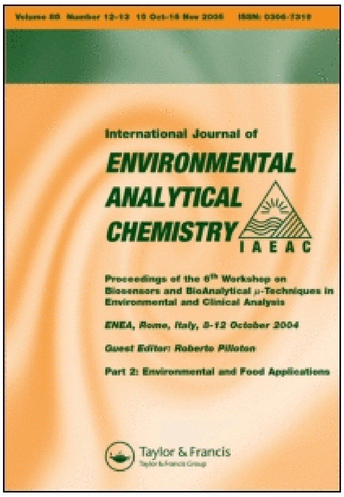

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Fast Interpretation Of 3D Liquid Chromatography Signals: Application To The Study Of Organic Matter In Wastewaters

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FAST INTERPRETATION OF 3D LIQUID CHROMATOGRAPHY SIGNALS: APPLICATION TO THE STUDY OF ORGANIC MATTER IN WASTEWATERS

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The aim of this work is to propose a very simple method for the selection and interpretation of the relevant individual spectra of a set of absorbance spectra, as for example in a 3D chromatogram. The method is based on the rank computation of the matrix of experimental values, after or during a chromatographic run. From a classical chromatographic system with a rapid scanning spectrophotometer (diode array detector for example), a computer is used for data acquisition of absorbances of the whole UV spectrum, and later computation. Firstly, acquisition is performed until the end of the elution and then the program gives the number and the corresponding spectra of relevant fractions. If another run is needed, for a different sample for example, the procedure makes it possible to check whether the detection signal is independent of the previous spectra. Different applications are presented for the study of organic matter in wastewaters by low pressure liquid chromatography.

KEY WORDS: UV absorptiometry, gel chromatography, chemometry, water analysis, organic matter.

INTRODUCTION

The interpretation of 3Dimension liquid chromatography signals is theoretically easy because the fractions have to be well separated by the chromatographic column. But in practice the analyst often works at only one or two wavelengths because of the lack of rules for the choice of a detection method. In that case, the use of a 3D acquisition system is of little interest. However, it is very important to be sure that the user is able to control the detection system as well as the chromatographic part.

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This condition is very important for environmental studies, particularly if the analyst wants to work directly on raw samples for qualitative analysis or for the development of on line techniques.

The aim of this paper is to propose a fast interpretation method of the 3D response of a liquid chromatographic system. This method gives the relevant information on the set of spectra for each chromatographic assay, and allows the comparison of the results within different samples. The proposed method must be also simple and easy to apply for any analytical chemist. It is one of the simplest ways of interpreting UV spectra.[1].

The main illustration of this method is the qualitative study and the comparison of the organic matter of raw and treated wastewaters from a simple analytical method involving gel chromatography and UV absorptiometry. This experimentation complements other methods of UV examination of waters[2,3] and intends to show if a relation between the different forms of the organic matter of the waters and wastewaters and their UV absorptiometric spectrum can be established.

EXPERIMENTAL

Several samples of waters and wastewaters were chosen for this work. From the most concentrated sample to the least, we selected successively a landfill leachate, urban wastewater before and after a biological treatment, and synthetic natural water. For all these samples the same methodology was implemented after a 0.45 μ filtration:

— the measurement of the Dissolved Organic Carbon (DOC) and the layout of the UV absorption spectrum,

— the concentration of the sample if necessary,

—the analysis of the sample with low pressure gel chromatography for the molecular weight separation,

— the analysis of the chromatographic fractions (UV absorption spectra).

The DOC measurement was performed with a cold UV oxidation procedure, the concentration with a vacuum evaporation system (temperature 30°C), the chromatography with a Sephadex G–25 gel and the UV absorption spectrum with a spectrophotometer between 200 and 350 nm (with a 2 nm bandwith and a 10 mm quartz cell). The chromatographic column was 70 cm long and 1.6 cm of internal diameter, and the flow rate was 30 ml/h. The eluent was a 75.4% KH_2PO_4- 24.6% Na_2HPO_4 (0.0667 M each) solution corresponding to a 0.1 M ionic strength and a 6.4 pH. For the leachate chromatography, the conditions were slightly different[4]: a column 100 cm long and 2.06 cm of internal diameter, distilled water as eluent with a flow rate of 140 ml/h, and the sample diluted to a DOC value between 150 and 200 mg/l.

Therefore, for each sample the DOC value, the 3D chromatogram, and for some samples the DOC of the main fractions were obtained.

The identification of the relevant fractions was carried out through the individual spectra of the 3D chromatogram. The use of this method can be generalized for the study of the composition of mixtures and the comparison of absorption spectra. To this end:

— the matrix A is built from the absorbance data of the n fraction files (for m wavelengths λ):

$$A = \begin{pmatrix} A_1(\lambda_1), A_1(\lambda_2), & \ldots & A_1(\lambda_m) \\ A_2(\lambda_1), A_2(\lambda_2), & \ldots & A_2(\lambda_m) \\ \vdots & & \vdots \\ A_n(\lambda_1), A_n(\lambda_2), & \ldots & A_n(\lambda_m) \end{pmatrix}$$

— the rank of the matrix A is computed either with a simple procedure[5] based on a variant of the Gaussian method, or with another method using the singular value decomposition[6]. The rank of the matrix is equal to the number of independent rows corresponding to the reference spectra. Any spectrum of the chromatogram can be restituted from the reference spectra: the coefficients of the linear combination are computed from the following relation with a least squares method:

$$AB(\lambda_i) = \sum_{j=1}^{p} a_j.SR_j\,(\lambda_i)$$

where $AB(\lambda_i)$ is the absorbance of a fraction, for a wavelength λ_i, and aj the coefficient of the jth reference spectrum whose absorbance value for λ_i is $SR_j(\lambda_i)$.

At the end of the procedure, the analyst is able to easily determine the qualitative nature of the organic material of any fraction, from the knowledge of all the coefficients a_j.

RESULTS

Landfill leachate

The sample was collected from a 70 m^3 lysimeter filled with household refuse. Then, the previously reported analytical scheme was applied. Its DOC value was 350 mg/l. Figure 1 shows the UV spectrum of the diluted leachate (20 times) which is typical for any wastewater: its shape is monotonous and decreases in mainly two parts, the first around 220–230 nm and the second near 260–270 nm.

Figure 2 presents the 3D chromatogram which is obtained with the 21 eluted fractions. Its examination shows 2 main sets of fractions. The first one corresponds to exclusion of macromolecules (MW > 5000), which could be humic substances, and the second corresponds to smaller molecules such as carboxylic acids, commonly found in landfill leachates[4,7].

The rank method gives the UV spectra of the relevant fractions, presented in Figure 3. Four fractions were detected which correspond to elution volumes of 180, 340, 380 and 430 ml, or K_{av} of 0, 0.56, 0.72 and 0.92, respectively. The K_{av} value represents the fraction of the stationary gel volume where the concerned molecules are diffusing. The first one corresponds to the first peak of the chromatogram (macromolecules) and the others belong to the second part of the chromatogram (small molecules) and are very close to each other. Different shapes are observed among the four spectra, especially between the first one and the others, which suggest the presence of the different types of molecules, and allow to get some insight on the analysis of the chromatogram.

Urban raw and treated wastewaters

Two types of samples (instantaneous) were taken from a biological wastewater treatment plant, before and after the treatment. The DOC were, respectively, 52 mg/l and 15 mg/l. Their UV spectra (Figure 4) are obviously different and seem to be homothetic with an absorbance ratio very close to the respective DOC values (note that this observation could be made with the absorbance of any wavelength). Figure 5 shows the chromatograms of the two samples recorded at 210 nm which is classically chosen for this type of analysis. The profiles are very different, as the chromatogram of the raw sample (r) reveals 5 peaks (with 2 important ones), whereas the treated sample (t) shows mainly one large peak, at a similar retention time to the last peak of the raw sample. It means that during the biological treatment, the components of high molecular weight were biotransformed, so the compounds of smaller molecular weight were dominant in the treated wastewater.

The spectra of the relevant fractions are presented in Figure 6. Three spectra correspond to the chromatogram of the raw wastewater (ra, rb, rc) and two to the treated wastewater (ta

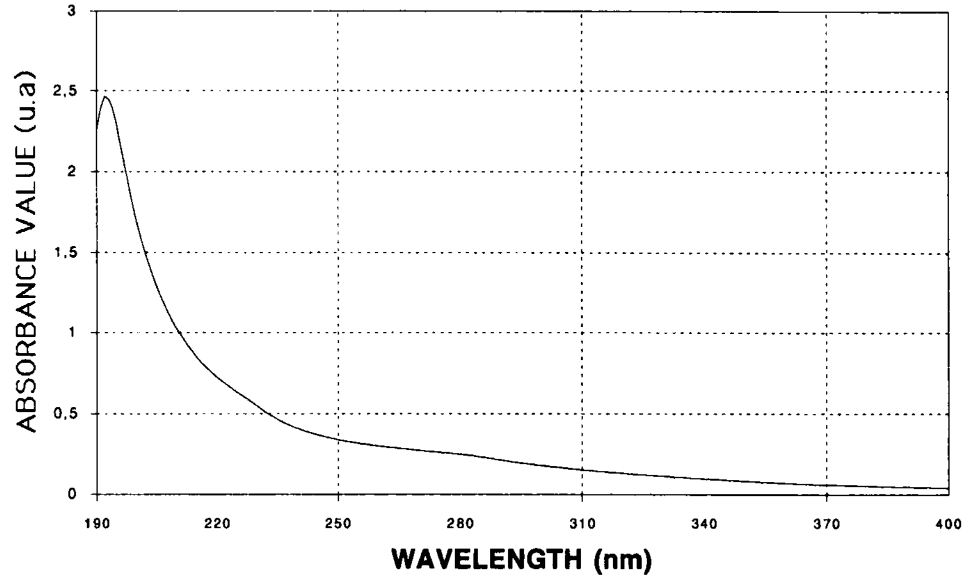

Figure 1 UV spectrum of landfill leachate (pathlength 10 mm).

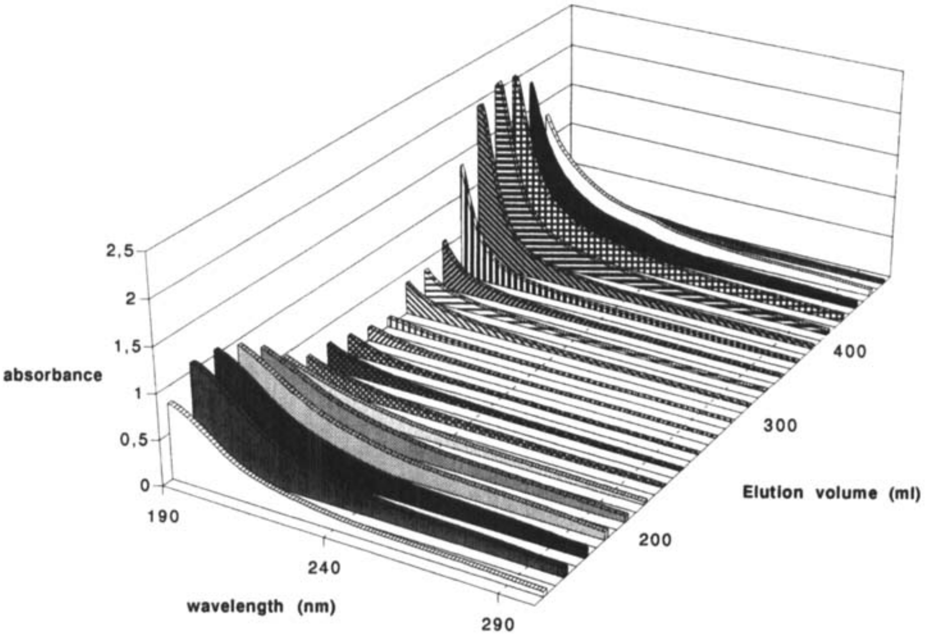

Figure 2 3D chromatogram of landfill leachate (pathlength 10 mm).

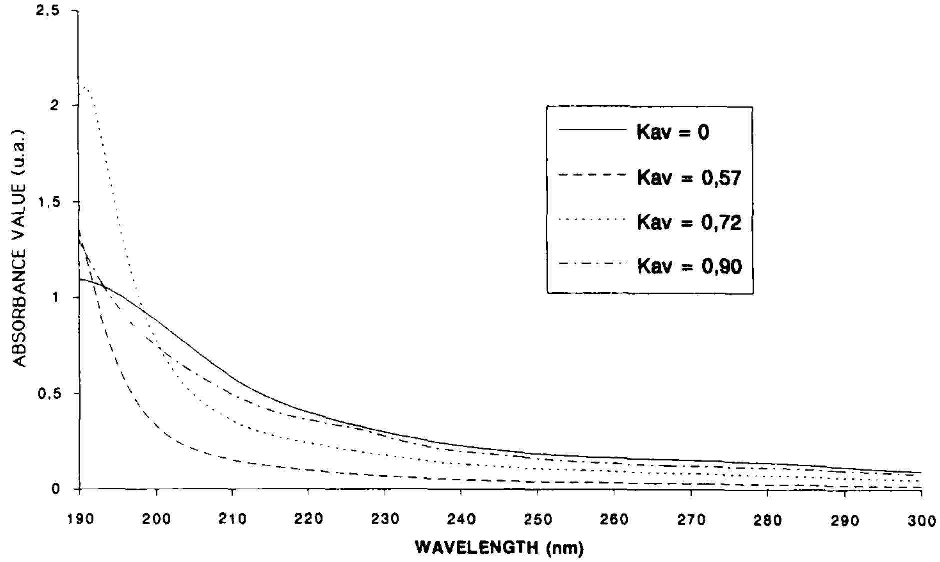

Figure 3 UV spectra of fractions of landfill leachate selected with the rank method (pathlength 10 mm).

and tb). The corresponding elution volumes are 79, 104 and 130 ml (K_{av} of 0.28, 0.61 and 0.96) for the raw sample, and 78 and 134 ml (K_{av} of 0.27 and 0.98) for the treated one. The comparison of the shapes and the K_{av} of the relevant fractions shows that the extreme fractions are quite similar. The first one, which corresponds to components of molecular weight around 1000, has a shoulder around 210–220 nm and a non negligible absorbance up to 300 nm. The second is very different with a high peak at the beginning of the spectrum due to the presence of small components. The intermediate fraction of the raw sample is typical with a shoulder around 220–230 nm. The molecular weight is about 500.

Proposition of a general method

It is now possible to propose a general method for the examination of chromatograms of new samples from the selected reference spectra. First, for each UV spectrum acquisition of a new fraction, the file of absorbances is added to the reference matrix (the p lines of which correspond to the absorbances of the p reference spectra). Second, the rank of the new matrix is computed, and if the rank increases we can conclude that the new spectrum is independent from the reference spectra: it must be considered as a new type of organic material. Otherwise, if the rank is unchanged, two conclusions are possible:

— the new spectrum does not belong to the list of the reference spectra and its contribution can be computed (then the analyst is able to identify the characteristics of the new fraction),

— the list of reference spectra is modified and the new one is included. It means that this spectrum is considered as a new reference one and corresponds to a more extreme state, or to an unexpected change in the system.

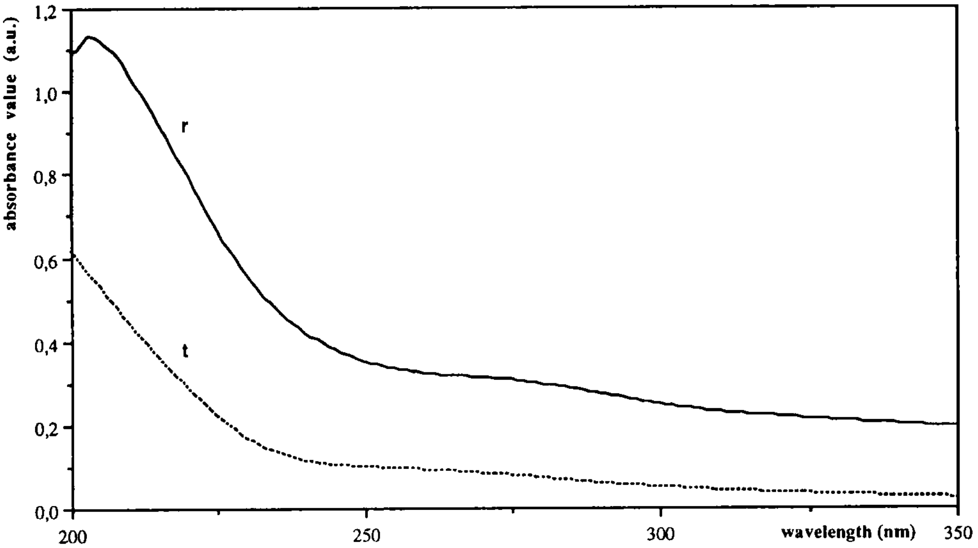

Figure 4 UV spectrum of raw (r) and treated (t) wastewaters pathlength 10 mm).

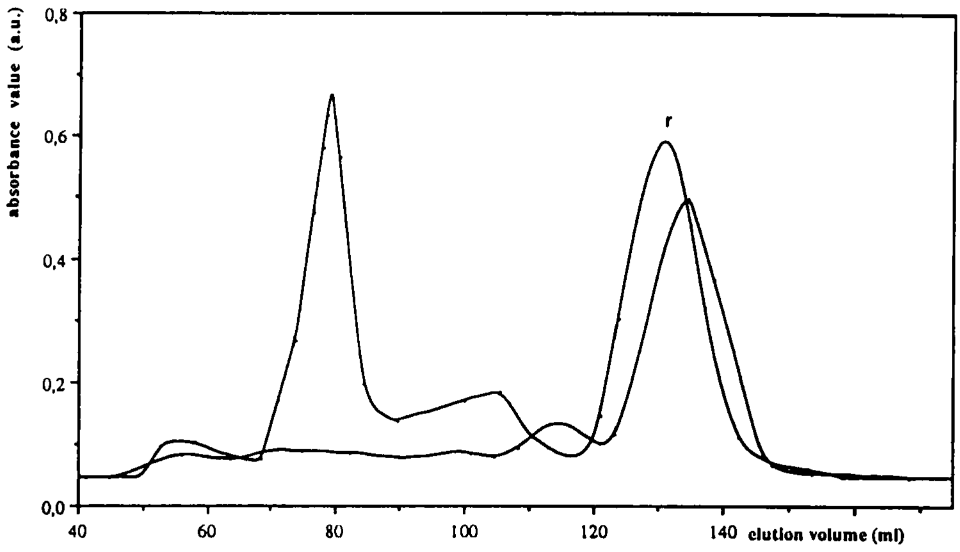

Figure 5 Chromatogram of raw (r) and treated (t) wastewaters (wavelength 210nm, pathlength 10 mm).

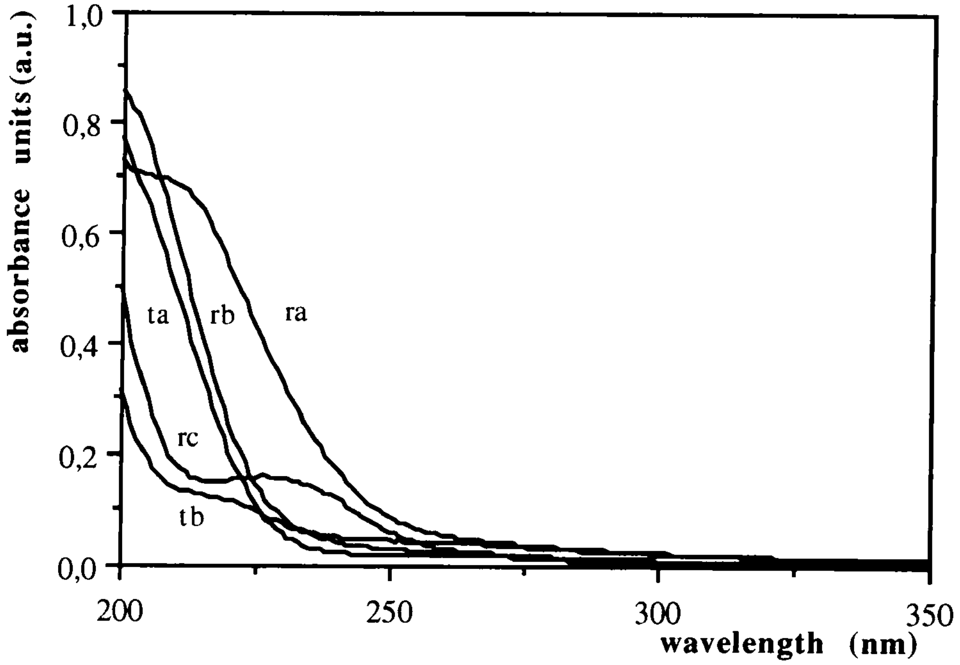

Figure 6 UV spectra of fractions of raw (ra, rb, rc) and treated (ta, tb) wastewaters selected with the rank method (pathlength 10 mm).

In the application of this method, another experimentation was performed in order to verify whether any similarities could be found between the previous samples of wastewaters and natural water. We chose synthetic natural water made from 4 g of *Populus sp.* dry leaves and 2 l of distilled water. After 24 hours the leaves were removed and the water was then treated with the procedure described.

The UV spectrum of the sample shown in Figure 7 is characterized by a big shoulder between 250 and 270 nm. As a result of the rank method, 3 fractions were identified with Kav values of respectively 0.14, 0.27 and 1.00, despite a complex chromatogram. The spectra of the relevant fractions show a shoulder between 250 and 280 nm, especially the second one which is likely to contain phenolic compounds.

For the comparison of the shapes of the different reference spectra, the rank method was applied to all spectra of fractions of the previous chromatograms (about 120 fractions). Table 1 presents the results (selected spectra and error estimation) for the two methods which are used for the rank determination, the classical Gaussian elimination[5] and the singular value decomposition[6]. The last one is best suited for the rank determination because of the nature of the error estimation which is more robust than that of the Gaussian method. Both methods split each spectrum as a sum of two: the first one spanned by the selected reference spectra and the second as the residual spectrum with a minimal norm. The error is computed as the ratio between the maximum of the norms of all residual spectra and the maximum of the norms of all spectra. The two methods differ only in the evaluation of the norm of a spectrum: it is the maximal absorbance for the Gaussian method and the square root of the sum of the square of the absorbances for the singular values decomposition. If we consider the variation of the error value with the rank (Table 1), a gap appears in both procedures, corresponding to an accepted error lower than 4%.

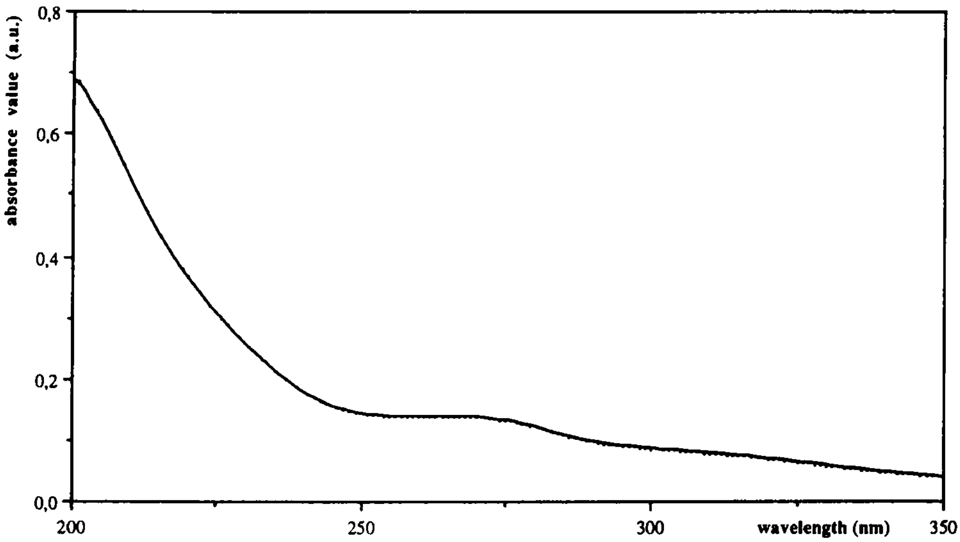

Figure 7 UV spectrum of synthetic natural water (pathlength 10 mm).

Table 1 Rank determination among 120 spectra of selected fractions of chromatograms: five spectra give an error lower than 4%.

	Gaussian method		*Singular values method*	
Rank	*Selected spectra**	*Error %*	*Selected spectra**	*Error %*
1	1	41.4	3	58.2
2	1,2	38.4	3,2	31.0
3	1,2,3	17.5	3,2,4	25.2
4	1,2,3,4	10.9	3,2,4,1	8.8
5	1,2,3,4,5	3.9	3,2,4,1,5	2.6
6	1,2,..,6	2.4	3,2,..,5,7	1.8
7	1,2,..,7	1.9	3,2,..,7,9	1.4
8	1,2,..,8	1.8	3,2,..,9,8	0.9
9	1,2,..,9	1.5	3,2,..,8,6	0.5
10	1,2,..,10	1.2	3,2,..,6,10	0.3

*the numbers given to the selected spectra are arbitrary and correspond to the order of apparition in the Gaussian method

Therefore, five reference spectra were obtained (Figure 8): two for the leachate (K_{av} = 0 and 0.56), two for the raw wastewater sample (K_{av} = 0 and 0.98), and one for the natural water sample (K_{av} = 0.27). These spectra are the same with the two methods of rank determination.

The main conclusion is that the computation of the rank makes it possible to concentrate the information, as only five spectra are needed for the explanation of all the others. It is also shown that the spectra of the treated wastewater fractions do not appear, the organic matter of this sample being not as typical as the others, from a qualitative point of view. Further studies will be required for the characterization of the different relevant fractions.

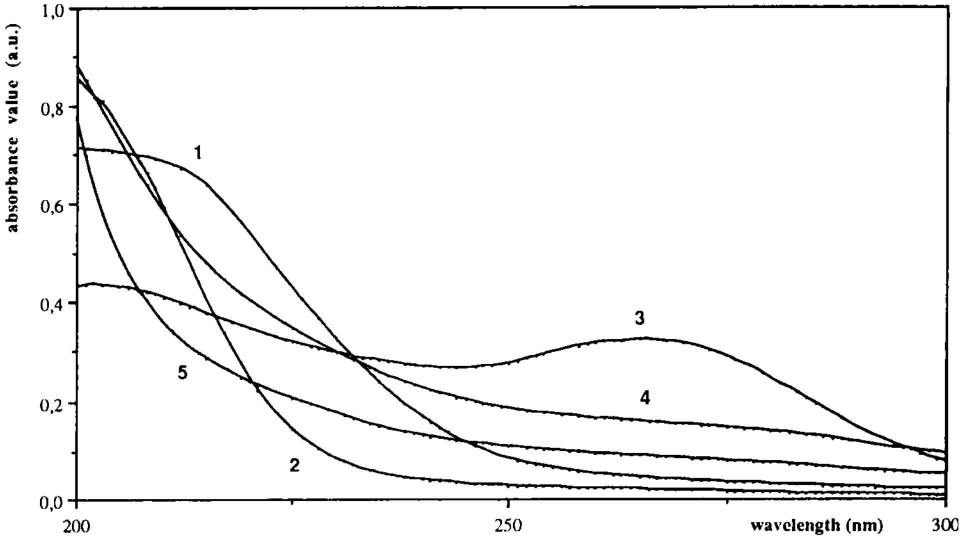

Figure 8 UV spectra of selected fractions (with the rank methods) of all samples (pathlength 10 mm): two coming from the raw wastewater (1,5), two from the landfill leachate (2,4) and one from the synthetic natural water (3).

Thus, the answer to the initial question about the similarity of the organic materials of waters is not complete but we have easily shown how some simplifications could be made for further studies.

CONCLUSION

The simple procedure presented here is able to identify the relevant fractions of a chromatogram (if their spectra are different) and to compare the spectra of the fractions for different samples. It is useful for the interpretation of chromatograms of complex mixtures such as environmental samples but also for the development of chromatographic methods. The mathematical separation must be considered as an aid for chemical separation. It can also be directly applied to the comparison and the interpretation of the UV spectra of waters and wastewaters.

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