Research Report

Extraction of crude oil components with affinity for ice surfaces.

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Extraction of crude oil components with affinity for ice surfaces

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Summary

Some crude oils are believed to contain natural inhibiting components that can prevent hydrate plugging of oil pipelines in petroleum production. In this work, several extraction methods are tested to extract crude oil components with affinity for ice surfaces to see whether ice can be used as a model for natural gas hydrates. By allowing oil to adsorb onto ice crystals, we obtain ice extracts that are further analysed by HPLC, GPC and GC-MS. The ice extracts are found to contain high amounts of non-polar compounds, but when the non-polar compounds are disregarded, the ice extracts contain a relatively larger amount of polyfunctional compounds compared to other types of extracts from crude oil, such as acid fractions. However, the amount of organic material adsorbed onto the ice surface is too low for further sample preparations, and therefore inadequate for detailed molecular analysis of ice-adsorbing compounds. By comparing the extraction yields with other methods in which hydrate particles were used as adsorption material, it seems that the ice extracts contain very little adsorbed polar material. Thus, the ice surface does not seem to be a good model for natural gas hydrate surfaces.

Contents

1 Introduction

Gas hydrates consist of gas molecules that are trapped in a framework of water molecules [1]. These hydrate structures can grow into larger hydrate clusters that may agglomerate into plugs. The hydrates have similarities with ice, but depending on the pressure they are stable at higher temperatures. The temperature and pressure conditions inside pipelines in the petroleum industry can often be within the region where hydrates form. The formation of hydrate plugs in petroleum pipelines and installation can create problems leading to shut-downs and economic loss, and the oil companies use large amount of inhibitors to prevent the hydrate formation. However, some crude oils have shown to create no problems even when operating within the hydrate stable region, while other crude oils have a strong tendency to form large plugs. Several authors have suggested that the plugging tendency of the crude oil is dependent on the presence or absence of natural inhibiting components (NICs) [2, 3, 4, 5]. One likely mechanism would be adsorption of specific compound types onto the hydrate surface, preventing small hydrate particles from agglomerating into larger plugs. Analysis of non-plugging and plugging crude oils, with focus on finding components with specific affinity for the hydrate surface, is thus important for obtaining more information about the hydrate morphology in different crude oil systems.

Crude oil is a very complex mixture, and the natural inhibiting components are probably present in a low concentration. Thus, we need to extract a fraction of the crude oil containing the active components. Acid fractions extracted from crude oils have been studied in order to investigate if this fraction can be active as plug inhibitor, and in some cases have been found to have this effect [6, 7]. Due to the pressure and temperature conditions, it is difficult to make natural gas hydrates for adsorption studies in the laboratory. Therefore we use models for the natural gas hydrates. Freon (trichlorofluoromethane) makes Structure II hydrates, the same hydrate structure as petroleum associated natural gas, and the freon hydrates can be formed at 0°C at 1 bar [8]. Freon hydrates have previously been used as a model for natural gas hydrates, and the results from these test are presented in a paper by Borgund et al. [9].

In this work, ice is tested as a model for natural gas hydrates for extracting surface active components from crude oil. Tiny ice crystals are made to obtain a large surface area of active sites for adsorption of crude oil components.

2 Experimental

2.1 Crude oil samples

Two crude oils were used in the experiments. B4c is a biodegraded and non-plugging oil, and S7b is a non-biodegraded and plugging oil. They both originate from the Norwegian continental shelf and were supplied by Norsk Hydro ASA. More information on the oils can be found in earlier work by Borgund et al [7, 9] and Barth et al. [10].

2.2 Making the ice extract

Several methods have been tested to obtain very small ice crystals, and a method involving spraying water into liquid nitrogen was found to provide the best material. In this procedure a spray flask is used to spray water into a bath of liquid nitrogen situated inside a climate cabinet at approximately -4°C, see picture in Figure 1. The small water droplets are immediately converted into ice crystals, and the crystals are crushed in a mortar before the liquid nitrogen evaporates. Very small ice crystals, like snow, are formed.

The ice crystals are added to a pre-cooled crude oil for extraction inside the climate cabinet at approximately -4°C. In the extraction, crude oil components are adsorbed to the ice-surface, and the remainder of the crude oil is rinsed off with a solvent. Three different extraction methods are tested, and they are presented in Section 2.2.1. Experimental variables like solvent types and contact time are also tested, and are presented in Section 2.2.2.

The mixture of adsorbed oil and ice is then left to decompose at room temperature, see Figure 2, and it separates into a water phase at the bottom and an organic phase at the top.

2.2.1 Extraction procedures

Stirring of ice and crude oil

A mixture of crude oil and ice is stirred for one hour, before the crude oil is filtered off. The ice is then washed with cold toluene, and left to decompose at room temperature.

Figure 1: *Tiny ice crystals are made by spraying water into liquid nitrogen.*

Figure 2: *The ice extract is left to melt at room temperature.*

Mixing in a rotating wheel

Mixtures of crude oil and ice are sealed in sample vials, and they are run in a rotating wheel for three days, see Figure 3. The crude oil is filtered off, and

the ice is washed with cold toluene, cyclopentane or isooctane. The ice with the adsorbed components is then left to decompose at room temperature.

Figure 3: *The oil samples were run in a rotating wheel for three days.*

Column packed with ice

Oil is passed through a column packed with ice, see Figure 4 to the left. Excess oil is rinsed off with cold toluene (or cyclopentane) until the solvent is colourless (see Figure 4), and the ice is then transferred to a beaker for melting at room temperature.

2.2.2 Experimental variables

Solvent types

Toluene is a very strong solvent for oil components, and there is a possibility that it can wash away components that are adsorbed onto the ice surface. Three different solvents were tested in the experiments using the rotating wheel; toluene, cyclopentane and isooctane. Cyclopentane and isooctane are not as strong as toluene, and components that are adsorbed to the ice surface are not assumed to be washed away when these weaker solvents are used. Thus, if toluene does not wash away more than cyclopentane or isooctane, we can assume that toluene is an appropriate solvent for these experiments. The amounts of material in the organic phases after ice extraction using different solvents for washing off excess oil, are therefore compared to see whether the yield from using toluene is lower than the other solvents. In previous studies [9], where freon hydrates were used as adsorption material, toluene was used to wash away the excess oil. By using toluene as the solvent, it is easier to make a comparison between the ice extractions and the freon hydrate

Figure 4: *Ice extraction by the use of a column packed with ice. Left: Ice and oil in the column. Middle: The oil has passed through the ice. Right: The ice is washed with toluene to remove excess oil.*

extractions. The solvents toluene and cyclopentane are also compared in the experiments where oil is run through a column packed with ice.

Contact time

Different contact times have been tested. In the previous freon hydrate experiments [9], a contact time of one hour was used. The crude oil and ice were therefore also stirred for one hour. In the experiments using the rotating wheel, the rotation is quite slow, and the contact time is set to three days. Extending the contact time, gives a possibility for the components that immediately adsorb onto the ice surface, to be exchanged by other components. Equilibrium condition can be established, where the components in the ice extract are an average of the components in the crude oil. In the experiments where oil is run through a column packed with ice, the whole amount of oil uses approximately one hour to pass through the column.

2.3 Analysis of the extracted sample

2.3.1 Quantification

After the ice has melted, a small amount of diethyl ether was added, the organic phase is separated from the water phase, dried with anhydrous Na_2SO_4

and quantified for content of non-volatile constituents on a Cahn electrobalance (range 0.0001-2 mg). In the quantification procedure 5 or 10 μl of the solution is placed on a weighing pan and the solvent is evaporated for 20 minutes. The amount of non-volatile organic material as a fraction of the aqueous phase (i.e. the amount of ice added to the crude oil) is then calculated.

2.3.2 Column fractionation

Some of the ice extracts are fractionated on silica columns (commercially available Silica 1cc columns from Waters Sep-Pak) into four fractions. The volume of different solvents used for the fractionation is showed in Table A.1 in Appendix A. To concentrate the samples for further analysis, the fractions are evaporated under a gentle N_2-flow. In order to have a specific solvent composition for the fractions, they are evaporated to dryness and redissolved in a small amount of the respective eluent used in the fractionation. The amounts of organic material in the fractions are then quantified by the Cahn electrobalance (see Section 2.3.1).

2.3.3 FT-IR analysis

Infrared spectroscopic analysis show the functional groups present in the organic samples. The FT-IR analysis is performed on a Nicolet Protege 460 FT-IR spectrometer with a Diamond Attenuated Total Reflection (ATR) - Dura sampler cell (from SensIR). A small amount of sample (one droplet) is placed on the ATR diamond, and the solvent is evaporated before the spectra are recorded. The spectra are recorded in the frequency range between 600 to 4000 cm^{-1}, using 32 scans and a resolution of 4 cm^{-1}.

2.3.4 HPLC analysis

HPLC analysis provides a chromatogram that shows a division of a sample into different compounds groups, where the components in the sample are separated according to their polarity. Due to the complexity of crude oil fractions, individually compounds cannot be detected. However, the HPLC chromatogram can be used to indicate the amounts of different compound types that are present.

The HPLC analyses are performed on a cyano analytical column with an Evaporative Light Scattering Detector (ELSD) and a UV detector (UVD). More information about the HPLC system can be found in Appendix B.1.

2.3.5 Gel Permeation Chromatography (GPC) analysis

GPC analysis gives the molecular weight distribution of a sample. The 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 Appendix B.2.

2.3.6 GC-MS analysis

GC-MS is used to obtain structural information about the ice extracts. The MS spectra obtained from these analysis, can be used to identify compounds. The large amount of compounds present in crude oil often make GC-MS analysis difficult, so an ice extract needs to be fractionated into smaller fractions before analysis. Polar and non-volatile samples can be difficult to analyse with GC-MS, and this problem is overcome by derivatisation procedures that make compounds less polar and more volatile. More information about the GC-MS system can be found in Appendix B.3.

2.3.7 Iatroscan analysis

Iatroscan analysis consists of thin-layer chromatography and a flame ionisation detection (TLC-FID), and a sample is divided into three compounds classes; saturated hydrocarbons (saturates), aromatic hydrocarbons (aromatics) and polar compounds (polar comp.). The procedure is standardised and described in the Norwegian Industry Guide to Organic Geochemical Analysis (NIGOGA) [11].

3 Results and discussion

3.1 Quantification of adsorbed oil

The amounts of organic material found from the different extraction procedures are shown in Table 1.

In the first tests crude oil and ice were mixed by stirring. Two parallel tests of the crude oils S7b and B4c were run, denoted by the date 3.12.04 in Table 1. The results showed large variations between the parallels, probably due to various amounts of co-extracted crude oil in the different samples. Hence, it was concluded that a better procedure was needed.

Table 1: *Amounts of organic material found by the different extraction procedures.*

Oil	**Date**	**Oil used (g)**	**Org. mat. (mg)**	**Water (g)**	**Org.mat./ water (mg/g)**	**Extr. time**	**Solvent**
S7b 1	3.12.04	10.6	1863.6	4.4	424.8	1 hour	a
S7b 2	3.12.04	10.6	732.1	4.7	157.0	1 hour	a
B4c 1	3.12.04	10.2	277.9	4.1	67.6	1 hour	a
B4c 2	3.12.04	10.2	37.0	3.1	11.9	1 hour	a
B4c 1	10.12.04	6.3	86.1	2.9	29.4	3 days	a
B4c 2	10.12.04	6.3	400.1	3.9	103.2	3 days	a
B4c 3	10.12.04	6.3	293.2	4.1	71.9	3 days	b
B4c 4	10.12.04	6.3	48.4	3.0	15.9	3 days	b
B4c 5	10.12.04	6.3	56.7	4.5	12.6	3 days	c
B4c 6	10.12.04	6.3	170.4	5.2	33.0	3 days	c
B4c 1	4.03.05	16.9	19.7	10.1	2.0	1 hour	a
B4c 2	4.03.05	17.0	8.8	9.0	1.0	1 hour	a
B4c 3	4.03.05	16.4	20.6	6.0	3.5	1 hour	a
B4c 4	4.03.05	17.0	1.9	3.4	0.6	1 hour	a
B4c	18.03.05	91.1	2.13	9.63	0.22	column	a
B4c	27.05.05	86.0	2.58	10.36	0.25	column	c
B4c	12.08.05	90.2	15.70	35.34	0.46	column	a
B4c	12.08.05	90.2	70.99	37.47	1.89	column	a

a: toluene
b: isooctane
c: cyclopentane

Ice extractions using a rotating wheel for stirring of samples, were performed on the oil B4c. The results are shown in Table 1, denoted by the date 10.12.04. Three different solvents were tested. Toluene is a very strong solvent, and it was suspected that some of the adsorbed components could be redissolved in the solvent phase. From the results in Table 1, toluene does not seem to wash away more components than the other solvent. Large variations in the amounts of organic material are still observed when comparing parallel samples, and the procedure had to be improved.

Extractions with one hour stirring of crude oil and ice was tested with

the oil B4c, and the results from these experiments are shown in Table 1, denoted by the date 4.03.05. In these experiments the ice phase was washed more thoroughly to remove excess oil after the oil was filtered off. Very small amounts of organic material were collected from these experiments compared to the experiments at the date 3.12.04 and 10.12.04 in Table 1.

Due to the small amounts of organic material in the ice-extracts and the large variations between parallel samples, an extraction procedure using a column packed with ice was tested. When a mixture of crude oil and ice is stirred in a beaker, problems with insufficient mixing due to high viscosity of the cold fluids may be encountered. Using a column packed with ice makes it possible to run crude oil through the ice crystals. In this procedure it is possible to use large amounts of oil and ice. By using a large amount of crude oil compared to the ice, we expect to have enough surface active compounds to cover the whole surface of the ice crystals. Thus, surface active compounds that easily adsorb to the ice surface, will occupy the sites at the surface instead of other components in the crude oil, and more selectively adsorption of the most surface active compounds will be achieved. When a column packed with ice is used for the extraction of crude oil components, it is also easier to wash away excess crude oil. This is done by flodding the solvent through the ice-packed column after the surface active crude oil components have been allowed to adsorb onto the ice surface.

The results from the experiments using a column packed with ice, are shown in Table 1 denoted by date 18.03.05 (toluene as the solvent), 27.08.05 (cyclopentane as the solvent) and 12.08.05 (toluene as the solvent). The amounts of material found in the ice extracts were very low, both when toluene and cyclopentane were used as the solvent, and the results show less variation than before. However, the two parallel samples from 12.08.05 give quite different amounts of organic material. This variation might be a result of temperature instability in the climate cabinet that occurred this day. The ice composition might have been changed due to different temperature conditions, and the surface area of the ice can have changed. Thus, the results from these experiments might not be representative, and there might be some uncertainties regarding these extracts (12.08.05).

Information from further analysis of the ice extracts are of interest for knowing more about the composition of the ice extracts. Several of the ice extracts from experiments using a column packed with ice for the extraction have been analysed, including the extracts from 12.08.05 that might be considered to be somewhat uncertain.

3.2 Column fractionation of ice extracts

The ice extracts from the date 10.12.04 in Table 1, were fractionated on silica columns to obtain more information about what type of components that are present. The fractionation results showed that most of the ice extracts consisted of non-polar material, which probably comes from co-extracted oil. The amount of more polar material found in the second and third fractions were considerable lower, but could still easily be detected. The most polar fraction, fraction four, contained very little organic material, and it was not quantified. Overall, the results from the fractionations did not show any systematic variations depending on which solvent was used for the filtration of excess oil in the ice extraction procedure. A histogram with the amount of organic material found in the three first fractions are shown in Figure A.1 in Appendix A.

Some of the residual oils (i.e. the excess oil washed off the ice with the solvents toluene, cyclopentane and isooctane) were fractionated to compare with the ice extracts. In these tests we wanted to see if the ice extracts are enriched in a specific part of the crude oil with affinity for the ice surface. The ice extracts did not contain more polar material than the corresponding residual oils, which indicates that the ice extraction does not isolate any specific surface active compounds. However, the mechanism of the adsorption onto the ice surface is still unknown, and we do not know if the extraction time of three days, that was used for these extractions, might result in an equilibrium condition. The oil components might substitute each other at the surface, and eventually the components adsorbed at the surface can be an average amount of the crude oil, and not the most surface active components.

3.3 Functional groups of compounds in the ice extracts, FT-IR results

Some of the fractions after the column fractionation of ice extracts described above, were analysed by FT-IR. Very little differences in the spectra were observed for the three solvents used in the filtration procedures of the ice extract. It was especially interesting to see that the ice extracts from using isooctane and cyclopentane did not contain any peaks that was not found in the ice extract using toluene as the solvent. Thus, these results confirm the indication that toluene is an appropriate solvent for washing the ice extracts. The FT-IR spectra are shown in Figure C.1 in Appendix C.

3.4 Results from HPLC analysis of ice extract

The ice extract of the oil B4c denoted by the date 18.03.05 in Table 1 was analysed with HPLC. Chromatograms from using the ELS detector and the UV detector are shown in Figure 5, and the difference between the two detectors can be seen. The ELS detector is universal, and most types of compounds can be detected. The only compounds that will not be detected are low-boiling compounds that evaporate together with the solvent, e.g. certain phenolic compounds. The UV detector can detect compounds that absorb radiation in the UV-range, and the phenolic compounds that are difficult to analyse with the ELS detector, can easily be detected by UV-absorption.

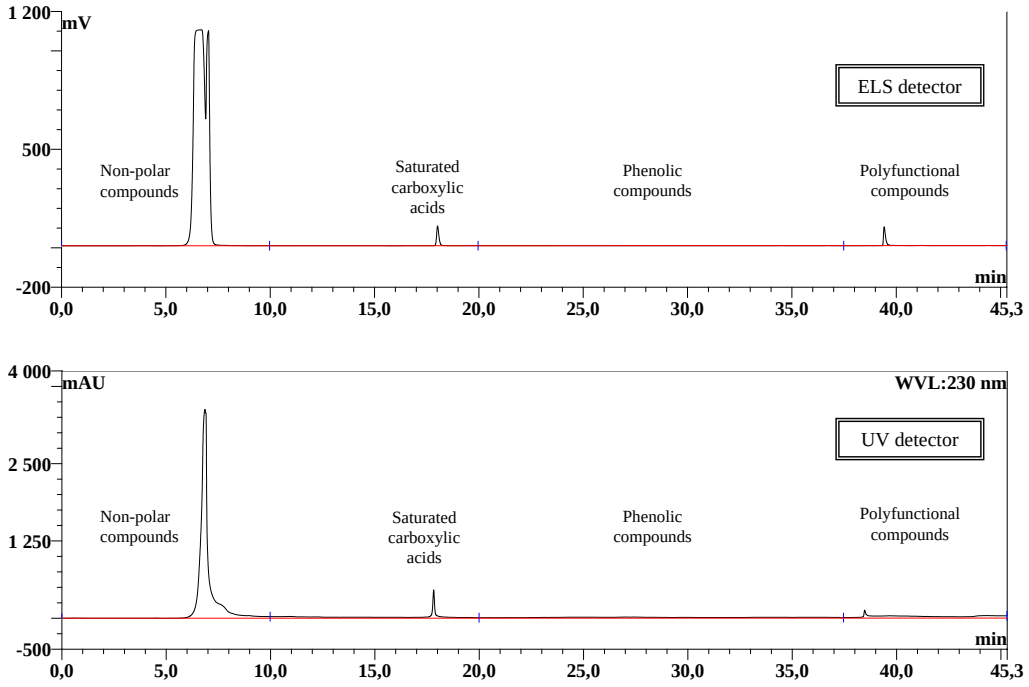

Figure 5: *HPLC chromatograms of the ice extract of B4c from 18.03.05. Top chromatogram: ELS detector, lower chromatogram: UV detector.*

The HPLC chromatograms in Figure 5 show that the ice extract contains significant amounts of non-polar compounds, which most likely are co-extracted hydrocarbons. Peaks are also found in the area corresponding to carboxylic acids and polyfunctional compounds, but these peaks have low intensities. No peaks are found in the area of the chromatogram corresponding to phenolic compounds.

3.5 Molecular weight of compounds in the ice extracts by GPC analysis

GPC analysis of whole extract

GPC was used to find the molecular weight range of the ice extract from the oil B4c denoted by the date 12.05.05 in Table 1 (parallel no 2). The molecular weight calculated from the maximum intensity of the GPC-chromatogram peak was 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 for the same oil (approximately 500 g/mole) found in Borgund et al. [9].

GPC analysis of HPLC fractions

The same ice extract as above (parallel no 2, 12.08.05) was run preparatively on HPLC, and five fractions were collected according to the fraction-scheme described in Appendix B.1. These five fractions were analysed by GPC. The GPC analysis of the HPLC-fractions showed that most of the compounds in the ice extract fractions have molecular weights between 420 and 450 g/mole, and we find some minor peaks correlating to larger molecular weights. These results show that the ice extract 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 ice extracts.

The GPC results both from the whole extract and their fractions are found in Appendix D.

3.6 Structural analysis of the ice extracts, GC-MS results

Fractions from HPLC analysis of an ice extract from B4c (no 2, 12.08.05 in Table 1) were also analysed by GC-MS. The first fraction probably contains co-extracted hydrocarbons, and it was analysed by GC-MS without derivatisation. A lot of alkanes and UCM (Unresolved Complex Mixture) were found in the chromatogram of the first fraction.

The second fraction probably contains carboxylic acids, and is therefore derivatised with chloroformates before analysis on GC-MS. The chromatogram showed that the sample contained hexadecanoic acid and octade-

canoic acid, and some peaks that could not be identified. In addition to the peaks from the sample, the chromatogram contained a lot of system peaks from the derivatisation procedure. Fraction 3, 4 and 5 have not been analysed.

Information about the GC-MS instrumentation is found in Appendix B.3, and the GC-MS results are found in Appendix E.

3.7 Comparison with other extraction methods

3.7.1 SARA analysis by Iatroscan

In order to assure that a specific fraction of the crude oil is extracted, one of the ice extracts (of the oil B4c denoted by the date 18.03.05 in Table 1) was analysed by Iatroscan (SARA analysis) and compared to a maltene fraction and a hydrate extract [9] of the B4c oil. The values from the SARA analysis are normalised with respect to the maltene fraction, and the results are shown in Figure 6.

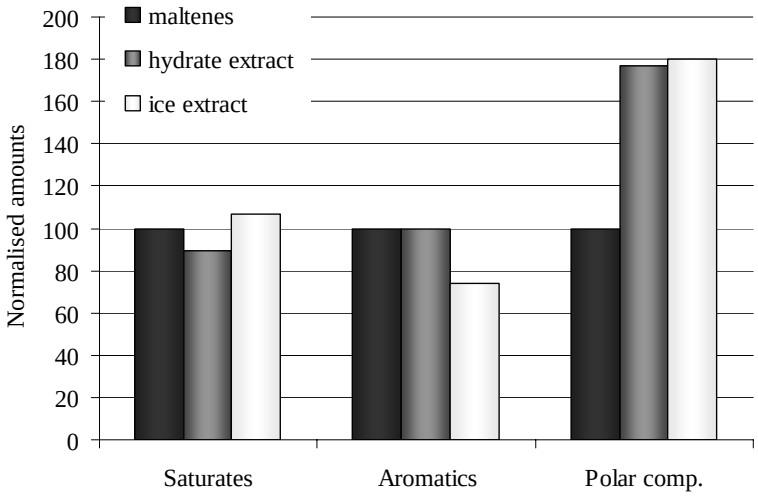

Figure 6: *Results from SARA analysis from maltene fraction (black), hydrate extract (grey) and ice extract (white) of the oil B4c. The values are normalised with respect to the maltene fraction.*

The first group of columns in Figure 6 represent the percent of saturated compounds, the group in the middle represent aromatic compounds and the last group represent the polar compounds. The amount of polar components is much larger in the hydrate and ice extracts compared to the maltene

fraction. Thus, the results show that the hydrate and the ice extracts are enriched in polar material compared to the maltene fraction of the same oil.

3.7.2 HPLC for comparison of different extraction methods

The ice extract analysed by HPLC using the ELS detector was compared to a freon hydrate extract [9] and an acid fraction [7] from the same oil. When we compare the HPLC results from the different extracts, the first and the fifth fractions from the chromatogram are disregarded. These fractions are presumed to be of less importance because the first fraction most probably consists of co-extracted hydrocarbons, and the fifth fraction is the time when the solvent-system is returning to its starting composition. The amounts of material found in the second, third and fourth fraction are calculated as a relative amount of the sum of these three fractions.

A comparison of the three extraction procedures; extraction of acids and extraction on freon hydrates and ice, is shown in Figure 7. The histogram shows that the ice extract contains a relatively smaller amount of carboxylic acids than the acid fraction and the freon hydrate extract, and a relatively larger amount of phenolic compounds and polyfunctional compounds.

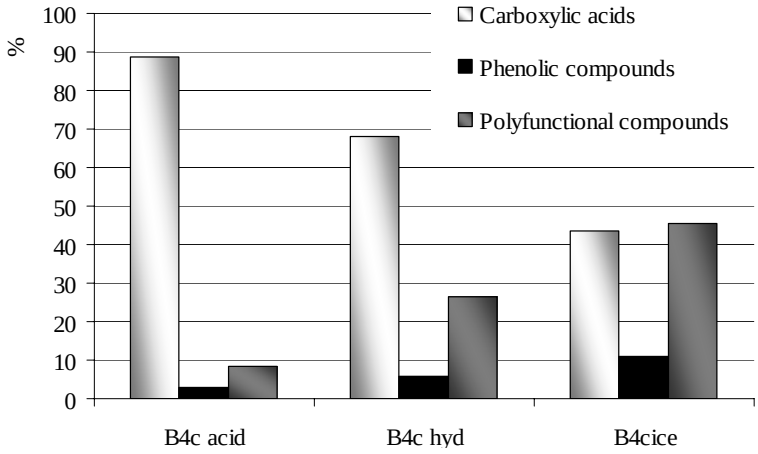

Figure 7: *Comparison of three different extraction procedures using HPLC results. The extracts are labelled; acid: acid fraction, hyd: freon hydrate extract, ice: ice extract.*

An acid fraction from the oil B4a, which is similar to the oil B4c, has previously shown to contain natural inhibiting components [6], but the activity of the acid fraction depends on the concentration. Thus, we know that

the acid fraction from B4a contains an active component. However, we do not know which part of the acid fraction is important. The natural inhibiting components in crude oil are most likely surface active components that are able to change the wettability of the hydrate surface. We are especially interested in the polyfunctional part of the HPLC chromatogram, because components found in that fraction probably are the most surface active.

From the comparison of the extraction methods in Figure 7 we can see that the relative amount of polyfunctional compounds is larger in the ice-extract compared to the freon hydrate extract and the acid fraction. This indicates that the relative composition of the ice extract might be important in the search for natural inhibiting components. However, in these results, the non-polar fraction is not considered at all, and the non-polar fraction, which 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.

The ice extraction procedure does not seem to be as effective as the freon hydrate extraction procedure in extracting active components from crude oil. The amount of organic material adsorbed onto the ice in the column experiments (< 2 mg/g water) is very low compared to freon hydrate extracts (approximately 71 mg/g water) [9]. One reason for the difference in the amount of organic material adsorbed to the surface might be the differences in the procedure regarding the crystallisation of the solid material. In the freon hydrate extract experiments, the hydrates grow inside the solution containing the crude oil, whereas for the ice extraction, the ice crystals are prepared before they are mixed with the crude oil, and the crude oil components can be adsorbed onto the outside the ice crystals. Thus, for the freon hydrate extractions, inclusion of surface active material during growth can be possible, and a larger amount of extract can be obtained. Another reason for the difference in amount of organic material can be that ice is not a good model for hydrates. This can result in the surface active components not to adsorb onto the ice.

4 Concluding remarks

Several methods have been tested to extract components from crude oil with affinity for the ice surface. The best method of the ones tested seems to be extraction in a column filled with ice. However, the amount of material found

in the ice extracts is very low, and the major part of the extract consists of co-extracted crude oil. Since the total amount of ice extract is very low and it contains large amounts of co-extracted hydrocarbons, the amount of actually adsorbed material is very small. Disregarding the co-extracted hydrocarbons, the relative amount of polyfunctional compounds in the ice extract is large compared to the freon hydrate extract and acid fraction from crude oil. However, the amount of polar organic material extracted during the ice extraction is simply too small for the method to be effective in the search for natural hydrate plug inhibiting components in crude oil.

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 could be improved. Based on our experiments, ice is not a good model for hydrate surfaces, due to very low amounts of organic material found in the extracts.

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Appendix

A Column fractionation of ice extracts

Table A.1: *Volume of different solvents for fractionation on a silica column.*

Fr.	Solvents (v/v)	Volume of solv., ml
1	hexane:DCM 90:10	6
2	DCM	26
3	DCM:MeOH 93:7	12
4	MeOH:DCM 70:30 +	ca. 2
	MeOH:formic acid 95:5	ca. 10

DCM: dichloromethane
MeOH: methanol

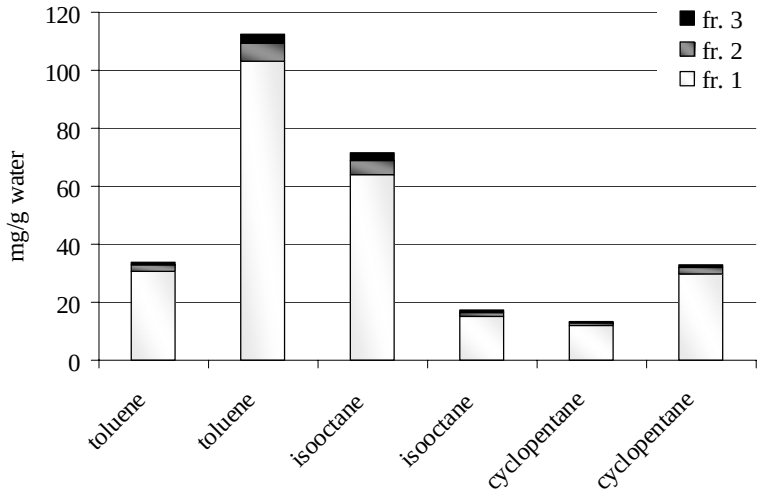

Figure A.1: *The amount of organic material in the different fractions after fractionation of ice extracts (ice extraction of B4c at the date 10.12.04).*

B Chromatographic methods

B.1 HPLC system

The HPLC analyses are performed on a cyano analytical column (BDS Hypersil Cyano column (4.6 mm * 25 cm, particle size 5 μm) Thermo Electron Corporation) with a guard column (BDS Hypersil Cyano, 10*4 mm, 5 μm, Thermo Electron Corporation). A Dionex P680 HPLC Pump and a Rheodyne 7725 manual injector with a 20 μl loop are used. Two types of detectors are used; a light scattering detector (ELSD, Sedex 55 Light Scattering Detector, operation temperature: 40°C, Nebulizing gas: nitrogen) and a UV detector (UVD340U Dionex, diode-array detector). The same column and HPLC system was also used in another work by Borgund et al. [12], and more information can be found here. However, the present report has a slightly different gradient programme.

The solvents used are hexane, dichloromethane (DCM) and methanol (MeOH), with the following gradient programme (flow rate: 0.5 ml/min): 3 v% DCM in hexane held for 10 minutes, increased to 30 v% DCM in hexane in 15 minutes, increased to hexane:DCM:MeOH 40:55:5 (v/v/v) in 15 minutes, and the system is re-equilibrated with a change to 100% DCM in 5 minutes, changed to 3 v% DCM in hexane in 10 minutes, held for 10 minutes. The samples are dissolved in DCM:MeOH 93:7 (v/v) to a concentration of approximately 10 mg/ml.

The chromatogram is divided into five sections. Standards having a wide range of polarities and different functionalities are run to decide the type of compounds that can be found in the different sections. The first section, from 0 - 10 minutes, probably contains co-extracted oil, like hydrocarbons. In the second section, 10 - 20 minutes, carboxylic acids will be eluted. Phenolic compounds can be found in the third section, 20 - 35.5 minutes, the fourth section, 35.5 - 45.3 minutes, contains polyfunctional structures, and the fifth section, 45.3 - 65 minutes, is the section where the solvent composition is returned to its starting composition.

B.2 GPC system

The GPC analysis is performed using a column that is packed with polystyrene/ divinylbenzene co-polymer (PLgel 3 μm MiniMix-E 4,6 mm * 25 cm, from Polymer Laboratories), and the molecules are separated according to molecular size and shape. Distilled tetrahydrofuran (THF), stabilised with KOH

pellets, is used as the mobile phase and the flow rate through the column is 0.2 ml per minute. The detector is the ELS detector that is also used for the HPLC analyses. The molecular weights of the samples are calculated from a standard curve based on model compounds; nine polystyrene standards with molecular weights from 162 to 20000 g/mole and the standard squalane (422.8 g/mole). Samples and standards were dissolved in THF. The molecular weights are calculated from the retention times corresponding to the maximum intensity of the chromatographic peaks.

B.3 GC-MS system

The samples are analysed by GC-MS on a HP5890 Series Gas Chromatograph Plus, with one Flame Ionisation Detector (FID) and one Mass Sensitive Detector (MSD), HP5971, and a WCOT fused Silica, ultra 2, 25 m column with i.d 0.2 mm and film thickness 0.33 μm. The injection temperature is 250°C, the detector temperatures 300°C (FID) and 280°C (MSD), and the temperature program is: 60°C for 1 minute, 10°C/minute to 250°C, 20°C/minute to 300°C, held for 3 minutes, and 20°C/minute to 320°C, held for 10 minutes. Helium is used as a carrier gas and 1 μl of the prepared sample is injected.

Samples containing acidic compounds need to be derivatised before GC-MS analysis. A derivatisation using chloroformate as described by Liebich et al. [13] is used in this work. This derivatisation procedure is also used on freon hydrate extracts in the paper from Borgund et al. [9].

C FT-IR results

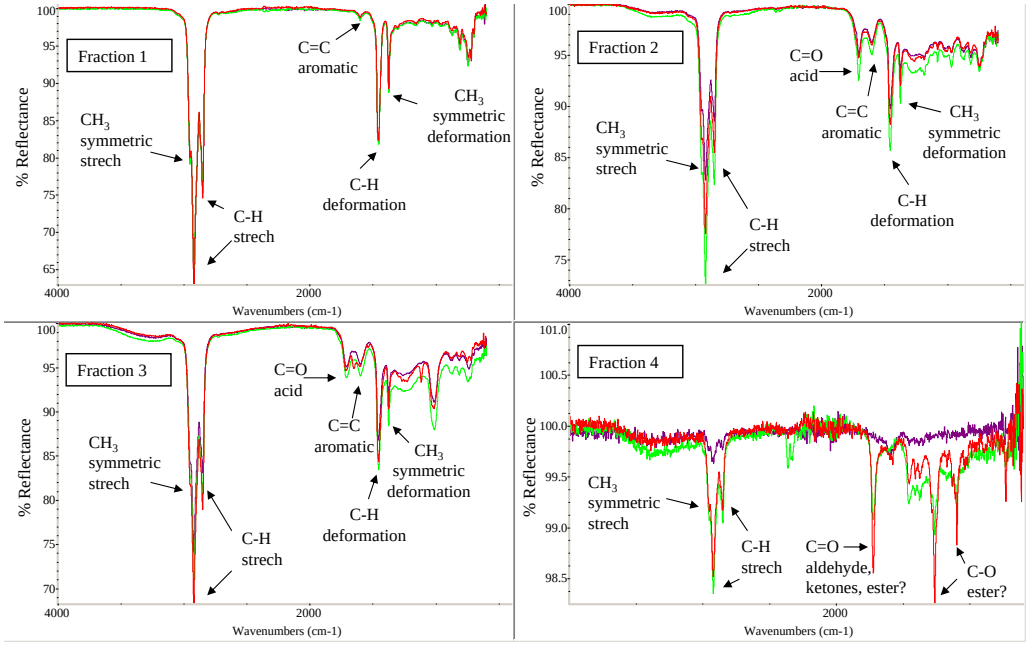

Figure C.1: *FT-IR spectra of the fractions after column fractionation of three ice extracts from the oil B4c, 10.12.04; red: toluene as the solvent, purple: isooctane as the solvent and green: cyclopentane as the solvent.*

The first fraction (non-polar) contains mostly absorption bands from C-H bonding at approximately 2850 - 2950 cm^{-1}, 1375 and 1455 cm^{-1}, and a small band from C=C bonding at approximately 1600 cm^{-1}. In the second and third fraction, bands from C=O at approximately 1705 (second fraction) and 1710 (third fraction) cm^{-1}, probably from acids, are found in addition to the bands found in the first fraction. The fourth fraction contains very small amounts of material. The FT-IR spectra show peaks at 1724 and 1263 cm^{-1} that can come from ester bonds. These peaks are found both when the solvents toluene and cyclopentane are used.

D GPC results

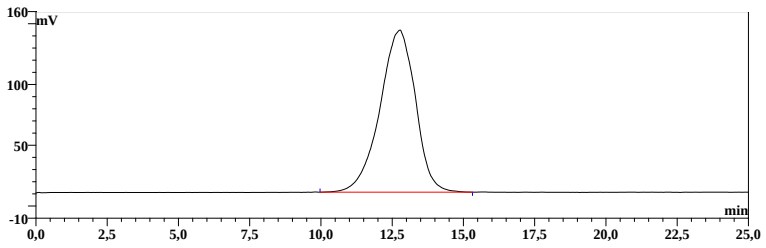

Figure D.1: *GPC chromatogram of an ice extract from B4c (no 2, 12.08.05).*

Table D.1: *Molecular weights of the HPLC fractions from an ice extract of B4c (no 2, 12.08.05). Some fractions had several peaks in the chromatogram, and the largest peak is written in bold.*

Sample	**Fr. 1** g/mole	**Fr. 2** g/mole	**Fr. 3** g/mole	**Fr. 4** g/mole	**Fr. 5** g/mole
B4c	**450**	1090	940	2440	780
(no 2 12.08.05)	-	**750**	**440**	1750	**430**
	-	-	-	1070	-
	-	-	-	**420**	-

The first fraction is the largest fraction in the ice extract, and it has a nice GPC chromatogram (Figure D.2, Fraction 1). The molecular weight from the maximum intensity of the peak in the chromatogram was calculated to approximately 450 g/mole. This is slightly lower than the molecular weight of the whole acid fraction (480 g/mole). The first fraction probably contains co-extracted hydrocarbons.

The other fractions from the HPLC fractionation had very low concentration, and the peaks from the GPC chromatograms of these fractions have very low intensity. When the concentration of a sample is very low it can be difficult to determine the molecular weight due to uneven shape of the peak. The low concentration can also make it difficult to separate peaks from background noise. The GPC chromatogram of the second fraction (in Figure D.2) has two peaks that is not baseline separated. The first peak, which has the highest intensity at the maximum of the peak, correspond to a molecular weight of approximately 1090 g/mole. The other peak has a larger peak area and corresponds to a molecular weight of 750 g/mole. The third fraction also has two peaks, where the largest peak correspond to a

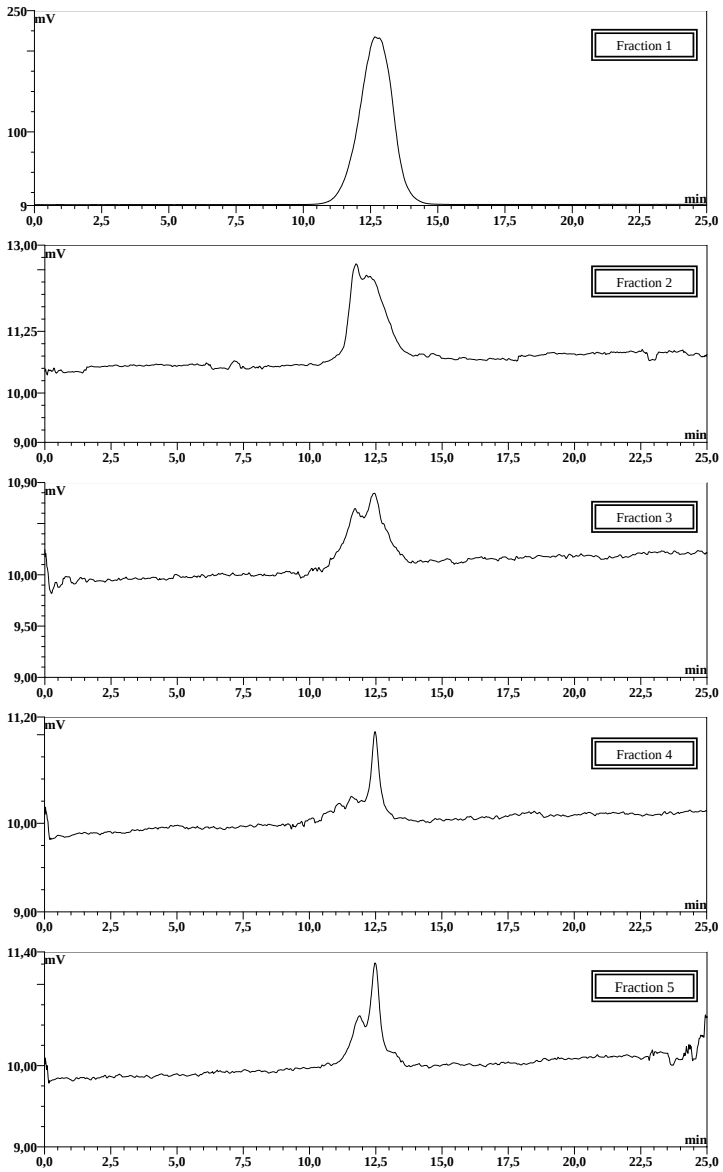

Figure D.2: *GPC chromatograms from HPLC fractions of an ice extract from B4c (no 2, 12.08.05).*

molecular weight of 440 g/mole. The fourth fraction contains one large peak and several smaller peaks in front of this one. The largest peak corresponds to a molecular weight of 420 g/mole. The chromatogram of the fifth fraction also has two peaks, and the largest peak corresponds to a molecular weight of 430 g/mole.

E GC-MS results

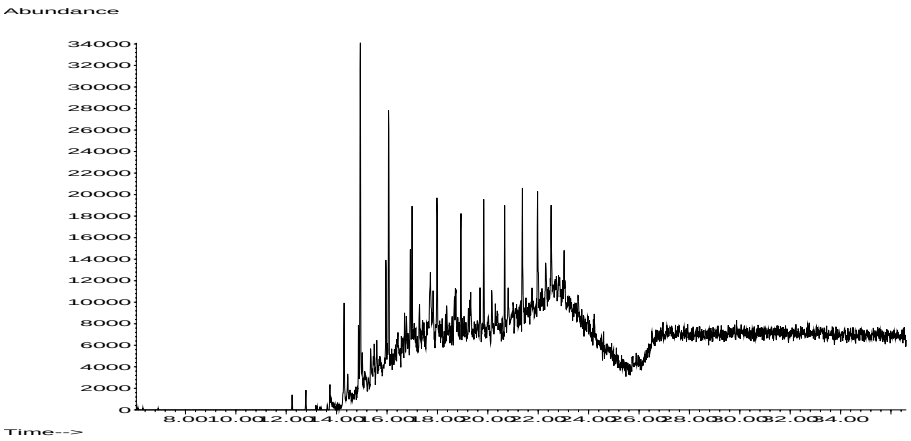

Figure E.1: *GC-MS chromatogram from HPLC fractions no 1 of an ice extract from B4c (no 2, 12.08.05).*

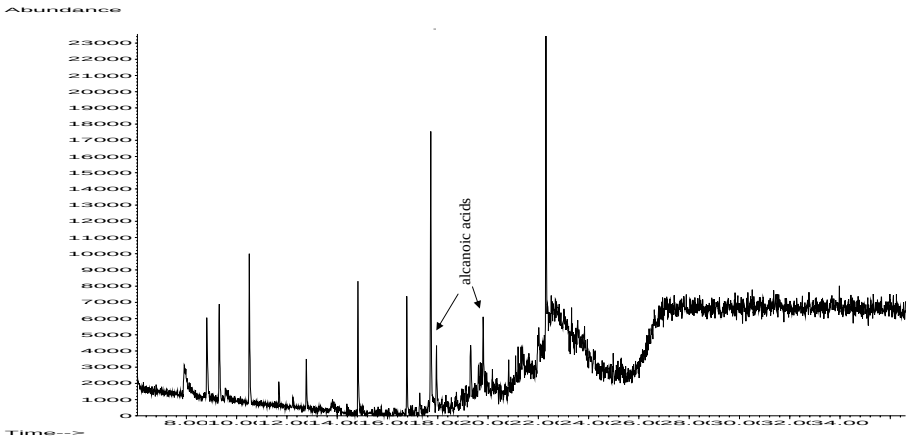

Figure E.2: *GC-MS chromatogram from HPLC fractions no 2 of an ice extract from B4c (no 2, 12.08.05).*