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Evaluation of a Newly Developed Enzyme-Linked Immunosorbent Assay for Determination of Linear Alkyl Benzenesulfonates in Wastewater Treatment Plants

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A recently developed enzyme-linked immunosorbent assay (ELISA) for the determination of linear alkyl benzenesulfonates (LAS) and long chain sulfophenyl carboxylates (SPCs) has been evaluated for its application in wastewater control analysis. This ELISA based on the use of polyclonal antibodies in an indirect format shows an IC_{50} of 28.1 ± 3.2 μg L^{-1} and a limit of detection (LOD) of 1.8 ± 0.6 μg L^{-1} in buffer. The assay uses antibodies raised through a *pseudo*heterologous immunization strategy using an equimolar mixture of two immunogens, *N-(4-alkylphenyl)sulfonyl-3-aminopropanoic acid* covalently coupled to *keyhole limped hemocyanin* **(SFA-KLH)** and *sulfophenyl carboxylate* **$13C_{13}$** coupled to KLH **($13C_{13}$-SPC-KLH)**. The immunizing haptens have been designed to address recognition versus two different epitopes of the LAS molecule. To assess the performance of this immunoassay in complex real samples, a cross reactivity study was carried out, and the possible interference of other surfactants commonly detected in wastewater, including nonylphenol ethoxylates (NPEOs), nonylphenol (NP), octylphenol (OP), and coconut fatty acid diethanol amides (CDEA), have been evaluated. Additionally, a study of the matrix effects of different types of wastewater was achieved. This ELISA has been evaluated and validated by measuring the LAS content of 22 samples collected from the influents and the effluents of six wastewater treatment plants (WWTP) located in Catalonia, Spain. A solid-phase extraction followed by liquid chromatography coupled to mass spectrometry detection (SPE-LC-MS) has been used as a validation method of the new ELISA test.

Introduction

Surfactants are one of the major organic pollutants found in water bodies because of their extensive use. The linear alkyl benzenesulfonates (LASs) global consumption is continuously increasing, for example, the world consumption in 1988 was estimated around 1.3×10^9 kg, and 10 years later in 1998 it was around 2.4×10^9 kg (*1*, *2*).

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Commercial LAS consists of a mixture of at least 20 individual compounds and isomers (*3*). The environmental fate of LAS is of interest due to their consumption and their direct disposal to wastewater. The water treatment processes can generally remove up to 98% of the LAS content in the raw influent (*4*, *5*), during physicochemical treatments. The precipitation and adsorption displace up to 70% of the LAS in water to the sludge (*6*), being as how the aerobic degradation is the major elimination route (*7*). Biodegradation is faster for the higher alkyl chain LAS (*8*, *9*). However, due to the high concentrations in primary influents, some quantities of LAS together with its aerobic breakdown intermediates, the sulfophenyl carboxylates (SPCs), can reach receiving waters. SPCs are polar compounds formed by the LAS ω-oxidation of the alkyl chain terminal carbon followed by successive β-oxidations (*10*), and their complete degradation ends in the opening of the aromatic ring. SPCs can be generated by the self-purification of wastewater under aerobic conditions (*11*) and during wastewater treatment processes due to an incomplete degradation of LAS. The concentration of SPCs in WWTPs effluents usually is approximately the same or higher in the influents (*12*, *13*). The SPCs having medium carboxylic chain lengths (C6−C8) are the most abundant products in water (*14*) and the long chain in sludge.

Different papers have reported the synergistic toxic effects between LAS and pesticides, such as parathion and dieldrin (*15*), or metal bioaccumulation (*16*). Due to their high usage, an efficient detection of their presence in wastewater is necessary.

The quantitative analysis of LAS and SPCs in environmental matrices requires generally the application of LC-MS (*17*−*19*).

The official semiquantitative method for the rapid screening of LAS is the colorimetric determination of methylene blue active substances (MBAS) (*20*).

Nowadays, this method can be performed automatically involving a sequential flow analysis; however, this method presents as a main drawback an extremely high overestimation. In a previous work of our group was compared LAS determined by MBAS and by LC-MS (*13*), being the contribution of LAS to the total MBAS from 11% to 28%. These results were in agreement with other studies comparing MBAS and LC-MS methods (*21*). Immunochemical techniques are selective, sensitive, rapid, and cost-effective. In this work, the performance of an ELISA based on an indirect competitive assay with a limit of detection of 1.96 μg L^{-1} and a IC_{50} of 35.48 μg L^{-1} (*22*) has been evaluated for the rapid determination of the LAS content in WWTP as an indicator of the proper functioning of these plants.

The main objectives of the present work were as follows: (1) To study the performance of a new immunoassay for the rapid estimation of the LAS content in wastewater samples. In this sense, the possible interference of main surfactants identified in wastewater and the matrix effects of different wastewaters were studied. (2) To compare the functioning of different WWTP by the application of this new ELISA, in order assess the LAS concentrations in the influents and effluents of six WWTPs, with different proportions of mixed urban and industrials inputs. (3) To compare the data obtained by the ELISA method with those from a chromatographic SPE-LC-MS.

To our knowledge this is the first paper using an ELISA test for determining LAS in wastewater effluents.

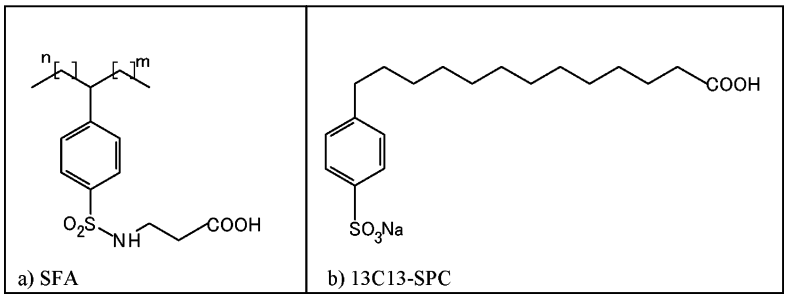

FIGURE 1. Chemical structure of the haptens of immunogens: (a) SFA (sulfonamide) and (b) 13C13-SPC sulfophenylcarboxylate).

Experimental Section

Chemicals. The commercial mixture of linear alkyl benzene sulfonates (LAS) with a low dialkyltetralinsulfonates (DATS) content (<0.5%) was supplied by Petroquimica Española S.A. (San Roque, Cádiz, Spain) in a single standard mixture with the proportional composition of the different homologues as follows: C_{10}, 3.9%; C_{11}, 37.4%; C_{12}, 35.4%, and C_{13}, 23.1%. The coconut fatty acid diethanol amide (CDEA) used is a mixture of the homologues C_7 (7%), C_9 (7.5%), C_{11} (60.9%), C_{13} (18%), and C_{15} (6.6%) and was supplied by H. Fr. Schröder from the Institut für Siedlungswasserwirstschaft (Aachen, Germany). The high purity standard (98% pure) of 4-*tert*-octylphenol (OP) and standards of 4-nonylphenol (NP) were obtained from Aldrich (Milwaukee, WI). The standard of nonylphenol polyethoxylates (NPEO) as a mixture of chain isomers and oligomers with an average of 6 ethoxy units was purchased from the Kao Corporation (Barcelona, Spain). All solvents (water, acetonitrile, and methanol) were HPLC grade and were purchased from Merck (Darmstadt, Germany), and filter membranes were purchases from Scharlau (Barcelona, Spain).

ELISA Buffers and Instrumentation. PBS is a 0.2 M phosphate buffer with 0.8% saline solution (pH 7.5). PBST is PBS with 0.05% Tween 20. The coating buffer is a 0.05 M carbonate-bicarbonate buffer (pH 9.6). The citrate buffer is a 0.1 M solution of sodium citrate (pH 5.5). The color substrate solution is 3,3′,5,5′-tetramethylbenzidine (0.01%) and H_2O_2 (0.004%) in citrate buffer. Enzymatic reaction was stopped by adding 2 M H_2SO_4. ELISA polystyrene microtiter plates were purchased from NUNC (Maxisorb, Roskilde, Denmark). Microtiter plates washing steps were carried out using a SLT 96PW microtiter washer (SLT Labsinstruments GmbH, Salzburg, Austria). ELISA absorbances were read on a SpectramaxPlus (Molecular Devices, Sunnyvale, CA) at a single wavelength mode at 450 nm. The competitive curves were analyzed with a four parameter logistic equation using the software SoftmaxPro v2.6 (Molecular Devices) and GraphPad Prism (GraphPad Sofware Inc., San Diego, CA). Data presented correspond to the average of two well replicates.

Immunoreagents. The preparation of the polyclonal antibody As98 and the coating antigen $7C_7$-CONA used in this ELISA has been described before (*22*). The immunization protocol was performed on female New Zealand white rabbits, and the As98 was obtained through a heterologous immunization procedure by combining the two immunogens shown in Figure 1. The **SFA** hapten maximizes recognition of the alkyl moiety while preserving the complexity of the different alkyl chains present in the LAS technical mixture. The **$13C_{13}$-SPC** hapten addresses recognition of the common and highly antigenic phenylsulfonic group. The coating antigen $7C_7$-CONA is 4-(1-carboxy-7-heptyl)phenylsulfonic acid covalently attached to conalbumin via the active ester method. Goat antirabbit IgG-horseradish peroxidase (anti-IgG-HRP) was provided by Sigma (cat. number R2004, St. Louis, MO).

WWTPs and Sample Collection. Six WWTPs located in Catalonia, Spain, were selected for this study. The WWTPs ranged from completely urban influents to mixed discharges with a high industrial input. The selected plants were as follows: Besós (WWTP-1), Igualada (WWTP-2), Terrassa (WWTP-3), Martorell (WWTP-4), La Llagosta (WWTP-5), and Depurbaix (WWTP-6). One of them (WWTP-1) performs only a physicochemical treatment, while the others have primary and secondary treatments. WWTP-1 receives urban discharges with an inflow about 500 000 m^3 day^{-1}. WWTP-6 is the newest one; it receives urban discharges of 420 000 m^3 day^{-1} and gives service to 2 000 000 of inhabitants. On the other hand, WWTP-2, WWTP-3, WWTP-4, and WWTP-5 received mixed influents, specially WWTP-2 with a high input of industrial wastes from tannery industries. A total number of 22 samples from the influents and effluents of the six WWTP were taken in May and June 2004. Samples were collected in glass bottles and were stored at 4 °C immediately after sampling. The extraction was carried out during the 24 following hours after the sampling in order to avoid any degradation of target compounds, and a part of each sample was preserved in the freezer.

Sample Pretreatment and Solid-Phase Extraction. The main degradation products found after the secondary treatment are 6C6 and 7C7 SPCs whose cross reactivities were very low (see Table 1). For this reason, the extraction protocol followed here was focused on the isolation of LAS and the majority of the surfactants content in the samples. Since chromatographic analysis of SPCs was not the objective of the present work, the extraction procedure has not been carried out in acidic conditions for the isolation and analysis of SPCs. All samples were filtered through a 0.45 μm membrane filter, and the preconcentration step was according to a previous protocol (*19*). A solid-phase extraction (SPE) with LiChrolut RP-18 (500 mg, 6 mL) sorbent (Merck, Darmstadt, Germany) was performed using an automated sampler processor ASPEC (Automated Sample Preparation with Extraction Columns) XL from Gilson (Villiers-le-Bel, France), fitted with a 817 switching device and an external 306 LC pump. The SPE cartridges were conditioned passing 7 mL of acetonitrile, 5 mL of methanol, and 5 mL of LC-grade water through the cartridges at a flow rate of 3 mL min^{-1}. Two hundred milliliters of filtered samples was percolated through the SPE-cartridges at 5 mL min^{-1}. After the preconcentration step the C_{18} cartridges were dried, and the elution was carried out using acetonitrile (2 × 5 mL). After elution the extracts were evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 mL. After solid-phase extraction the extracts were analyzed by SPE-LC-MS and in parallel reconstituted with the assay buffer and analyzed by ELISA. The already extracted wastewater samples were kept and treated as "blank samples" (absence of LAS) in order to use them to evaluate the matrix effect of the different types waters on the ELISA (see below).

TABLE 1: Cross Reactivity Percentage Observed with the LASs-SPCs ELISA (As98 and 7C7-CONA)

group	analyte	CR%
LAS	LAS commercial mixture	**100**
LAS isomers[a]	C8	**56**
	C10	**155**
	C12	**95**
	C14	**13**
	C16	**4**
	C18	**4**
	C20	**3**
SPCs[a]	2C3	<3
	2C4	<3
	2C5	**3**
	3C4	<3
	3C5	**3**
	3C6	<3
	5C5	**6**
	6C6	<3
	7C7	**5**
	9C9	**379**
	12C12	**7138**
	13C13	**5550**
phenyl sulfonic acids[a]	*p*-toluene sulfonic acid	**4**
	xylene sulfonic acid	**3**
	ethylbenzene sulfonic acid	<3
	benzene 1,3-disulfonic acid	<3
naphthalene and benzene sulfonates	1-naphthalene sulfonate[a]	**5**
	1,5−naphthalene disulfonate[a]	<3
	1,3,5-naphthalene trisulfonates[a]	**4**
	4-methylbenzene sulfonates	**3**
	4-chlorobenzene sulfonate	**4**
	3-nitrobenzene sulfonates	<3
	2-amino-1-naphthalene sulfonate	**4**
	1-amino-5-naphthalene sulfonate	< 3
coconut diethanol amides	CDEAs technical mixture	<3
nonionic surfactants	nonylphenol	<3
	octylphenol	<3
	nonylphenol ethoxylates (technical mixture) NPEOs	<3
	nonylphenol ethoxylate NP_1EO	<3
	nonylphenol ethoxylate NP_2EO	<3

[a] CR% values from ref *24*.

Liquid Chromatography−Mass Spectrometry Analysis. The extracts were analyzed using a reference method based on LC-MS. The protocol used here had been widely reported and arranged to different studies for LAS analysis (*13*, *17*, *19*, *23*, *24*). This protocol based on LC-MS was carried out using an HP 1100 auto sampler with a 100 μL loop and an HP 1090 A LC binary pump, both from Hewlett-Packard (Palo Alto, CA). The separation was performed under gradient elution conditions described in previous work (*19*), using methanol (A) and water (B). LASs were detected under negative ionization conditions. To obtain a more detailed analysis of the samples, other surfactants and related compounds such as nonylphenol (NP), octylphenol (OP), and nonylphenol ethoxylates (NPEO) were also investigated. LASs, NP, and OP were detected under negative ionization (NI) conditions, while NPEOs were analyzed under the positive ionization (PI) mode. In both modes the compounds are separated using the following solvent programming: initial conditions were 30% A, in 10 min it is linearly increased to 80%, then increased linearly in 5 min to 90%, and finally increased again to 95% in 5 min more. Detection was carried out, using an HP 1040M diode array UV−vis detector coupled in a series with an LC-MSD HP1100 mass-selective detector, equipped with an atmospheric pressure ionization source and ESI interface. The operating parameters of ESI-MS were as follows (NI/PI): drying gas flow, 12/11 L min^{-1}; drying gas temperature, 375/325 °C; nebulizer pressure, 55/50 psi (1 psi = 6894.76 Pa); capillary voltage, 4500/4000 V; fragmentation voltage, 90/60 V. Quantitative analysis was performed in a selected ion monitoring (SIM) mode, using external calibration. Five-point calibration was performed, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 6 injections. The confirmation of compound identity in environmental samples was done in a full scan mode.

Operational Conditions of the Optimized ELISA. Microtiter plates were coated overnight with 7C7-CONA in coating buffer (0.08 μg mL^{-1}, 100 μL well) at 4 °C. After incubation, the plates were washed with PBS (4 times, 300 μL well) and LAS standard solutions, or samples were added to the coated plates (50 μL well), followed by the antiserum As98 (1:6000, 50 μL well). After 30 min of incubation at room temperature (RT), the plates were washed with PBST (4 times, 300 μL well), and a solution of goat antirabbit IgG-HRP (1:6000 in PBST) was added (100 μL well) and incubated 30 min more at room temperature. The plate was washed with PBST as described before, and the color substrate solution was added (100 μL well). After 30 min, at room temperature in darkness the enzyme reaction was stopped with a solution of 2 M of H_2SO_4 (50 μL well). Finally, the absorbances were measured at 450 nm. Data presented in the present work correspond to the average of at least two well replicates.

Standard curves were prepared testing eight levels of increasing concentrations of a LAS mixture standard. The standard curves were fitted to a four parameter equation according to the following formula: $A = B + (T - B)/(1 + 10\wedge((\mathrm{Log}IC_{50} - \mathrm{Log}\ c)\cdot HS))$ where A is absorbance, T is the maximal absorbance, B is the minimum absorbance, IC_{50} is the concentration producing 50% of the maximal absorbance, c is concentration, and HS is the slope at the inflection point of the sigmoid curve.

Matrix Effect Studies. The influence of the different types of wastewater on the ELISA was investigated using the wastewater samples already extracted (in order to avoid an initial LAS, see above) and diluted in different proportions of PBS. Standard curves were prepared, by adding increasing concentrations of the standard LAS mixture. The ELISA was carried out as described before, and the curves were obtained, adjusted to a four parameter equation, and used to compare their parallelism with the standard curve prepared in the assay buffer.

Cross Reactivity Studies. In the previous work by Ramón et al. (*22*), the possible interference of close structurally related compounds such as phenylsulfonic acids was evaluated. In the present work, the cross reactivity study was extended to other commonly used surfactants present in wastewater.

TABLE 2: Concentrations of Target Compounds in Wastewater Samples [μg L^{-1}] by LC-MS

	May-2004												June-2004									
compds	In-1	Eff-1	In-2	Eff-2	In-3	Eff-3	In-4	Eff-4	In-5	Eff-5	In-6	Eff-6	In-1	Eff-1	In-2	Eff-2	In-3	Eff-3	In-4	Eff-4	In-5	Eff-5
LASs	892	283	515	25	667	57	962	229	762	21,8	774	115	812	217	398	32	751	43	867	52	629	59
CDEAs	175	33	117	36	65	bdl[a]	145	54	73	27	102	41	145	20	287	153	45	bdl[a]	132	55	121	32
NP	bdl[a]	1.8	1.2	2.0	bdl[a]	0.8	bdl[a]	0.8	bdl[a]	0.5	1.7	2.9	0.7	5.1	0.5	2.2	bdl[a]	0.6	bdl[a]	0.9	bdl[a]	1.6
NPEOs	40	bdl[a]	175	8	64	18.6	82	7.4	87	bdl[a]	30	bdl[a]	55	4,2	113	12	67	4.7	53	12	94	21
OP	7.3	bdl[a]	0.8	bdl[a]	bdl[a]	bdl[a]	9.8	11	2.6	bdl[a]	1.1	bdl[a]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]	NA[b]

[a] bdl: below limit of detection. [b] NA: not analyzed.

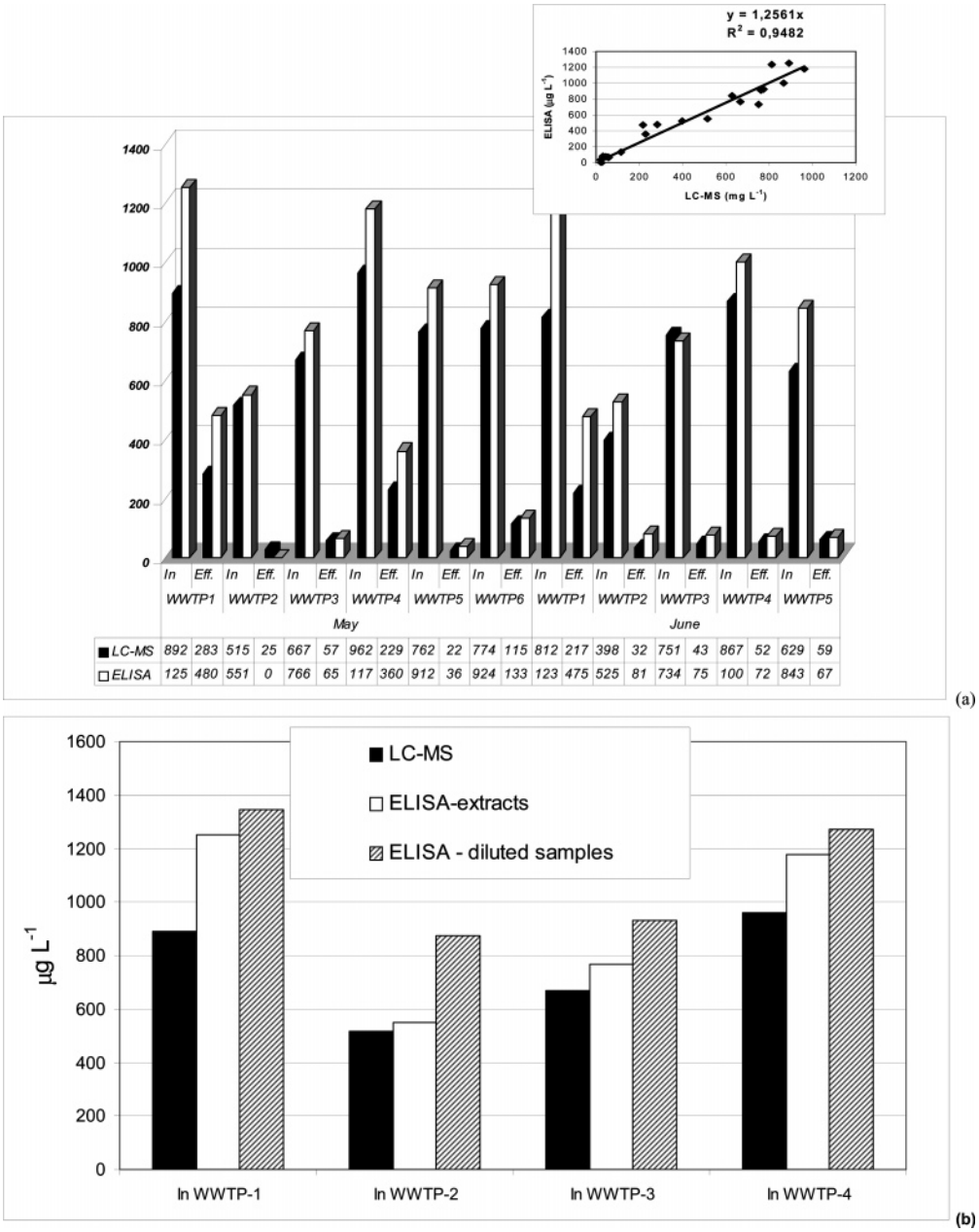

FIGURE 2. (a) Comparison of LAS concentration expressed in μg L^{-1} measured in the sample extracts by ELISA and by LC-MS and the correlation between both techniques. (b) Comparison of LAS concentration expressed in μg L^{-1} measured by LC-MS and by ELISA performed directly on samples diluted in assay buffer. Concentrations measured by ELISA correspond to LAS IR equiv. In. is influent and Eff is effluent.

Standard solutions of coconut diethanol amides (CDEAs) technical mixture, nonylphenol, octylphenol, nonylphenol ethoxylates technical mixture (NPEOs), nonylphenol ethoxylate (NP_1EO), nonylphenol ethoxylate (NP_2EO), 4-methylbenzene sulfonate, 4-chlorobenzene sulfonate, 3-nitrobenzene sulfonate, 2-amino-1-naphthalene sulfonate, and 1-amino-5-naphthalene sulfonate, were prepared in PBST (0.16−25000 nM) and measured with the ELISA. For some analytes it was possible to build a standard curve that fitted to the four-parameter equation mentioned above. The cross-reactivity values were calculated according to the equation

$$\text{LAS immunoreactivity equivalents} = (IC_{50}\ \text{LAS}/IC_{50}\ \text{tested compounds})\cdot 100$$

The results of these studies studies are summarized in Table 1.

Results and Discussion

SPCs with medium carboxylic alkyl chain lengths (C6−C8) are reported to be the most persistent and abundant intermediate degradation products of LAS in water, while long chain SPCs remain retained in sludge (*11*, 25). The present work was focused on the detection of LAS in wastewater, where long chain SPCs are not expected to be present. The studies made here have been addressed to evaluate the WWTP capabilities to remove LAS; however, considering the potential interference of these SPCs, all the results obtained with this ELISA will be reported as LAS immunoreactivity equivalents (LAS IR equiv). In this sense, the first purpose has been to expand the cross reactivity studies by examining the specificity of the immunoassay in front of other surfactants commonly detected in wastewater. The results of these studies are summarized in Table 1, where it can be observed the low interference produced by other

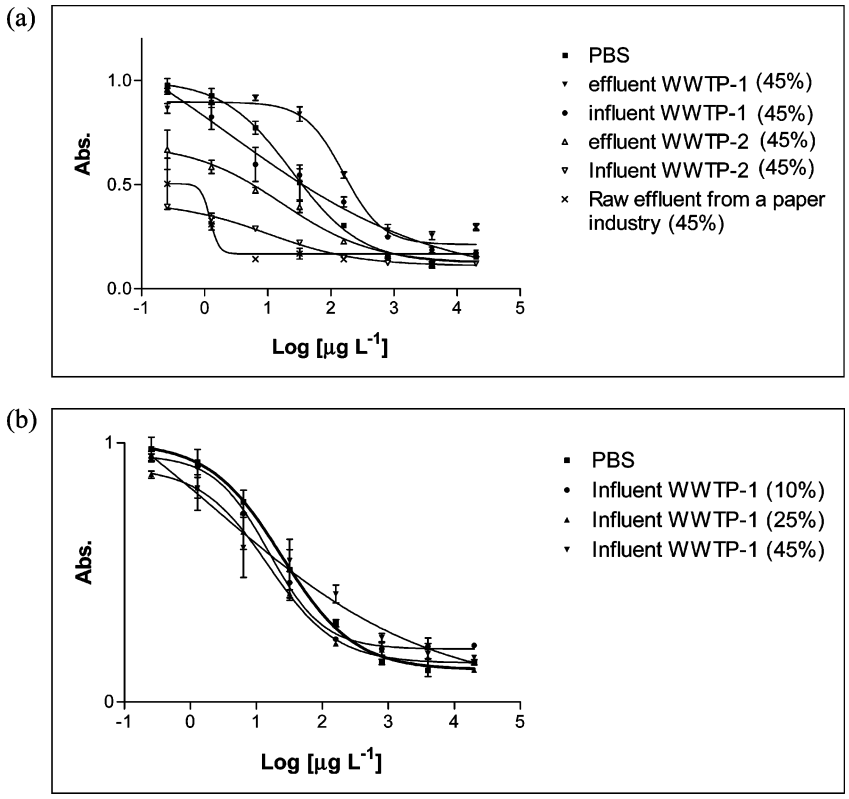

FIGURE 3. (a) Matrix effect standard curves for LAS IR equiv by ELISA in 45% of wastewater diluted in PBS. (b) Matrix effect standard curves using different percentages of dilution of wastewater from WWTP-1.

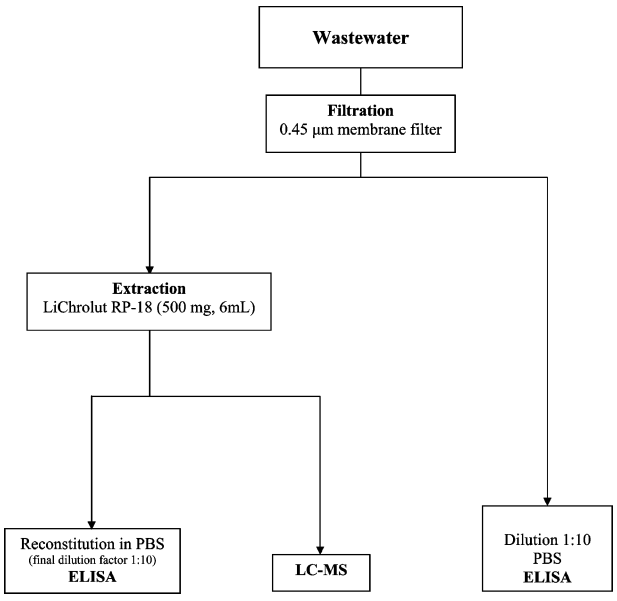

FIGURE 4. General operational scheme.

related compounds, such as benzenesulfonates, NPEOs, or CDEAS, which its concentration in wastewater is usually high (see Table 2).

All the WWTP samples were initially characterized to know the anionic and nonionic surfactants content. Analyses were performed after solid-phase extraction by LC-MS, and the results are summarized in Table 2. Simultaneously, the extracts were diluted with PBS and analyzed by ELISA. Despite the overestimation obtained by the immunoassay, a good agreement between the LC-MS and the ELISA results can be observed (see Figure 2a). In general, the overestimation measured using this immunoassay can be attributed to the sum of cross reactivity produced by SPCs and other cross reactants. 12C12-SPC and 13C13-SPC are the major cross reactants for this ELISA. High concentration of these compounds should not be expected in the samples, just residual concentrations (pg L^{-1} to ng L^{-1} range). First of all, because the major degradation intermediates found in different matrix samples from LAS commercial mixtures are those with a medium length of the linear alkyl chain (*11, 26*), formed by the ω-oxidation of the terminal carbon of the alkyl chain followed by successive β-oxidations. Different works remark the no detection of long chain SPCs, which indicates that the first oxidations are very rapid processes (*26*). However, residual concentration of these compounds can contribute to the final degree of overestimation.

Good correlation was obtained comparing ELISA and LC-MS, and the average of overestimation of the ELISA kit was 1.26 times on average (see Figure 2a). The contribution (on average) of the LAS measured by LC-MS to the total LAS IR equiv, by ELISA 79%, ranged for the different samples from 66% to a 93%. Comparing these results with those reported for the MBAS method (*7, 21*), the new ELISA used here is much more specific than MBAS, even when the ELISA was performed directly without a previous extraction step (Figure 2b). The major overestimation corresponded to the influents samples from WWTPs 1, 4, and 5, being that the samples with the highest concentrations of LAS were in accordance with the results of LC-MS (see Table 2).

On an attempt to measure water samples without any previous treatment by ELISA, we performed some experiments in order to assess potential nonspecific interferences produced by these sample matrices. With this purpose, "blank samples" and water samples from different plants, previously extracted to ensure the absence of LAS, were diluted in different proportions of PBS (22.5%, 45%, etc.) and used to prepare standard curves. The parallelism of the curves corresponding to the proper diluted matrix samples (1:10 dilution factor) and the standard curve prepared in the assay buffer can be observed in Figure 3b. A raw influent sample

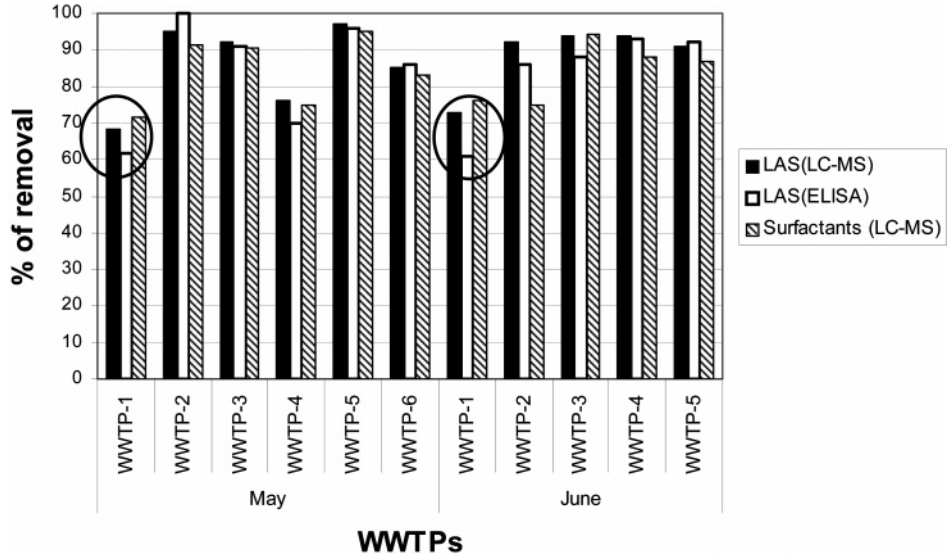

FIGURE 5. Percentages of removal calculated using the influents and effluents concentration of total LAS IR equiv and the sum of nonionic and anionic surfactants quantified by LC-MS in comparison to the percentage of removal calculated using the influent and the effluent concentration as LAS IR equiv by ELISA. Circles indicated the samples with the highest difference of the percent of removal calculated with LC-MS results and by ELISA results.

from the paper industry was also included in this study as a sample with a strong matrix effect for comparison purposes. As it can be observed at 45% concentration of the samples a strong matrix effect was still observed in most of the samples (Figure 3a); however, when the assay was conducted using a 1:10 dilution factor, the curves do not differ significantly to those constructed using just the assay buffer (see as an example WWPT 1 in Figure 3b). The only exceptions were those samples corresponding to plants with a high industrial influent, such as WWTP-2 or the raw influent. On the other hand, it should be noticed that, considering the commonly quantified concentrations of LAS in wastewater and the detectability and operational range of this ELISA, the application of a dilution factor (1:10) will be always necessary to place the samples within the dynamic interval of the immunochemical method.

ELISA analysis of some samples (influents of WWTP 1, 2, 3, and 4) were carried out, directly after dilution with the assay buffer, by SPE-ELISA and by SPE-LC-MS (see scheme in Figure 4). The comparison of the results obtained by these procedures showed a overestimation when the samples were measured directly, although this difference accounted for only about 10−12% for most of the samples. Only the WWTP-2 sample, with a high industrial inflow, showed a higher difference (37%) (Figure 2).

Regarding the WWTPs effluents, in general the concentrations levels measured by both LC-MS and ELISA were below 200 μg L^{-1}. A greater overestimation was observed for the samples corresponding to the effluents from WWTP-1 when measured by the ELISA and that is the only plant using physicochemical treatment of the water. The results obtained by ELISA may reflect a contribution to the LAS equiv due to other pollutants present in the sample.

Moreover, longer chain homologues should be degraded more rapidly than that of the shorter homologues (*27*). Second, longer LAS homologues should be expected to be in suspended material or sludge, not in water (*14*).

Figure 5 shows the total percentage of surfactants removal (measuring anionic and nonionic surfactants by LC-MS) and particularly for LAS measured by LC-MS and by ELISA. The anionic surfactants removal percentage is a good control parameter of the wastewater treatment efficiency. These results were obtained according to the concentration values in the influents and the effluents of the WWTPs. The results recorded by LC-MS and ELISA are very similar. Only for WWTP-1 were the percentages of removal according to ELISA lower than those for LC-MS (bars with circles in Figure 5). This WWTP is the only one without secondary treatment.

Considering the results presented here it can be concluded that the LAS ELISA used here can be a fast and cost-effective method to control the efficiency of WWTPs. This ELISA assay could be an excellent alternative to the still used unspecific classical methods, such as the methylene blue active substances (MBAS). The detectability, specificity, and high throughput sample capability of this microplate-based immunochemical method offers the possibility to perform efficient screening and monitoring programs. Moreover, the rapid determination of LAS in wastewater can be used as an alarm or an indicator of the WWTPs performance.

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