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Assessment of the applicability of HLB values for natural phospholipid emulsifiers for preparation of stable emulsions

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Received October 27, 2019, accepted May 16, 2020

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Pharmazie 75: 365-370 (2020) *doi: 10.1691/ph.2020.9174*

Natural phospholipid (PL) excipients are native, biocompatible and relatively inexpensive alternatives to synthetic emulsifiers. A well-known PL excipient is lecithin, which primarily contains phosphatidylcholine (PC) and (depending on the purity grade) also contains a well-defined mixture of other PLs with a fatty acid composition, which reflects their natural source. Since all of these lipid species are emulsifiers, natural PLs can be considered as a mixture of (co-) emulsifiers. Many different HLB values for lecithins are given in the literature, which is why this needs to be clarified. To assess this, HLB values of thirteen different plant derived PLs differing in PC content were determined using a centrifugation stress method to determine the relative stability of an emulsion with the respective emulsifier and different oil phases. It could be shown that the studied PLs can be characterized by a broad HLB range, which may be linked to the composition of the PLs and the oil used. In order to emphasize the results of the HLB determination, it could be demonstrated that stable emulsions could be prepared with a wide range of oils using the plant-based PLs and that the preparation method of the emulsions is important in order to obtain stable emulsions. Therefore, assigning a specific exact HLB value to natural PLs instead of a wider range is inappropriate. The broad HLB ranges indicate the suitability of the studied PL emulsifiers for the preparation of oil-in-water emulsions for a wide range of oils: It is recommended to experimentally evaluate the suitability of these natural emulsifiers for the preparation of stable emulsions and to benefit from the wide range of HLB values of these emulsifiers instead of relying on inaccurate and confusing HLB values in the literature.

1. Introduction

Natural phospholipids (PLs) prepared from for example soybean or egg yolk are widely used as excipients in the food, pharmaceutical and cosmetic industry (van Hoogevest and Wendel 2014). They have amphiphilic properties and are used as multifunctional excipients in many types of formulations such as fat emulsions, mixed micelles, liposomes and suspensions as emulsifiers, solubilizers, liposome formers and wetting agents (Pardun 1989; van Hoogevest and Wendel 2014).

Natural bilayer forming PLs have advantages over the more aggressive, micelles forming synthetic surfactants such as polysorbates and polyoxyethylene derivatives. PLs are a main constituent of cell membranes and are therefore toxicologically safe, biocompatible and biodegradable.

The PL molecule consists of a glycerol backbone which is esterified in positions 1 and 2 with fatty acids such as palmitic acid, stearic acid and oleic acid, and in position 3 with a phosphate residue which is esterified with an alcohol. This alcohol is choline in phosphatidylcholine (PC), ethanolamine in phosphatidylethanolamine (PE), glycerol in phosphatidylglycerol (PG) and inositol in phosphatidylinositol (PI). The PL without esterified alcohol is defined as phosphatic acid (PA). PLs with only one fatty acid are defined as monoacyl PLs or lyso PLs. Natural lecithins/PLs are produced by extraction from e.g. soybeans, sunflower seeds and egg yolk with non-toxic solvents such as ethanol. The PLs can be purified using chromatographic methods in order to obtain PL fractions with different PC content (20–98 %) and a different ratio of polar PLs to non-polar lipids. PL excipients are reproducibly produced using standardized raw materials and validated extraction and chromatographic procedures. The native PLs are available on a large scale, are environmentally friendly and the production costs are low compared to synthetic PLs (van Hoogevest and Wendel 2014).

Natural PLs have a fatty acid composition that is typical for the source from which they were extracted (van Hoogevest and Wendel 2014). Although the natural PLs are heterogeneous with regard to the content of PLs with different polar head groups (i.e. phosphate esterified with the alcohol) and the fatty acid composition, the PLs have a reproducible (interbatch) composition within precisely defined specifications. In this publication, the following nomenclature according to international pharmacopeias is used and recommended, which describes the various commercially available natural lecithins/PL excipient products that differ in PC content. Lecithin is a complex mixture of acetone-insoluble phosphatides (i.e. PLs), which consist mainly of PC, PE, PI and PA and is separated from the crude vegetable oil source together with various amounts of other substances such as triglycerides, fatty acids and carbohydrates (United States Pharmacopeial Convention 2014). The term “lecithin” is used when the product contains less than 80 % by weight total PLs; the term “phospholipids” is used when the product contains 80-100 % by weight PLs. The specific PL is mentioned (e.g. PC) if the product contains more than 90 % by weight of the specific PL.

For the characterization of emulsifying agents for the preparation of any kind of application, the most common indicator for the selection of emulsifiers for producing emulsions is the so-called HLB (hydrophilic-lipophilic balance). The use of HLB values was introduced by Griffin (for non-ionic detergents) in 1949/1954 and has been modified and expanded since then. Related to the addition of ionic emulsifiers to the scale and the required HLB of the lipophilic phase used for the emulsion, some inconsistencies of this parameter were highlighted (Abbot 2017; Schott 1990). To date only a few alternatives to using HLB values (such as the Hydrophilic Lipophilic Deviation) have been presented, which have their own distinct flaws. Nowadays, HLB values are still used to classify surface-active substances due of the lack of alternative, easily accessible and reliable parameters.

Due to the heterogeneous composition of the natural PLs, which contain different PL species with different physicochemical properties, they therefore act as a mixture of emulsifiers and co-emulsifiers. Many different HLB values for lecithins and PLs are mentioned in the literature (see Table 1).

Table 1: Examples of HLB values of phospholipids as described in the literature

Phospholipid	HLB	Reference
Egg phosphatidycholine	Approximately 7	Chung et al. 2001
Sunflower lecithin	Approximately 8	Cabezas et al. 2016
Lysolecithin Lecithin, concentrated Lecithin with minor hydrolysis	6.5 – 8.0 4.0 – 4.5 5.0 – 5.5	Reddy Jala et al. 2016

Table 1 shows that the literature tends to assign PLs to either specific HLB values – or narrow HLB ranges, which are not well described in terms of the analytical method used to determine these values – and the exact chemical composition of the phospholipids mentioned. In this regard Van Nieuwenhuyzen and Sjuhaj (1998) refer to an HLB value of 4 for a standard lecithin with 16 % PC, 13 % PE and 10 % PI. Further confusion is caused by the nomenclature of lecithin. In American literature lecithin is used for pure phosphatidylcholine, whereas the USP definition of lecithin refers to a mixture of phospholipids with mainly phosphatidylcholine (USP, Lecithin Official Monographs USP 39/ NF 34 First Supp 01.12.2016)

The relatively narrow assigned HLB values, which are mostly below 7, suggest the use of lecithins as water-in-oil emulsifiers, although literature and industry uses them primarily as oil-in-water (o/w)-emulsifiers. These confusing literature values are not very helpful for the users of PLs who are interested in selecting the excipients as emulsifiers to prepare a particular type of emulsion. The objective of this study was, therefore, to critically assess these assigned HLB values for thirteen plant derived PLs compared to SPAN® 20 (Sorbitan monolaurate) as reference and to investigate the correlation between the experimentally determined HLB values of these plant-based PLs and their suitability for stabilization of oil-in-water emulsions (see Rahm 1966; Schott 1990; Vaughan and Rice 1990).

2. Investigations and results

2.1. Determination of the HLB value of plant derived phospholipids

The HLB values of the PLs were assessed according to a modified method published by Robbers and Bahia (1961). For details on the sample preparation and centrifugation reference is made to the Experimental section. From the obtained phase separation data of each emulsion series the most stable emulsion, characterized by a minimum of phase separation after centrifugation was selected for calculation of the respective HLB value. This procedure was performed using three different lipophilic phases with varying required HLB values to ensure robustness of the calculated HLB values.

The modifed method was calibrated by assesseing the HLB values of a well known emulsifier (SPAN® 20) with defined HLB value using three different oil phases with varying required HLB value. The resulting correlation between calculated HLB values and observed degree of phase separation with SPAN® 20 (represented by the position of the interphase of the oil and water phases in the centrifuge tube) is shown in Fig. 1. The phase separation in ml accounts for the volume of separated oil phase by centrifugation from the 10 ml of previously prepared emulsion.

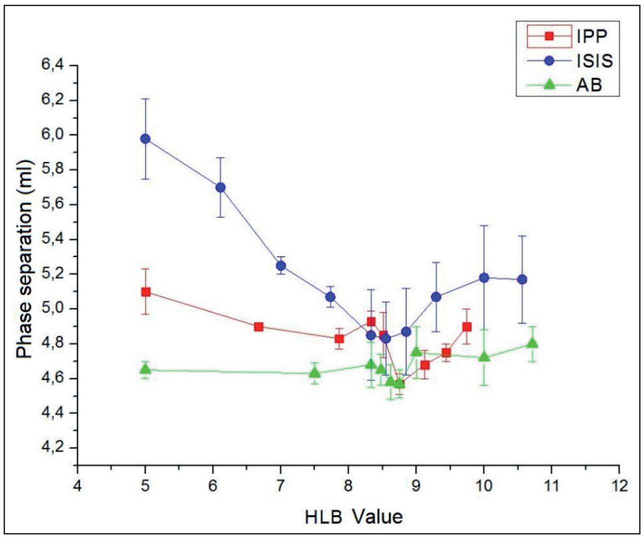

Fig. 1: Phase separation curves of SPAN® 20 (n = 3) using the HLB assessment method as described in the Experimental section.

In general, the precision and accuracy of the method were both comparable to the original method and distinctive minima for SPAN® 20 with all three analyzed lipophilic phases at its known HLB value of 8.6±0.1 were observed (Needs 1976). When the same method was applied to TWEEN® 80 (polysorbate 80) with three different oil phases a HLB value of 15.2±0.3 was obtained in agreement with literature values (Needs 1976) (Fig. 2).

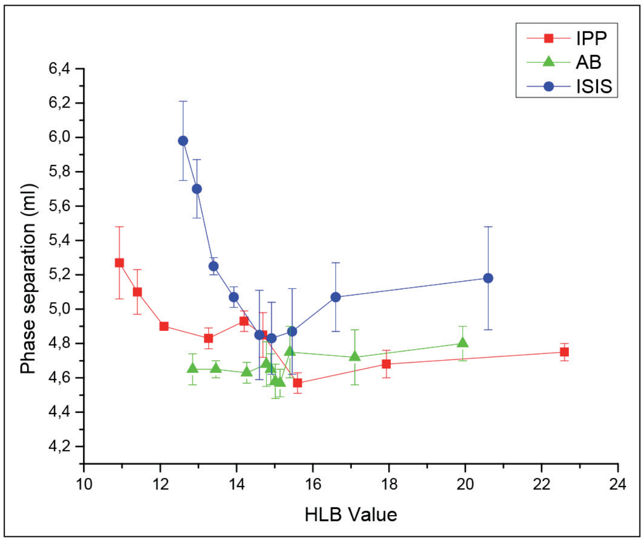

Fig. 2: Phase separation curves of TWEEN® 80 (n = 3) using the HLB assessment method as described in the Experimental section.

In contrast to the results obtained for the reference emulsifiers, all analyzed PLs presented a different behavior reflected by their phase separation curves. In Fig. 3, phase separation curves for the typical phospholipid LIPOID S 75 as natural PL for the three different oils are provided.

Figure 3 demonstrates minor differences in the determined phase separation lines of the three oils used. Emulsions prepared with isosteraylisostearate (ISIS) as lipophilic phase exhibited the tendency to separate at a higher level compared to isopropylpalmitate (IPP) and alkyl benzoate (AB). Especially when using AB, it is clearly shown that highly stable emulsions can be created over a wide range of HLB values. Possible explanations for this

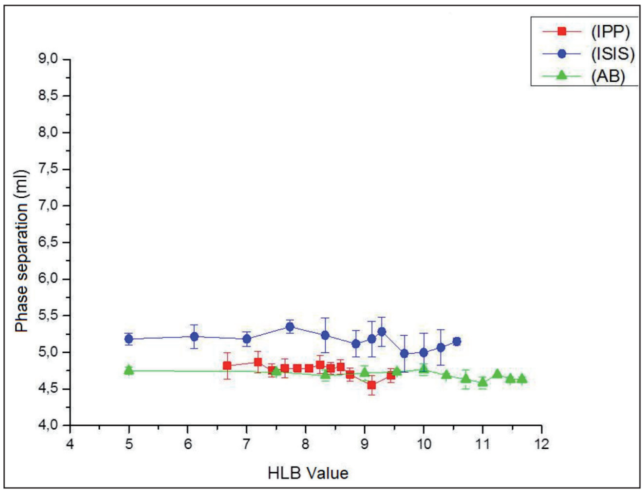

Fig. 3: Phase separation curves for phospholipid LIPOID S 75 (n = 3) using the HLB assessment method as described in the Experimental section.

behavior may be the different affinity of the applied lipophilic phases to the natural PLs (Rydhag and Wilton 1981). This may be due to the differences in their respective structure and the resulting interactions at the interface. These physicochemical effects are challenging to understand and their explanation was not in the focus of this study. More insight may be gained by analysis of the respective layers at the interface via Langmuir-Blodgett trough, IR-spectroscopy or force field based computer modeling. One method alone will most likely not be sufficient to provide a deeper understanding of the effects.

Although the influence of the used oil phase on the characteristics of the curves could be seen, no distinct minimum, as observed for SPAN® 20 and TWEEN® 80, could be determined over a wide range of HLB values. This questions the assignment of a certain narrow range of HLB value to the examined PLs mentioned in the literature.

As no significant minimum of phase separation could be identified for most of the analyzed natural PLs (in accordance to the presented figures) these results clearly indicate that the PLs tested do not have a distinct HLB value but are best described as emulsifiers having a broad range of HLB values. The emulsions used to determine the HLB value were comparably stable over a wide range of the studied ratios between hydrophilic and lipophilic phase. Therefore, the entire phase separation curve should be used to describe the HLB characteristics of natural phospholipids.

SPAN® 20 shows a clear minimum that indicates its HLB value, in contrast to the results obtained for natural PLs that have no clear minimum. As a result, the method and its experimental setup can in principle generate clear minima in a phase separation line, which ilustrates the reliability of the method, but the behavior of PLs at the interface between two immiscible phases differs significantly from the well referenced emulsifier (SPAN® 20). Therefore, it is more appropriate to assign broader HLB ranges to natural PL emulsifiers instead of an exact number or narrow ranges. The majority of the analyzed PLs exhibited minimal phase separation, which corresponds to an HLB range, which is around the expected literature values between 7.5 and 10.5. This underlines the preference for using most of these PLs as emulsifiers for the preparation of o/w-emulsifiers.

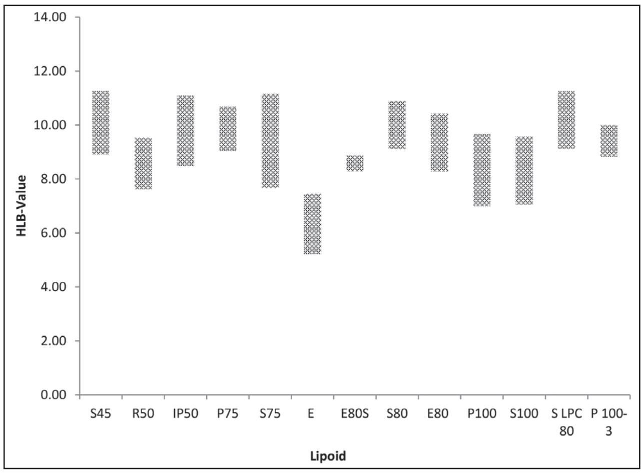

Fig. 4: HLB ranges of the plant-based PLs using the HLB assessment method as described in the Experimental section.

In Fig. 4, the results of the HLB determination of the studied lecithins are summarized, the raw data of the HLB determination are shown in Appendix I.

When comparing the HLB ranges of the analyzed natural phospholipids, most of them are within the range of emulsifiers for oil-in-water emulsions. An exception is LIPOID E, which is more an o/w emulsifier with a range from 5.2–7.5. This can be correlated with the higher amount of PE in LIPOID E compared to the rest of the natural PLs PE, which has a smaller hydrophilic head group compared to PC, and reduced swelling capacity, which makes it more suitable for preparation of oil-in-water (o/w)-emulsions (Rydhag and Wilton 1981). In addition, unsaturated PE molecules have an inverted cone shape and fit better into the curvature of the interface of water-in-oil emulsions (Muschiolik and Bunjes 2007). In general; natural PLs with a higher concentration of PE exhibit a lower HLB range and can possibly be used for both types of emulsions. S LPC 80, a plant (soybean)-based lyso-phospholipid with a high amount of Lyso-PC is as expected showing the highest HLB-range and is therefore best used for preparation of o/w-emulsions.

2.2. *Stability testing of emulsions prepared with natural phospholipid emulsifier*

These particular HLB ranges can be a first indicator for predicting the stability of natural PL-based o/w-emulsions. In order to further investigate the influence of the variance in composition of the phospholipids, oil-in-water emulsions were prepared with three different PLs. Although the HLB experiments showed in some cases the use of natural PLs as w/o-emulsifiers as well, o/w-emulsions were chosen for a better comparison of the results.

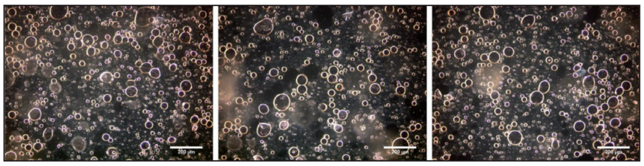

Fig. 5a: Microscopic image of LIPOID S45, E and S 100 (from left to right) based o/w emulsions at t = 0

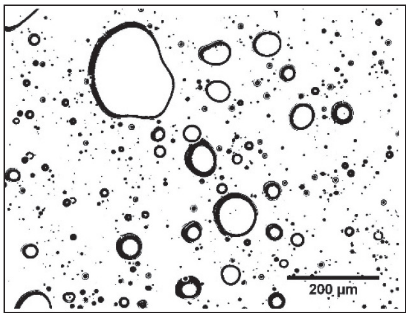

Fig. 5b: Processed image of a LIPOID S45 based o/w-emulsion at t = 0 A B

The correlation between the PLs with experimentally determined broadest HLB range and the usefulness for preparing stable o/w-emulsions with these phospholipids was studied. In order to produce a fairly normal o/w emulsion, 20% (w/w) MCT was chosen as an often used oil phase with a required HLB of 11. To test

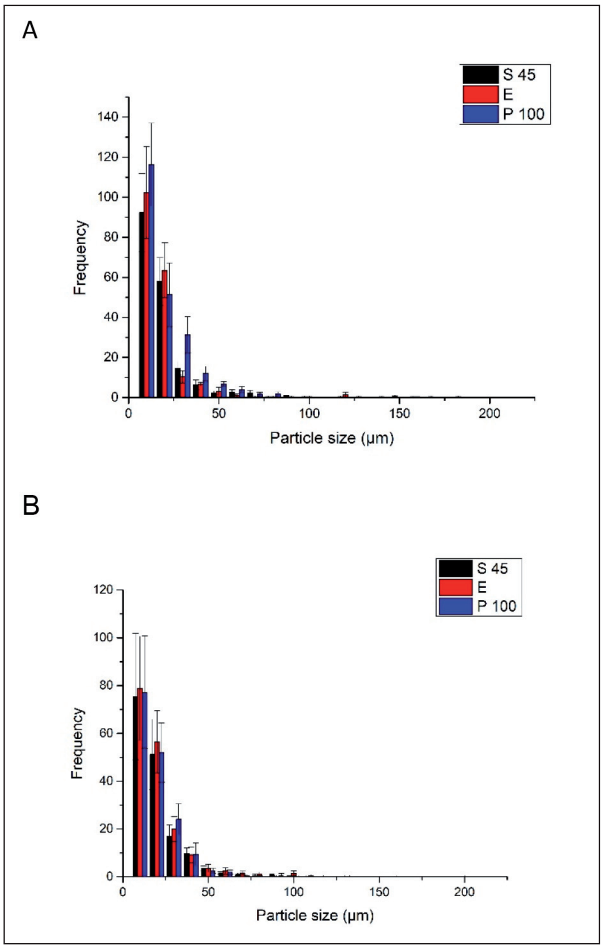

Fig. 6: Histograms of droplet size of S 45, E and P 100 (n = 5) at t = 0 (A) and at t= 2 months (B)

the physical stability of these o/w emulsions, the oil droplet distribution of the test emulsions was analyzed by dark field microscopy (Fig. 5a) and an automated image analysis (Fig. 5b). The counted droplets were classified according to their size (diameter) and displayed as histograms for comparison. The applied microscopic and analytic methods provide an approach to automation which, to the knowledge of the authors, is not described in the literature.

Due to the challenging statistic and preparation a stack of images (n=5) was used to generate a cumulated frequency of droplet size. Fig. 6 shows histograms of three o/w emulsions prepared with three different natural PLs with the greatest variety in respect of their determined HLB ranges. These PLs were LIPOID S 45 (HLB range = 8.9–11.3), LIPOID E (HLB range = 8.3–8.9) and LIPOID P 100 (HLB-range = 7.1–10.0).

All emulsions prepared with the natural PLs showed a non-normal size distribution of the oil droplets. The size distribution resembles a logarithmic distribution, as has already been described for emulsions. This could not be confirmed statistically, presumably due to the arbitrary defined classes of particle size and the technical limit for a particle diameter of 2 µm. To investigate whether the emulsion originated from populations with the same distribution, the nonparametric Wilcoxon-Mann-Whitney test was used. All three emulsions showed no differences from each other as the small variances in Fig. 6 suggest ($p<0.05$).

The o/w-emulsions were stored at room temperature and the droplet size analysis of the emulsions was repeated after 1 and 2 months of storage at room temperature. After one month of storage at room temperature (detailed results not shown) no significant differentiation between the three natural PLs based emulsions was detectable. Figure 6 shows the repeated analysis after two months of storage, in which all types of emulsions are still of the same population. The frequency parameter of the Y-Axis describes the average amount of detected droplets in each image of the image stack. The minor differences in the amount of small droplets (< 30 µm) were explained above. With larger droplets, the observed difference is attributed to statistical randomness in the selected image frame.

3. Discussion

A centrifugation assay was used to assess the HLB value of thirteen natural phospholipids with varying degrees of purity from different natural plant sources. Although all recorded values were within the expected range, no accuracy of the values could be confirmed with regard to the literature value. The determined HLB value was strongly dependent on the required HLB of the lipophilic phase used for preparation of the test emulsion. Higher required HLB values of the oils used for the determination of the HLB led to higher HLB ranges of the PLs pointing at a flaw in the HLB equation.

Only minor variances in the phase separation curves of the natural PLs studied were registered over a wide range of HLB values. It was observed that the variability of the results increased with higher purity of the phospholipids. This is likely due to the nature of plant-based phospholipids and the presence of additional substances in the phospholipids acting as co-emulsifier in the emulsion. It should be stated that the situation at the interface between water and oil phases during the experimental setup is different from the practical use of the emulsifiers, since at least two emulsifiers are used to determine the HLB-Values. However, analysis of the entire separation curve (see Fig. 3) suggests that stable o/w emulsions can be prepared using natural PLs.

In contrast, more defined emulsifiers such as SPAN® 20 or TWEEN® 80 showed a clear minimum in the phase separation curve that was used to determine their HLB value. Since the HLB value was originally developed and defined for more defined ethoxylated emulsifiers (and has been extended to some ionic emulsifiers as well) (Griffin 1949, 1954), it is not inherent that all classes of emulsifiers, especially mixtures of emulsifiers, may be correctly characterized through it. Although ethoxylated emulsifiers are also a mixture of substances, the more complex (but reproducible) composition of a greater variety of substances in natural PLs may give them a broader usability as an emulsifier. Therefore, the use of wider HLB ranges for natural PLs is recommended instead of a precise value or narrow range as in most of the literature.

These determined HLB ranges were verified by comparing the stability of emulsions prepared with three natural PLs with the most diverse range. It could be shown that these o/w-emulsions could be prepared with comparable stability using a new approach of automated microscopic droplet evaluation. This suggests that the determined HLB ranges for the PLs could be further extended by additional experiments.

To analyze the application of the determined HLB ranges, a method for microscopic droplet analysis was applied to assess the stability of emulsions prepared with natural PLs with different HLB ranges. All three emulsions prepared with plant based PLs that differed in HLB range showed the same physical stability as assessed using the Wilcoxon-Mann-Whitney statistical test method for a non-parametric particle size distribution. Natural plant-based PLs can be considered proficient emulsifiers over a wide range of ratios of different lipophilic and hydrophilic phases. To characterize their potential to form stable o/w or w/o emulsions, the attributed HLB value alone is not sufficient.

There are few other methods for characterizing surfactants and, particularly, for surfactant mixtures that are already available, but none of them is currently widely used. Although the HLB is mostly easy to evaluate, there is a need to sensitize people for the disadvantages of experimentally determined HLB values and ranges for natural mixtures of plant-based emulsifiers such as PLs.

In conclusion, natural lecithins and phospholipids from soybean, sunflower and egg yolk were tested for their HLB values in a centrifugation assay. Contrary to the common conception of HLB in the literature, wide ranges of HLB values were found instead of a single numeric values. Most of the lecithins analyzed exhibited

Table 2: Lipid composition regarding main polar lipids, of the studied phospholipids batches as derived from the certificates of analyses of Lipoid GmbH (n.d. … not determined).

Phospholipid	PC [g/100 g]	PE [g/100 g]	Lyso-PC [g/100 g]
Soybean			
LIPOID S 45	57.4	16	1.5
LIPOID S 75	70.6	7.5	2.5
LIPOID S 80	77	3.9	5
LIPOID S 100	96.7	0.1	0.7
LIPOID S LPC 80	12.8	n.d.	82.7
Non-GMO Soybean			
PHOSPHOLIPON 50 IP	54.1	n.d.	n.d.
LIPOID P 75	69.2	5-10	3
LIPOID P 100	97.4	0.1	0.3
LIPOID P 100-3	96.4	n.d.	1.1
Rapeseed			
LIPOID R 50	52.4	11.9	n.d.
Egg Yolk			
LIPOID E	70	12.8	2.6
LIPOID E 80	85	9.5	3
LIPOID E 80 S	70.1	15	2

a HLB range suitable for preparation of o/w emulsion. Lecithins with increased PE content (approx. 10% and greater) showed additional preferences for use as w/o emulsifiers. Microscopic images of generic o/w-emulsions with natural PLs from different HLB ranges showed their capability to from stable emulsions beyond their known HLB value. Considering the complexity of the interfacial packing of natural emulsifiers and the challenge of predicting the ability of such mixtures to form stable interfaces and emulsions, it is recommended to conduct emulsion stability screening experiments with phospholipids rather than relying on approximate HLB literature values to select phospholipids for a certain emulsion type.

4. Experimental

4.1. Materials

All PLs (see Table 2) were donated by Lipoid GmbH (Ludwigshafen am Rhein, Germany) and used without further purification. The composition of the utilized PLs is shown in Table 2. Isopropyl palmitate (IPP), alkylbenzoate (AB), medium-chain triglycerides (MCT) and isosteraylisostearate (ISIS) were purchased from Croda Europe (Cowick Hall, UK). SPAN® 20 and TWEEN® 80 were obtained from Sigma Aldrich (Schnelldorf, Germany).

4.2. HLB-Determination of PLs

HLB Determination was performed as described by Robbers and Bahtia (1961) with minor modifications since the original method was used to determine the unknown required HLB value of the oil phase and not the emulsifier. The following equation was used to calculate the HLB value of the tested phospholipids. Three lipophilic phases with different required HLBs were selected to analyze the natural PLs with unknown HLB value.

$$HLB_b = \frac{HLB_{Oil}*(m_a+m_b)-HLB_a*m_a}{m_b}$$

HLB calculation for unknown emulsifier

List of Symbols

HLB (Oil) = required HLB value of the respective oil

m_a = mass quantity of the emulsifier with known HLB[%]

m_b = mass quantity of the PL [%]

HLB_a = known HLB value of the emulsifier (SPAN® 20)

HLB_b = unknown HLB of PL

Stock solution (25 mg/ml) of the respective lecithin in the oil phase (isopropylpalmitate (IPP), isostearyl stearate (ISIS) and alkyl benzoate (AB)) were prepared at room temperature in an ultra-sonic bath Sonorex Super RK 102 H from Bandelin electronic (Berlin, Germany), as well as a stock solution of 25 mg/ml SPAN® 20 in deionized water. 0.5% (w/w) of these solutions was mixed into a 50:50 oil and water mixture to create a stock emulsion via homogenization with an Ultra-Turrax (IKA, Staufen im Breisgau, Germany) at 13000 rpm. These stock emulsions containing either lecithin or SPAN® 20 were then mixed together at different volume ratios (e. g. 1:9 ml) for 20 s with the Ultra-Turrax at 13000 rpm. These 10 ml of different volumes of the stock emulsions represented the range of HLB values to be tested for determination of the most stable balance between water and oil phase for the respective PL. The obtained series of emulsions (n = 3) was transferred in a Heraeus Biofuge primo R (Hanau, Germany) and centrifuged for 8 min at 1500 rpm. The resulting phase separation in the centrifuge tubes was measured via a caliper. The composition(s) of the mixture(s) showing the least phase separation was used to calculate the HLB value of the phospholipid.

4.3. Stability testing of microscopic evaluation of PL emulsion

All of the o/w-emulsions were prepared with medium-chain triglycerides (MCT) as lipophilic phase of 20 % (w/w) and with 2 % of the respective PL as emulsifier. After hydrating the natural PLs in water for 30 s both phases were united and stirred with an Ultra-Turrax for 2 min at 13000 rpm.

All emulsions were stored at room temperature and were transferred *via* a pipette on a microscopic slide. To overcome deformation and modification of the present emulsion droplets at the cover slip, a microscopic slide was used with a curvature of 5 mm to provide enough space for the larger droplets in the emulsion to overcome this problem. The imaging with a mounted camera (ColorView 3.1, SIS, Norwich, UK) took place at the edge of the curvature because the contrast was acceptable in this area due to the deflection of light at this specific point. Dark field microscopy via Leica DM RXE (Wetzlar, Germany) was chosen to optimize the contrast which is a critical point in emulsions because both phases only slightly vary in their refractive index. Images (see Fig. 4) show that the indirect light from the dark field enhances the refraction caused by the interface of the inner lipophilic phase.

The acquired images were processed using Fiji distribution (Schindelin et al. 2012) of ImageJ Software (Schneider et al. 2012).to perform an automated particle counting. This approach was chosen because manual counting is time consuming and lacks reproducibility. Software based evaluation can only be performed on unambiguous parameters so the images had to be converted in binary images. This was accomplished combining several image modification steps (Fig. 4b) into a self-programmed macro allowing rapid procession of stacks of images. These images could be processed using the integrated particle counting routine with defined parameters for which the minimal particle diameter was crucial as the resolution of light microscopy is limited to ~2 µm. Accordingly, this reduces sufficiently the disturbances of microscopic artifacts on the software routine.

Conflicts of interest: None declared.

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Appendix: I

Actually measured HLB values of natural PLs in the respective lipophilic phase (n = 3)

Lipoid	HLB (IPP)	RSD [%]	HLB (ISIS)	RSD [%]	HLB (AB)	RSD [%]
S 45	8,8	1,3	9,7	3,6	11,3	0,0
R 50	7,6	0,9	9,5	2,5	8,6	4,0
IP 50	8,5	2,6	8,5	1,4	11,1	1,3
P 75	9,0	1,4	10,2	1,6	10,7	2,8
S 75	7,7	0,8	9,8	2,6	11,2	1,4
E	5,8	0,4	5,2	5,3	7,5	1,2
E 80 S	8,9	2,3	8,9	0,2	8,3	0,2
S 80	9,1	0,0	9,8	1,9	10,9	1,5
E 80	8,3	1,1	10,2	1,7	10,4	0,1
P 100	7,6	2,4	7,0	0,6	9,7	2,7
S1 00	7,5	3,9	7,0	1,0	9,6	0,2
P 100-3	9,9	1,4	8,8	1,2	10,0	2,1
S LPC 80	9,1	0,2	10,1	0,8	11,3	1,8