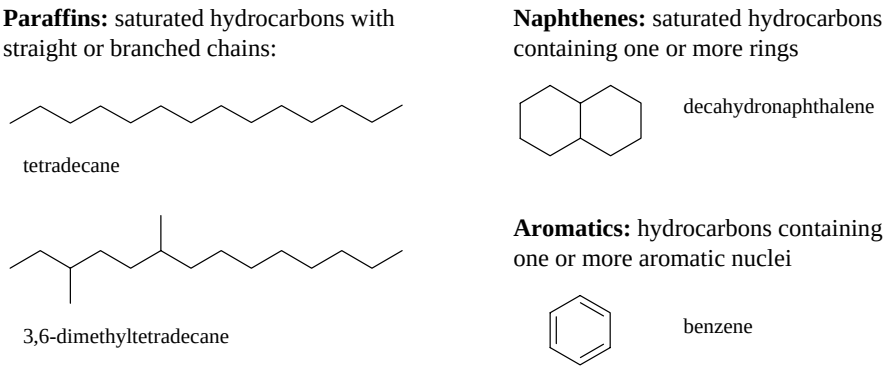

Figure 3.1: Examples of different hydrocarbons in crude oil; paraffins, naphthenes and aromatics.

3.1.2 Nonhydrocarbon constituents

Components containing oxygen, nitrogen and sulfur are termed hetero compounds. These compounds appear throughout the whole boiling range of the crude oil, but they tend to concentrate in the heavier fractions [10]. The polar compounds in petroleum containing nitrogen, sulfur and oxygen are often called NSO-compounds or resins.

Oxygen compounds in crude oil are typically alcohols, phenols, acids, ketones, esters, ethers and anhydrides, and many of them are acidic. Typical nitrogen compounds found in crude oil are pyridine and quinoline, that are basic, and pyrole, indole and carbazole that are non-basic [10]. Sulfur compounds often have harmful effects, like increased corrosion, and they need to be removed due to environmental concerns. Examples of crude oil compounds containing oxygen, nitrogen and sulfur are shown in Figure 3.2.

In addition to the resins, asphaltenes are also found in the heavier frac-

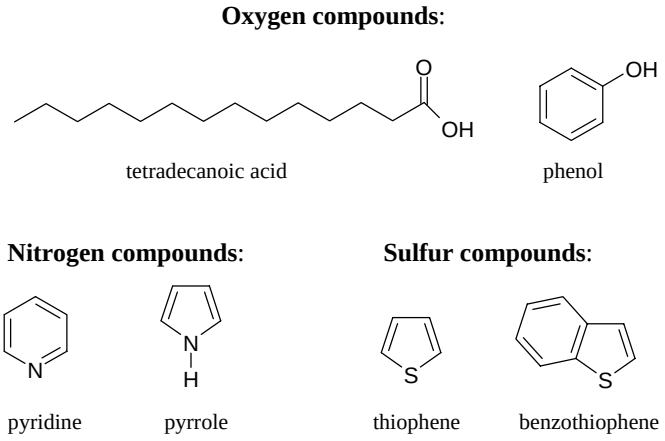

Figure 3.2: Examples of crude oil components containing oxygen, nitrogen or sulfur.

tions. The asphaltenes are not special compounds or compound groups, but a solubility fraction. The asphaltenes consist of crude oil components that cannot be dissolved in low boiling liquid hydrocarbons, like pentane, hexane and heptane [10]. The composition of the asphaltenes is dependent on the liquid hydrocarbon used for the asphaltene extraction, and asphaltene data always has to be reported together with the extraction procedure. In the literature asphaltenes are referred to as macromolecules consisting of many aromatic rings, hydrocarbon chains and heteroatoms (and trace metals), and the molecular weight is probably in the range 2000 $\pm$ 500 g/mole [10].

3.2 Biodegradation of crude oil

Biodegradation is microbial alteration of the crude oil. Bacteria are under some conditions able to degrade some of the compounds present in crude oil, using them as a source of carbon [22]. The n-alkanes are attacked first, probably because they are easiest for the bacteria to consume. After the n-alkanes, the bacteria start to consume compounds with one methyl branch,

and then more highly branched compounds. Later polycyclic alkanes are attacked, starting with the most degradable ones, and at the end aromatic hydrocarbons. Degradation of the lighter hydrocarbons leads to enrichment of other compounds in the crude oil, like heavy polar compounds and asphaltenes. Biodegradation also leads to formation of compounds like acids, mostly cyclic acids (naphthenic acids), that can be manufactured by the bacteria [24] and demethylated hopanes, possibly components of bacteria cells [22]. The production of acids during biodegradation might be due to either oxidation of hydrocarbons in the crude oil, or the acids can come from the cell walls of the micro-organisms [24–27].

The level of biodegradation can be determined by investigating which compounds that are degraded. Peters and Moldowan [28] have developed a scale for evaluating the biodegradation level from 1 to 10, 1 representing light biodegraded and 10 representing severe biodegraded oils. The scale of Peters and Moldowan is used to characterise the crude oils in this thesis. Crude oils that are not biodegraded at all (non-biodegraded) are given the level 0. The Peters and Moldowan scale focus on the heavy and severe biodegradation. Other scales for biodegradation level have been developed, e.g. a scale by Wenger et al. [29] that focus more on the lower levels of biodegradation, and characterisation of biodegradation by the use of carbon isotopic ratio that has been used by Vieth and Wilkes [30]. Recent results from our group have shown that early biodegradation may occur in a very variable manner, depending on the micro-organisms present [31].

Biodegradation results in reduction of the crude oil quality and economic value. The enrichment of heavy polar components leads to an increase in density, viscosity, acidity and content of sulfur, asphaltene and metals [24, 28, 29, 32]. The increased acidity due to biodegradation further reduces the

value of the oil and may contribute to corrosion, e.g. [25,33,34] and emulsion problems, e.g. [29].

The biodegradation process is not well understood. Previously aerobic bacteria was thought to be the main contributor to biodegradation [22, 32, 35]. However, recent research has shown that anaerobic biodegradation also occurs, e.g. [36–42].

In order for biodegradation to occur, some conditions are needed. Most of the biodegradation takes place at the oil-water contacts, where the bacteria can live in the water phase, which contains nutrients, and consume components from the oil phase [32, 41, 43, 44]. Thus, a water phase in addition to the crude oil is needed. In order to easier get hold of the crude oil components, the bacteria often produce biosurfactants, see Section 3.3.2. Another important factor for biodegradation is the temperature in the reservoir. Generally the degradation level decrease with increasing temperature up to 80°C [32,41,43,45]. Over 80°C reservoirs are sterilised, and no bacterial activity occurs [45].

In general, biodegradation occurs in shallow reservoirs with temperatures below 80°C, and most of the degradation processes are anaerobic, see review article by Larter et al. [46]. The biodegradation reduces the value of the crude oil by the lower hydrocarbon content and the higher amounts of acids, asphaltenes and other heavy polar components.

3.3 Surface active compounds in crude oil

Surface active compounds in crude oil are important because they affect the phase behaviour when an oil comes in contact with one or several other phases, like water or solid surfaces. For instance, surface active crude oil

components are important for behaviour during processing of crude oil, e.g. formation of emulsions [47, 48] and foam [49]. Surface active compounds are also believed to be of special interest regarding the hydrate plugging problem in the petroleum industry [5]. In addition to production problems, adsorbed surface active components influence the wetting properties of reservoir rocks, which again influence the oil recovery from the reservoirs [50].

Several classes of hetero compounds in crude oil show surface activity, but the carboxylic acids are especially important for the interfacial activity of crude oil [51]. Recently, very surface active biosurfactants formed during petroleum biodegradation have also been identified [52, 53].

3.3.1 Petroleum acids

Acids are a natural part of petroleum, and have been extracted and analysed in several contexts [24, 25, 51, 54–67]. The amount of acids in petroleum is low, generally less than 4 wt% [51, 59, 67]. However, they are important due to their interfacial activity and their emulsifier and corrosive properties [51, 61, 68–70].

The acids are present because the crude oil formation process has not proceeded to a sufficient degree to defunctionalise them, or because the crude oil has been biodegraded by bacteria, see Section 3.2 [23, 24, 26]. Thus, the amount of acids is much higher in biodegraded oils than in non-biodegraded oils.

Naphthenic acids comprise a large part of the petroleum carboxylic acids. The naphthenic acids are a complex mixture of alkyl substituted acyclic and cycloaliphatic carboxylic acids, and they have the general formula $C_nH_{2n+Z}O_2$, where n stands for the number of carbon atoms and Z specifies the hydrogen deficiency, see Figure 3.3. When Z is 0, the formula represents an acyclic

fatty acid. More information about naphthenic acids can be found in a review article by Clemente and Fedorak [71] and references therein. The cyclic structures that are found to a large degree in the naphthenic acids are not easily identified on molecular basis.

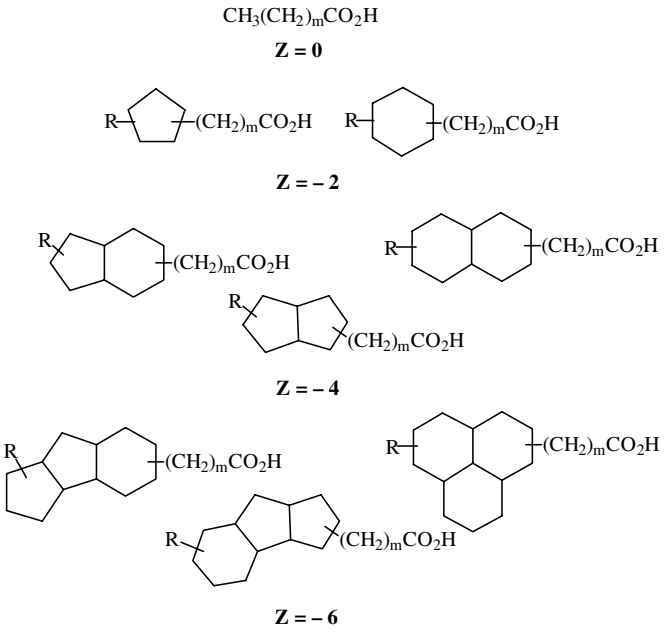

Figure 3.3: Naphthenic acid structures where R is an alkyl chain, Z describes the hydrogen deficiency and m is the number of CH_2 units, from Clemente and Fedorak [71].

The petroleum acid fraction does not only consist of cyclic or acyclic alkanoic acids. Compounds containing heteroatoms like sulfur and nitrogen are also present, as well as phenols [25, 65, 68, 72, 73].

3.3.2 Biosurfactants

Micro-organisms cause the biodegradation of petroleum. These organisms live in the water phase, and they consume crude oil components that mostly are found in the oil phase (see Section 3.2). In order to enhance the avail-

ability of the crude oil components, the micro-organisms can produce biosurfactants [74–77]. Surfactants are surface active agents, which amongst other properties can reduce the surface and interfacial tension of liquids. The surfactants contain one hydrophilic part (head) and one hydrophobic/lipophilic part (tail), where the hydrophilic head can interfere with the water phase, and the hydrophobic tail stays in the oil phase at an oil-water interface [78]. Biosurfactants have high surface active properties and are produced by microbial activity [78, 79].

Biosurfactants have several application areas, where the oil industry is the major market, for instance in bioremediation of petroleum contamination [80–82], oil tank cleaning [83] and microbial enhanced oil recovery [84]. Other applications are agriculture, cosmetics, pharmaceuticals, detergents, personal care products, food processing etc. (see review article by Banat et al. [85] and references therein). Advantages with the biosurfactants, are that they can be biodegraded [86–88] and they are non-toxic or less toxic [85, 89]. One disadvantage is the high production costs of biosurfactants [75, 90].

Several classes of biosurfactants can be found, like glycolipids, lipopeptides, phospholipids, fatty acids and neutral lipids [91]. In this thesis two biosurfactants have been studied; surfactin which is a lipopeptide, and rhamnolipid which is a glycolipid.

Surfactin has a molecular weight of approximately 1050 g/mole, and contains a seven-membered ring of amino acid units, made up of four different amino acids, linked with a hydroxy fatty acid. The number of C-atoms and the branching in the fatty acid might vary, as well as the the amino acid substitution in the peptide ring [92–95]. More information about surfactin can be found in the mini-review by Peypoux et al [96] and others [92, 97–101]. The structure of a surfactin molecule is shown in Figure 3.4. In our tests

we used a commercial surfactin (purity approx. 98%), purchased from from Sigma.

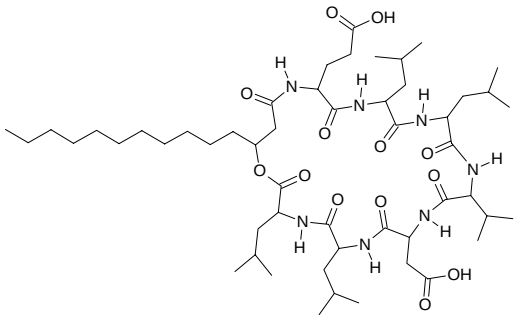

Figure 3.4: The structure of a surfactin molecule, containing a seven-membered ring of amino acid units, made up of four different amino acids (leucine, glutamic acid, valine and aspartic acid), linked with a hydroxy fatty acid. (Figure 1, Paper III.)

Rhamnolipid is a glycolipid, and consists of the sugar structure rhamnose and hydroxy fatty acids. One or two sugar units might be present, in addition to one or two fatty acids [102], giving molecular weights from 330 to 650 g/mole. The structure of a rhamnolipid is shown in Figure 3.5. More information about rhamnolipids can be found in the review article by Desai and Banat [102] and others [103–105]. Our sample of rhamnolipid (0.25 % in water) was recieved from Professor I. Banat, University of Ulster [103].

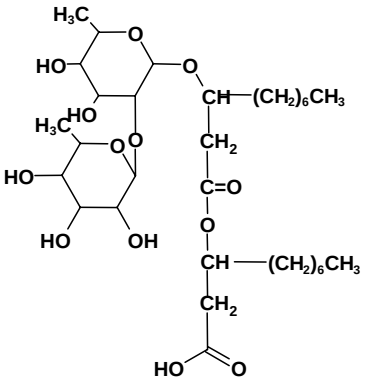

Figure 3.5: The structure of a rhamnolipid molecule, consisting of two rhamnose units and two hydroxy fatty acids.

Chapter 4

Methods for fractionation and analysis

4.1 Bulk analysis of crude oil

To examine the general nature of petroleum the percentages of carbon, hydrogen, nitrogen, oxygen and sulfur are often characterised [10]. The atomic ratio of the various elements compared to carbon (i.e. H/C and O/C) can be used to indicate the overall character of the crude oil. However, this analysis does not give any information about the structure of the components present in the crude oil.

The amounts of acids and bases in crude oil are often measured by titration. The titration procedures only give the total amount of titratable acids and bases, and they do not give any information about the molecular composition of the compounds. Standard methods have been developed for total acid number (TAN), e.g. ASTM664-89 [106], and total base number (TBN), e.g. ASTM2896-88 [107] with modifications by Dubey and Doe [108].

4.2 Fractionation of crude oil

Crude oil can be divided into fractions in different ways. In distillation fractionation for instance, compounds are separated according to their boiling points, and fractions of broad boiling ranges are obtained [10]. Solvents can also be used to fractionate crude oil, usually by having two phases - a solvent phase and an oil phase [10]. One type of solvent extraction is asphaltene separation. In this procedure 40 volumes of low-molecular-weight paraffinic hydrocarbons, like n-pentane, n-hexane or n-heptan, are added to the crude oil. The fraction that can be solved in the hydrocarbon solvent is called maltenes, and the fraction that is not soluble is called asphaltenes.

The maltene fraction can be further fractionated into sub-fractions by the use of adsorption chromatography. This is performed on a column filled with adsorption material. When a sample is applied onto the column and transported through by a solvent, the components in the sample are adsorbed to the column material to various extents, depending on their chemical nature. By varying the polarity of the solvent, the components elute from the column in different fractions [10]. A standard fractionation procedure is the SARA fractionation, where the crude oil is separated into Saturates, Aromatics, Resins and Asphaltenes [109]. A scheme for a simplified fractionation procedure is shown in Figure 4.1.

In this work, crude oil extracts are fractionated on micro silica columns to remove the non-polar components and separate the rest of the extract into two polar fractions, the first polar fraction containing less polar compounds (typically carboxylic acids) than the second polar fraction (typically polyfunctional compounds). Two types of columns are used, and the fractionation procedures are described in Paper III.

Solid Phase Extraction (SPE) cyano columns are also used to fraction-

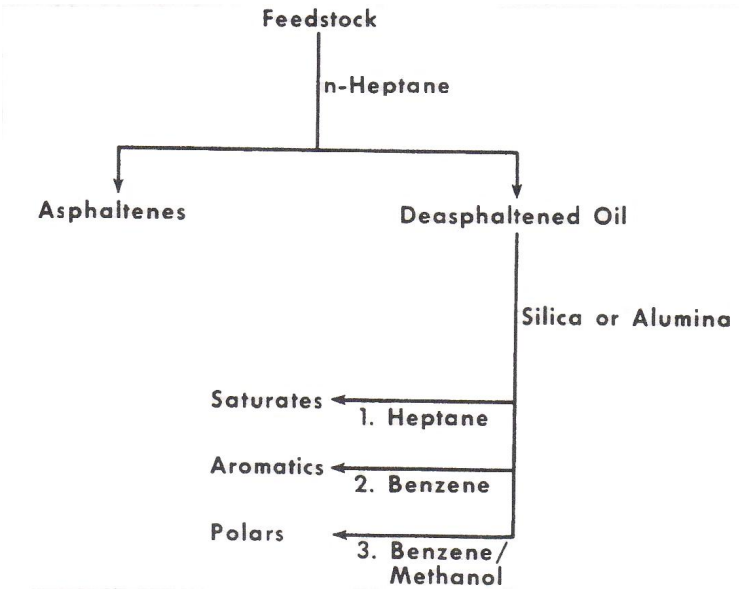

Figure 4.1: A simplified fractionation procedure, from Speight [10].

ate crude oil extracts. A method for separation of acid fractions into four sub-fractions has been developed, and this procedure is described in Paper IV. A picture of the SPE column during the fractionation of an acid fraction is shown in Figure 4.2. This method was primarily developed to fractionate large amounts of sample in a way similar to the HPLC fractionation using cyano columns, see Section 4.4.2. Even after optimisation of the eluent composition, the SPE columns cannot reproduce the fractionation on the HPLC cyano columns. The produced sub-fractions are not sufficiently uniform to be useful in precise analysis on GPC, FTIR and GC-MS to obtain structural information. However, the SPE procedure can be used to rapidly separate large amounts of the acid fractions into rough sub-fractions for studies of physical and chemical properties.

Iatroscan is a method that quickly and inexpensively quantifies the relative amounts of material present in different fractions. Samples are spotted near the end of tubes coated with a thin layer of stationary phase, and solvent

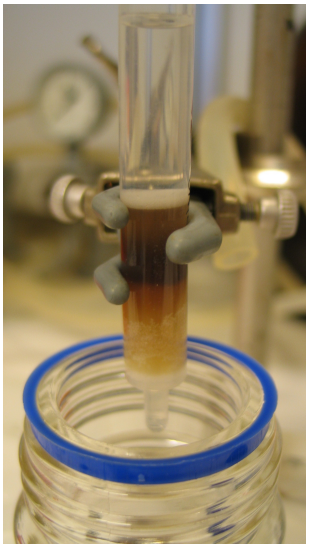

Figure 4.2: SPE fractionation of an acid fraction. The picture is taken during the fractionation when the third fraction is being eluted off the column.

are allowed to climb up the tube. Some of the molecules are mobilised by the solvent. A series of eluting solvents of increasing polarity is used to mobilize all the compounds. The amount of material can be determined using a flame ionisation detector [22].

Acids can be extracted from crude oil by various methods. Several authors report the use of liquid-liquid extractions, where alkaline solutions are used to extract the acidic components from crude oil [25, 51, 55, 59, 110, 111]. An alternative method for extracting the acids from petroleum is using an ion-exchange resin [24, 54, 60, 62, 67, 112]. The separated acid fractions still comprise a wide range of structures and acid strengths [6]. In this thesis, acids have been extracted from crude oils both by liquid-liquid extraction (Paper I and V) and ion exchange extraction (Paper V).

4.3 Spectroscopic methods

Infrared spectroscopy (IR) provides information on the functional groups present in samples. Individual compounds cannot be identified using Fourier Transform InfraRed (FTIR) spectroscopy, but information about structural features of the whole sample can be obtained, e.g. the presence of linear or branched carbon chains, aromatic rings, carbonyl groups and other specific functional groups. The FTIR interpretations in this thesis are based on literature from Williams and Fleming [113] and Coates [114]. In this work, the Attenuated Total Reflection (ATR) technique is used. The technique is used for hydrate extracts in Paper III and acid fractions in Paper IV and V.

Nuclear Magnetic Resonance (NMR) has a large potential for characterisation of structures of heavy petroleum components. The NMR method can measure the aliphatic and aromatic carbon content, as well as hydrogen distributions [6]. However, there are limitations to the interpretation of NMR spectra due to the complexity of petroleum [23, 115].

4.4 Chromatographic methods

Molecular analysis of crude oil is very challenging due to the large number of different molecules present. Even after fractionation, the number of different molecular structures in most fractions is too high to be determined. All chromatographic methods thus give only partial information about the composition at a molecular level.

4.4.1 Gas Chromatography

Gas chromatography (GC) is often used on saturated hydrocarbon fractions of crude oils [22]. In gas chromatography a sample is injected into a column situated inside an oven. Molecules in the sample are vaporised and an inert gas carries them through the column, where they are separated according to boiling point and affinity to the column stationary phase. Heavy molecules generally move more slowly than lighter molecules, and polar molecules move more slowly than non-polar. The temperature in the oven can be increased to enhance the volatility and mobility of heavier molecules, in order to get them more easily through the column. When the molecules come out of the column, they are recorded in a detector, and a chromatogram with peaks that ideally represent single compounds is recorded. However, if two or more peaks come out of the column almost simultaneously, they may overlap. The compounds in a sample can be identified by comparing the retention time to retention times of known standards.

Fractions from petroleum that are more polar than the saturated hydrocarbons are more complicated to analyse by GC, due to high boiling points and the large number of compound types [6]. Compounds with high boiling points and polarity that are difficult to get through the GC column can be converted into compounds with lower boiling points and polarities in a process called derivatisation [116]. Petroleum acids, for instance, can be converted into more volatile and less polar esters. However, even after fractionation of the crude oil, and derivatisation of fractions, insufficient separation is often found in gas chromatograms. Especially biodegraded oils, containing a large variety of heavy, polar compounds, give a hump in the chromatogram from Unresolved Complex Mixture (UCM) [27, 117–119].

4.4.2 High Performance Liquid Chromatography

High molecular weight and low volatility of the target compounds can make the use of GC difficult, and liquid chromatography may be preferred. High Performance Liquid Chromatography (HPLC) is very useful for separation of oil fractions containing functionalised compounds. In HPLC the mobile phase is a liquid, and the compounds in a sample can be separated according to the affinity for the stationary phase (column material) and the liquid mobile phase [116]. A simple sketch is shown in Figure 4.3. HPLC can be used in normal phase and reverse phase mode. In normal phase the stationary phase is polar, and by using a non-polar mobile phase the least polar sample components come out first from the column. Reverse phase mode uses a non-polar stationary phase, and polar components are eluted first from the column.

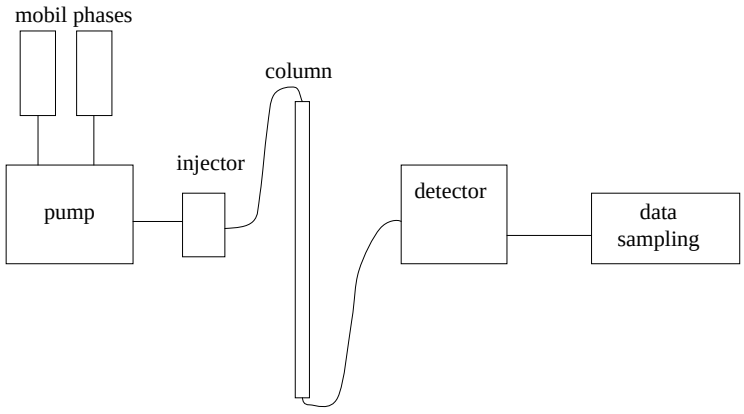

Figure 4.3: A simple scheme for HPLC procedure.

HPLC has been used for chromatographic separation of petroleum by several authors, e.g. [120–122]. However, better HPLC methods for separation, especially for the more polar parts of the crude oil, are needed. A new method for HPLC separation of petroleum acids has been developed and is

presented in Paper IV.

The work in Paper IV presents an HPLC method using normal phase chromatography on a cyano bonded phase column, which provides a stable and fast separation of organic acids from crude oils into four well-defined fractions that correspond to the main types of acidic compounds found in the oils; weak acids with no acidic protons, saturated carboxylic acids, phenols and polyfunctional acids. The method has been developed both in analytical scale for characterisation of acid fractions, and in preparative scale to provide sufficient sample amounts for further analysis using other spectroscopic or chromatographic methods. Gradient programmes for the analytical and the semi-preparative columns are presented in Paper IV.

Two detector types are used, an Evaporative Light Scattering Detector (ELSD) and a UV detector. In the ELS detector the mobile phase is evaporated, the sample is turned into droplets and the amount of scattered light is detected. This detector is universal, and most types of compounds can be detected. The only compounds that will not be detected are compounds that evaporate together with the solvent, in this case certain phenolic compounds. In the UV detector, compounds that have UV-absorption can be detected. The phenolic compounds that are difficult to analyse with the ELS detector, can easily be detected by UV-absorption. The UV detector is therefore used complementary to the ELS detector. HPLC chromatograms from acid fractions from the oil B4c are shown in Figure 4.4 to demonstrate the separation into different fractions, using both an ELS detector and a UV detector.

When comparing different samples, the chromatogram is divided into four sections; F_A - non-polar compounds, F_B - saturated carboxylic acids, F_C - phenolic compounds and F_D - polyfunctional compounds. The percentage of each area in the chromatogram is used to calculate the amount of material in

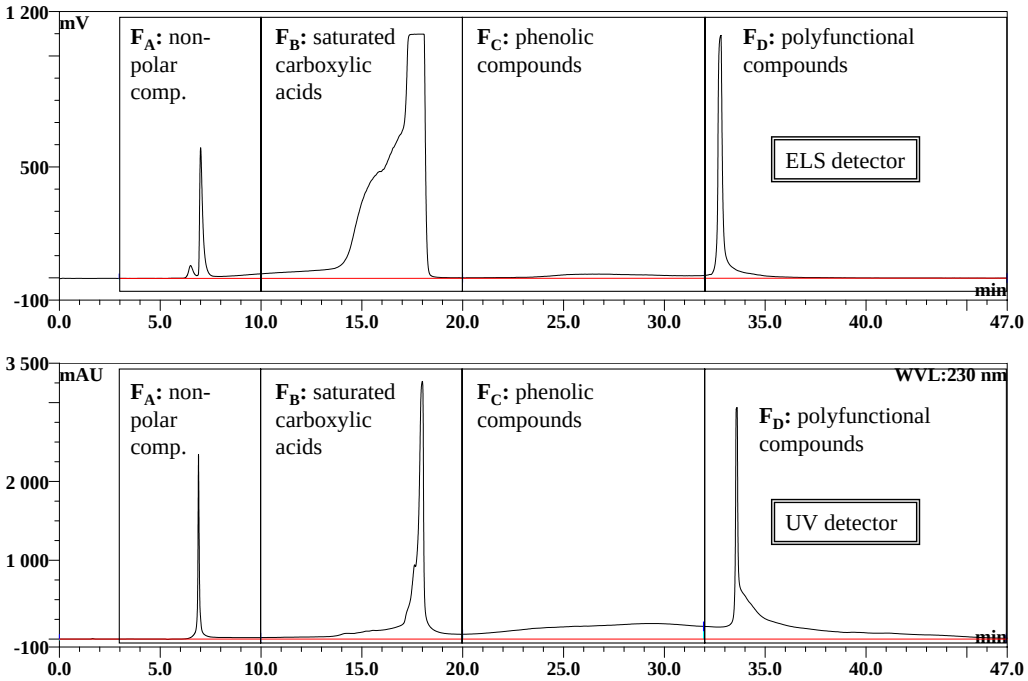

Figure 4.4: Chromatograms from using an analytical cyano column of the acid fraction from the oil B4c (ion exchange extraction); upper: ELS detector and lower: UV detector. (Figure 2 and 4, Paper IV.)

the different fractions of the crude oil. This is performed on the basis of the amount of acids extracted from the corresponding oil. The semi-preparative cyano column gives chromatogram with five fractions (F_A, F_{B1}, F_{B2}, F_C and F_D), as shown in Figure 4.5.

4.4.3 Gel Permeation Chromatography

Gel Permeation Chromatography (GPC) is a method that separates compounds according to their molecular weights. In this technique the chromatographic column is packed with gels of varying pore size [10]. A sample is injected to the column, and small molecules have longer residence time in the pores, while the larger molecules are too large to spend time in the pores, and they are eluted first. From using a calibration curve made from stan-

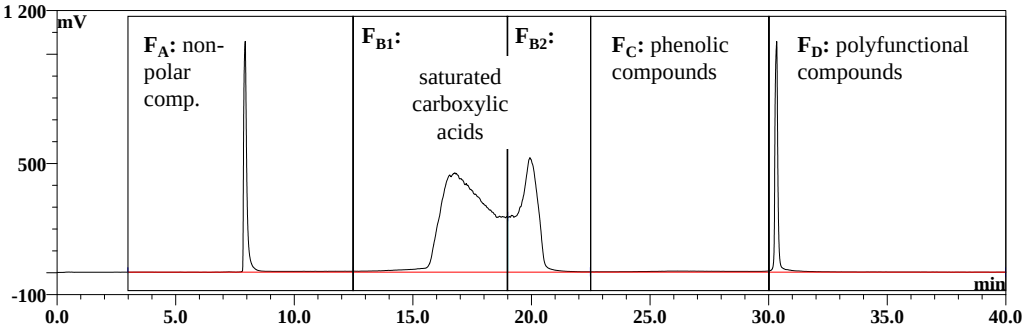

Figure 4.5: A chromatogram from semi-preparative HPLC of the acid fraction from B4c (ion exchange method), using ELS-detection. (Figure 5, Paper IV.)

dards with known molecular weights, the number of average molecular weight distribution of petroleum fractions can be determined. However, petroleum samples contain constituents of widely different polarity that may interact with the gel surface. This could effect the elution time of compounds in the column, making the molecular weights obtained from GCP somewhat uncertain. However, the method still is the most suitable way of getting molecular weight profiles of these complex samples.

In this work, GPC analysis is performed using a column that is packed with polystyrene/ divinylbenzene co-polymer, and the molecules are separated according to molecular size and shape. The molecular weights of the samples are calculated from a standard curve based on model compounds. More information about the GPC system can be found in Paper I, III and IV.

4.5 Hyphenated techniques

Hyphenated techniques like GC-MS (Gas Chromatography-Mass Spectrometry) and LC-MS (Liquid Chromatography-Mass Spectrometry) have been used for analysis of crude oil, e.g. [24, 56, 60, 123, 124]. In these techniques,

the compounds in the different peaks from the GC or LC chromatograms can be identified by MS analysis. The compounds are broken up into charged fragments (ions) in the MS part of the instrument. Every compound has a special fragmentation pattern, which is closely related to the chemical structure, giving a unique fingerprint for that compound [22, 125].

In this work, GC-MS is used for analysis of the hydrate extracts, see Paper III. Due to high molecular weights, many of the compounds in the samples are outside optimal GC-MS scope. The samples are therefore hydrolysed to decompose large molecules into smaller, identifiable compounds. After hydrolysis, the water phase and the organic phase are derivatised with chloroformate as described by Liebich et al. [126]. This procedure is tested for the biosurfactant surfactin (Paper III), and surfactin can easily be identified. The GC-MS procedure is then used on polar fractions of hydrate extracts to search for lipopeptides in the oils. The detection limit for surfactin on GC-MS is tested, and samples of surfactin of 1, 10 and 100 ppm in ethyl acetate are analysed. The detection limit is between 1 and 10 ppm, meaning that sample concentrations of 10 and 100 ppm are easily detected, but not sample concentrations of 1 ppm.

Some LC-MS analyses have been performed at the University of Newcastle upon Tyne on a Surveyor HPLC system, Thermo Finnigan, with a UV (Surveyor PDA) and a MS detector (LCQ Advantage ion trap). Both APCI (Atmospheric Pressure Chemical Ionisation) and ESI (Electron Spray Ionisation) are tested. The instrumentation allows direct infusion of samples into the MS ion source as well as separation of samples in an HPLC column prior to analysis in the MS. Hydrate extracts and acid fractions, sub-fractions from these, fractionated on an analytical cyano column (see Section 4.4.2), and the standards surfactin and rhamnolipid are used for analysis. In ad-

dition, a reversed phase C18 column (BDS Hypersil, 250 mm × 4.6 mm, 5 μm) is used for separation of the samples prior to MS analysis. These results have not been published.

4.6 Crude oil - hydrate interactions

4.6.1 Plugging tendencies of crude oils

Crude oils have different tendencies to form hydrate plugs within realistic petroleum pipeline temperature and pressure conditions. Several methods can be used to evaluate the plugging tendency of a crude oil, for instance the use of a high pressure sapphire cell. A high pressure cell from Sanchez Technology is situated at Norsk Hydro Research Centre, and allows pressure up to 500 bar and temperature down to -40°C. The experimental setup is described by Fadnes [2].

This high pressure cell is used for the plugging tendency tests in Paper II. The evaluation are primarily performed by visual inspection, and a crude classification of the oils is obtained; dispersive systems and plugging systems. In a dispersive system all the hydrates are present as a dispersion, and in a plugging system the hydrates form aggregates.

4.6.2 Wettability of crude oil/brine systems

Previously, Høiland et al. [5] developed a method for investigation of the wettability of freon hydrates in crude oil/brine emulsions. An emulsion containing colloidal solid particles will have one liquid that is more likely to wet the solid than the other liquid [127], and the more poorly wetting liquid becomes the dispersed phase. This means that the wettability of the solid can

influence the type and stability of an emulsion. In the method of Høiland et al., hydrates are considered to act as emulsion-stabilising colloids. Thus, if the hydrate particles are oil wet, the emulsion system most likely will be oil continuous, and if the hydrate particles are water wet, the emulsion system most likely will be water continuous. A schematic illustration of spherical particles at a planar oil-water interface is given in Figure 4.6.

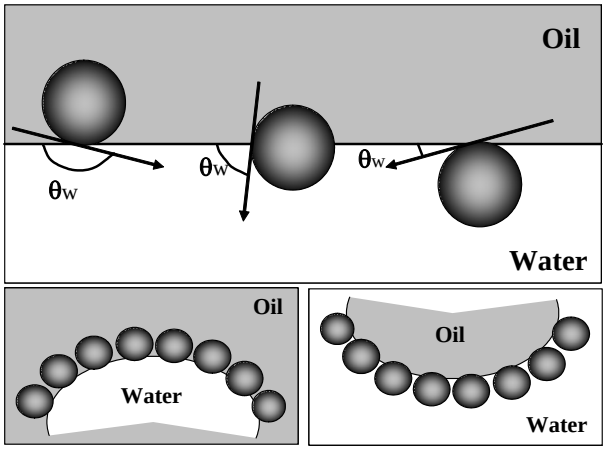

Figure 4.6: Spherical particles at planar oil/water interface. Oil wet particles (contact angle $> 90°$) will reside in the oil phase (left), and tend to stabilize water-in-oil emulsions. Water wet particles (contact angle $< 90°$) reside mainly in the water phase (right), and tend to stabilize oil-in-water emulsions. (Adapted from Binks [128].) (Figure 1, Paper II.)

The wettability of a hydrate particle in contact with brine and crude oil will probably be influenced by the presence of surface active compounds that can be adsorbed onto the hydrate surface. Schulman and Leja [129] have previously reported that surfactants may adsorb to the particle surface and alter the wettability of the particle. Crude oils contain a large variety of components, and the amounts of surface active species vary for different crude oils. Hence, the wettability alteration of hydrate particles will be different for various crude oils.

In the method of Høiland et al. [5], the point of phase inversion from

oil-continuous to water-continuous is found by gradually increasing the volume fraction of brine. The point of phase inversion is found both for systems with freon hydrates, and without hydrates for the same crude oil, to avoid the influence from other compound classes in crude oil affecting the properties of crude oil/brine emulsions (asphaltenes, resins and naphthenic acids [48]). The difference between the two inversion points (with hydrates - without hydrates), $\Delta\varphi_w^{inv}$, is calculated for the crude oil systems. A positive value of $\Delta\varphi_w^{inv}$ indicates presence of oil wet hydrate particles, negative $\Delta\varphi_w^{inv}$ value indicates the presence of water wet particles, and $\Delta\varphi_w^{inv}$ values close to zero indicate the presence of intermediate wet particles. Crude oils are likely to contain components with different affinities for adsorption onto the hydrate surface, resulting in a range of wettability states, from water wet particles to intermediate wet and oil wet particles, depending on crude oil composition. The generation of oil wet freon hydrates correlates well with a low hydrate plugging tendency. Oil wet hydrates reduce the possibility of strong attractive hydrogen-bonding between hydrate particles. This results in flocculation and dispersion rather than agglomeration and hydrate plugs. Dispersions can be transported in the petroleum pipelines, while hydrate plugs can block fluid transport completely.

By investigation of the wettability of freon hydrates, the method developed by Høiland et al [5] can differentiate oils with regard to hydrate morphology. Additives can be tested to see if the wetting properties of a plugging crude oil can be altered by addition of surface active components, and this is presented in Paper II. Chemical additives are introduced to water wet or intermediate wet crude oils to see whether the wettability of the crude oil/brine systems are altered. A change to a more positive $\Delta\varphi_w^{inv}$ value indicates that the chemical additive affects the system to change into a more

oil-wetted state. Both oil soluble and water soluble additives are used. Surfactin and rhamnolipids are water soluble, and naphthenic acids extracted from crude oils are oil soluble. All the additives are added in moderate to low concentrations (6500 ppm for the naphthenic acids).

Chapter 5

Main Results

5.1 Characterisation of the crude oils

The data set used in this thesis consists of 19 crude oils, spanning from heavy biodegraded oils enriched in asphaltenes to light non-biodegraded oils and condensates. Most of the oils originate from the Norwegian continental shelf and are supplied by Norsk Hydro ASA. The oils are labelled with a letter, B - biodegraded oil or S - sweet, non-biodegraded oil, followed by a number indicating production field and a letter denoting different wells or different batches within one field.

Out of the 19 crude oils 4 are identified as having low tendency to form hydrate plugs. These so called non-plugging crude oils are believed to contain natural inhibiting components that prevent hydrate particles from agglomerating into a large plug, see Section 2.3.3. The oils have been characterised with regard to biodegradation level, asphaltene content, density and the amount of extractable acids, acidic compounds (TAN) and basic compounds (TBN), and these results are reported in Paper I and V. The plugging tendency of crude oils and the wettability of the freon hydrate sur-

face in crude oil/brine emulsions for various crude oils have been reported by Høiland et al [5] and in Paper V. Some of the crude oil properties are given in Table 5.1.

Table 5.1: Characterisation of the crude oils; the level of biodegradation on the Peters and Moldowan [28] scale (from Paper I), and wettability of hydrate particles in crude oil and plugging tendency of the crude oils (reported in Høiland et al [5] and Paper V).

Oil	**Biodegr. level**	**Wetta-bility** [a]	**Plugging tendency**
B1a	2	water	high
B1c	2	n.a.	n.m.
B2a	6	n.m.	low
B2b	6	oil	low
B2c	5	int.[c]	n.m.
B3a	8	water	high
B4a	8	oil	low
B4b	8/2 [b]	int.	high
B4c	2	oil	low
S1a	0	n.m.	high
S2a	0	n.m.	high
S2b	0	water	high
S3a	0	int.	high
S3b	0	int.	high
S4a	0	n.m.	high
S4b	0	water	high
S5a	0	water	high
S6a	n.a.	water	high
S7b	0	int.	high

[a] Denotations from wettability tests: oil: oil wet hydrate particles, water: water wet particles, int.: intermediate wet particles. [5].
[b] The B4b oil is a mixture of a more biodegraded oil and a less biodegraded oil.
[c] The oil might contain large amounts of water. The results may not be trustworthy.
n.a. not available.
n.m. not measured

The crude oils with low tendency to form hydrate plugs are all biodegraded, but not all the biodegraded oils are non-plugging, so a direct correlation between biodegradation and plugging tendency are not found. Still, the results indicate that biodegradation is an important factor for the presence of natural inhibiting components (NICs) in the non-plugging crude oils.

5.2 Natural inhibiting components in crude oils

The natural inhibiting components probably have surface active properties, and petroleum acids comprise some of the surface active constituents of crude oils, see Section 3.3.1. An acid fraction from a non-plugging crude oil was previously injected into a plugging crude oil and tested for plugging tendency at realistic conditions at Norsk Hydro ASA (reported in Paper II). The test showed that the plugging crude oil was changed into a non-plugging crude oil. This indicates that the acids extracted from that particular crude oil hold inhibiting compounds. However, the concentration of the acid fraction, the nature of the non-plugging crude oil from which the acids are extracted, and the nature of the plugging crude oil they are injected into, have been shown to be important factors influencing the success of changing a plugging crude oil into a non-plugging crude oil. Hence, it is likely that only a part of the acid fraction works as plug inhibitors, and the solvent effect of the crude oil might also be of importance. The addition of the biosurfactant rhamnolipid has also shown to alter a plugging crude oil into a non-plugging oil (reported in Paper II). Biodegraded crude oils probably contain biosurfactants, and these surface active molecules might be able to influence the plugging properties of the crude oils.

The plugging tests at realistic conditions are very time-consuming, and

they require the use of high pressure equipment such as the sapphire cells located at the laboratories situated at Norsk Hydro ASA in Bergen. A faster screening method for evaluation of the plugging tendency of crude oil systems was developed by Høiland et al. [5], and it is based on the wettability of hydrate particles in crude oil systems, see Section 4.6.2.

Chemical additives are introduced in low concentrations to crude oil/brine emulsions in an attempt to modify intermediate or water wet hydrate surfaces into an oil wet state, and the results are presented in Paper II. For most systems the additives affect the emulsion behaviour. However, the same chemical additive can give different results when different crude oils are used, as is shown in Figure 5.1. These results show that for one of the crude oils (to the right in Figure 5.1), all the acid fractions could make a change towards a more oil wet state, while for the other crude oil (to the left), only one of the acid fractions managed to give a change towards a more oil wet state. The reason for the difference regarding the crude oil systems can be that one of the oils initially gives intermediate wet hydrate particles, while the other gives water wet particles.

The addition of the biosurfactant rhamnolipid gives a change to a more oil wet system. The concentration of the additive is shown to be important, as several systems show a more oil wet state when the concentration of the additive is increased. More details can be found in Paper II.

The results from some of the wettability tests are compared to results from plugging tests at realistic conditions, and a positive correlation is found for 9 out of 11 systems (presented in Paper II). One difference between the wettability tests and the realistic conditions is the hydrate forming component, which is freon in the wettability tests and a natural gas mixture in the realistic tests. Both form Structure II hydrates, and the physical properties

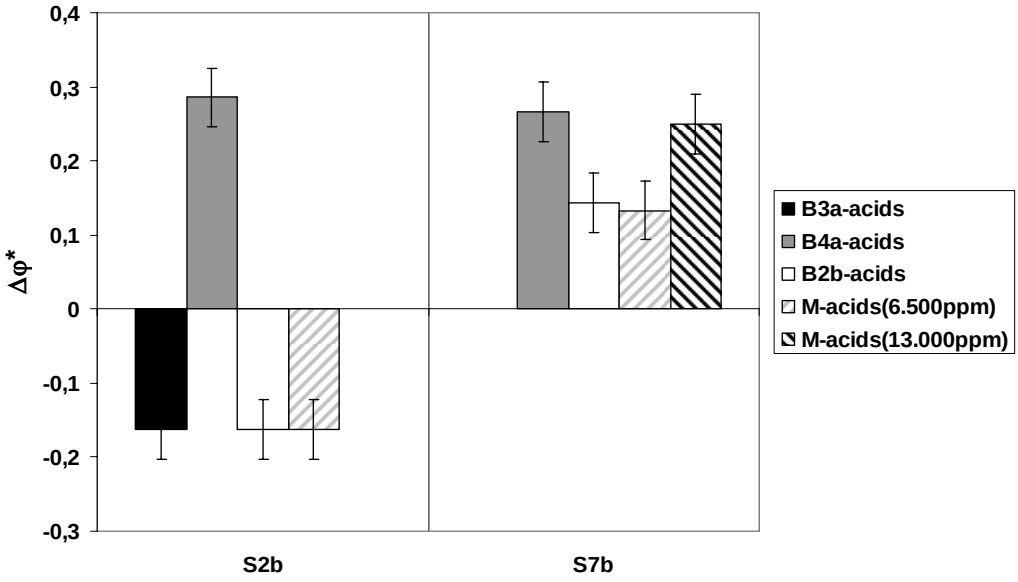

Figure 5.1: The effect of naphthenic acids on emulsion behaviour, given as changes in inversion points, normalised values [5]. (Figure 8, Paper II.)

of the hydrates formed are not assumed to be different. However, the two hydrate formers can have an impact on the surface energy in the system, and this might make a difference in systems with low oil/water surface tensions.

5.3 Acid fractions as hydrate plug inhibitors

5.3.1 Acids in biodegraded crude oils

Acids extracted from crude oils are analysed in Paper I and V. The results show that there is a difference between acids extracted from biodegraded compared to non-biodegraded crude oils. Both the acidity (TAN) and the amount of extracted acids are larger in biodegraded oils.

The results in Paper I also show that compounds in the acid fractions from biodegraded oils have lower molecular weights than acids from non-biodegraded oils, and a comparison between TAN and the molecular weights

gives an indication of the presence of multifunctional compounds in the biodegraded oils. The infrared spectra of acid fractions from biodegraded oils hold more carboxylic acid functionalities and saturated hydrocarbon structures, compared to non-biodegraded acid fractions which are more phenolic in character. GC-MS analyses of acid fractions from biodegraded crude oils show UCM, indicating that many complex structures are present.

The results from comparing acids from biodegraded and non-biodegraded crude oils strongly suggest that the acids in the biodegraded oils are produced in the microbial degradation process, and this agrees with findings from others [24–26].

5.3.2 Comparison of methods for acid extraction

The amount of acids extracted from the different crude oils are presented in Paper I and V. In Paper V the two extraction methods (see Section 4.2) are compared. The results show that the amount of extracted acids is much larger using the ion exchange method than the liquid-liquid extraction in this sample set. The oil B4a is extracted by both methods, and the amount of acids found by the ion exchange method is three times the amount that is found by the liquid-liquid method. The results from Paper I show that the liquid-liquid extraction method has a low recovery of acids in the extract. The ion exchange procedure has previously been shown to have a very good recovery [62], and the results confirm that the ion exchange method is a far more effective method.

Analyses of acid fractions using HPLC also show differences between the two extraction methods, see Figure 5.2 below. This means that the variations between the two extraction methods are not just a matter of different amounts of total material, but also what types of material has been extracted.

The ion exchange fraction contains larger amounts of the saturated carboxylic acid fraction. Furthermore, the amount of non-polar components is lower in the ion exchange fraction compared to the liquid-liquid fraction. Thus, the extra amount of organic material found by the ion exchange method is not co-extracted crude oil, which would show as a large amount of non-polar compounds.

5.3.3 Analysis of acid fractions

The results from Paper I show that GC-MS analysis cannot give much structural information on the acid fraction from biodegraded crude oils. Acid fractions are therefore analysed using HPLC (presented in Paper V). A comparison of the estimated amounts of material found in the different sections of the HPLC chromatograms of the acid fractions is shown in Figure 5.2.

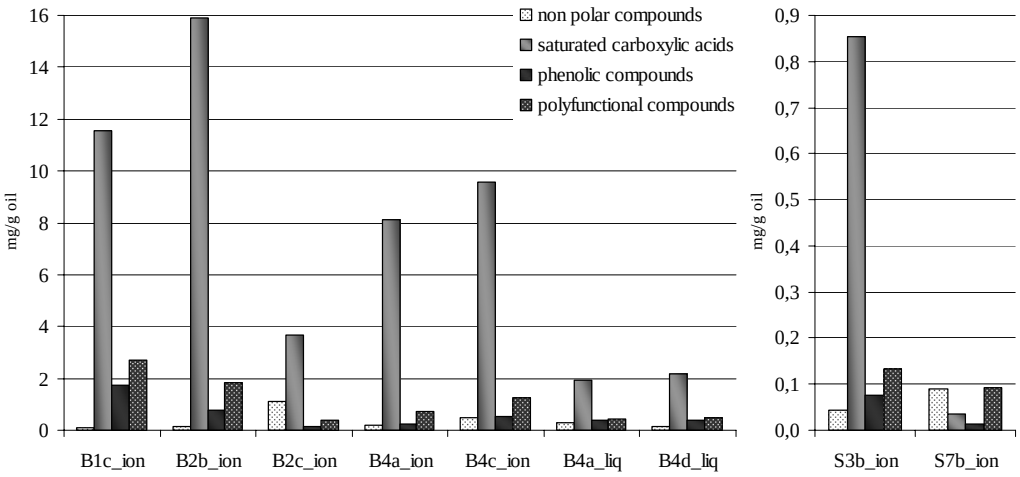

Figure 5.2: The estimated amounts in the sub-fractions of the acid fractions from different oils using the analytical HPLC column and the ELS detector. The oils marked with "ion" have been extracted by the ion exchange method, and the oils marked with "liq" have been extracted by the liquid-liquid extraction. The amount of material found in the non-biodegraded oils is much lower than in the biodegraded oils, so the y-axes are shown with different scales. (Figure 5, Paper V.)

Many of the biodegraded oils extracted by the ion exchange method show a similar compositional trend, with largest amount of saturated carboxylic acids and more polyfunctional compounds than phenols. Of the two non-biodegraded oils that are investigated, one of them has a similar relative composition as the biodegraded oils. Thus, the HPLC results show that there are groups of oils with similar acid composition, but the criteria for which oils group together is not just whether the oils are biodegraded or not. However, all the oils show that the saturated carboxylic acids are major constituents.

The UV detector is primarily used to obtain information about the phenolic compounds (fraction F_C) in the samples (see Section 4.4.2), and the results show that no distinct peaks from phenolic compounds are found, but a broad band with low intensity. UV spectra from various parts of the chromatogram are also studied, e.g. spectra from the F_C fraction shown in Figure 5.3. These spectra show that the B1c and S3b ion extracts have more absorbance in the region above 250 nm than the B2b and B4a ion exchange extracts, indicating that some of the oils might contain additional compounds with aromatic character in the F_C fraction. And it is interesting to note that the oils containing the increased absorption bands are known to be plugging oils.

FTIR analysis of sub-fractions from acid fractions (after fractionation on a semi-preparative column) indicate a non-polar fingerprint in fraction F_A, saturated carboxylic acids in fraction F_{B1} and F_{B2} and a lower relative amount of carbonyl (C=O) and increased complexity of the spectrum in fraction F_D. The GPC-analyses show that fractions F_{B1} and F_{B2} have molecular weights ranging from 400 - 600 g/mole, most likely carboxylic acids with more than 30 carbon atoms. The polyfunctional compounds in the samples,

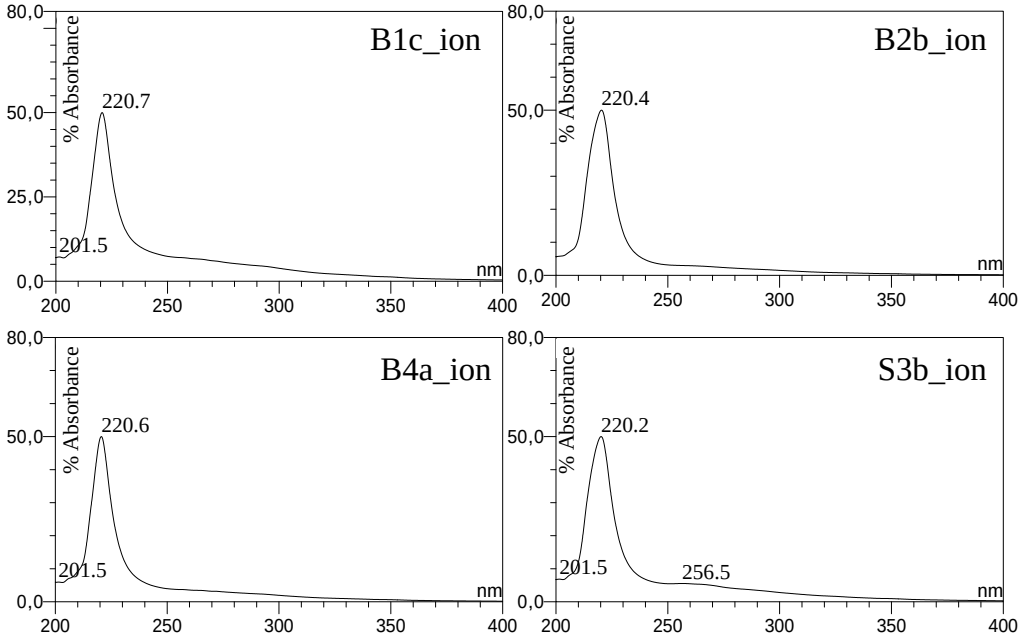

Figure 5.3: UV spectra from fraction F_C of acid fractions from using analytical HPLC column. (Figure 7, Paper V.)

fraction F_D from HPLC, are believed to contain the most surface active compounds, and they have a relatively high molecular weight range, from 700 - 800 g/mole. They correspond well with the molecular weight of some types of biosurfactants, like rhamnolipids. These GPC-results generally show that acid fractions mostly contain compounds of intermediate molecular weights. Thus, neither high-molecular compounds such as asphaltenes nor simple, low-molecular petroleum acids and bases are prominent in the acid fractions.

The results from the analysis of the acid fractions have not provided molecular identification of specific compounds. However, an elimination of possible compounds types as Natural Inhibiting Components (NICs) can be obtained, e.g. the asphaltenes are probably less important. The focus of attention should be aimed at functionalised fractions of intermediate molecular weights.

5.3.4 Fractionation of extracted petroleum acids

The extracted petroleum acids are fractionated into sub-fractions by SPE-columns as described in Paper IV. In order to investigate which part of the acid fraction is active as a hydrate plug inhibitor, the sub-fractions can be studied by the use of wettability tests as described in Paper II. Some preliminary tests have been done, indicating that each of the sub-fractions has an effect, even though some of the fractions have very low concentrations. This work is continued by Kristin Erstad [130].

5.4 Adsorption of compounds onto freon gas hydrate surfaces

The extracts from contacting oil with freon hydrates contain components with affinity for the hydrate surfaces. In this method, the aim is to obtain an extract that contains the specific components that adsorb onto the hydrate particles. The results from the freon hydrate extractions are presented in Paper III.

5.4.1 Quantification of hydrate extracts

In the analysis of the freon hydrate extracts the focus is on the polar fractions, due to a considerable amount of co-extracted hydrocarbons. These co-extracted hydrocarbons are removed by column fractionation, and are assumed not to represent adsorbed material. The results from the fractionation of hydrate extracts are given in Figure 5.4.

The polar 1 fraction contains significantly more material than the polar 2 fraction. With the exception of oil S1a, a considerably higher amount of

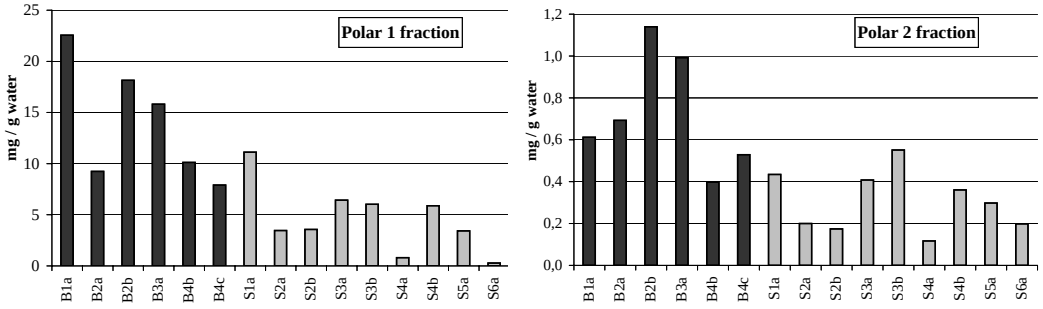

Figure 5.4: The amount of organic material in the hydrate extracts after column fractionation of the hydrate extracts into one non-polar (hydrocarbons, not presented) and two polar fractions by the use of a silica column. Left: Polar 1 fraction, Right: Polar 2 fraction. Black bars (B-oils): Biodegraded oils, and grey bars (S-oils): Non-biodegraded oils. (Figure 3, Paper III.)

polar material is extracted from the biodegraded oils compared to the non-biodegraded oils for both fractions.

5.4.2 Analysis of the freon hydrate extracts

FTIR analyses show some differences between the hydrate extracts from different crude oils. The extracts from the B2 and B4 oils are different from the other oils, both in the first and second polar fractions. The bands identified at approximately 1735 and 1250 cm^{-1}, probably corresponding to C=O and C-O stretching in an ester compound present in the B2 and B4 oils in the second polar fractions, are of special interest because these peaks can indicate the presence of biosurfactants in the extracts. Spectra of the second polar fraction of one biodegraded oil and one non-biodegraded oil are shown in Figure 5.5.

The average of molecular weights in the hydrate extracts, determined by GPC, vary from 400 to 600 g/mole. The molecular weight range for some of the sub-fractions of the hydrate extracts are also determined, and vary from 500 to 1000 g/mole. The GPC results show that the major part

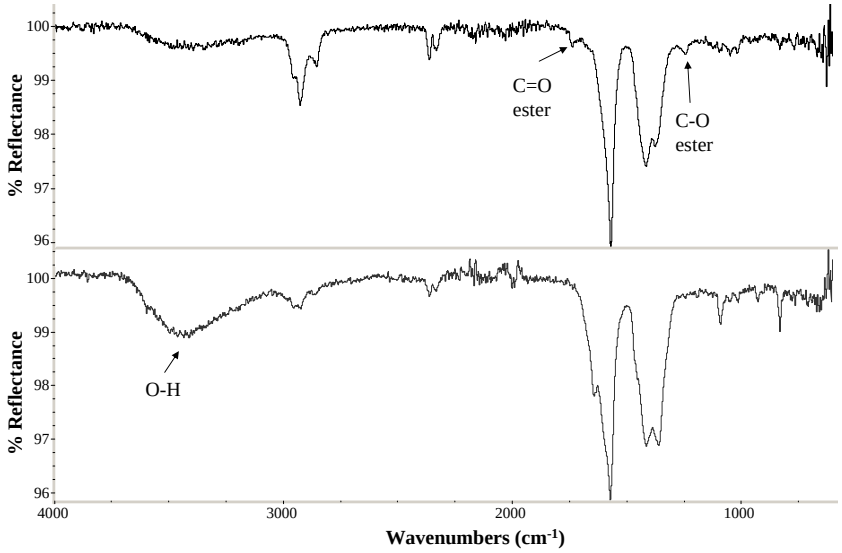

Figure 5.5: FTIR spectra of the polar 2 fraction of the hydrate extracts of one biodegraded oil, B2a (upper spectrum) and one non biodegraded oil, S2a (lower spectrum). (Figure 5, Paper III.)

of the components in the hydrate extracts are of intermediate molecular weights. Thus, like for the acid fractions, neither high-molecular compounds such as asphaltenes nor simple, low-molecular petroleum acids and bases are prominent in the adsorbed matter.

5.5 Searching for biosurfactants in freon hydrate extracts

Surfactin is used as a representative biosurfactant standard in the freon hydrate experiments. Surfactin elutes in the second polar fraction in both fractionation methods presented in Paper III and is identified by FTIR and GC-MS. The FTIR spectrum of surfactin is compared to spectra found in the literature [97, 101]. The GC-MS analysis is performed after hydrolysis and derivatisation of the surfactin sample, and the GC-MS chromatograms

of surfactin are shown in Figure 5.6. In the aqueous phase three of the four amino acids present in surfactin are easily identified, and hydroxy fatty acids are found in the organic phase.

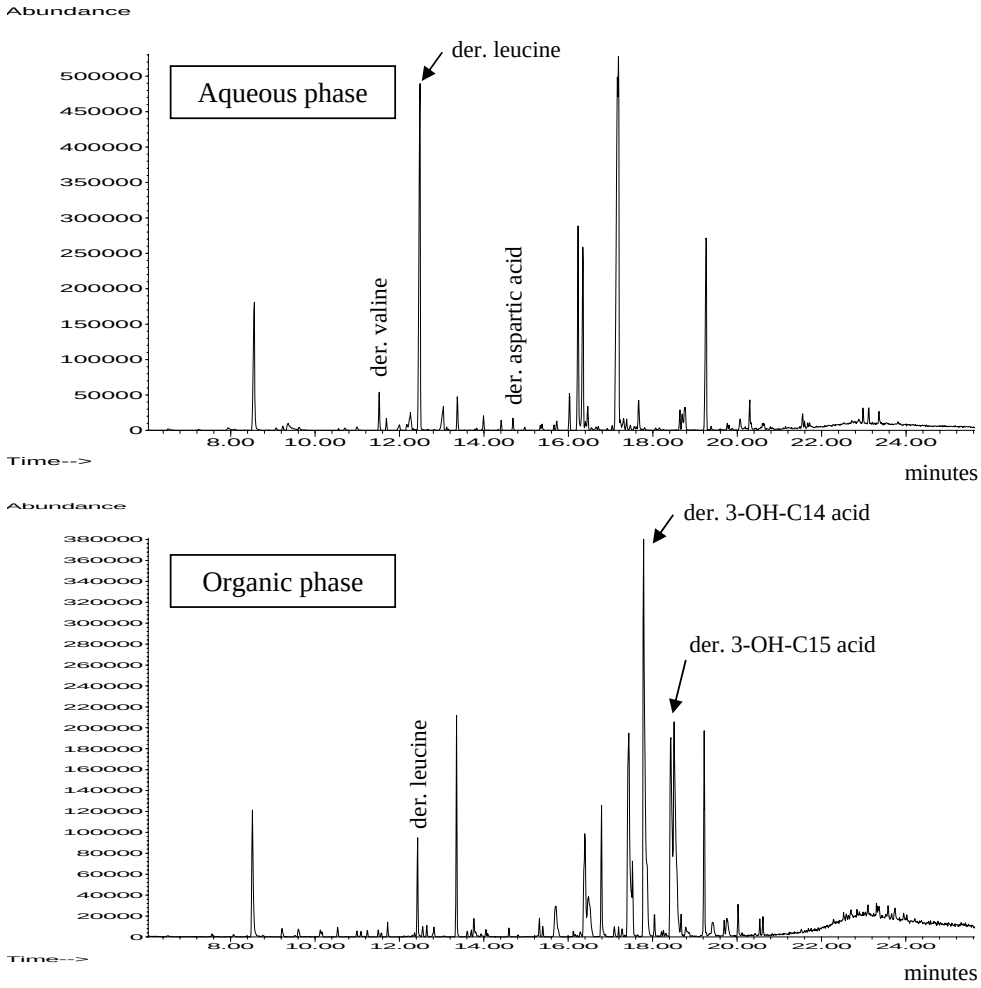

Figure 5.6: Total ion chromatograms of; upper chromatogram: the aqueous phase after hydrolysis of surfactin, and lower chromatogram: the organic phase after hydrolysis of surfactin. Both phases are derivatised with chloroformate. (Figure 10, Paper III.)

In order to validate the procedure, one crude oil is spiked with surfactin in a concentration of 1350 ppm prior to hydrate extraction. The hydrate extract is analysed in the same way as the other non-spiked hydrate extracts, and

surfactin could be identified.

The results indicate that units incorporated in biosurfactant structures of the lipopeptide type are recovered and identified in the applied procedure. However, neither amino acids or hydroxy fatty acids are found in the hydrate extracts. The results indicate that molecular structures of lipopeptide type are either a) not present in the hydrate extracts, b) present in concentrations below the detection limit of the GC-MS (i.e. less than 10 ppm, see Section 4.5), or c) not liberated by the applied procedure. However, oil degrading bacteria produce a number of different biosurfactants depending on type of bacteria and growth conditions, so biosurfactant compounds cannot yet be eliminated as the determining factor for hydrate particle wettability in oil/water/gas systems.

5.6 Adsorption of compounds onto ice surfaces

5.6.1 Analysis of ice extracts

The results from the ice extractions are shown in the Research Report which is included in this thesis. The amount of organic material adsorbed onto the ice surface is generally low when a good washing procedure for removal of excess oil is applied (<3.5 mg/g water).

One of the ice extract is analysed with HPLC, and chromatograms from using an ELS detector and a UV detector are shown in Figure 5.7. The HPLC chromatograms show that the ice extract contains significant amount of non-polar compounds, which most likely are co-extracted hydrocarbons. Peaks are also found in the areas corresponding to saturated carboxylic acids and polyfunctional compounds, but these peaks have low intensities.

GPC is used to find the molecular weight range of one of the ice extracts.

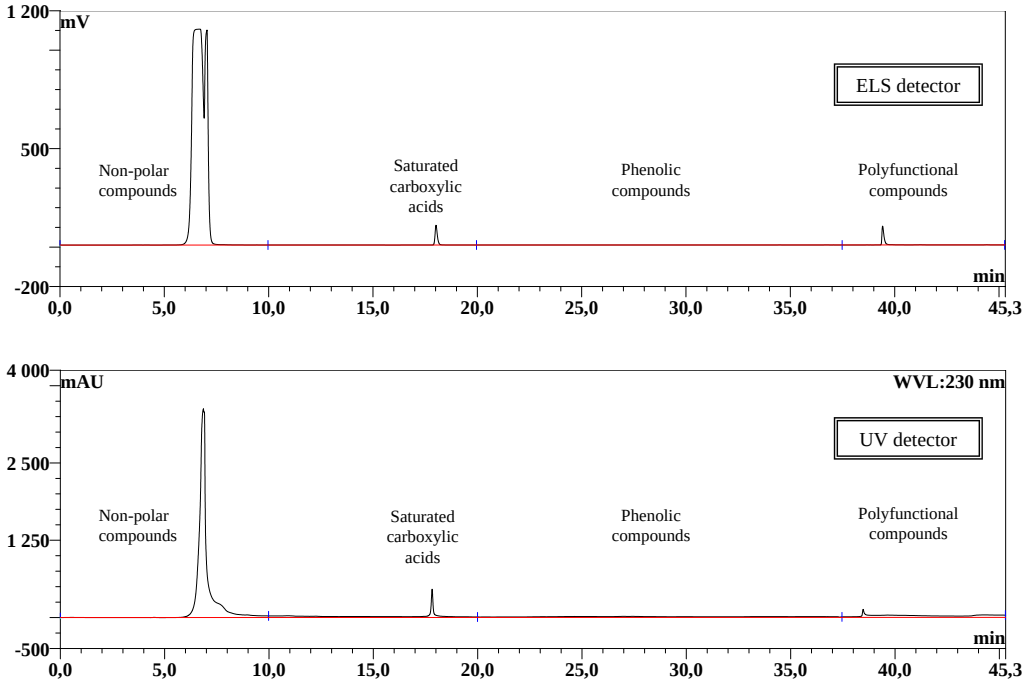

Figure 5.7: HPLC chromatograms of the ice extract of B4c. Top chromatogram: ELS detector, lower chromatogram: UV detector. (Figure 5, Research Report.)

The molecular weight calculated from the maximum intensity of the GPC-chromatogram peak is found to be approximately 480 g/mole. This result shows that the molecular weight of the ice extract is similar to the molecular weight of the freon hydrate extract and acid fraction (ion exchange method) for the same oil (approximately 500 g/mole) reported in Paper III and V. GPC analyses of HPLC fractions of an ice extract show that most of the compounds in the ice extract fractions have molecular weights between 420 and 450 g/mole, and there are some peaks correlating to higher molecular weights. These results show that the ice extract mostly contain compounds of intermediate molecular weights, like the acid fractions and the freon hydrate extracts.

5.6.2 Comparison of ice extractions with other extraction methods

HPLC analysis using an ELS detector is used to compare the relative composition of an ice extract, a freon hydrate extract and an acid extract of the same oil (B4c), see Figure 5.8. In the comparison of the different extracts the first fraction from the chromatogram is disregarded. The first fraction is presumed to be of less importance because it most probably consists of co-extracted hydrocarbons. The estimated amounts of material found in the second, third and fourth fractions are calculated as a relative amount of the sum of these three fractions.

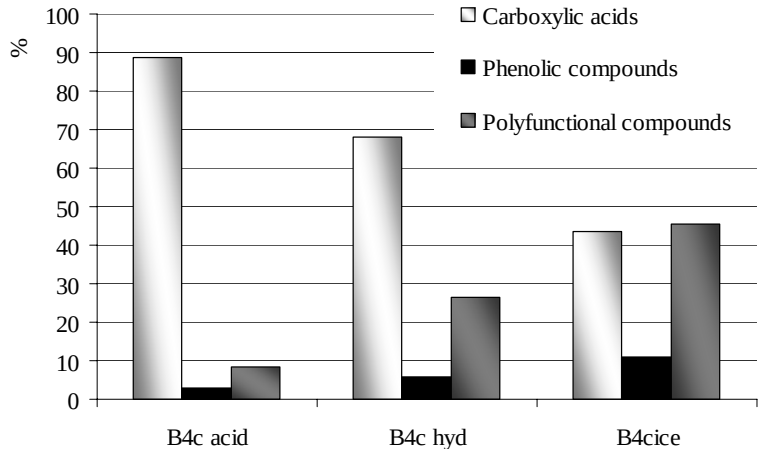

Figure 5.8: Comparison of three different extraction procedures using HPLC results. The extracts are labelled; acid: acid fraction, hyd: freon hydrate extract, ice: ice extract. (Figure 7, Research Report.)

The histogram in Figure 5.8 shows that the ice extract contains a relatively smaller amount of saturated carboxylic acids than the acid fraction and the freon hydrate extract, and a relatively larger amount of phenolic compounds and polyfunctional compounds. The polyfunctional compounds probably contain the most surface active components of the sample, and is

most interesting regarding natural plug inhibiting components in crude oil. The relatively larger amount of polyfunctional compounds in the ice extract compared to the freon hydrate extract and the acid fraction indicates that the relative composition of the ice extract might be significant. However, in these results, the non-polar fraction is not considered at all, and the non-polar fraction that probably contains co-extracted hydrocarbons, is very large in the ice extract. Thus, even if the relative composition of the ice extracts might be interesting, the amount of oil necessary to extract active components from the ice extract is too large for this method to be considered to be effective.

Extraction of surface active components from crude oil by the use of ice does not seem to be as effective as using freon hydrates. One reason for the low yields of extracted material might be that ice surface is not a good model for hydrates, resulting in surface active components not adsorbing onto the ice surface to the same degree or by the same mechanisms. Another possible explanation for the lower yields of material from the ice extractions can be that the ice crystals are prepared before they are mixed with the oil, and the crude oil components can only be adsorbed onto the outside of the crystals. In the freon hydrate extractions, on the other hand, the hydrates grow inside the solution containing the crude oil and inclusion of surface active material during growth is possible.

From the results it is difficult to decide whether ice is a good model for natural gas hydrates, or not. It does not seem like ice is an effective surface for extraction, but it is possible that the extraction method can be further developed and improved. Based on our experiments, ice is in practice not a good model for hydrate surfaces, due to very low amount of organic material found in the extracts.

5.7 Correlation of extract to crude oil composition

Crude oil compositional properties, such as asphaltene content, total acid number (TAN), total base number (TBN), density, level of biodegradation and wettability of hydrate particles are correlated to the amounts of acids extracted, the amounts of organic material found in the different fractions from HPLC of acid fractions (total amount and relative amount), the amounts of hydrate extract as a whole, and the amounts after fractionating it into the polar fractions. These correlations are described in more detail in Paper III and V.

For the acid fractions (Paper V), a strong negative correlation between the relative amount of material found in fraction F_C using the ELS detector and the wettability of hydrates formed in the crude oil is found (R = -0.94). The negative correlation means that high amounts of phenolic compounds (fraction F_C) will give lower values for the wettability, which again indicate water wet hydrates and generation of hydrate plugs. Based on the correlation one cannot say whether this is a cause-effect relationship, or whether the absence of compounds eluting in the phenolic fraction is indicative of some other compositional factor that determines the wettability. The UV spectra from HPLC of ion exchange acid fractions show that crude oils with high tendency to form hydrate plugs probably contain components that are not present in measurable concentrations in crude oils with low plugging potentials. This strengthens the idea that the relative amount of material in fraction F_C is important for the wettability of the crude oils. However the system is very complex, and this correlation can be indicative of some other compositional factor that determines the wettability. Thus, at the moment

no clear conclusions can be reached.

The results from the hydrate extractions (Paper III) show that the amount of hydrate extract and polar fractions have a poor correlation with most of the crude oil compositional properties, which supports that the hydrate extract is different from the fractions obtained by other fractionation schemes (asphaltenes, petroleum acids, etc.). This supports the premise that the hydrate extract consists of compounds with special affinity to the hydrate surfaces. Density of the oil has the strongest correlation to the amount recovered in the polar 1 fraction with R = 0.84, so this fraction may represent a certain part of the heavier oil components. The biodegradation level has the highest correlation with the polar 2 fraction yields, which thus may be linked to biodegradation products. The amount of material extracted onto the hydrates do not correlate with the wettability of the corresponding crude oils. Biodegraded crude oils that generate water wet hydrates, e.g. B1a and B3a, give similar yields of adsorbed compounds as the crude oils generating oil wet hydrates (B2b and B4c). Hence, it is likely that the type and structure of the adsorbing material is more important for hydrate wettability, and thus the hydrate plugging tendency, than the amounts of material adsorbed. This observation corresponds well with the conclusions in a previous work by Høiland et al. [110], where petroleum acid types and structures were found more important to wettability alteration of silica surfaces than the acid concentrations in the oils. For the two oils that form dispersed, oil wet freon hydrates, B2b and B4c, FTIR analysis of the hydrate extracts shows that they deviate from the rest of the data set with respect to their internal relation between functional groups. This supports the idea that the type of compounds present may be more important than the amount. However, more complex relationships between the solvent properties and polar

compound content for each crude oil can also be envisioned.

5.8 LC-MS analysis of extracts

The MS detector was first tuned for the rhamnolipid standard to make the instrumental settings optimal for searching for this type of biosurfactant in the samples. Negative ionisation, using Electron Spray Ionisation (ESI) source, was found to be best for the rhamnolipid standard, and rhamnolipids consisting of two sugar units and two hydroxy fatty acids, one sugar unit and two acids and two sugars and one acid were identified using reference data from two articles by Deziel et al. [131, 132].

The samples were difficult to ionise by ESI, and left a sticky layer in the ionisation chamber of components that were not ionised. Samples (HPLC fractions from an acid fraction) were run by APCI (Atmospheric Pressure Chemical Ionisation) and direct infusion (an MS tuning previously used by Dr. Helen Talbot at the University of Newcastle for hopanoid samples was applied [133]). Some peaks were found, but they could not be identified. However, the results indicated that compounds with either several acid groups, or hydroxy groups were present. Running a sample through a C18 column prior to MS analysis gave no other peaks than contamination from plastics, and again a lot of sticky material was found in the ionisation chamber, indicating that the samples were not properly ionised.

Surfactin was found both from using the ESI and the APCI ion sources, with help from articles by Vater et al. [53], Hue et al. [134] and Leenders et al. [135]. The APCI ion source was chosen for the analysis of the samples. Samples were separated on a C18 column with a gradient profile of the solvents water, methanol and acetone. Some peaks that in principle should

be possible to interpret were found in the chromatograms, but no structures have been identified. The identification of compounds from the Ion Trap MS is very difficult and time consuming, especially when we do not know much about the compounds we are searching for. Identification of standards like rhamnolipid and surfactin is possible, but compounds in complicated samples, like crude oil fractions, are much more difficult. Further work is needed to provide clear results.

5.9 Summary of main results

19 crude oils have been investigated to learn more about the presence of natural hydrate plug inhibiting components. These crude oils have been thoroughly characterised with respect to chemical composition and properties, e.g. acid and base content, asphaltene content, biodegradation level and the wettability of the freon hydrates that are generated by each crude oil. Due to the complexity of crude oil, structural analysis is very complicated, and fractions have been extracted to ease the characterisation by working with a fraction containing fewer components than the whole crude oil. In the search for natural plug inhibiting components, fractions containing surface active compounds have been extracted; acid fractions, freon hydrate extracts and ice extracts. One acid fraction has shown plug inhibiting properties, but the concentration of the fraction, the crude oil which the acids are extracted from and the solvent effect of the bulk crude oil, are important factors. Probably only a part of the acid fraction is active, and the amount of this specific compound group is most likely different in various crude oils. Alternatively, a combination of several compound groups can be important.

Fractionation of the acids is needed to simplify the detailed analysis. A

new HPLC method using a cyano column gives reproducible fractionation of acid fractions into four distinct sub-fractions; non-polar compounds, saturated carboxylic acids, phenolic compounds and polyfunctional compounds.

Freon hydrate extracts contain a specific fraction of the crude oil with affinity for the freon hydrate surface. A difference between biodegraded and non-biodegraded oils is observed, in a general higher amount of material in biodegraded oils. However, the amount of material cannot differentiate between plugging and non-plugging crude oils. A method for identification of the biosurfactant surfactin has been tested on the freon hydrate extracts, and surfactin cannot be found in the hydrate extracts.

Ice surfaces do not seem to be a good model for hydrate surfaces, because very low amount of material is extracted, and an ice extract contains a lot of co-extracted crude oil.

In general the analysis of the extracts from crude oil investigated in this thesis show that the extracts primarily contain components of intermediate molecular weights. This means that neither high-molecular compounds such as asphaltenes, nor simple low-molecular petroleum acids and bases are present to a large extent. FTIR analysis can to some degree differentiate between freon hydrate extracts from plugging and non-plugging crude oils. However, this analysis technique does not give any molecular structural information. GC-MS is not suited for the extracts used in this thesis due to high molecular weights and low volatility. LC-MS analysis with Ion Trap MS has been tested at the University of Newcastle, but was not optimal for our samples.

Chapter 6

Concluding remarks and further work

The work in this thesis have shown that the task of extraction and characterisation of components in crude oil with affinity for hydrate surfaces is more complicated than originally expected. Biodegradation has shown to be a necessary, but not sufficient, condition for formation of oil wet hydrate surfaces. For one oil, the acid fraction has shown to contain components that change the wetting properties of hydrate surfaces. However, analyses of both acid fractions and hydrate extracts have not yet been able to identify specific molecules or structures. This means that these structures either are present in very low concentrations, like biosurfactants, or that the wetting properties are dependent on interactions between several fractions, like acids and bases or acid and bulk crude oil (the bulk crude oil is then functioning as a solvent for the acid).

General hypothesis based on the results:

- Crude oil acid fractions contain natural inhibiting components that

prevent gas hydrate agglomeration.

- Biodegradation is necessary but not sufficient for the presence of these compounds.
- Acid fraction composition is critical for the anti-agglomerant effect.
- Polyfunctional compounds have the strongest interaction with hydrate surfaces

Further work with biosurfactants is of special interest, as well as further developments of methods for SPE fractionation and evaluation of wetting properties. The wetting properties of sub-fractions from acid fractions can be tested as performed on whole acid fractions (Paper II) in order to find which part of the acid fraction is active in making an oil wetted system. Eventually these tests can show if a combination of different parts of the acid fraction is necessary for inhibition of freon hydrate systems. The number of samples analysed is still quite limited, and the continuing mapping of new samples when they become available will be of great value for confirming or rejecting the hypothesis presented in this work.

So far, molecular identification of inhibiting structures has not been achieved. LC-MS (Time of Flight MS) will soon be available at the Department of Chemistry, University of Bergen, and this will hopefully give more molecular identifications of compounds in our samples. A high pressure cell is also being installed at the University of Bergen. Formation of natural gas hydrates, and adsorption of crude oil components with affinity for the hydrates might be possible to achieve. An interdisciplinary cooperation between organic petroleum chemistry and physical petroleum chemistry will be very useful in developments of new methods for characterisation of hydrate inhibiting systems.

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