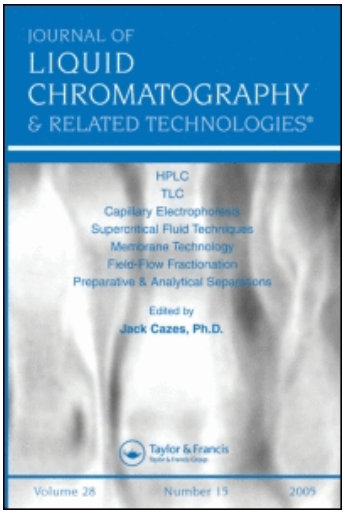

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HPLC METHOD FOR THE STUDY OF ANAEROBIC DEGRADATION OF POLYETHYLENE GLYCOLS

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ABSTRACT

This paper describes a rapid gradient reversed-phase high-performance liquid chromatography method for the characterisation of PEGs, HO-(CH2-CH2-O)n-H, in water. This method allows the routine analysis of a mixture of these compounds, in the range n=1-50 (up to PEG 1500) in unesterified samples, at room temperature. The method has been used for the study of anaerobic digestion of PEG-200 and PEG-300 in an one litre laboratory digester.

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INTRODUCTION

Polyethylene Glycols are used in the production of surfactants, lubrificants and cosmetics (1-3). They are, therefore, present in waste or surface waters as catabolic products of these compounds (4). Many polar compounds cannot be eliminated by various treatment steps such as: biochemical, chemical and physico-chemical tratment (soil filtration, ozone treatment or activated carbon processes) (5,6). The recalcitrant nature of PEGs by aerobic degradation led researchers to investigate the biodegrability under anaerobic conditions (1,7-9). The information regarding its biodegradation is scarce and conradictory (2,8). The study of degradation process is based mainly on the bacterial growth observation (8), chemical oxigen demand (COD) (1) or GC analysis of the final products (2,7,9). The anaerobic degradation mechanism in medium with PEGs as the sole source of carbon and energy was not established. In this field, the determination of the highest size polimer of PEGs, that are biodegradable, as well as the bacteria with a higher degrading capacity are very important.

The direct analysis of the PEGs' molecular distribution in an anaerobic digestor is very useful for studying the fundamental aspects of this process. The GC or GC/MS methods have a very limited applicability with these problems, taking into account that these compounds are polar and thermolabile (6). A newly established HPLC method would be suitable to solve the many analytical aspects.

The use of a refractive index detector (10,11) is limited to isocratic methods and therefore to a limitated range of polymers. The reported gradient elution methods are using detection to 185nm (12), which is a inaccesible value for many detectors, or the use of the coupled columns (13).

Other HPLC methods are working with high temperature (14), derivatizated samples (15,16) or a mass spectrometer as a detector (17). The use of mass spectrometer can improve the detection limit with two orders (18) but higher than room temperature is used and therefore can produce thermal degradation of the polymers.

The size-exclusion chromatography (SEC) methods gave separation of PEGs into approximate groups (19).

We have found that the use of the nitric acid (12) for solvent absorbance equalisation at 190 nm, 2-36% acetonitrile gradient and the use of a short RP-C18 column (15 cm) makes possible the rapid separation of PEGs up to 1500 (n=1-50) at room temperature. The detection limit is 0.5 ug and the elution time 40 min. This method has been used in the study of laboratory anaerobic digesters.

EXPERIMENTAL

All chemicals and solvents were of analytical grade. For sample filtration, a syringe filter of type nylon, 0.45um, 25mm in diameter was used.

The PEGs used in this experiment were from: Panreac, 08110 Barcelona, Spain (PEG-200, PEG-300, PEG-400 and PEG-1500) and Merck-Schuchardt, 8011 Hohenbrunn bei München, Germany (PEG-600 and PEG-1000).

A Gilson HPLC system was used (Gilson Medical Electronics, Middelton, WI 53562 USA) equipped with: Pump 305(A) and 306(B), Manometric Module 805, Dinamic Mixer 811C and an 20ul Injector (Reodyne, Cotati POB 996, California 94931, USA).

A Dinamax UV-1 Absorbance Detector (Rainin Instrument CO, Mack Road, Woburn, MA 101801, USA)

equipped with a dual-pathlength flowcell (9mm and 1mm lightpaths) set at 190nm was used. The output of the detector was monitored by a Macinntosh Classic II Computer (Aplle Computer, Inc. California USA).

The analyses were made using a 15cm, 4.6mm id, Nucleosil 5u, C-18, 120A column (Tecnocroma, 08190 Barcelona, Spain) at room temperature. The acetonitrile in water gradient was used, as shown in Table 1. As an absorbance equalisation for water to 190 nm we used 3ppm nitric acid.

RESULTS AND DISCUSSION

The resolution. In Figure 1 the separation of the PEGs polymers with n between 1 and 50 are shown. This sample was obtained by a mixture of PEG-200, 300, 400, 600, 1000 and 1500, 50 mMolar each. The work conditions are as in Experimental. Only for Ethyleneglycol (EG) which is eluting at 1 min., very close to dead time (0.7 min.), the retention factor (capacity factor) is lower than 1. All other are higher, which is an important condition for good reproductability (20). As was shown (21,22), the retention factor for adjacent oligomers decreases with the increase of mobile phase strength which is equivalent to resolution decreasing.

In Table 2, column 2, 3 and 4 respectively, the range of n, the value of n corresponding to major polimer (nm) for every PEG and the resolution calculated near nm (Rm) are shown. The ranges of n were calculated taking into account all peaks with the area higher than 5% from a major polymer.

Up to PEG-600, the resolution is better than 1.5, which is a minimum value for complete separation of the adjacent peaks (23). For PEG-1000 the valley hight (the

Table 1. Gradient of Acetonitrile in Mobile Phase

time(min.)	A(%)	B(%)
0	96	4
10	70	30
41	28	72

A=1mM Phosphoric acid, B=1mM Phosphoric acid+Acetonitrile (1:1), Flow 1.5 ml/min

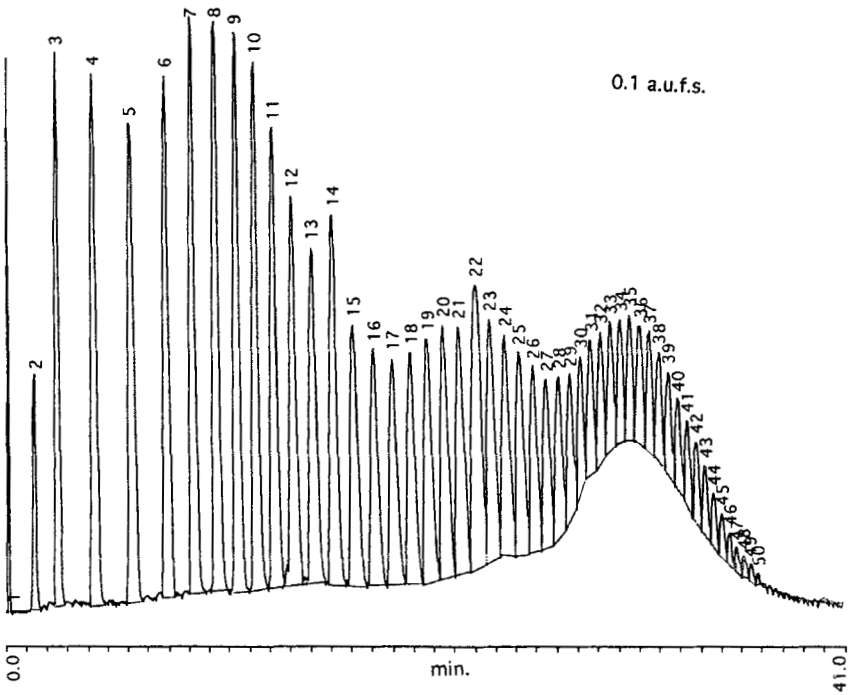

FIGURE 1. The separation of PEGs polymers, n = 1-50. (50 mM PEG-200, 300, 400, 600, 1000 and 1500). Conditions as in Experimental

Table 2. Range of n, n for major polymer (nm), Resolution close to nm (Rm) and Estimated Detection Limit for the first peak (DL)1 and for the first six peaks (DL)6.

PEG	range n	nm	Rm	(DL)1	(DL)6
TEEG	4	4(100%)	-	0.5	-
PEG-200	2-8	4(30.2%)	3.34	1.65	10.86
PEG-300	2-11	6(18.3%)	2.68	2.75	6.2
PEG-400	4-15	9(16.4%)	2.03	3.00	6.3
PEG-600	7-20	13(13.3%)	1.60	3.75	6.7
PEG-1000	14-32	23(9.2%)	1.00	5.5	6.8
PEG-1500	25-50	34(7.5%)	0.41	6.6	8.5

ratio of the valley level to the peak height relative to the base line) is smaller than 15% and for PEG-1500, it is around 50%. Therefore the study of individual polymers can be made with good precision, up to PEG-1000, and with medium precision for PEG-1500.

In Figure 2 the chromatogram obtained for PEG-1000 is shown.

Detection Limit (DL). The Detection Limit was tested with Tetraethyleneglycol (TEEG), n = 4, M = 194. For a ratio signal/noise 3.5 we found a value of 0.5 ug/injection equivalent to 25 ppm (w/v) TEEG in water (Injection = 20 ul).

Taking into account the polymer distribution as an n function, a Detection Limit for comerccial PEGs as a Detection Limit for major polymer, (DL)1, and as Detection Limit for the first six polymers, (DL)6, were evaluated (Table 2, column 5 and 6 respectively). In comparison with early reported results, the (DL)6 is better than using refractive index detector (40 ug, PEG-

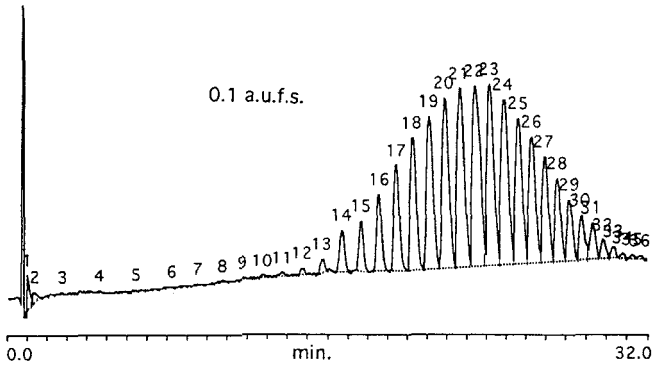

FIGURE 2. The PEG-1000 (10 mM) chromatogram. Conditions as in Experimental

400) (10,11) but is less (but comparable) than using methods involving sample derivatization (1 ul PEG-600) (16).

The linearity. Because the feed digestor is made with PEGs of 1% (w/v) concentration, the range of individual polymers is 0 - 0.1% (w/v) and corresponds with 0 - 20 ug/injection. Therefore, we have tested the liniarity with TEEG (n = 4) in the range 0-24 ug/injection. The calibration curve is shown in Figure 3.

The relation between the response (peak area) and quantity injected is given by the equation:

$$A = 7790.94 \times q + 375.05 \qquad (1)$$

where: A is peak area (uV x sec.) and q is the TEEG quantity injected (ug).

The correlation coeficient (r2) was 0.9982. The medium R.S.D. for differente concentrationes calculated with this calibration curve was around 3.00%

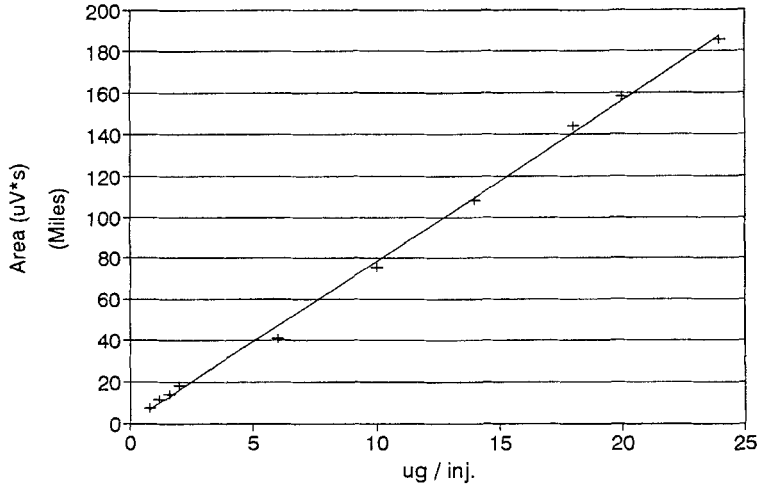

FIGURE 3. The calibration curve obtained for TEEG (n = 4, M =194)

The degradation of PEGs in an anaerobic digester. We have been studying the degradation of the PEGs as a sole source of carbon and energy in a laboratory stirring anaerobic digestor with the semicontinuous feed (daily) with PEG-200 and PEG-300 respectively of 1% concentration (w/v). The anaerobic bacteria were methanogenic cultures obtained from a municipal sludges digester. The volume of the digester was of 1 litre and was maintained at a constante temperature (36 C).

In order to determine the degradation process, an assesement was made of the molecular distribution of the PEGs for digestor samples, 24 h after feeding, and then compared with the initial configuration.

The degradation rate (r) for every polymer was calculed as:

$$r(\%) = (Qi-Qf)x100/Qi \qquad (2)$$

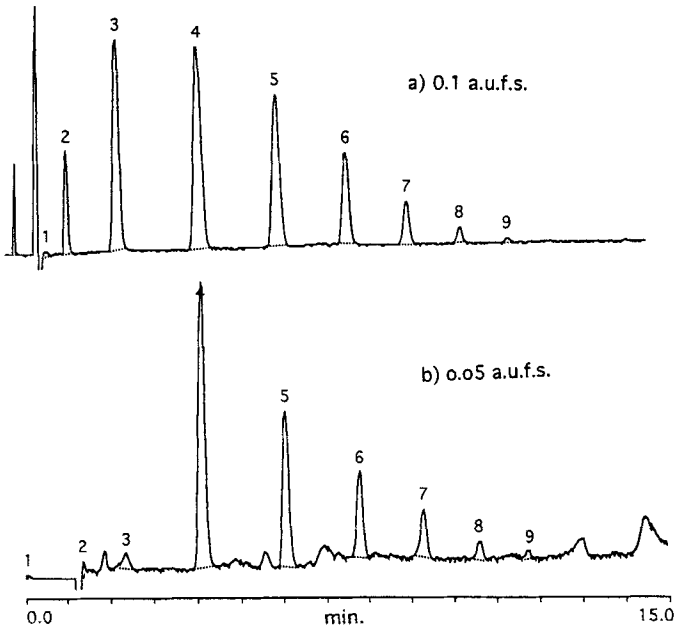

FIGURE 4. PEG-200 chromatogram. (a) initial, (b) from the anaerobic digestor. Conditions as in Experimental

where Qi and Qf are the initial and final quantity of polymer and were calculated from the peak area. R is a degradation rate for a correspondent PEG and was calculed in the same way from the sum of polymers.

An other parameter measured was the volume of gas results by anaerobic degradation (CH_4 + CO_2) which is also a indicator about degradation rate. When the gas production were smaller as 0.5 l a new quantity of bacterial culture was inoculated. In the Figure 4 the chromatogram for initial PEG-200 (a) and for a sample collected from the anaerobic digestor (b) at ten days after bacterial inoculation is shown.

The daily degradation rate r and R and gas production for PEG-200 from different days is presented

Table 3. Polymeric degradation rate, r, Total degradation rate, R, and Gas production (l) (daily) for PEG-200

n	r(%)				
	day 1	day 4	day 7	day 8	day 10
2	>96.48	>96.48	>96.48	>96.48	>96.48
3	97.26±0.41	98.72±0.18	97.69±0.06	>99.13	>99.13
4	52.73±2.36	58.80±0.41	81.29±0.62	87.34±0.02	91.96±0.52
5	59.67±2.01	76.70±0.40	92.40±0.26	95.07±0.02	96.27±0.54
6	60.79±2.11	71.17±0.34	92.90±0.34	94.63±0.04	95.74±0.63
7	58.47±3.02	68.46±0.91	89.980.76	88.50±0.02	93.18±0.90
8	57.87±3.10	65.32±2.6	79.08±2.20	83.81±0.02	>86.48
R(%)	68.71±1.56	76.01±0.21	89.89±0.75	93.49±0.06	95.49±0.57
Gas(l)	0.270±0.003	0.450±0.004	0.670±0.007	0.670±0.007	0.650±0.01

Table 4.Polymeric degradation rate, r, total degradation rate, R, and gas production (l) (daily) for PEG-300.

n	r(%)				
	day 1	day 4	day 7	day 8	day 10
2	>80.14	>80.14	>80.14	>80.14	>80.14
3	>95.58	>95.58	>95.58	>95.58	>95.58
4	46.67±2.18	58.95±1.57	93.60±1.72	93.78±0.83	>98.07
5	84.51±1.23	91.83±0.78	98.70±0.18	98.64±0.45	>98.67
6	81.94±0.50	90.87±0.94	98.58±0.47	98.48±0.37	>98.88
7	74.18±1.16	80.85±1.14	94.67±0.20	96.87±0.02	>98.88
8	72.69±1.01	78.51±0.15	91.12±1.09	95.75±0.88	>98.47
9	68.21±2.50	68.95±1.22	87.32±1.38	91.92±1.33	>97.56
10	70.67±2.30	61.80±0.53	79.89±6.4	91.59±1.21	>95.15
11	71.52±8.35	63.66±4.08	87.14±2.51	88.20±1.36	>89.39
R(%)	74.53±3.25	80.60±0.11	94.25±0.13	95.75±0.41	>98.5
Gas	0.250±0.002	0.430±0.004	0.680±0.007	0.710±0.007	0.680±0.01

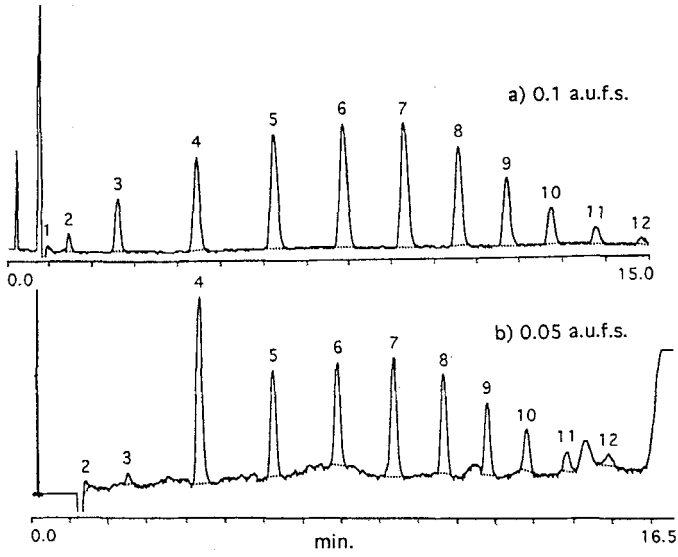

FIGURE 5. PEG-300 chromatogram. (a) initial, (b) from the anaerobic digestor. Conditions as in Experimental

in Table 3. The same data for PEG-300 are shown in Table 4.

In Figure 5 the chromatogram for initial PEG-300 (a) and a sample colected from digestor 13 days after bacterial inoculation (b) are shown.

Ten days after bacterial inoculation (100 ml, 5g/l suspension solids) the daily degradation ratio R for PEG-200 was changed from 68.71% to 95.49%. The degradation of PEG-300 was similar but with a degradation range of 74.00% to higer than 98.5%. Taking into account that a degradation rate higer than 98.5% is out of detection limit, the degradation of the PEG-300 after 10 days can be complete.

For every PEG, a linear corelation between the quantity of a PEG degraded and the volume of gas eliminated was observed (Table 3 and 4).

We found that daily degradation rate for PEG-200 and PEG-300 depending on polymeric length. The elution time for $\underline{n}$ = 1, in the digestor samples is superimposed with the elution time corresponding to acetic acid and ethyl alchol present as degradation products (7). Therefore the EG ($\underline{n}$ = 1) was not measured. For $\underline{n}$ = 2 and n = 3 the degradation is very strong, about 100%. This is in acordance with the data of Chemical Oxigen Demand (COD) reported for Diethyleneglicol (DEG), Triethyleneglycol (TEG) and PEG-400 (1). For n = 4, in both situations we found a lower rate relative to the polymers with n > 4. As a result of this, the polymeric distribution for the samples colected from the digestor are very diferrent in comparison to the initial PEGs (Figure 4 and 5).

For PEG-200 the polymeric degradation rate for $\underline{n}$ >4 are very similary. In the situation of the PEG-300 the polymers $\underline{n}$ = 5 and $\underline{n}$ = 6 have the degradation rate higher than $\underline{n}$ > 7.

The order of dgradation rate, $\underline{r}$, observed for diferrent values of $\underline{n}$ are:

PEG-200: 2 = 3 >> 5,6,7,8,9 > 4
PEG-300: 2 = 3 >> 5,6 > 7,8,9,10,11 > 4

These results can be suported by the exitence of two types of bacterium strain, one degrading short oligomers (up TEG, n=3) and the other one degrading higher polymers, with depolimerization action out of the cell (2), in contrast with the hypothesis regarding the penetration of the cytoplasmic membrane by longer polymers to the bacterium cell (8,24). It must be noticed the smaller r value for n = 4 polymer. Experiments are in progress in our laboratory to examine the degradation of higher PEGs.

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