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How to decide whether a substance is a surfactant?

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Introduction

Both the EU Detergent Regulation[1] (EU DetReg) and the EU Customs Tariff regulation[2] contain a surfactant definition, but the two regulations do not provide any guidance on how to apply the respective criteria. For this reason, the CESIO working group Test Methods of Surfactants, comprised of leading phys-chem experts in the field of surfactants, has prepared this guidance document on how to use those definitions in practice. It should be noted that the current definition of the EU DetReg, which is likely to be transposed into the updated Detergents Regulation, is difficult to apply in practice. Therefore, with this guidance we hope to create some common understanding on the testing methods and phys-chem criteria which can be applied to identify a surfactant within the confines of the current definitions.

1. Current Definitions

EU DetReg (2004):

> 'surfactant' means any organic substance and/or mixture used in detergents, which has surface-active properties and which consists of one or more hydrophilic and one or more hydrophobic groups of such a nature and size that it is capable:

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[1] Council. 2004. Regulation (EC) No 648/2004 of the European Parliament and of the Council of 31 March 2004 on detergents

[2] International Customs Tariff Regulation. Section VI – Chapter 34

- of reducing the surface tension of water (to below 45 mN/m as specified later in the “Questions and agreed answers” document[3])
- and of forming spreading or adsorption monolayers at the water-air interface,
- and of forming emulsions and/or microemulsions and/or micelles,
- and of adsorption at water-solid interfaces.

Customs Tariff Regulation

“organic surface-active agents” are products which when mixed with water at a concentration of 0.5% at 20°C and left to stand for one hour at the same temperature:

- Give a transparent or translucent liquid or stable emulsion without separation of insoluble matter; and
- Reduce the surface tension of water to 45 mN/m or less.

2. Challenges of the current definitions

Apart from the fact that there are no details given how to perform the experiments necessary, the main difficulty in applying the existing definitions is that at least two of the five criteria of the current Detergent Regulation are not at all clear, and they are connected by “and”, making interpretation all the more difficult. Materials which meet the first four criteria could very well fail to qualify as surfactants, since it is not clear how to use the last criterion (adsorption on solids) of the DetReg.

Reduction of surface tension to <45 mN/m (DetReg, Customs Tariff)

For most of the surfactants used in laundry and cleaning formulations, the surface tension is in the range of 25-35 mN/m, which means this requirement is met by a safety margin of 10-20 mN/m. This does not come as a surprise, since surface activity is a phys-chem property determined by the length and nature of the hydrophobic tail in relation to the size and nature of the hydrophilic headgroup. Therefore, experts having a sufficient amount of experience do not really need to measure surface tensions – one can pretty much predict surface tension by looking at the molecular architecture of the substance studied.

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[3] Questions and agreed answers concerning the correct implementation of Regulation (EC) No 648/2004 on detergents

There are, however, a few cases which require some closer attention:

- Very hydrophilic surfactants (i.e. those having a large hydrophilic headgroup and a rather short alkyl tail) show reduced surface activity; hence, to be on the safe side, the surface tension should be measured. The use of classical equipment[4] (Wilhelmy plate[5], du Noüy ring[6]) to determine the surface tensions should not pose any problem.
- Large hydrophilic polymers with very few hydrophobic groups, which is the typical architecture of so-called associative thickeners, cannot bring a sufficient number of hydrophobic groups to the air/water interface to efficiently lower surface tension. Therefore, it is difficult to predict the 3D structure of the molecules and whether the surface tension criterium could be met. Since by using the classical equipment (Wilhelmy plate, du Noüy ring) the surface age is not controlled, such measurements are prone to artifacts. Special care needs to be taken when measuring surface tension, since the classification as surfactant should be based upon the properties of the substance and not on deficiencies of unsuitable methods.
- In case of not sufficiently soluble substances, there are two challenges: On the one hand, a separation of some hydrophobic material at the air/water interface is quite likely, making a determination of surface tension difficult; especially using classical equipment (Wilhelmy plate, du Noüy ring) will lead to nothing but artifacts. On the other hand, the challenge concerning the surface tension criterion is not that surface activity of the material might be too low - the challenge is that surface activity could be quite high, but the methods originally developed for soluble surfactants cannot be applied. In these cases, the method of choice (but more or less only available in a limited number of specialized academic laboratories) would be a Langmuir film balance[7], which would need to be employed to determine the surface activity of lipids forming so-called insoluble monolayers (e.g. spread from a chloroform solution onto a water surface). Such purely academic studies should be outside the scope of this regulation and hence will not be discussed further.

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[4] OECD (1995), Test No. 115: Surface Tension of Aqueous Solutions, OECD Guidelines for the Testing of Chemicals, Section 1, OECD Publishing, Paris

[5] Wilhelmy L. (1863). Ueber die Abhängigkeit der Capillaritäts-Constanten des Alkohols von Substanz und Gestalt des benetzten festen Körpers. Ann Physik 195:177-217

[6] du Noüy PL. (1919). A new apparatus for measuring surface tension. J Gen Physiol 1:521-524

[7] Gaines G.L. Insoluble Monolayers at Liquid-Gas Interfaces, Interscience Publishers, New York 1966

While the Customs Tariff Regulation clearly specifies that the surface tension cut-off criterion of <45 mN/m should be met at a concentration of 0.5%, the DetReg does not add much precision, which leaves room for interpretation. Although the European Commission made clear in the “Questions and agreed answers” document that the surface tension cut-off criterion of <45 mN/m applicable under the DetReg is the exact same one as applicable under the Custom Tariff Regulation, it did not confirm the concentration of 0.5%. This introduces confusion, especially as the “legally existing” methods like the OECD 115 recommend a concentration of 0.1% to measure surface tension. Hence, either (i) the definition of the DetReg should be adapted to allow for a range of concentration (0.1-0.5%) to be consistent with the Custom Tariff Regulation and with the “legally existing” methods that have been long in place, or (ii) the definition shall be clearly aligned with the one of the Custom Tariff Regulation (concentration = 0.5%) and new preferred methods shall be recommended like the Pendant Drop Tensiometry method, that is applicable at any test concentration. The second option (ii) is the one supported by CESIO, as further explained in the sections below (cf. sections Preparation of the samples and Suggested experimental procedures).

Spreading or adsorption layer at the air/water interface (DetReg)

It is not clear what the idea behind this criterion is, since the consequence of such an adsorbed surfactant layer is a reduction in surface tension – but this is already the previous criterion on reducing surface tension. How should this adsorption layer be assessed experimentally, if not by measuring surface tension? There are reflectometry methods to study the internal structure of such adsorption layers at the air/water interface; however, they require highly specialized experts e.g. for X-ray reflectometry, available at some academic laboratories or at major research institutions (e.g. Neutron Reflectometry at ILL in Grenoble[8]). Therefore, since the reduction of surface tension is already a criterion, this “adsorption layer” criterion is probably not needed, as it only describes the structural origin of the surface tension reduction criterion. Otherwise, this criterion can be considered to be fulfilled without further ado, in case the surface tension criterion was met.

One idea behind using the term “spreading” in this criterion is probably to exclude more hydrophobic substances which are forming an oil lens at the air/water interface – but such cases could easily be covered (i.e. excluded) by using the remark “without separation of insoluble matter” as discussed below. Concerns

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[8] Campbell R. A., Wacklin H. P., Sutton I., Cubitt R., and Fragneto G. (2011) “FIGARO: The new horizontal neutron reflectometer at the ILL,” Eur. Phys. J. Plus, vol. 126, no. 11, pp. 1–22

mentioned in the European Commission's "Questions and agreed answers" document[9] that hydrophilic substances may reduce surface tension without forming an adsorption monolayer are unjustified; even at a concentration as high as 10%, acetic acid (which is mentioned there as an example) has a surface tension of 59.1 mN/m[10], which is closer to water (72.8 mN/m) than to the limit of 45 mN/m.

Formation of emulsions and/or microemulsions and/or micelles (DetReg)

The "micelle" requirement of this criterion excludes alcohols such as pentanol or hexanol, which might sufficiently reduce surface tension, but do not act as surfactants in terms of solubilization or formation of micelles. In case of clear aqueous solutions, the formation of micelles could be proven by using Dynamic Light Scattering; this, however, requires specific equipment as well as highly specialized experts. It would be much easier to just use a (green) laser pointer: in case the laser beam can be seen in the clear sample, there must be micelles – single low molecular weight molecules do not scatter light.[11]

However, the main idea behind this criterion is to cover not only hydrophilic surfactants giving clear micellar solutions in water, but also the more hydrophobic ones which are not water-soluble, but only water-dispersable. Therefore, amphiphilic materials which are not soluble in water, but able to act e.g. as w/o-emulsifiers are included in the surfactant definition. The inclusion of such hydrophobic substances poses several challenges.

The fundamental properties of **microemulsions**, i.e. thermodynamically stable, homogeneous mixtures of oil, water and surfactant, are extensively described in the literature[12], [13], [14]. Typical properties of microemulsions are a transparent or translucent appearance and a droplet size in the range of tens of nm. Typically, microemulsions require the existence of both oil and surfactant – so for a single substance, the formation of a microemulsion will hardly be possible. Only in case of

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[9] Questions and agreed answers concerning the correct implementation of Regulation (EC) No 648/2004 on detergents

[10] Yamada T., Ono N. (2015) A Study on Micromixing Utilizing Marangoni Effect Induced on Gas-Liquid Free Interfaces, J. Micro Nano-Manufacturing 3(2):021003

[11] K. Brandt, J. Venzmer: Micellar Formulations – Old Wine in New Skins? SOFW Journal 03/20, Vol 146, 2020, 34-37.

[12] J. H. Schulmann, W. Stoeckenuis, L. M. Prince, J. *Chem. Phys.* **63** (1959) 1677

[13] L. M. Prince, in: Microemulsions: Theory and Practice, Academic Press, Ney York 1977

[14] P. Winsor, in: Solvent Properties of Amphiphilic Compounds, Butterworth, London 1954

multiconstituent substances, there could be an o/w-microemulsion consisting of micelles (formed by the more hydrophilic species) swollen by the more hydrophobic components of the substance.

An **emulsion** is “a heterogeneous system of two or more liquid phases, consisting of a continuous liquid phase and at least one other liquid phase dispersed into the first in the form of small droplets”[15]. According to this definition, an emulsion, at least temporarily, can be created from all, even completely incompatible, liquids – it is just a matter of shear force and time scale of observation, whether a homogeneous emulsion or (eventually complete) phase separation is observed. Therefore, without guidance on how to prepare the emulsion and without a stability criterion, this part of the surfactant definition is hardly useable. In fact, this part of the definition could be the basis of “reverse engineering”: Depending on whether the substance tested should be a surfactant or not, the mixing conditions could be chosen in order to provide the expected result. Also, an inherently unstable emulsion poses challenges when it comes to the determination of surface tension: An oil film at the air/water interface will more or less dominate the results, depending on the method to measure surface tension.

Adsorption at solid/water interfaces (DetReg)

This last criterion of the Detergent Regulation is the most unclear for several reasons. Most importantly, the solid is not specified: It could have a variety of surface properties (hydrophobic or hydrophilic, cationic (e.g. mica) or anionic (e.g. silica)), and by choosing different materials (such as activated carbon, minerals, or PTFE powder) drastically different results will be obtained. In addition, the logic behind this criterion is not clear: In case the other four criteria were met, is one adsorbing solid sufficient for a material to qualify as surfactant, or is one non-adsorbing solid sufficient for a substance to disqualify as surfactant? Depending on how this criterion is being used, it does not allow to discriminate between a surfactant and a non-amphiphilic substance, e.g. almost everything adsorbs to activated carbon, but this does not mean that the molecules which are being adsorbed to activated carbon are surfactants. Also, because of Coulomb repulsion, it would be possible to show that cationic surfactants are not surfactants according to DetReg, since they do not adsorb to cationic solids (e.g. mica). The same holds true for anionic surfactants, which do not adsorb to anionic solids (e.g. silica). Therefore, this criterion could be deleted without any consequences; during the past two decades, it was (or in fact had to be) ignored anyway.

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[15] EN ISO 862:1995

Transparent or translucent liquid or stable emulsion without separation of insoluble matter (Customs Tariff)

The addition “without separation of insoluble matter” is an important necessity, but difficult to assess experimentally. The idea is that an oily substance floating on top of water should not qualify as surfactant. The absence of such an oily layer is also crucial for the surface tension measurements using the only methods mentioned in OECD TG 115: Wilhelmy plate and du Noüy Ring. These classical methods are – because of an undefined surface age – especially sensitive to artifacts caused by hydrophobic materials accumulating at the air/water interface.

Preparation of the samples

A concentration has not been defined in the Det Reg; in order to be consistent and not to create confusion, the 0.5% from the Customs Tariff regulation should be used, just like the corresponding surface tension limit of 45 mN/m. For “typical” surfactants, i.e. hydrophilic and micelle-forming, the preparation of an aqueous solution is straight forward – no special care needs to be taken. But there are more difficult candidates, which could be rather hydrophobic (or contain more hydrophobic components) and/or are solid. In those cases, it is indispensable to both define a mixing procedure and a stability criterion, since “separation of insoluble matter” should lead to an exclusion as surfactant, also because such a separation makes a determination of surface tension pretty much impossible. Intensive, high shear mixing could force a substance incompatible with water to form an emulsion; this is, however, nothing the substance would do “voluntarily” – and therefore should rather be avoided; low-shear mixing using a magnetic stir bar would be recommended. Another question is how to deal with solids: Should they be molten before mixing in case they are sufficiently stable? Or should the dispersion be made above the melting point to facilitate the dispersion process? However, in both cases, ensuring the chemical stability of the substance is important. Otherwise, an unsuitable mixing process would be an excellent option to rule out that a solid surfactant is – legally-speaking - a surfactant. In this context, (anionic) surfactants having a Krafft point above room temperature also pose a challenge, since “separation of insoluble matter” means in this case “separation of crystals”.

Some substances are hard to disperse in water; therefore, their delivery form might contain a considerable amount of a water-miscible solvent (e.g. glycols). This is intentionally added just for ease of handling by the formulators. Such substances shall also be tested in a neat form; this follows the rationale that the classification of a substance should be a property of the substance itself rather than its delivery form.

Some nonionic surfactants have a cloud point <20˚C, typically measured at 1% in water; hence, they are insoluble in water at room temperature. If possible, the solution should be prepared at temperatures below cloud point.

The evaluation (visual observation, surface tension measurement) of the samples should be done at room temperature; for practical reasons, this could be 20 to 22 ˚C.

Some substances are insoluble at certain pH values, e.g. soaps at pH<8. This applies also to other ionic surfactants containing ionic groups of weak acids or bases. Therefore, the pH value of the aqueous mixture is relevant for both the determination of solubility and the measurement of surface tension. Since surface tension can depend on pH, there are several options to choose the “correct” pH: This could be either the pH of the substance as placed on the market, or the pH at which the substance is used in a typical application, or the pH of the substance after use. If pH adjusters are used, they must be chosen from the list described in the Ecotox test standards (e.g. OECD 201 or OECD 301).

3. Suggested experimental procedures

Preparation of aqueous samples

A 0.5 wt% aqueous solution is prepared by adding 0.45 g of the test substance to 89.55 g of demineralised water in a 100 mL beaker or glass jar (h = 10 cm; Ø = 4 cm) with screw top. The mixture is thereafter stirred for one hour at room temperature (approximately 20 – 25 ˚C) using a magnetic stirrer (l = 29 mm; Ø = 7 mm) between 500 and 1000 rpm. Solid substances should be molten first in order to facilitate preparation of a representative sample. At the end of the dissolution process, the evaluation time of one hour starts.

Evaluation of aqueous samples

a) **Clear micellar solution**: In this case, the formation of micelles needs to be proven, either by Dynamic Light Scattering or by using a (green) laser pointer.

b) **Microemulsion and** c) **Emulsion:** Concerning the separation stability of the mixture, it is reasonable to evaluate the optical appearance after one hour (just before measuring the surface tension); special care needs to be taken to look for separation either on top (creaming) or at the bottom (sedimentation).

Recommended procedure for evaluating the stability of emulsions:
An aliquot of the prepared sample is transferred to a cuvette and the turbidity (preferred: turbidity ratio at two widely separated wavelengths) is measured

at the beginning of the test period and after standing for 1 h by using e.g. a nephelometer. If the change in turbidity units (preferred: turbidity ratio at two widely separated wavelengths) is >25%, the sample is regarded as not forming a stable emulsion. Otherwise, the stability criterion is fulfilled.

Surface tension measurement

Only if the stability criterion (micelle, microemulsion, or emulsion, and no separation of insoluble matter) is fulfilled, surface tension is to be measured. The classic methods (Wilhelmy plate and du Noüy ring) to determine surface tension are described in OECD 115. However, the state-of-the-art in both academic and industrial laboratories for more than 20 years is Drop Shape Analysis (a.k.a. Pendant Drop Tensiometry). This is much preferred, especially for turbid samples, since every measurement starts with a fresh surface, making this method more reliable and less prone for artifacts. A norm (DIN 53013) on the use of the Pendant Drop method to determine surface tension of surfactant solutions is currently being developed; as of today, there is only a norm from the area of paints and varnishes (EN ISO 19403-3[16]).

Experimental report

The evaluation report for each sample should include the following points:

- Used materials, concentrations, conditions, pH value, pH adjustment to reach solubility if appropriate
- Optical appearance directly after mixing
- In case of clear solution check for micelles using laser pointer
- Optical appearance (separation?) after storage for one hour at 20 °C
- Turbidity ratio measurements (1 hr vs. fresh) for an emulsion in case no oily layer is visible on top
- Surface tension, ideally determined via the Pendant Drop method; in case of turbid samples picture of magnified drop (used for the surface tension measurement) to substantiate the validity of the result.

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[16] EN ISO 19403-3. 2020. Paints and varnishes - Wettability - Part 3 : Determination of the surface tension of liquids using the pendant drop method

4. Final Remarks

This document shows that it is not really possible according to the current DetReg to give a legally binding answer whether a substance is a surfactant or not. As discussed above, an omission of the criteria “spreading or adsorption layer at the air/water interface” and “adsorption to solids” and the addition of the remark “without separation of insoluble matter” would solve these issues; additionally, such an update of the surfactant definition in DetReg would also harmonize the regulations in Europe (DetReg and Customs Tariff). This is the reason CESIO has already proposed an amendment of the surfactant definition.