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CHARACTERIZATION OF NON-IONIC DETERGENTS OF THE POLYETHOXYLATED TYPE FROM WATER SYSTEMS

II. ISOLATION AND EXAMINATION OF POLYETHOXYLATED MATERIAL BEFORE AND AFTER PASSAGE THROUGH A SEWAGE PLANT

P. JONES* and G. NICKLESS

Department of Inorganic Chemistry, School of Chemistry, The University, Bristol, BS8 1TS (Great Britain)

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SUMMARY

The XAD-4 extraction system evaluated in Part I of this series was used to pre-concentrate polyethoxylated material from waste water produced by a small relatively unindustrialized city. Samples were taken from the inlet and outlet of the main sewage plant and the river into which the effluent discharged. A three-stage isolation procedure was developed and the final extract of each sample separated into a non-ionic detergent component and a polyethylene glycol (PEG) component. The concentrations of non-ionic detergent found in the water system were low (less than 1 $\mu g\ ml^{-1}$) in the sewage influent and about 100 times lower (approximately 8 $ng\ ml^{-1}$) in the river water. Comparing the same samples, the PEG concentration as a percentage of the non-ionic detergent increased from 3% to about 30%.

Further characterization of the extracts was attempted using thin-layer chromatography and spectroscopic methods, and two main types were identified in the non-ionic detergent component. These were alkylphenol ethoxylates (APE) and linear aliphatic alcohol ethoxylates, which can be further subdivided into secondary alcohol ethoxylate (SAE) and primary alcohol ethoxylate (PAE). APE was found to be the most persistent, while the aliphatic alcohol ethoxylates broke down much more rapidly in the sewage plant.

INTRODUCTION

In Part I of this series[1], the macroreticular resin Amberlite XAD-4 was shown to be a useful adsorbent for the extraction of polyethoxylated detergents and polyethylene glycols from water. The evaluation was carried out using standard solutions of model compounds made up in distilled water. It should now be possible to use

* Present address: Department of Chemistry, University of the West Indies, St. Augustine, Trinidad, West Indies.

the XAD-4 resin for the pre-concentration of these compounds from real water systems. By processing large volumes of water, sufficient material can be collected for a more complete characterization using a variety of analytical techniques. The most important of these will be liquid chromatography and ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. The characterization of the residues could give information on biodegradation processes, the most important aspects being rate and mechanism of breakdown and the accumulation, if any, of breakdown products. The success of the study will depend very much on the ability to isolate the compounds of interest from other organic material co-extracted from the water. If the clean-up procedures are not very efficient, interference will be a major problem, particularly with spectroscopic examinations.

The secondary alcohol ethoxylate type of non-ionic detergents (SAE) are likely to be important domestic detergents, and could be a major replacement for the "harder", less easily biodegradable alkylbenzene sulphonates. The main purpose of this investigation was to find out as much as possible concerning the fate of the SAE as it proceeds through the water system. It is likely that alkylphenol ethoxylates (APEs) closely related to SAEs (see Part I) will be present in the water system through industrial use. Straightforward clean-up and isolation procedures will not separate these two types and renders the investigation of SAEs much more difficult.

A number of important biodegradation studies have already been carried out on these detergents. The "die away" tests most relevant to this work can be seen in the studies by Patterson *et al.*[2–4] on non-ionic detergent degradation. These investigations, under controlled laboratory conditions showed the following:

(1) SAEs with linear alkyl chains degrade very rapidly, particularly if the ethylene oxide chain is not very long. The main mechanism appears to be the fission of the molecule into the hydrophobic and hydrophilic parts with rapid oxidation of the hydrophobic group.

(2) APEs degrade much more slowly but can be very dependent on conditions such as pH. There appears to be no fission mechanism but rather simultaneous attack on the alkyl chain, benzene group and ethylene oxide chain.

(3) Polyethylene glycols (PEGs) result from the fission of SAEs. These PEGs biodegrade more slowly than the parent non-ionic detergent and particularly slowly if the molecular weight of the PEG is greater than 1000.

(4) Also associated with the breakdown of the above three compounds is the formation of carboxyl groups in some of the oxidative processes.

These findings give a useful indication of the possible products to be found in a water system where these types of domestic detergents are used. It must be remembered, however, that actual biodegradation conditions can vary significantly from those set up in the laboratory.

The river Avon in southern England was the system chosen for study, in particular a sewage treatment plant downstream from the city of Bath. A number of samples were taken from the influent and effluent and from the river. These samples were processed with XAD-4 resin and the polyethoxylated material isolated by liquid chromatography and solvent extraction. Finally, thin-layer chromatographic (TLC), UV, IR and NMR techniques were used in an attempt to quantify and characterize the various components in the residues.

EXPERIMENTAL

Sampling

Each sample was collected using a metal bucket lowered into the flowing water, and decanted into a glass container through a glass-wool plug inserted in the stem of a metal filter funnel. The samples were then taken to the laboratory for immediate processing with the XAD-4 resin.

Water extraction procedure

This utilized the same set-up as that designed for the evaluation of the XAD-4 resin in Part I. A small narrow glass column was filled with the resin (*ca.* 5 g), purified by solvent extraction to remove interferences. This column was then screwed to a much larger glass column, 2 m in length and of 2½ l capacity, acting both as a reservoir and pressure head. The water sample, pre-filtered through a large plug of glass-wool, was poured into the reservoir and the flow-rate adjusted to 100 ml min^{-1}. Normally, the large column has to be completely filled before this flow-rate is reached.

In this way, water samples varying from 3.5 to 50 l have been processed. Care is needed with samples which have large amounts of suspended solids, such as sewage influent samples. Inadequate filtration will lead to partial blocking of the glass-wool plugs and a reduction in the flow-rate.

Isolation of the polyethoxylated material

It is likely that the polyethoxylated material will be a minor component of the organic material adsorbed on the resin column, and these compounds will therefore have to be isolated from a large amount of unwanted material. In many instances, slight differences in physical properties can be exploited to separate wanted from unwanted compounds using liquid chromatography. Unfortunately, owing to the polymeric nature of the polyethoxylated compounds under investigation, physical properties of individual oligomers such as polarity and water solubility will vary over a wide range. This will make it very difficult to exclude unwanted organic compounds of both a non-polar and a polar nature. Because of this problem much time was spent in finding the right conditions necessary for an efficient isolation procedure. This phase of the analysis was helped considerably by making use of a "model extract" available from the work of Roberts[5] in his investigations of the river Avon for pesticide residues. By passing 20,000 l of river water through activated charcoal, Roberts obtained nearly 2 g of organic residue, of which approximately 6% was polyethoxylated material. Small portions of this "model extract" (5–10 mg) were used to investigate a large number of chromatographic solvent extraction procedures to find the correct conditions for the efficient separation of the XAD-4 extracts. The finalized method required a three-stage procedure, as follows, for reasonable isolation and separation of the polyethoxylated material.

Elution and solvent extraction. The organic compounds adsorbed from the water sample by the XAD-4 resin were eluted off with four solvent systems to give two fractions:

(1) 20 ml of methanol–water (1:1) followed by 20 ml of methanol;

(2) 50 ml of acetone, followed by 20 ml of acetone–*n*-hexane (1:1).

Each fraction was collected in a small beaker and evaporated to dryness in

a stream of filtered air on a hot water-bath. Fraction 2 contained most of the polyethoxylated material, but significant amounts were also present in fraction 1. The residue from fraction 1 was treated with 10 ml of acetone, decanted into a small tube, centrifuged, and the clear acetone layer poured into the beaker containing fraction 2. This was repeated once more with a further 10 ml of acetone. Fraction 2 was again evaporated to dryness and the residue should then contain all of the polyethoxylated material. The residue in fraction 1 was very polar, water-soluble material, and appeared to be mainly humic and fulvic acids.

Liquid–solid chromatography. This is the most critical section of the separation procedure. The polyethoxylated material contained in fraction 2 from the XAD-4 elution will still be a relatively minor part of the residue. Experience with the "model extract" suggested that most of the unwanted organic compounds extracted from the river water would be medium to non-polar in character. There would therefore be overlap with the medium polar oligomers of SAE and APE (*i.e.*, those with only 1, 2 or 3 ethylene oxide units per molecule). Silica gel was found to be the best chromatographic adsorbent, and the correct choice of eluting solvent strength is important for efficient separation.

Silica gel (20 g, 200–325 mesh) was activated at 300° for 1 h. After cooling in a sealed container, it was packed into a glass column (20 cm long, 2 cm diameter) as a slurry with ethyl acetate–benzene (7:3). A 2-ml volume of this solvent was then added to the residue from fraction 2. The residue was normally a very viscous dark brown "paint layer" on the bottom of the beaker. By swirling gently, a certain portion of the film would dissolve and the solution loaded on to the column. The remaining insoluble residue contained highly polar polyethoxylated material and must be dissolved and loaded on to the column when more polar solvents are used later in the eluting procedure. A typical elution procedure is shown in Table I. Most of the unwanted organic compounds came through in fractions 1 and 2 and were discarded. Fractions 3 and 4 were combined and kept for TLC examination only as the amount of polyethoxylated material was usually less than 1% of the total. Fractions 5 and 6 were combined and usually contained greater than 98% of the total polyethoxylated material present in the original extract, together with some highly polar compounds imparting a faint yellow colour to the solution.

TABLE I

TYPICAL ELUTION PROCEDURE FOR AN XAD-4 EXTRACT ON SILICA GEL

Fraction No.	*Eluting solvent and type of residue obtained*
1	25 ml of ethyl acetate–benzene (7:3). A yellow band eluted off, containing most of the non-polar to medium polar compounds in the residue.
2	A further 25 ml of ethyl acetate–benzene (7:3). More yellow-coloured material eluted.
3	30 ml of pure ethyl acetate. Further amounts of yellow-coloured residue, containing a trace amount of polyethoxylated material.
4	20 ml of ethyl acetate–acetone (1:1). Less yellow-coloured residue eluted, and further trace amounts of polyethoxylated material.
5	70 ml of pure acetone. Large amounts of polyethoxylated material, no yellow colour.
6	30 ml of acetone–water (3:2). More polyethoxylated material plus some yellow-brown residue.

Liquid–liquid chromatography. The combined fractions 5 and 6 obtained from liquid–solid chromatography needed further separation for two reasons: (1) the fractions still contained significant amounts of other organic compounds which were found to be mainly acidic in character, and (2) meaningful results could only be obtained from spectroscopic examinations if the polyethoxylated material is divided into the non-ionic detergent component and the polyethylene glycol component.

Both of these problems were solved in one operation using liquid chromatography based on a procedure by Nadeau and Waszeciak[6]. With this procedure, using water as a stationary phase (on Celite) and chloroform–benzene as the mobile phase, very good separations of PEG from SAE and APE can be obtained. By simply changing the stationary phase to dilute sodium hydroxide solution, unwanted acidic compounds can be removed with the PEG fraction.

A 4-g amount of Celite 545 (coarse grade), previously coated with sodium hydroxide (0.25%, w/w) was shaken with 1.3 ml of water to give a nominal loading of 25% (w/w) of 0.05 *M* sodium hydroxide. The Celite was then packed as a slurry with benzene–chloroform (3:2) into a 1-cm I.D. glass chromatography column. The residue from the combined fractions 5 and 6 obtained in the previous section was dissolved in a small volume of the same solvent and loaded on to the column. Elution was continued with the benzene–chloroform and the first 30-ml fraction contained all of the non-ionic detergent material (SAE and APE). The PEG and acidic material remaining on the column were eluted with 20 ml of methanol. The fraction containing the non-ionic detergent compounds was evaporated to dryness and the residue re-dissolved in a known amount of chloroform (usually 0.5 ml). The other PEG-containing fraction was evaporated to dryness, the residue extracted three times with small amounts of chloroform, combined and evaporated to 0.5 ml. These fractions were then examined by TLC and IR, UV and NMR spectroscopy. The whole separation scheme is shown in Fig. 1.

Methods used for characterization

Thin-layer chromatography

The method of Patterson *et al.*[7] as described in Part I was used for all TLC investigations. Solvent A (ethyl acetate–acetic acid–water, 4:3:3) was used for quantitative information, where a compact spot is obtained for non-ionic detergents (SAE and APE combined), and an elongated spot of lower R_F value for PEG. Solvent B (ethyl acetate–acetic acid–water, 70:15:15) was used for information on the molecular weight distribution of SAE and APE. APE gives a "string" of well resolved spots and SAE a long unresolved streak. These patterns will be superimposed if both are present in the residue. All records were made immediately after spraying because fairly rapid fading occurred.

UV, IR and NMR spectroscopy

UV spectroscopy. Only APE absorbs in the UV region, giving a peak at 277 nm with a characteristic shoulder at 285 nm (Fig. 2). Chloroform was the normal solvent (UV cut-off 240 nm), and after measurement the sample can be re-concentrated in a stream of air back to 0.5 ml. A quantitative measurement was not attempted at this stage as it was not known which model compound would give a

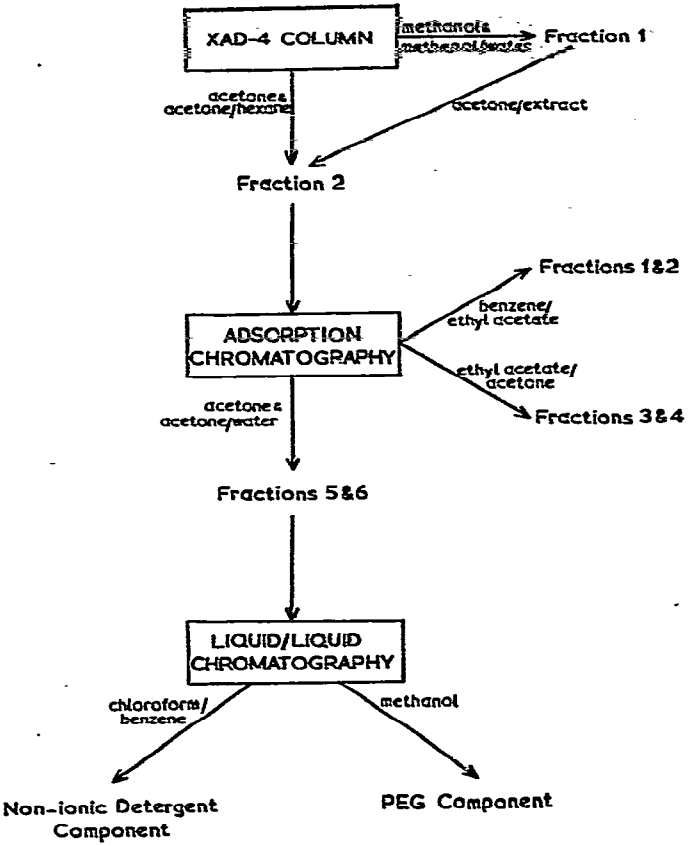

Fig. 1. Complete separation scheme for polyethoxylated material.

reasonable standard for accurate results. Quantitative measurement is also made difficult by the presence of a sloping baseline, as even trace impurities give a significant "toe" into the 300-nm region. In fact, the slope of the baseline gives a good indication of the efficiency of the separation procedure. In early work, slopes of 70–80% were not uncommon.

IR spectroscopy. Infrared spectra were measured after evaporating a small amount (usually 50 μl) of the chloroform fraction on to the centre of a sodium chloride disc. Slow movement of the syringe plunger while playing a light stream of air over the needle tip in contact with the disc, is the best way of achieving this.

NMR spectroscopy. All samples were sent to Dr. Shuttleworth at Unilever Research, Port Sunlight, Great Britain, for NMR studies. Deuterochloroform was the solvent, and Fourier transform was used to achieve sufficient sensitivity.

RESULTS AND DISCUSSION

The area chosen for study was centred around a sewage works discharging into the River Avon, approximately 2 miles downstream from the city of Bath. Domestic

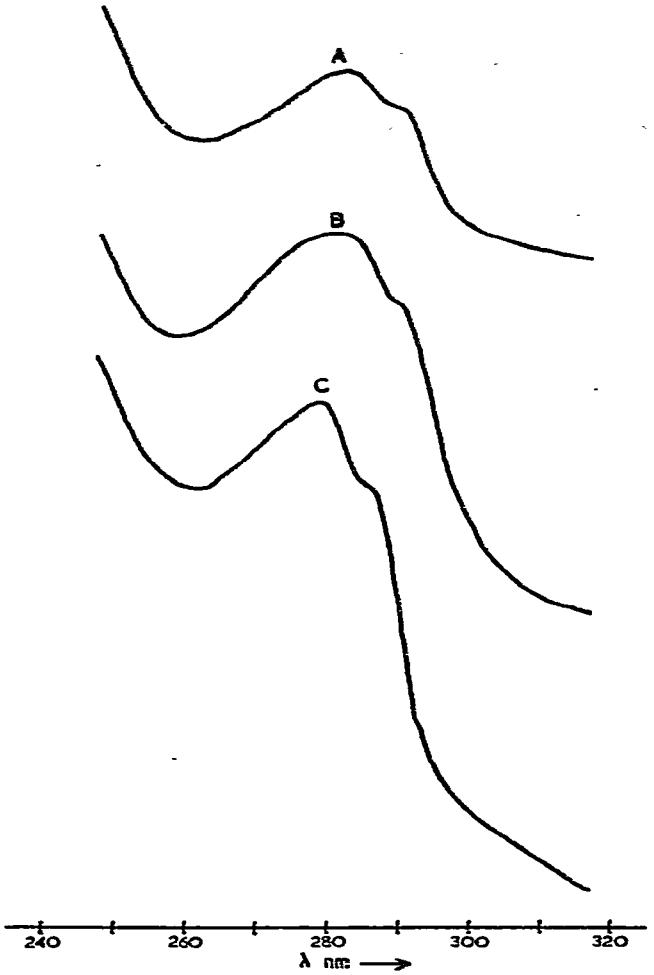

Fig. 2. UV spectra of (A) a commercial alkylphenyl ethoxylate, APE 9EO, (B) sewage effluent extract and (C) Roberts river extract[5].

consumption of detergents based on secondary alcohol ethoxylate was known to be fairly light in the area, and so low concentrations of polyethoxylated material were expected.

Concentrations of non-ionic detergent and PEG found in water samples

Water samples were collected, processed by the XAD-4 resin, and the extracted residue was subjected to the full separation procedure. Silica gel TLC with developing solvent A was used to obtain approximate concentrations of non-ionic detergent and PEG. Tergitol 15-S-9 (an SAE detergent) and PEG 400 were used as control standards. The results are given in Table II.

The concentrations reported for the non-ionic detergent component represent the total present, that is, mainly SAE plus APE, although significant amounts of other types may be present, such as primary alcohol ethoxylate (PAE) indicated in

TABLE II

CONCENTRATIONS OF NON-IONIC DETERGENT AND PEG FOUND IN WATER SAMPLES

Sample identification (volume processed)	*Weight of acetone-soluble XAD-4 extract (mg)*	*Weight of non-ionic detergent and PEG calculated from TLC (mg)*		*Approximate concentration of non-ionic detergent and PEG in the water sample (ppm)*	
		Non-ionic	*PEG*	*Non-ionic*	*PEG*
(1) Sewage influent (14 l)	88.0	10.0	0.25	0.7	0.02
(2) Sewage influent after storing for 3 days (3.5 l)	29.6	1.5	0.8	0.4	0.2
(3) Sewage effluent (30 l)	17.7	2.0	0.6	0.07	0.02
(4) River water downstream of sewage outlet (46 l)	33.5	0.4	—*	0.008	—

* Present, not measured.

the NMR spectra (see later). The decrease in concentration of non-ionic detergent from influent to effluent was approximately 10-fold and biodegradation must have played a large part in this. The decrease in concentration was much less when measured on a portion of the same effluent sample stored under essentially anaerobic conditions for 3 days. A significant amount of the non-ionic detergent measured in the river water probably originated upstream of the sewage outfall, as evidenced by the amount found in the "model extract" of Roberts[5] taken 1 mile up river from the discharge (see Experimental).

Characterization of polyethoxylated material extracted by the XAD-4

For this particular study, characterization not only requires the identification of the major types of polyethoxylated material present, but also the analysis of the chain-length distribution of the polyethoxylate group. Using this information, the fate of any particular non-ionic detergent could be followed as it progressed through the aquatic system, and linked if possible with the appearance of a metabolite such as PEG. The investigation of a real water system is likely to produce a non-ionic detergent residue containing two or more major components. As such the study of SAE in the river Avon was made more difficult by the presence of APE in every sample.

TLC investigation

Examination with solvent A was primarily used for quantitative information already discussed, but some indication of PEG chain length was also obtained with this solvent. All extracts gave an elongated spot in the PEG region centred at R_F 0.4. Comparison with PEG 400 and PEG 1000 model compounds showed that the average molecular weight of the PEG in the water samples was approximately 500 with a range between 300 and 1000.

Examination with solvent B should have given some useful information on the effect of biodegradation on chain-length distribution of SAE, but the presence of APE made this very difficult to ascertain. The observations on the TLC of the extracts are best described under separate headings.

Sewage influent. This showed a distinct spot pattern similar to APE 9EO, but almost completely masked by an unresolved streak, which could be an aliphatic alcohol ethoxylate of the SAE type.

Sewage effluent. The APE spot pattern was now much clearer and relatively more concentrated in the short chain region than found with the above extract. This is both an indication of rapid biodegration of the SAE type relative to APE, and preferential attack on the long-chain oligomers of APE.

As a comparison, the pattern observed with the sewage effluent sample, stored for 3 days, showed a spot pattern not quite as blurred as the fresh sample, and a large reduction in intensity in the short-chain region. There was also a large increase in PEG concentration.

River water. A clear spot pattern of the APE type was again seen but the short-chain region was masked by an unresolved streak.

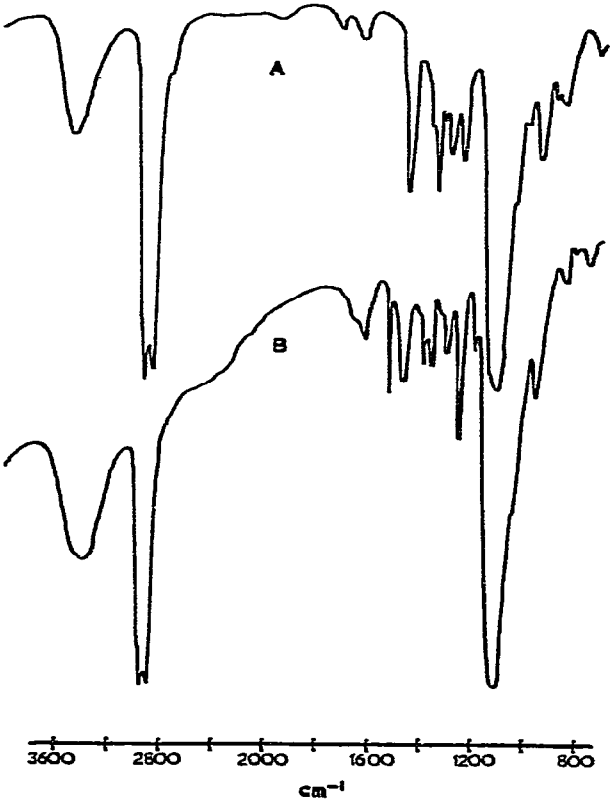

Fig. 3. IR spectra of two commercial detergents: (A) Tergitol (SAE 9EO) and (B) Marlophen 810 (APE 9EO).

Spectroscopic investigation

UV spectroscopy. The presence of APE in all samples was confirmed, as shown in Fig. 2. Quantitative information could be obtained with the right choice of model compound, and therefore alcohol ethoxylate detergent concentrations calculated by difference.

IR spectroscopy. As with the TLC study, diagnostic information from the IR spectra is limited because of the presence of two major components in the extracts. The IR spectra of a commercial APE and SAE are shown in Fig. 3. The general appearance of both types is very similar with the broad strong peak at 1100–1120 cm^{-1}, characteristic of a polyethoxylate grouping. The only clearly recognizable difference between them is the very sharp aromatic peak present in the APE spectrum at 1500–1505 cm^{-1}.

The spectra of the non-ionic detergent components of the water extracts are

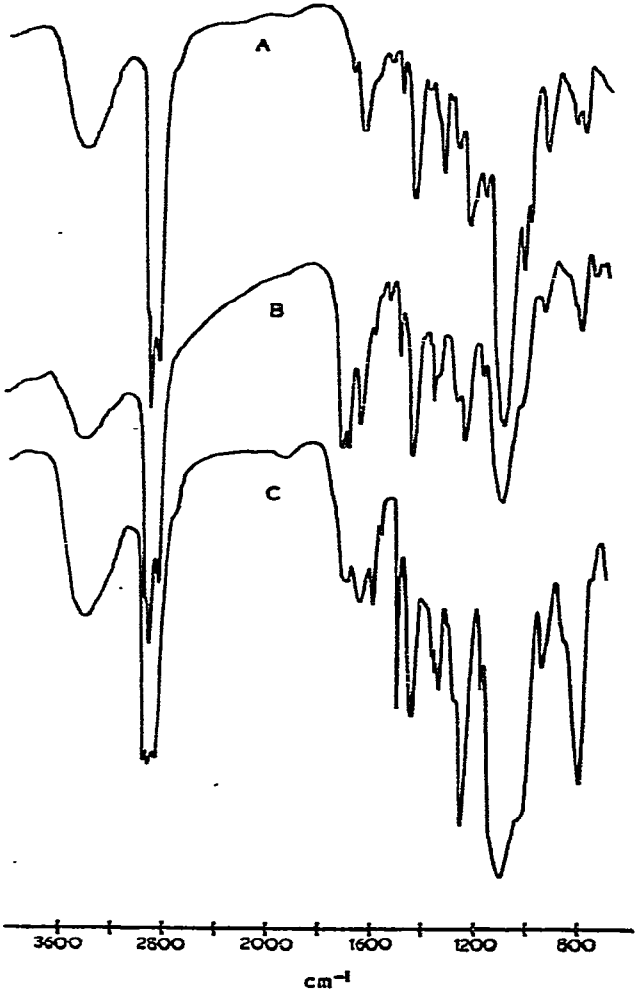

Fig. 4. IR spectra of (A) sewage influent extract, (B) sewage effluent extract and (C) Roberts river water extract.

shown in Fig. 4. Owing to the small amount of sample available, varying amounts of scale expansion had to be used. Again, the general appearance of the three spectra is similar and closely resembles the spectra in Fig. 3. The relative height of the aromatic peak at 1505 cm^{-1} does seem to have increased from influent to effluent and is very strong in the Roberts river water extract. Owing to the very small sample weight, the river water extract taken below the sewage outfall was kept for NMR examination only.

NMR spectroscopy. The sample sizes are such that Fourier-transform NMR spectroscopy must be used, and also great skill in interpretation is needed when mixtures are present. For these reasons, the samples were examined at the Port Sunlight Laboratories of Unilever Research. Their analysis is given in Table III.

TABLE III

NMR EXAMINATION OF WATER RESIDUES

Sample	*Alkylphenol ethoxylate*	*Other ethoxylate*	*Miscellaneous*
(1) Influent	Present, probably nonyl. A major component	Primary alcohol ethoxylate. Approx. equal to APE in moles	Probably alkyl aryl sulphone. Approx. equal to APE
(2) Influent stored for 3 days	Present, probably nonyl	Possible primary or secondary alcohol ethoxylate	Probably alkyl aryl sulphone. About twice as much as APE
(3) Effluent	Trace	Possibly secondary alcohol ethoxylate	—
(4) River water downstream of outlet	Trace	Not detected but might be trace	Probably (alkyl-$OCH_2CH_2O)_3$-P=O plus phthalate ester
(5) River water upstream of outlet (Roberts extract)	Present, a major component. Probably mixed nonyl and octyl	Probably secondary alcohol ethoxylate	—

The NMR results tend to support the findings of the TLC study, but the most surprising aspect was the presence of apparently large amounts of primary alcohol ethoxylate (PAE) in the influent sample, masking any evidence of SAE. As a result of these findings it is best to report polyethoxylated detergent other than APE as aliphatic alcohol ethoxylate (PAE plus SAE). According to Patterson *et al.*[4], PAE degrades even faster than SAE and might explain why NMR shows no evidence of PAE after passage through the sewage works. The NMR survey also shows that the isolation procedure cannot completely eliminate compounds such as alkyl aryl sulphones and phthalate esters.

CONCLUSION

Concentrations of polyethoxylated material in the area chosen were relatively low (compare the work of Patterson *et al.*[2,3]), and so were a good test of the pre-

concentration and isolation procedures. For example, 0.4 mg of non-ionic detergent needed to be isolated from 34 mg of organic material in the river water extract, a ratio of 85:1. Significant amounts of other material may still be present in some extracts, as indicated in the NMR study, and preparative TLC may give better results than column liquid–solid chromatography.

The polyethoxylated material found in the water was characterized into four types, namely APE, SAE, PAE and PEG. Carboxylated degradation products will not be detected with this present isolation technique, being eliminated in the liquid–liquid chromatography section (see Fig. 1). Further characterization of SAE in terms of chain-length distribution, and hence information on biodegradation processes, was not possible because of the presence of APE in all extracts and PAE in the influent extract. High-performance liquid chromatography with different detectors should give further information on this, otherwise separation of the non-ionic detergent component into aromatic and aliphatic fractions is needed, although the presence of PAE will still be a problem. Even so, the results clearly indicate the greater persistence of APE with SAE (and PAE) degrading very rapidly in the sewage plant and very little appearing in the river water.

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