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Review

Pretreatment of oily samples for analysis by flow injection-spectrometric methods

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ABSTRACT

This review presents a critical discussion of selected reports dealing with the pretreatment methods of oily samples and the determination of their organic and inorganic constituents using flow systems and spectrometric methods. Special emphasis is given to the on-line couplings with detection systems based on UV–visible spectrophotometry and spectrofluorimetry, atomic absorption spectrometry either with flame or electrothermal atomization as well as inductively coupled plasma optical emission spectrometry or inductively coupled plasma-mass spectrometry. Simple dilution with organic solvents, digestion with concentrated acids under thermal heating, microwave or ultrasound radiation and emulsification procedures are mostly used. The empirical preparation of certain organized assemblies like micelles, emulsions and specially microemulsions added to the confusion of some of the terms, demand a brief description of their characteristics, the correct formulation and some of their applications to the manipulation and treatment of oily samples.

The analytical capabilities of combining flow manifolds with spectrometric methods for the determination of specific parameters in oily samples apparently have not been sufficiently exploited yet.

Contents

1. Introduction

For the purpose of this paper, "oily" refers to those samples which contain certain amount of fat or oil, like lipids, oils, fuels and others. Lipids for instance are small, naturally-occurring hydrophobic molecules which include fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, saccharolipids, and their derivatives [1]. These kinds of compounds play key roles in many metabolic and biochemical processes such as energy production and storage, the formation and functioning of cellular membranes and signal transduction, among others [2]. In addition to their biological significance, it should be pointed out that some of the lipids are of great interest in food industry, cosmetic preparations or in the chemical industry for fabrication of soaps, lubricants or fuels, like gasoline (distilled from crude oil) and biodiesel (composed by fatty acid alkyl esters, obtained from vegetal oils and animal fats). Oils for example, either edible or lubricants, are slippery and viscous liquids, of mineral, vegetable or synthetic origin, which are generally immiscible with water but soluble in various organic solvents [3].

Development of methods for the comprehensive analysis of lipids is therefore fundamental in the academic, clinical and industrial fields. However, lipids are not the easiest substances to analyze. Some of the spectrometric techniques are regarded as most promising analytical tools for their analyses due to advanced instrumental developments, the extensive use

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of computers and appropriate chemometric procedures. Nuclear magnetic resonance (NMR) spectrometry, mass spectrometry (MS) and some vibrational techniques (Fourier transformed infrared (FTIR) or Raman spectrometry) preceded by chromatographic separations are universal methods for lipid analysis [1]. Such techniques give invaluable information about the structure of these complex compounds and promise to revolutionize the understanding of lipid biology and their relationship with certain diseases.

Many publications related to this subject are focused on the automation of methods used for the determination of some organic components like bitter substances, sterols, pesticides, aniline, glycerol and others, by hyphenating flow injection (FI) with gas or liquid chromatographic techniques. The aim of this review however, is to critically discuss selected reports, treating the determination of organic and inorganic constituents of oily samples by FI-spectrometric methods. In this sense, UV–visible spectrophotometry and spectrofluorimetry, atomic absorption spectrometry (AAS) either with flame (FAAS) or electrothermal atomization (ET AAS) as well as inductively coupled plasma (ICP)-optical emission spectrometry (OES) or ICP-MS, are currently used. Several books [4–8] and reviews [9–21], although not dedicated exclusively to oily samples, comprehensively deal with these subjects.

Flow techniques in general, are recognized as powerful and useful methodologies for the automation of many analytical procedures. Their application to the on-line pre-treatment of complex matrixes and subsequent detection of different parameters by spectrometric techniques is well documented in the literature. However, the complexity of oily samples, their viscosity and the high organic load impose serious difficulties for their direct analysis. Improper manipulation and processing of such samples may lead to contamination and/or analytes losses. Also, the introduction of organic matrixes into the spectrometric detectors is associated with serious instrumental problems, thus most of these methods require some sample pre-treatment, although sampling rate depends most of the time of these procedures. In some cases however, it is almost impossible to avoid the utilization of organic solvents thus, the adaptation of flow manifolds to the analysis of oily samples, originates methodologies in which the inherent advantages of the flow techniques may not be as evident as the ones observed in the analysis of aqueous solutions. Nevertheless, the advantages over the traditional batch procedures, usually complicated, laborious and time consuming, using significant amounts of organic solvents, justify the use of flow techniques in this kind of analysis.

Connecting spectrometric detectors on-line with continuous flow, FI and sequential injection (SI) manifolds, avoids some of the problems mentioned above, bringing along advantages like versatility, simplicity, low cost, low consumption of reagents and samples, low waste generation as well as high precision and high sample throughput. These advantages have been exploited in the edible oils and biodiesel fields, namely in quality control and stability assays [21–42], as well as in the determination of non-metallic [43–45] and metallic constituents [46–53] of oils and petroleum-related samples. Some of the referred methodologies inject small volumes of sample in a stream of organic solvent to perform the on-line mixing through a confluence point, while some others involve sample pre-treatments that can vary from no treatment at all [45,46] to simple dilution with an organic solvent [26–41,47–55] and going to more complex processes like combustion [56,57], extractions/leaching [58–61] or digestions with concentrated acids at high temperature and pressure in open or closed systems with thermal heating or under microwave (MW) or ultrasonic (US) radiation [47,57,62,63]. Sometimes, filtration or other physical or chemical separation methods must also be previously implemented.

The use of surfactants leads to the formation of organized assemblies like micelles and microemulsions [16–20,64–86] allowing calibration with aqueous standards without the need of sample mineralization, thus easing samples manipulation and treatment. Although there are huge differences regarding their properties and uses, the terms “emulsion” and “microemulsion” have been often indistinctly used in the analytical literature when two immiscible liquids disperse into each other. Therefore, in this paper, we will also briefly describe the characteristics of these assemblies and some of their applications to the determination of certain constituents of oily samples by FI-spectrometric methods.

2. Pretreatment of oily samples

According to the aims of an analysis (determination of organic substances or metallic elements), the treatment of the oily samples may vary considerably. Also the diversity of these samples nature imposes different pretreatment procedures. The analyses of viscous oil-based organic materials present certain problems, mainly due to the difficulty in making them compatible with the employed instrumentation in spectrometric techniques. In addition to the many problems related to the complexity of the matrix, there are no certified reference materials available for these samples. For this reason the accuracy of the developed methods has to be accomplished by comparison with results obtained with independent techniques, particularly with respect to sample preparation. In this sense, a variety of approaches have been reported, with their advantages and limitations. Among them: (1) dilution with organic solvents; (2) analyte transfer to aqueous solutions by: extraction or leaching, ashing or combustion followed by dissolution of the residue in diluted mineral acids and complete mineralization with oxidizing acids using thermal heating, US or MW radiation; and finally, (3) emulsification.

2.1. Dilution with organic solvents

The dilution with an organic solvent (the so called direct method) is the easiest, simplest and fastest sample preparation procedure, used for samples of different nature like edible oils, biodiesel or petroleum derivatives.

An edible substance is one which can be suitable for consumption without harm to humans. In general, edible oils refer to vegetable oils like corn, sunflower, soy, palm or olive; their quality could deteriorate during production, storage and marketing or may be adulterated for economic gain. Checking and maintaining the quality of edible oils is of increasing interest. The straightforward way to assess the purity of edible oils is by UV spectrophotometric analysis in the wavelength range 200–300 nm [9]. Of particular interest are the extinction coefficients at 232 nm (K_{232}) and 270 nm (K_{270}) for which standard values are set. An increase in these values is a sign of adulteration or cross-contamination. The presence of peaks in the 260–270 nm region of the spectrum is due to diene and triene absorption, whereas no peaks are expected for unaltered extra virgin olive oil. Also, the combination of FI with an expert system [23] allows the determination of four quality parameters (total acidity, peroxide value, K_{232}, and K_{270}) of olive oils using independent measuring systems. The expert system is able to compare the results obtained in the determination of the four parameters with the expected values, in accordance with the European Community regulations. There are modern analytical methods which are able to unequivocally classify the oil samples by quality grades and also to detect any fraud [25].

The parameters which give important information about oil quality are: anisidine [26] and iodine [27] values, 2-thiobarbituric acid reacting substances [28], acidity expressed as free fatty acids

Table 1
Quality control of edible oils by on-line spectrometric methods.

Parameter (Quality evaluated)	Sample	Method of detection	Sample processing	Sampling frequency (h^{-1})	RSD (%)	Ref.
Anisidine value (Oxidative rancidity)	Olive oil	FI-spectrophotometry	Dilution off-line with n-propanol	110	1.0	[26]
Iodine value (Identify oil insaturation)	Olive oil	Parallel FI-multichannel spectrophotometry	Dilution off-line with n-propanol. Incubation for 10 min	60	0.4–1.1	[27]
TBARS (Oxidative rancidity)	Olive oil	Parallel FI-spectrophotometry	Dilution on-line with n-propanol Incubation for 5 min. Stopped flow for 25 min	20	4.6	[28]
Free fatty acids (Organoleptic properties)	Vegetable oils and biodiesel	SIA-spectrophotometry	Dilution on-line with ethanol	12	NR	[29]
	Olive oils	FIA-spectrophotometry	Dilution on-line with toluene	12	4.9–7.8	[30]
	Olive oils	FIA-spectrophotometric titratrion	Dilution on-line with n-propanol	30–100	<2	[31]
	Olive oils	FIA-spectrophotometric titratrion	Dilution on-line with n-propanol	12–60	<5	[32]
	Olive oils	FIA-spectrophotometric titratrion	Dilution on-line with n-propanol	21–74	2.2	[33]
	Palm oil	FIA-spectrophotometric titratrion	Dilution off-line with n-propanol			
Peroxide value (oxidative rancidity)	Olive, fish and vegetable oils	FI-spectrophotometry	Dilution off-line with n-propanol	30	1.5–2.2	[34]
		Parallel FI-multichannel spectrophotometry		83	2	[35]
	Olive oil		Dilution on-line with n-propanol Incubation for 5 min	24	0.23	[36]
	Olive and vegetable oils	Continuous flow-molecular specific FTIR	Dilution on-line with toluene and hexanol			
Hydroperoxides (oxidation products)	Olive and vegetable oils	FI-spectrofluorimetry	Dilution off-line with ethanol	25	0–4.7	[37]
	Soybean oil	HPLC-CL	Stopped flow for 60 s None	NR	3	[38]
Polyphenols (antioxidants)	Olive oil	FI-liq–liq extraction spectrophotometry	Dilution on-line with n-hexane	18–29	2.8–4.5	[39]
	Olive oil	FI-liq–liq extraction spectrophotometry	Dilution off-line with n-hexane.	11	2.7–5.3	[40]
Total antioxidant capacity	Olive oil	FI-CL	Dilution off-line with methanol/water	180	2.8	[41]

NR = Not reported.

(FFA) concentration [29–33], peroxide value [34–36], as well as the presence of oxidation products like hydroperoxides [37,38] and antioxidants like tocopherols and polyphenols [39,40].

The auto-oxidation of olive oils occurs when the lipid molecules react with oxygen in the presence of a catalyst like heat, light or metal ions to form lipid peroxy-radicals (ROO^-), which in turn react further to give hydroperoxides (ROOH). The extent of oxidation is therefore followed by directly measuring the lypohydroperoxides concentration in oil samples by, spectrofluorimetric [37] and chemiluminescent [38] methods. However, hydroperoxides are unstable compounds and decompose further to produce a complex mixture of volatile, secondary oxidation products like aldehydes, ketones, hydrocarbons, alcohols, and esters responsible for the deterioration of flavor termed oxidative rancidity [24]. Therefore, despite the fact that the oil quality is poor, the hydroperoxides may not be detected.

The pleasant flavor and the stability against oxidation of olive oils are partially attributed to their natural content in tocopherols and polyphenols, compounds with high antioxidant action. Modified FI configurations have been developed for the direct determination of polyphenols in olive oils [39,40], although it is more useful and practical to estimate the total antioxidant capacity (TAC), which accounts for possible synergistic or antagonistic effects among the antioxidant compounds present in the samples [41]. As these compounds are strong free radical scavengers, the TAC could be assessed through light emission inhibition due to hydrogen peroxide scavenging by antioxidants.

Table 1 summarizes some specific features of the methods generally used to assess edible oils quality, showing that olive oil analysis have received most attention. Apart from the non-aqueous spectrophotometric titrations still in use for FFA monitoring [31–33], these parameters are determined by spectrophotometric, spectrofluorimetric or chemiluminescent methods, most of them, automated versions of existing batch systems. The versatility of FI manifolds allows their adaptation for the manipulation of viscous and non-aqueous oil solutions and to deal with slow reactions like those which need long incubation times [26–28,35]. To comfortably be introduced into FI systems, viscous samples generally are appropriately diluted off-line or on-line with organic solvents like methanol, ethanol, propanol, toluene, xylene or hexane [26–41]. From 12 to up to 100 samples h^{-1} are processed in such systems, which fulfill the requirement of routinely analyze a large number of samples.

In all these methods however, the experiments are conducted independently. The determination of various parameters at a time may spare time of specialized personnel and uses less instruments and reagents. In this sense, Wai et al. [42] used a potentiometric analyzer to determine the total oxidative value in palm oleins.

As for edible oils, the quality control of biodiesel is mandatory for the success of its commercialization. This fuel is obtained mainly by the base- or acid-catalyzed transesterification reaction of vegetable oils and fats with monohydric alcohols, such as methanol, to give the corresponding mono-alkyl esters. As a result, it may contain several by-products like mono-alkyl esters and glycerol, residual catalysts (Na, K, sulfate) and many metallic species (Ca, Mg, Cu, Zn, Pb) from soil and water. Their concentrations must be maintained under certain permissible limits in order to avoid environmental pollution and/or damages to certain parts of the engines. There are comprehensive critical publications up-dating the methods for biodiesel characterization [21] as well as those for monitoring biodiesel production [22]. The determination of the biodiesel fuel quality is a matter of successfully commercialize the product, thus the quantification of bound and free glycerol [43], sulfate [44], methanol [45] or different metallic species [46–53] and of other by-products have received increasing attention. Different chromatographic (gas-chromatography, thin

Table 2
Determination of different species in edible/crude oil and petroleum-related samples by FI-Spectrometric methods.

Sample analyzed	Element determined	Analytical technique	Samples processing (Calibration)	Sample throughput (h^{-1})	DL ($\mu g\,L^{-1}$)	RSD (%)	Ref.
Biodiesel	Sulfate	SIA-spectrophotometry	Dilution on-line with methanol:water (Aqueous standards	15	1420	NR	[44]
Biodiesel	Methanol	FI-spectrophotometry	No treatment (methanol in xylene)	NR	2 $\mu g\,g^{-1}$	4.5	[45]
Olive, soybean, peanut oils	Fe(III)	SIA-spectrophotometry	No treatment (Organometallic standard)	20	310	<3.5	[46]
Lubricating oil	Cu, Cr, Fe, Pb	FI-FAAS	Dilution on-line with deodorized kerosene (Multielemental organometallic standard)	50	NR	2–8	[47]
Gasoline/lubricating oil	K	FI-FAAS	Dilution off-line with K-free gasoline. Oil diluted on-line with petroleum spirit (Organometallic standards)	40–100	NR	<3	[48]
Lubricating oil	Metal-based additives	FI-FAAS	Dilution on-line with kerosene (Aqueous standards)	30	NR	<5	[49]
Gasoline	Hg	CV-ETAAS	Dilution off-line with ethanol (Aqueous standards)	4	0.08–0.14	<2.4	[50]
Petroleum-related	Mo, Ni, V	μFI-ICP-MS	Dilution on-line with xylene (Organometallic standards)	100	<1.0	<3.5	[51]
Fuels and light petroleum	Multielements	μFI-ICP-MS	Dilution on-line with xylene (Organometallic standards)	NR	0.1–9.0	NR	[52]
Petroleum-related	Multielements	FI-ICP-MS	Dilution on-line with xylene (Multielement oil-based standards)	NR	0.3–2.8	<3.0	[53]
Lubricating oil	Insolubles	FI-spectrophotometry	Dilution on-line with kerosene. (Most loaded sample diluted with unused oil)	30	NR	2.4	[54]
Lubricating oil	Insolubles	FI-spectrophotometry	Dilution on-line with kerosene. (ASTM standards of insolubles in pentane)	45	0.07%	1.0	[55]
Lubricating oil	Cu Fe	FI-spectrophotometry	Dry ashing. Dissolution in HNO_3 (Aqueous standards)	12	0.77 0.85	1.0 0.6	[56]

NR = Not reported.

layer chromatography, liquid chromatography) and spectrometric (mainly spectrophotometry preceded by extraction procedures) methods have been used for this purpose.

Elemental analysis of different edible oils and petroleum matrices without any pretreatment [45,46] or diluted in organic solvents [47–55] is normally carried out using on-line spectrophotometric [44–46,54,55], AAS [47–50] or ICP-MS [51–53] methods. Selected applications of these methods to the determination of metallic species in oily samples are given in Table 2. Petroleum-related samples like lubricating oil and gasoline, as well as some vegetable oils are of interest for trace element analysis. Beside the matrix complexity and heterogeneity of such samples, dilution with organic solvents looks appropriate as sample pretreatment since most of the publications follow this procedure. However, while equalizing the viscosity of the samples, the dilution reduces the overall sensitivity of the analysis, degrades the detection limit and increases the probability of contamination or other type of analytical errors. Additionally, the direct introduction of an organic matrix into the detector leads to inaccurate results due to matrix effects and inappropriate calibration procedures. Organometallic compounds traditionally employed as standards, dissolved in purified oil or kerosene, are aimed to compensate for matrix effects, allow the use of simple and direct external calibration and assure a good accuracy of the analysis. These substances are generally unstable, relatively expensive and may lead to concentration changes resulting from the evaporation of the solvent and/or adsorption of the analyte on the walls of the long-term storage containers, particularly when working at the $\mu g\,L^{-1}$ levels [10]. Moreover, the use of organic solvents in ICP-MS could cause plasma instability, matrix interferences and carbon deposits on sampler and skimmer cones [53]. For some of the samples, e.g. lubricating oils, such procedures are not at all recommended due to the presence of insoluble solid inorganic matter arising from physical wear of the engine or from oil and additives degradation [10,11]. In these cases, the determination of insoluble components is highly recommended, because may further cause engine wear and clog the filters. Measurement of the scattered visible light by a diluted sample in a simple FI-spectrophotometric system could be used as a routine method for field monitoring of the residual life of the used oil [54,55].

Due to the above mentioned problems of these more or less direct methods, the transfer of the analyte to an aqueous solution seems to be advantageous. It is usually performed by combustion followed by dissolution of the residue in diluted acids [56,57], analyte leaching [58–61] or by complete mineralization of the oily product [10–13,57,62].

2.2. Analytes transfer to aqueous solution

Analytes which are not strongly bonded to the oily matrix can be efficiently extracted into aqueous solutions by irradiating acidified samples with either US or MW energy. This efficient and fast sample pre-treatment step is gaining acceptance among analytical chemists as an alternative to more drastic pretreatment procedures usually recommended for spectrometric determinations. There are several recent papers dealing with US- and MW-assisted extraction of trace elements from troublesome matrices [58–62]. The interaction of MW with samples has been extensively studied and explained in numerous publications and books. Interesting considerations about the mechanism of US effects in samples preparative steps have been recently discussed [15,58]. US is a radiation which, when transmitted through a substance in any of its aggregation states, can produce mechanical and chemical changes. It is therefore involved in many analytical systems, namely: homogenization, emulsification, leaching, slurry formation, nebulization, degassing, defoaming and others. Luque de Castro and Priego-Capote [15] comprehensively discussed some key applications that can help

analytical chemists to decide when US energy can benefit a certain step of their procedure. It appears however, that more in-depth work should be carried out to clearly understand for example, the effect of the irradiation (either MW or US) on speciation studies and help researchers to decide whether the energy-assisted step is beneficial or not to preserve species integrity.

Mineralization of oily samples in strong oxidizing acids allows a complete dissolution of the matrix. Although still in use, these procedures are time consuming and have a considerable risk of sample contamination, and analyte loss as volatile species. MW-assisted acid digestion in open or closed vessels located in the oven cavity or using focused MW devices, has been widely applied due to high efficiency of heat transfer and improvement on the efficiency of the sample mineralization [5,12]. Combination of mineral acids (HNO_3) and oxidizing agents (H_2O_2) with appropriate microwave oven power (500–700 W) and exposure time (max. 30 min accomplished in two or three steps) lead to clear sample solutions with a low carbon load. On-line oily samples decomposition in concentrated acids, followed by further metal and/or non-metal determinations, minimizes the risk of analyte losses and contamination, but usually increases blank values and cannot be supported by some analytical techniques like ICP-MS and ICP-OES; a subsequent step could be necessary to remove (or dilute) the acid excess [62]. Sometimes, the sample mass to be treated is limited to about 200 mg, fact that reduces the sample throughput, while the heterogeneous nature of the viscous samples lead to less repeatability (RSD 10–20%). These drawbacks have been overcome by implementing combustion techniques, which, due to a higher temperature achieved, are more effective for the conversion of matrix compounds to the correspondent oxidation products [16]. The relatively recent used MW-induced combustion (MIC) combines the advantages of MW-assisted digestion and combustion techniques, allowing the digestion of samples that are difficult to bring into solution like some edible oils or crude oil and its products [13,57,62].

Whatever the procedure, the digestion of the oily samples ensures that the organic matrix is completely mineralized and the total metal content is converted to simple water soluble species, allowing the use of aqueous standards for calibration purposes. In all cases, problems may arise due to trace contamination from large amounts of mineralizing acids used or losses of volatile analytes due to the high temperatures employed, especially when open digestion systems are used. Beside the abundant FI applications, the literature search shows that on-line systems have been scarcely used for the total mineralization of oily samples, probably due to the difficulty of obtaining a uniform flow pattern of sample solutions without back flush into the system, probably due to high built-up pressure within the conduits.

2.3. Oily samples emulsification

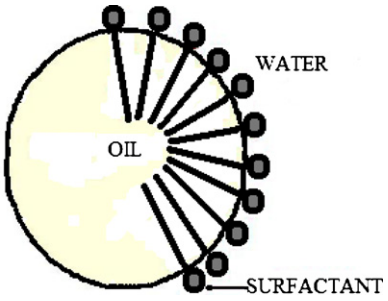
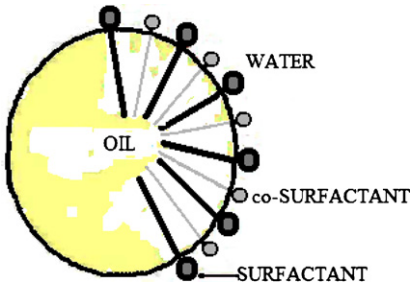
In the modern analytical chemistry, surfactants are considered special reagents as, when adequately used, are able to improve the characteristics of many chemical reactions and the performance of certain analytical methods**.** The more popular application of surfactants in analytical chemistry is probably their utilization to manipulate the surface tension of sample solutions in order to improve the nebulization efficiency in flames or plasmas as well as the wettability of the graphitic solid surfaces in ET AAS [63]. Although the changes observed in the physical properties of solutions by adding surfactants lead to more stable and reproducible analytical signals, the mechanisms to describe such behavior is still unknown. In these cases, trial and error techniques are used for optimization of parameters, because the signals depend on the experimental set-up, instrumental conditions, analyte and type of surfactant used, etc.

Under controlled conditions, surfactant-based organized assemblies may be obtained [8,16–19]. These are micro heterogeneous systems or self-assembling aggregates of molecules either of organic or inorganic nature within a surrounding medium. The hydrophobicity or amphiphilic nature of the oily samples allows them to form different organized assemblies when dispersed in an aqueous environment: emulsions or macroemulsions, normal and reversed micelles; microemulsions as well as lipid mono-, bi- and multi-layer assemblies. All these systems, except macroemulsions, possess unique properties which facilitate spectroscopic measurements: solubilize and concentrate analytes and/or reactants, alter physical, chemical and spectroscopic characteristics of the solutions, are relatively stable and non-toxic [8,17]. Such approaches do not require the destruction of the organic matter or extraction of analytes in large volumes of organic solvents. In general, the samples treated in this manner have physical and chemical properties similar to those of the inorganic standards in aqueous solution. Several types of organized assemblies were assayed, using cationic, anionic, zwitterionic/amphoteric and non-ionic surfactants.

Emulsions or macroemulsions, which consist of almost spherical droplets, are obtained when two immiscible liquids are homogenously mixed in the presence of a low concentration of an emulsifier agent like lecithin, certain waxes, alcohols (ethanol or propanol), surfactants, etc., which act as stabilizers. When a hydrophobic substance like fat, oil or a hydrocarbon is dispersed in a continuum phase (water), an oil-in-water (O/W) emulsion is obtained; a water-in-oil (W/O) emulsion is also formed when the fat surrounds the water molecules. The type of emulsion will depend on the ratio of the two phases and the type of emulsifier used. Most emulsions are white because their droplet diameter is greater than the light wavelength (Tyndall effect) and most oils have higher refractive indices than water. Emulsions are lyophobic colloids, which may remain stable for long periods of time, are kinetically stable but thermodynamically unstable (Table 3). In general, mechanical or manual agitation is used for their formation, regardless that the use of ultrasound for shaking allows obtaining a stable and homogeneous emulsion quicker in comparison with these mechanical processes. Although the macroemulsions have little analytical application, there are many examples of daily used emulsions: e.g. mayonnaise, butter and margarine in the food industry, creams and lotions in cosmetics or nanoemulsions (droplet diameter of 400–600 nm) of soybean oil used in medicine to distribute vaccines or for antipathogens treatment [18].

On the other hand, micelles are aggregates of surfactant molecules dispersed in an aqueous solvent with their ionic or polar head groups (hydrophilic heads) sequestering the hydrophobic tails (typically n-alkyl groups). This type of micelle, known as a normal phase or O/W micelle is formed when a minimum concentration of surfactant (called the critical micelle concentration, CMC) is reached. Inverse or W/O micelles are similarly formed but the polar head groups are directed at the center, forming a microreactor like environment filled with water molecules surrounded by the hydrophobic tails extending out into the bulk of an organic solvent. These microreactors can transfer species of experimental interest, quantitatively into the water phase. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength. This phenomenon is especially observed with non–ionic surfactants containing polyoxyethylene chains and can be attributed to the ethyl oxide segments in the micelle that repel each other at low temperature and attract each other at high temperature. When a micellar solution is heated, it becomes turbid over a narrow temperature range, known as its cloud point temperature (CPT). Above the CPT, such solutions separate into two isotropic phases: the surfactant-rich phase of a small

Table 3
Organized assemblies characteristics.

	Macroemulsion	Microemulsion
		
Droplet size (μm)	0.1–0.3 2–20	< 0.1
Color	Bluish white Milky white	Transparent
Stable	Kinetically	Thermodynamically
Unstable	Thermodynamically	Kinetically
Preparation	Intense agitation of sample with water, surfactant	Gentle mixing of sample with water, surfactant, co-surfactant and other additives
Colloid type	Lyophobic	Lyophobic and lyophilic (borderline)

volume and in a diluted aqueous phase, in which the surfactant concentration is close to the CMC. The phenomenon is reversible and upon cooling a single phase is obtained again. The cloud point is affected by salinity, being generally lower in more saline fluids. Cloud point extraction (CPE) is probably the most versatile and simple method for the preconcentration and extraction of hydrophobic species from aqueous solutions. Little has been published however about micelles applications to oily samples.

Due to their unique and advantageous properties, micellar systems have been successfully employed to enhance chemiluminescent [19,64], spectrophotometric [20,65] and spectrofluorimetric [66] reactions. As a result of the combination with FI methods, micellar-mediated reactions have been used to develop new methods for on-line extraction and preconcentration of metals (as ions or chelates) from different samples, followed by their quantification by spectrometric methods [64–71]. Recent advances in the fabrication of nanostructured arrays widely applied in optical, electronic, magnetic, catalytic and biosensing fields, highlighted the use of nanocrystal-micelles [72]. The nanocristals are encapsulated inside the core of surfactant micelles which allows further self-assembly into two- and three-dimensional ordered arrays. The nanocristal-micelles are biocompatible and thus, of great interest for bio-labeling. The property of CPE methodology to preserve the sizes and shapes of nanomaterials during the phase transferring has been recently exploited for reversible concentration/separation or dispersion of various nanomaterials in the aqueous phase [73]. An innovative recent work also used CPE on a microchip for preconcentration of phospholipids as a model of membrane-associated biomolecules [74]. These last approaches might have some analytical applications which should be exploited by young researchers to develop novel on-line procedures for lipids characterization.

Microemulsions are thermodynamically stable colloidal dispersions spontaneously formed when the components are brought together and stay stable as long as the ingredients are intact. They are obtained by gentle mixing of relatively large amounts of a surfactant and a co-surfactant in either oil or water and are optically clear as their droplets are smaller than the wavelength of visible light (10–100 nm). Microemulsions can have essentially infinite lifetime assuming no change in composition, temperature and pressure, and do not tend to separate; they lay on the borderline between lyophobic and lyophilic colloids (Table 3). Addition of surfactants facilitate the emulsification process by reducing the interfacial tension and increase the stability by introducing double layer forces and/or solvation forces between the dispersed particles [8,75,76]. The type of surfactant must be carefully chosen to attain a low interfacial tension at the oil/water interface and also its concentration must be high enough to provide a number of surfactant molecules needed to stabilize the microdroplets. Addition of a co-surfactant like sec-butanol [16,77–79] ensures flexibility of the interfacial layer and further reduces the interfacial tension thus the interface is fluid enough to promote the formation of a homogenous microemulsion phase. It is well known that in on-line (FIA or sequential injection analysis (SIA)) systems, the mixing of all components is made mainly through axial dispersion which, in the case of viscous samples, do not allow complete penetration of the sample plug with reagents zones. Thus, the emulsification process might not be complete and the components separate before measurement, leading to inaccurate results. US or MW radiation applied to the emulsifying mixture was recommended in order to allow accurate determination of Cr [77] or Al [78] by ET AAS in new and used lubricating oils or, of inorganic and methyl mercury in fish-eggs oil samples [79]. In all cases, time-based solenoid injectors were incorporated in the design of the on-line systems either to automatically introduce reagents or samples and standards. The optimal formulation of the emulsion was followed by measuring the drop size of the oleic phase for the different sample/reagents ratios.

The literature search shows that the preparation of microemulsions is generally based on an empirical manipulation of the physical and chemical conditions and on reagents availability. For further understanding and only for comparison purposes, Table 3 gives some characteristics of macro- and micro-emulsions, showing huge differences in their particle sizes, stability and preparation procedures.

The phase behavior of a surfactant-based organized assembly should be visually assessed with the aid of a ternary phase diagram (surfactant/oil/water), which indicates the lipophilic and the hydrophilic interactions at the O/W interphase (Fig. 1). This particular diagram shows the transition between phases when the content of the components mixture is varied. At high oil concentration (right corner), surfactant molecules form reverse micelles which solubilize water molecules in their hydrophilic core. Instead, at very low oil and surfactant concentration (left corner) a normal micellar solution is obtained. These are the two biphasic regions located on both sides of the diagram. Between these limits, in the center of the diagram is the macroemulsion domain. The diagram also presents a single-phase region containing a lamellar structure in which, water molecules are sandwiched between surfactant double layers, close to the surfactant vertex. This was identified by Winsor as a type IV phase [80]. However, as the water and surfactant content increases,

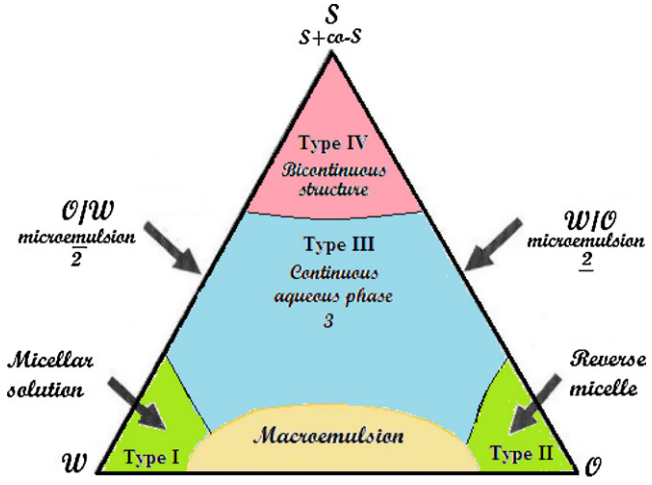


Fig. 1. Phase diagram of a microemulsion system. Oil-in-water (O/W) and water-in-oil (W/O) emulsions; S: surfactant; co-S: cosurfactant.

water droplets stabilized by the surfactant/co-surfactant mixture will first result in the formation of an isotropic clear region of W/O microemulsion. Further addition of water will cause the breakdown of the liquid crystal, forming another clear region containing droplets of oil stabilized by the surfactant/co-surfactant mixture (O/W microemulsion).

In order to identify these phases, Winsor introduced the ratio *R*, which is a measure of the affinity of surfactant for oil, or for water [80,81]. If $R < 1$, the surfactant has more affinity for water, thus the hydrophilic interactions will be stronger and a Winsor type I (WI) emulsion will be obtained (O/W normal micellar solution). If $R > 1$, the surfactant has more affinity for oil, thus the lipophilic interactions are stronger and a Winsor type II (WII) microemulsion (W/O inverse micellar solution) is obtained. They are also represented with the mnemonic symbols $\bar{2}$ and $\underline{2}$, respectively. When these interactions are in equilibrium (a microemulsion in equilibrium with both, excess water and excess oil), $R = 1$ and it is assumed that the optimum microemulsion formulation has been reached or a Winsor type III (WIII) system (indicated with the symbol 3) has been obtained. This situation corresponds to a three-phase system associated with the lowest possible interfacial tension and viscosity values. For a better understanding, the sponge-like model can explain the bicontinuous structure; if a sponge is filled with a liquid, resembles a bicontinuous structure since the liquid forms a continuous phase and the sponge material another continuous phase although they appear to be one [75,76]. The simplest methods for the determination of the different microstructures are particle (drop) size, conductivity (only applied to ionic surfactant systems), viscosity and interfacial tension measurements. Dielectric relaxation spectroscopy, NMR, light scattering or electron microscopy are also used for the characterization of microemulsions [18,19,81]. Depending on surfactant type and sample environment, types I, II, III or IV form preferentially, the dominant type being related to the molecular arrangement at the interface [81].

The equations which describe the optimum formulation of a microemulsion can be written as [75]:

$$\alpha - EON + bS - K \times \mathrm{EACN} - f(A) + c \times \delta T = 0 \quad \text{(for nonionic surfactants)}$$

$$\ln S - K \times \mathrm{EACN} - f(A) + \sigma - a\gamma \times \delta T = 0 \quad \text{(for ionic surfactants)}$$

where α is a parameter proportional to the surfactant "tail"; EON is the average number of ethylene oxide groups per molecule of a nonionic surfactant; *S* is the salinity of the aqueous phase expressed as weight percent of NaCl or the equivalent salinity of another electrolyte; $\ln S$ is the natural logarithm (ln) of the average salinity; EACN (equivalent alkane carbon number) is the number of carbon atoms in the alkane oil or its equivalent if a non-alkane oil is studied; $f(A)$ accounts for co-surfactant (alcohol) effect (its type and concentration); δT is the temperature variation from 25 °C, which will be constant during working day; while *b*, *c*, *K*, σ and $a\gamma$ are empirical constants which depend on the type of system and will never vary during the experimental work.

The transition between phases (WI to WIII or WIII to WII) is therefore induced by changing one of the variables (S, EON or EACN), with consequent changes in *R* value, and each can be used as a guide to the optimum microemulsion formulation [76,77]. These parameters must be systematically optimized by changing one of the variables while keeping the others constant. One of the most important parameters to be considered on the selection of the adequate surfactant is its hydrophilic–lipophilic balance (HLB). Its value goes arbitrarily from 0 to 20 and depends on the surfactant type and sample nature [8]. If HLB is <7 the surfactant is lipophilic and is likely to produce systems related to an $R > 1$ (W/O emulsions); if HLB is >18, *R* is <1 and the hydrophilic interactions will be stronger. For an optimum formulation an HLB in the range 8–18 is recommended in order to obtain stable O/W emulsions from lubricating oils [77–79]. This range is quite wide and very often, surfactants with the same HLB number exhibit different behavior. For example, HLB for Tween 85 is 11 and for Tween 20 is 16.7. Depending on the oil nature and the mixing ratio (surfactant:sample), variable HLB values may be obtained. It is obvious that other formulation variables should be considered, such as surfactant affinity difference (SAD), which includes the ethylene oxide number (EON), parameter strictly related to HLB according to the formulae [16]:

$$\mathrm{HLB} = \frac{100}{5} \times \frac{\text{weight of polyethylene oxide chain}}{\text{total molecular weight}}$$

In order to be employed in atomic spectrometry, the microemulsions must meet certain requirements: (1) to be stable during the time of analysis (usually exhibit long-term stability but are very sensitive to temperature changes); (2) be physically suitable for introduction into the atomizer; and (3) provide identical results as standards of identical analyte concentration [17]. Therefore, it is necessary to adequately select the emulsifying agent (the structure of its hydrophilic group which will interact with the analyte, the structure of its non-polar part which must be similar to the samples to be emulsified and its concentration, as it requires a high amount of surfactant (about five times the one needed to form macroemulsions). These indications are strictly related to the size of the dispersed droplets and their control can improve the reproducibility and the accuracy of the analytical signals.

Microemulsions have proved to be interesting to study for many different areas of chemistry because of the presence of unique compartmentalized environments for different kinds of reactions in a homogeneous, thermodynamically stable, isotropic media. Compared to micelles, microemulsions are more versatile because the compartment sizes can be altered at will in both the absence and the presence of additives. In most of the published work however, the choice of the procedure for preparation of the microemulsions is not at all based on the above considerations. In our opinion, the reagents are empirically mixed without any proven evidence of the optimum microemulsion formation. In this respect, Table 4 is giving some examples from published papers reporting the determination of metals in edible/lubricating oils and petroleum-related samples, pointing out the sample emulsification procedure. While some authors give experimental evidence of optimum formulation [77–79], some others claim that the emulsification takes place by directly mixing the samples with Triton X-100 and water [82], or

Table 4
Emulsification procedures for the determination of metals in edible/lubricating oils and petroleum-related samples using on-line spectrometric methods.

Sample	Element	Analytical technique	Surfactant	Emulsification procedure	DL ($\mu g\,L^{-1}$)	RSD (%)	Ref.
New and used lubricating oil	Cr	FI-ETAAS	SDS	On-line mixing: sample, hexane, NaCl, surfactant, sec-butanol	4.0	0.6–0.8	[77]
Fish-eggs oil	Total Hg and MeHg	FI-CV-AAS	Tween-20	Off-line mixing: sample, surfactant	0.11 0.12	2.2–9.0	[78]
New and used lubricating oil	Al	SIA-ETAAS	Various	On-line mixing: sample, hexane, NaCl, surfactant, sec-butanol	2.3	1.5–1.7	[79]
Olive, corn, sunflower oils	Multielements	FI-ICP-OES	Triton X-100	On-line magnetic stirring: sample, surfactant, water	NR	5.6	[82]
Soybean, peanut oils	As, Cd, Hg	FI-VG-ICP-MS	Triton X-100	Off-line mechanical mixing: sample, surfactant, acid	0.01–0.04	2.0	[83]
Cosmetics	Multielements	FAAS, ET AAS, CV-AAS	SDS*	Off-line US shaking: simple, surfactant, acid	0.02–28 $\mu g\,g^{-1}$	0.3–9.3	[84]
Biodiesel	Hg	CV-AFS	None	Off-line mixing: sample, surfactant, acid	0.2 $\mu g\,g^{-1}$	<8	[85]
Gasoline	Hg	CV-AAS	None	Off-line mixing: sample, propanol, acid	0.10	2.8	[86]

NR = Not Reported.
* SDS = Sodium dodecil sulfate.

first dilute the sample in an organic solvent and then mix it with an inorganic acid in the presence [83–85] or absence [86] of surfactant. Such procedures enabled long-time oily sample dispersion in the solution, but there is no experimental evidence of the formation of the microemulsion.

Although the calibration procedure is simplified by using aqueous standards and the organic content of samples is drastically reduced, these procedures result in sample dilution which consequently degrades the detection limits. However, the use of emulsified liposoluble matrices allows a direct and rapid analysis, avoiding the use of strong acids for complete mineralization or use of large volumes of organic solvents for sample dilution. Ultrasound-assisted emulsification is considered an interesting approach for accelerating the formation of emulsions. Nevertheless, this strategy has been scarcely used in on-line systems [77,78], although has been extensively applied to the determination of metals and metalloids in different cosmetics like oils, sunscreens or shampoos [84].

3. Conclusions

Although substantial improvements of existing FI methods have been carried out, novel developments in automated systems will find more applications in the analysis of oily samples analysis. As the concentrations of some of the parameters meant to control the quality of oily samples are usually high, FI manifolds developed so far are in general simple, allowing the on-line dilution of the viscous samples. Some others however, like the metallic species, are present at concentrations well below the detection capabilities of the spectrometric techniques. As already demonstrated for water samples, the use of micellar systems as alternatives to other separation and preconcentration techniques offer several advantages including low cost, safety and high capacity to preconcentrate even complex organic compounds, with high recoveries and good enrichment factors. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system.

However, it looks like the analytical capability of combining FI manifolds with spectroscopic methods for the determination of specific parameters in oily samples has not been sufficiently exploited. Compared to the huge number of publications in this field, the applications of surfactants-based assemblies are relatively modest, probably because the real interaction mechanisms at the interface it is not fully understood. According to the authors' opinion, there is a great potential for innovative applications by appropriately, on-line hyphenating micelles and microemulsions formation with available separation techniques and spectrometric detection in order to solve speciation problems. This methodology presents clear advantages over dilution in an organic solvent or acid digestion, in terms of simplicity of sample preparation, total analysis time, long-term sample stability and the use of inorganic standards for calibration. It also avoids the use of large volumes of strong acids and organic solvents. The correct formulation of the emulsions and the possibility of developing miniaturized FI systems might provide new applications in the field of nanomaterials and other real samples used in the modern technology.

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