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THE HYDROCARBON CONTENT OF 'WATER-SOLUBLE' FRACTIONS OF CRUDE OIL

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ABSTRACT

'Water-soluble' fractions of fresh Kuwait crude oil, prepared by the slow-stirring method, have been examined by both fluorescence and infra-red spectroscopy. The factors governing the performance of the method and the composition of the water-extracts have been studied. A large proportion of the hydrocarbon content is present as particulate material and even the aromatic content is lowered by filtration below 1 µm. Care in the preparation and handling of such 'water-soluble' fractions is required and the concentrations of dilutions are seldom predictable. These factors must be considered when toxicity-tests are carried out using such materials.

INTRODUCTION

The modelling in the laboratory of oil concentrations below a slick at sea is technically very difficult. Maintaining the droplet size-distribution and homogeneity of suspensions of oil during laboratory toxicity tests is similarly difficult. The use of 'water-soluble' fractions of oil has therefore been widely adopted for laboratory investigations of the biological effects of low concentrations of oil. Several methods of producing such 'water-soluble' fractions or 'water-extracts' have been described but that most widely used is the slow-stirring method of Anderson et al. (1974). It is recognized that the total hydrocarbon concentrations in the saturated extracts prepared by this method far exceed the theoretical true solubilities of these compounds, and that they must exist in the extract in a finely-divided, colloidal or micellular form. Indeed, some authors prefer to call such extracts 'water-accommodated' fractions.

A normal toxicity test involves the exposure of organisms to a series of concentrations of the material under test. In the case of water-extracts of oil, the final hydrocarbon concentration and composition bear an inconstant relationship to decreasing ratios of oil:water used in their preparation. Hence to prepare an extract of concentration, it is customary to dilute a saturated extract of known concentration. But if the saturated extract contains undissolved material, are the hydrocarbon concentrations of its dilutions adequately predictable and their compositions constant?

Before embarking on a series of experiments to investigate the biological effects of 'water-soluble' fractions of certain crude oils, it was therefore considered necessary to establish how reproducible the slow-stirring method was, how the aromatic and aliphatic hydrocarbons were distributed in the saturated extracts and whether such extracts were reproducibly dilutable. Preliminary results from these experiments are presented here.

METHODS

Preparation methods

In each case the slow-stirring method of Anderson et al. (1974) was used with 0.5 l of fresh Kuwait crude oil and 4.5 l of Crouch estuary sea water (approximate salinity 33.5‰) which had been filtered through Whatman GFC papers (pore size approximately 1.2 µm) but not sterilized. The filtered sea water was poured into a glass aspirator and oil from a previously unopened bottle carefully layered on to the water surface. The aspirator neck was closed but not sealed and room temperature was maintained at 19.5° ± 1.5°C. A Teflon-coated magnetic stirring bar, 6.4 cm long was used and the oil vortex held at 25% of the water column depth. Water samples were withdrawn through the aspirator tap and filtered using either slightly reduced pressure (2 x 4.7 cm diameter Whatman GFC papers) or slightly increased pressure (Gelman 0.45 µm and Nuclepore 0.2 µm membranes, both of 4.7 cm diameter). Care was taken throughout to minimize losses due to evaporation or adsorption onto surfaces. All containers were of glass and rinsed with glass-distilled grade dichloromethane before use.

Analytical methods

Samples being analysed by both fluorescence spectroscopy (UVF) and infra-red spectrophotometry (IR) were shaken before splitting. The

procedure used for UVF analysis was based on that adopted for the IGOSS project (IOC/WMO, 1976), fluorescence emission being measured at 360 nm with excitation at 310 nm. Both emission (excitation 310 nm) and synchronous ($\Delta\lambda$ = 25 nm) spectra were recorded for each sample. IR analysis was similar to the method of Gruenfeld (1973), the absorbance of the sample being measured at 2 930 cm^{-1}, corresponding to the stretching frequency of C-H bands in aliphatic $-CH_2-$ groups. IR spectra were recorded for each sample between 3 500 and 2 500 cm^{-1}. In both cases quantification was based on the response of solutions of the fresh Kuwait crude oil used to prepare the 'water-soluble' fractions.

RESULTS

The first series of experiments was carried out to distinguish the factors governing the performance of the method, its reproducibility and the distribution of hydrocarbons in 'dissolved' or particulate form. Samples were analysed by UVF only. Any significant changes in the synchronous spectra are reported.

(i) The effect of aspirator dimensions

Three sizes of aspirator were tested, having approximate dimensions of:

Nominal capacity (l)	Internal diameter (cm)	Water column depth (cm)	Oil depth (cm)	Stirring speed (rpm)
15	24.0	10.0	1.1	140
10	20.4	13.8	1.5	150
5	16.8	21.0	2.3	180

Within the variability of the methods of preparation, extraction and analysis, there are no significant differences in aromatic hydrocarbon content between extracts from the three sizes of aspirator (Table 1). However, there is an apparent trend toward higher concentrations with increasing area of oil/water interface, although this is most pronounced in unfiltered samples and those filtered only to < 1.2 µm. It would appear sensible to maximize the area of oil/water interface when preparing saturated extracts.

(ii) The effect of stirring time

Extracts were prepared by stirring separate batches of oil and water for different periods of time; samples were not withdrawn at successive intervals from the same batch, as this would have altered the oil:water ratio, water column depth, etc. The results (Table 2) indicate that

while less than 20 hours stirring is insufficient to produce a 'saturated' extract, greater than 40 hours stirring does not increase the aromatic hydrocarbon concentration, and may even decrease it, perhaps due to photolysis or the onset of microbial degradation. Examination of the synchronous spectra from the 0.45 μm - filtered samples showed an increase in the response with increased stirring time at 325 nm relative to that at 290 nm, indicating a change with time in the aromatic hydrocarbon composition of the extracts. A stirring time of 24-30 hours appears optimal for the preparation of saturated extracts.

(iii) The effect of separation time

It is customary to allow the water-extract to stand immobile below the oil layer before withdrawal in order to allow any oil droplets to separate upwards. Table 3a shows that there was little change in the concentration of unfiltered samples during the first 5 hours. It should be noted that at the end of the stirring period, none of the extracts throughout this series of experiments contained any droplets visible to the naked eye.

When successive samples are drawn off from the same batch of extract beneath the oil, the oil/water interface approaches the inlet and later samples can be expected to contain increasing concentrations of any rising droplets or particles. To test this, several 500 ml samples were withdrawn, with the stirrer motor still rotating into stoppered separating funnels. At the end of the separation time the lower 250 ml was drawn off. The hydrocarbon concentrations of these samples (Table 3b) were similar to those from extracts left standing under the oil, thus confirming the absence of rising droplets.

(iv) Changes due to filtration

At the end of the stirring or separation period, samples were drawn off and their hydrocarbon concentration determined in an unfiltered sample, or after passage through papers or membranes of differing pore size. The results (Table 4a) show that the aromatic hydrocarbon concentration was progressively reduced with decreasing pore size, demonstrating the importance of particulate material in the hydrocarbon content of the unfiltered extract.

To determine whether filtration reduced the aromatic hydrocarbon concentration by adsorption of 'dissolved' material on to the filter media rather than by exclusion of particulate material, 500 ml samples were passed through Whatman GFC papers of 4.7 cm or 12.5 cm diameter. The results (Table4b) show little evidence of significantly increased adsorption by the larger papers.

To ensure reproducibility of extracts used in toxicity tests, it appears necessary to pass water extracts through a filter of pore size $\leq$ 0.45 µm. Since the toxicity of oil extracts appears to be strongly correlated with the concentration of aromatic rather than aliphatic hydrocarbons the presence of particulate material in unfiltered water extracts raised the question of the distribution of aromatic and aliphatic hydrocarbons between 'dissolved' and particulate forms. A series of experiments was therefore carried out in which samples were divided and analysed both by UVF and IR.

(v) The distribution of aromatic and aliphatic hydrocarbons within water-extracts

Extracts were prepared in 10 l and 15 l aspirators using stirring times of 24-72 hours and separation times of 0-1 hours. Unfiltered samples ranged in concentration from 3 500-5 800 µg/l fresh Kuwait crude oil equivalents by IR analysis, and from 650-1 000 µg/l by UVF. There was no apparent correlation between the IR and UVF values for individual samples. This range of concentrations by IR analysis was less than the 10 040 µg/l reported by Anderson *et al.* (1974), using a similar analytical technique.

Filtration of these extracts through 0.45 µm membranes increased the variability of the IR values (2 300-5 900 µg/l) but not of the UVF values (620-820 µg/l); again there was no correlation between the IR and UVF values. The effect of filtration was investigated further (Table 5); the results indicate that pore sizes below 1.2 µm progressively reduced the aliphatic component but had much less effect on the aromatic concentrations. It is probable that the aliphatic component is largely adsorbed to particulate material. This conclusion is supported by the IR spectra (Figure 1a), in which the 2 930 cm^{-1} peak which measures the $-CH_2$ band stretch of aliphatic compounds becomes of decreasing relative importance with decreasing pore size. Even after filtration through a pore size of 0.45 µm, the concentration measured by IR does not allow prediction of the concentration by UVF, or *vice versa*.

(vi) Dilution experiments

If the aliphatic concentrations of filtered extracts vary so much, are dilutions reproducible and thus suitable for toxicity tests? To test this, saturated water-extracts were prepared and filtered through 0.45 µm membranes. This 'neat' extract was serially diluted with 1.2 µm filtered sea water whose hydrocarbon concentration ranged from 9-13 µg/l

aromatics and from 20-70 µg/l aliphatics, but on one occasion the concentrations were 26 and 270 µg/l respectively. The dilution series was as follows:

	Nominal concentration of 'neat' extract
416 ml of 'neat' extract made up to 1 000 ml: (Dilution 1)	0.416
304 ml of dilution 1 made up to 1 000 ml: (Dilution 2)	0.126
664 ml of dilution 2 made up to 2 000 ml: (Dilution 3)	0.042
608 ml of dilution 3 made up to 2 000 ml: (Dilution 4)	0.013

The remainder of each dilution was divided and solvent-extracted for either IR or UVF analysis. In Table 6 the predicted values are calculated from the dilution of each successive measured concentration, taking into account the hydrocarbon concentration of the sea water used in each case. The results show that the initial dilution usually produced an aromatic and aliphatic concentration well below that predicted. Thereafter, the aromatic concentration, though usually lower than predicted, was more predictable than the aliphatic which tended to be well above the predicted value. This could be explained if part of the hydrocarbon content of the 'neat' extract is unstable, perhaps held in an 'accommodated' form by high concentrations of some non-hydrocarbon compound derived from the oil. When sufficient diluting water is added, the concentration of this compound falls below a critical level and the concentration of hydrocarbons that can be held in this 'accommodated' form falls, forcing the excess to form particulates which adsorb to the container walls.

Alternatively, the addition of 'clean' particulates in the diluting water (filtered only < 1.2 µm) could sweep both aromatics from 'solution' and aliphatics from suspension, followed by adsorption to the container walls. It is important to note that when samples were split for analysis by IR and UVF, the solvent extractions could not be carried out in the original sample containers and hence would not include material adsorbed to the sample container walls. Adsorption to the walls would deplete not only the initial, but to a lesser extent all subsequent dilutions explaining the lower-than-predicted aromatic concentrations. However, such a process would not account for the often much higher-than-predicted aliphatic concentrations, unless aliquots were occasionally taken from liquid rich in particulates derived from the walls, even though the containers were shaken beforehand.

The IR spectra showed a progressive increase in the peaks at 2 960 cm^{-1} (due to the $-CH_3$ band stretch) and at 3 030 cm^{-1} (due to the -CH band stretch in aromatic compounds) relative to that at 2 930 cm^{-1} until by the final dilution they became the dominant peaks (Figure 1b). It appears that dilution progressively removes aliphatic compounds, which again would be explained by adsorption to the container walls of the particulates on which they are held. There was no marked change in peak height distribution from the 'neat' extract to the first dilution despite the great change in concentration; the UVF spectra remained the same throughout the dilution sequence.

DISCUSSION

Aspirator dimensions and stirring time affect the concentration and composition of the unfiltered 'saturated' sea water extract of Kuwait crude oil, but allowing the extract to stand below the oil layer makes little difference over the first 5 hours. Even with fixed aspirator dimensions and stirring time, the concentration of aromatic and aliphatic hydrocarbons in the unfiltered extract varies considerably. Filtration through a pore size of $\geq$ 0.45 µm, achieves reasonably reproducible concentrations of aromatic, but not aliphatic hydrocarbons. The latter are present mainly in particulate form and, even after filtration, serial dilution of extracts does not produce the predicted reductions in the concentrations of aliphatic hydrocarbons. When serially diluted, the initial dilution of the 'neat' extract gives lower than predicted concentrations of aromatic hydrocarbons. The hydrocarbon composition of serially diluted extracts changes progressively until very low concentrations (2-5 x background) consist almost entirely of aromatic hydrocarbons with benzenoid compounds becoming increasingly important. Thus, evaluation of the toxicity of such extracts and dilutions requires a thorough analysis of each test-concentration and comparisons can be made only between concentrations of the same hydrocarbon composition.

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Table 1 The effect of aspirator size on the aromatic hydrocarbon concentration of 'water-soluble' fractions (μg/l fresh Kuwait crude oil equivalents) [UNF = unfiltered]

Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Nominal volume: litres		
			15	10	5
24	0	UNF	1000 940 770	900	830
24	1	UNF	890 860 1000 970 840	870	700
24	0	1.2	950	830	800
24	0	0.45	640 690	560	660
24	0	0.2	740	630	650

Table 2 The effect of stirring time on the aromatic hydrocarbon concentration of 'water-soluble' fractions (μg/l fresh Kuwait crude oil equivalents) [UNF = unfiltered]

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Concentration
15	16	0	0.45	500
15	20	0	0.45	590
15	24	0	0.45	690
15	48	0	0.45	640
15	24	0	UNF	940,1000,770
15	40	0	UNF	890
15	48	0	UNF	650
15	24	1	UNF	1000,890,860,970,840
15	40	1	UNF	830
10	24	0	UNF	900
10	72	0	UNF	890

Table 3 Effect of separation-time after stirring on the aromatic hydrocarbon concentration of 'water-soluble' fractions (µg/l fresh Kuwait crude oil equivalents) [UNF = unfiltered]

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (µm)	Concentration
(a) Standing under the oil layer				
15	24	0	UNF	1000,940 - 770
15	24	1	UNF	1000,970,860,890, -
15	24	5	UNF	- - 940 810
10	24	0	UNF	900
10	24	1	UNF	870
10	24	42	UNF	750
10	24	333	UNF	710
10	24	0	0.2	630
10	24	42	0.2	580
5	24	0	UNF	830
5	24	1½	UNF	820
(b) Standing in stoppered separating funnels				
15	40	0	UNF	810
15	40	1	UNF	830
15	40	2	UNF	840
15	40	5	UNF	800

Table 4a The effect of filtration on the aromatic hydrocarbon concentration of 'water-soluble' fractions (μg/l fresh Kuwait crude oil equivalents) [UNF = unfiltered]

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Concentration
15	24	0	UNF	1000
15	24	0	1.2	950
15	24	0	0.45	690
15	24	1	UNF	970,860, - 840
15	24	1	1.2	930,820,800,820
15	24	1	0.45	790,730,760,760
15	24	1	0.2	730, - 740,740
15	40	0	UNF	890
15	40	0	1.2	830
15	40	0	0.45	610
15	40	0	0.2	570
10	24	0	UNF	900
10	24	0	1.2	830
10	24	0	0.45	560
10	24	0	0.2	630
5	24	0	UNF	830
5	24	0	1.2	800
5	24	0	0.45	660
5	24	0	0.2	650

Table 4b The effect of Whatman GFC filter-paper size on the aromatic hydrocarbon concentration of the filtrate (μg/l fresh Kuwait crude oil equivalents)

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Paper diameter (cm)	
				12.5	4.7
15	24	$2\frac{1}{2}$	1.2	880	820
15	24	1	1.2	730	800
15	24	1	1.2	850	820

Table 5 The effects of filtration on the aliphatic and aromatic hydrocarbon concentrations of 'water-soluble' fractions (μg/l fresh Kuwait crude oil equivalents) [UNF = unfiltered)

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Concentration by IR	Concentration by UVF	$\frac{IR}{UVF}$ ratio
15	24	1	UNF	4200	970	7.4
15	24	1	1.2	3700	930	4.0
15	24	1	0.45	2300	790	2.9
15	24	1	0.2	1100	640	1.7
15	24	1	UNF	5800	840	6.9
15	24	1	1.2	3400	820	4.1
15	24	1	0.45	2700	760	3.6
15	24	1	0.2	2100	740	2.8
15	24	1	UNF	-	-	-
15	24	1	1.2	5100	800	6.4
15	24	1	0.45	5700	760	7.5
15	24	1	0.2	3800	740	5.1
15	24	0	1.2	3000	950	3.2
15	24	0	0.45	3700	690	5.4
15	48	0	UNF	4300	650	6.6
15	48	0	0.45	2900	640	4.5
10	72	0	UNF	4000	890	4.5
10	72	0	0.45	2500	710	3.5

Table 6 Predicted and actual aliphatic and aromatic hydrocarbon concentrations in diluted 'water-soluble' fractions (μg/l fresh Kuwait crude oil equivalents) ('Neat' ≡ undiluted) (* - no response at 2930-3030 cm^{-1})

Aspirator volume (l)	Stirring time (h)	Separation time (h)	Filtration pore size (μm)	Concentration			
				IR		UVF	
				Predicted	Actual	Predicted	Actual
15	24	1	0.45	'Neat'	5900	'Neat'	620
				2480	1900	266	230
				610	1200	79	78
				430	560	35	34
				210	300	20	21
15	24	1	0.45	'Neat'	3800	'Neat	710
				1740	1500	313	310
				640	750	116	140
				430	870	67	65
				450	600	41	46
15	24	1	0.45	'Neat'	4300	'Neat'	820
				1830	1300	349	290
				440	380	97	97
				170	160	41	41
				100	110	22	28
15	24	1	0.45	'Neat'	5700	'Neat'	760
				2390	830	323	270
				280	210	90	95
				100	90	39	34
				69	170	18	18
15	24	1	0.45	'Neat'	2700	'Neat'	760
				1130	*	321	270
				-	*	88	110
				-	300	42	38
				110	100	18	15
15	24	1	0.45	'Neat'	3100	'Neat'	720
				1300	1200	307	250
				380	560	84	100
				200	180	41	36
				70	70	19	23

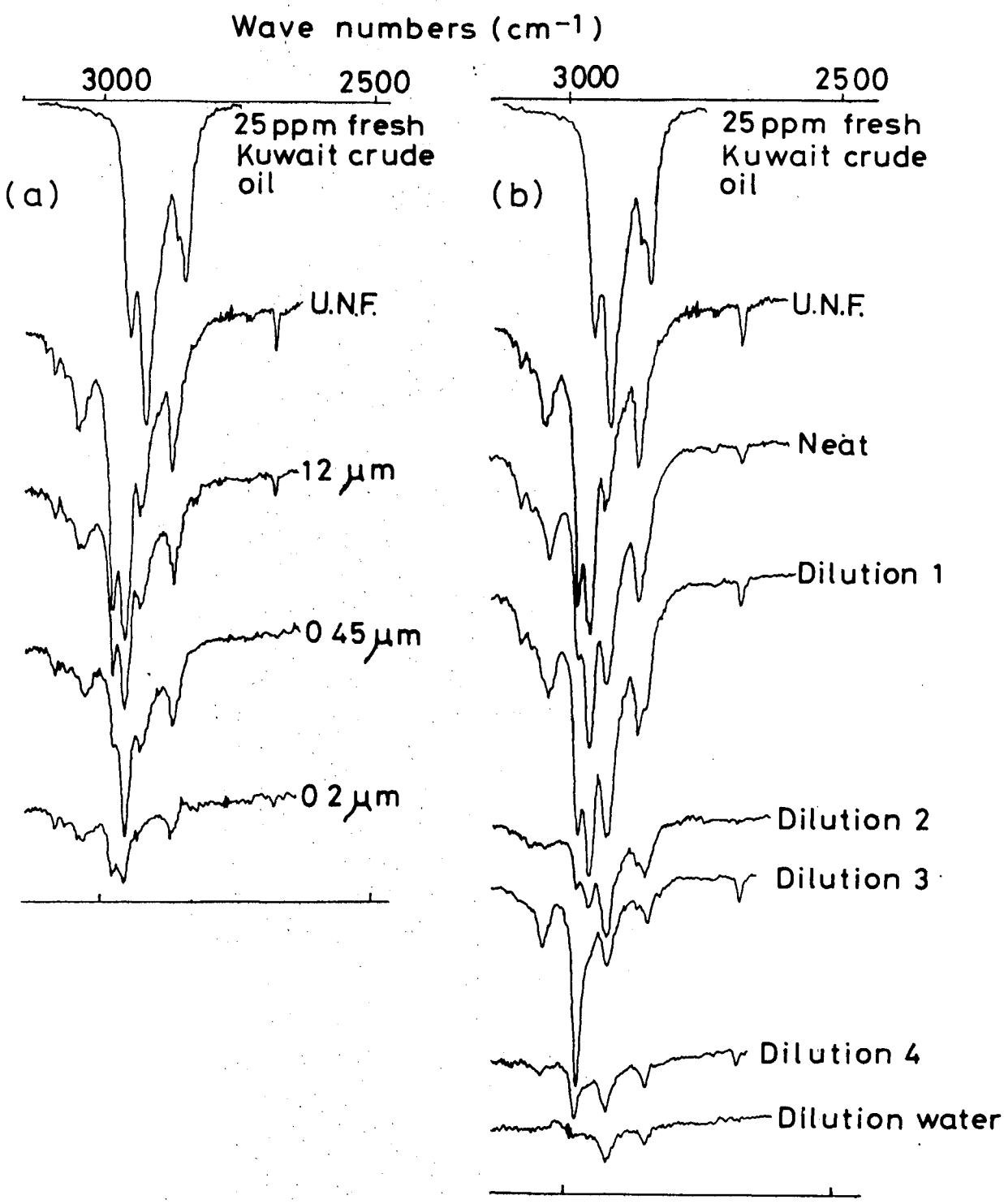

Figure 1(a) Change in IR spectra of "water-soluble" fractions with filtration through differing pore-sizes.

Figure 1(b) Change in IR spectra of "water-soluble" fractions with dilution.