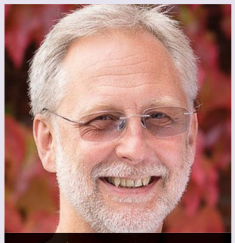
Stefan Ulvenlund

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Redefining *Poly* in Alkylpolyglucosides

Properties and applications of Alkylglycoside surfactants with elongated headgroup

KEYWORDS: Alkylpolyglucoside, APG, enzymatic synthesis, wetting, dispersion, polymeric headgroup.

Abstract

Conventional large-scale production of alkylglycosides ("alkylpolyglucosides", or APGs) yields surfactants comprising headgroups with low average degree of polymerisation (Dp≤2). The term "alkylpolyglucoside" is therefore, strictly speaking, a misnomer as far as conventional technology goes. In stark contrast, ethoxylated (PEG-based) non-ionic surfactants can be produced on industrial scale with virtually any length of alkyl chain and headgroup.

Enza Biotech AB has recently shown that enzymatic technology allows for facile production of alkylglycosides with long headgroups (Dp>>2) and long alkyl chains (≥16). The present paper summarises some of the physicochemical properties of these novel surfactants. The focus of the paper is on hexadecyloctaglucoside (C16G8), which is shown to have new and unique properties of direct relevance for formulation of cosmetics and personal care products, particularly as regards wetting and dispersion of hydrophobic particles.

INTRODUCTION

For the last 20-some years, alkylglycosides (often referred to as "alkylpolyglucosides", APGs) have rapidly grown in importance to become a key class of non-ionic surfactants in cleaning, personal care, cosmetics and manifold other areas of formulated products. Conventional, industrial-scale production of alkylglycosides is based on Fischer glycosylation. (1, 2) The method is used to prepare a wide range of APGs, in an economically feasible manner and from raw materials that are completely (or at least predominantly) sourced from sustainable origins. Nevertheless, the Fischer method has important inherent drawbacks that impose strict limitations on the molecular architecture of the reactants and products. In the context of the present paper, the most important limitation pertains to the length of the head-groups and alkyl chains in the products. More specifically, for reasons of reaction kinetics and physical incompatibility of the starting materials, the average degree of polymerisation in commercial APGs is actually lower than 2. Obviously, this means that the term *poly* in alkylpolyglucoside is in itself a misnomer. Furthermore, the incompatibility issues mean that it is challenging to use alkyl chains longer than 12 carbons in the syntheses, and the vast majority of available APGs consequently comprise chain lengths between 6 and 12 carbons. In order of increasing alkyl chain length, these APGs act as surfactants suitable as hydrotropes, wetting agents, foamers and detergents. However, other critically important applications, such as emulsification of oils and dispersion of hydrophobic particles, call for surfactants with longer alkyl chains. APGs with long alkyl chains are not only rare (3), but also riddled by solubility problems, since an increase of alkyl chain length decreases surfactant solubility for a given headgroup size. In actual fact, the Krafft temperature (the temperature at which the surfactant solubility starts to increase dramatically) is above room temperature already for tetradecylmaltoside (4). From a practical point of view, this means that APGs that combine a long alkyl chain with a short headgroup (as is the case when conventional technology is applied) will be very sparingly soluble at room temperature and thus require heating when used in aqueous systems. Increasing headgroup size lowers the Krafft temperature, and solubility considerations alone therefore provide compelling reasons to increase the headgroup size of APGs. The possibility to extend the headgroup simply expands the range of alkylglycosides that are soluble at ambient temperature. However, increasing headgroup size is also interesting for reasons of biocompatibility. Experiments conducted in human cell models have shown that the cell toxicity of ethoxylated (PEG-based) non-ionic surfactants decreases with increasing size of both the headgroup and the alkyl chain (5). This finding originally provided critical inspiration for our attempts to increase the headgroups size also for APGs.

As we have previously reported, enzymatic technology provides a possibility to circumvent key hurdles in conventional synthesis, and opens the possibility to produce APGs with dramatically increased headgroup size (6, 7). Enza Biotech AB has successfully applied this

technology to a range of substrates and produced APGs with alkyl chain lengths between 12 and 18, and with degrees of polymerisation between 4 and 20. However, we have not previously reported on the physicochemical properties of these novel surfactants. The present paper focuses on the properties of hexadecyloctaglucoside (henceforth abbreviated $C_{16}G_8$). Apart from fundamental physicochemical studies of surfactant self-aggregation and adsorption, we have investigated the ability of the novel alkylglycosides to wet and disperse micron-sized ("micronised") hydrophobic particles of corticosteroids in an aqueous solution. The reason for our interest in this type of particle dispersions is twofold:

(A) Micronised hydrophobic particles form very cohesive powders, which are extremely difficult to wet. This type of materials therefore represents a challenging test of the ability of a given surfactant to act as dispersant and wetting agent. Using proper particle sizing techniques (e.g. laser diffraction), these systems also allow for qualitative assessment of the relative wetting capacity of surfactants. This is readily explained by reference to Figure 1. As is illustrated in the figure, proper wetting and dispersion result in a system comprising only primary particles in the micron size range. Incomplete wetting, on the other hand, results in a system that contains both primary particles and larger, composite particles (aggregates and agglomerates of primary particles). Consequently, studies of particle size in dispersions of micronised powders can be used to build scientific understanding of the wetting ability of different surfactants and to rank order them in terms of efficiency.

(B) Efficient dispersion of cohesive particles using a minimum of surfactant is of direct relevance for manifold applications within cosmetics, pharmaceutics, cleaning and personal care. Preparation of physical sunscreens, soil removal during laundry and formulation of poorly soluble drugs provide pertinent examples. From an applied point of view, studies of dispersed micronised powders are hence of direct interest for formulators active in many different trades.

In order to compare the properties and performance of $C_{16}G_8$ with conventional technology, the studies have included existing conventional APGs, as well as a representative of the family of ethoxylated non-ionic surfactants, namely polysorbate 80 (the ethoxylated oleate ester of sorbitan). Polysorbate 80 was selected as comparator since it is approved for virtually all applications within the life sciences. It serves as an excellent reference system for benchmarking, since it is well-known in the formulation community and extensively used for dispersion of hydrophobic particles in a wide array of products on the market.

EXPERIMENTAL SECTION

Materials

α-cyclodextrin was obtained from Wacker. Hexadecyl-β-maltoside ($C_{16}G_2$) was of Anagrade quality (>98%) and sourced from Anatrace (Maumee, USA).

$C_{16}G_8$ was produced by Enza Biotech AB, by reacting α-cylodextrin with $C_{16}G_2$, using the enzyme cyclodextrin glycosyl transferase as catalyst. The process has previously been described in detail (6, 7). Chromatographic analysis showed the reaction product to consist mainly of the target product $C_{16}G_8$, together with *ca* 20% of the secondary coupling product $C_{16}G_{14}$ and <1% of $C_{16}G_n$ components with n≠8,14. The material was isolated and freeze-dried to yield a white powder, which was used without further purification. In calculations, a molecular mass of 1735 g/mol was used. This value is the appropriately weighted average of the molecular mass of pure $C_{16}G_8$ and $C_{16}G_{14}$. For reasons of convenience, the essentially binary mixture of $C_{16}G_8$ and $C_{16}G_{14}$ used in the studies is in the following referred to simply as "$C_{16}G_8$".

Polysorbate 80 of Super Refined™ grade was used for comparative purposes and was sourced from Croda. Polysorbate 80 is a polydisperse mixture consisting of hundreds of individual components. In calculations, a molecular mass of 1300 g/mol was used, in agreement with convention. (8)

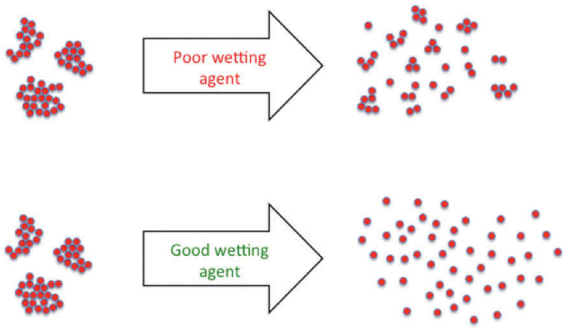

Figure 1. Schematic representation of the effect of the wetting ability of a surfactant on the dispersion of micronised particles in an aqueous vehicle. In the presence of a good wetting agent, the material is properly dispersed as primary particles, whereas a poor one (under otherwise identical conditions) gives a system that contains a significant portion of aggregates. Using suitable particle sizing techniques it is therefore possible to assess the ability of a given surfactant to act as a wetting agent.

Tensiometry

Tensiometric data were collected at 25 °C on a KSV Sigma70 instrument equipped with a DuNoüy ring made from platinum. The surfactants were dissolved in deionised water of Milli-Q quality.

Ellipsometry

The hydrophobic surfaces used in the experiments were prepared by cutting silicon wafers covered with a 300 Å silica layer into 1 cm wide stripes. After thorough cleaning, the strips were hydrophobised by placing them overnight in an air-free desiccator in the presence of dimethyloctylsilane. Ellipsometric studies were performed at 25 °C on a Rudolph Type 436 thin film ellipsometer. The light intensity minimum was first measured in air and then in the sample liquid, which was used to calculate the thickness and refractive index for the substrate oxide. The surfactant stock solutions were added in steps to concentrations of 2, 10, 20, 100 and 200 µg/ml. After the final addition, the cuvette was rinsed with water and the desorption was measured.

In order to calculate the adsorbed amount from the ellipsometric data, the refractive index was determined as a function of the concentration of surfactant (d*n*/dc), using an Abbe refractometer from Carl Zeiss.

Wetting and Dispersion of Hydrophobic Particles

The hydrophobic corticosteroid beclometasone dipropionate (BDP) was micronised by jet milling and used as test material in the wetting experiments.

50 mg of micronised BDP powder was first dispersed in 10 ml of a solution containing 20 mg/ml of surfactant and 0.15 M NaCl. In order to study the impact of agitation, the dispersion of the material was performed in two different ways. In the high-shear case (Method A), agitation was provided by means of an Ultra Turrax mixing rod. In the low-shear case (Method B), agitation was instead provided by means of a magnetic stirring bar. After 5 minutes of agitation, the dispersions were diluted to 1 litre with 0.15 M NaCl solution and then subjected to particle sizing by means of laser diffraction.

Laser Diffraction

Laser diffraction measurements were performed using a Malvern Mastersizer 2000, equipped with a Hydro 2000SM (A) sample dispersion unit. MilliQ water was used as the dispersant medium for all samples. The stirrer speed was set to 2000 rpm. For each sample added, 3 measurements were performed and an average particle size distribution calculated using the instrument software.

RESULTS AND DISCUSSION

Surfactant Adsorption and Self-Aggregation

Tensiometric data for $C_{16}G_8$ are shown in Figure 2. The intersection of the two linear parts of the data gives a cmc of 22 µM (38 mg/L). At concentrations above the cmc, the surface tension is stable at 49 mN/m. Using the linear part of the data at concentrations below the cmc, the average molecular cross-sectional area can be calculated from the Gibbs adsorption isotherm. This calculation gives a value of 78 Å^2 per molecule.

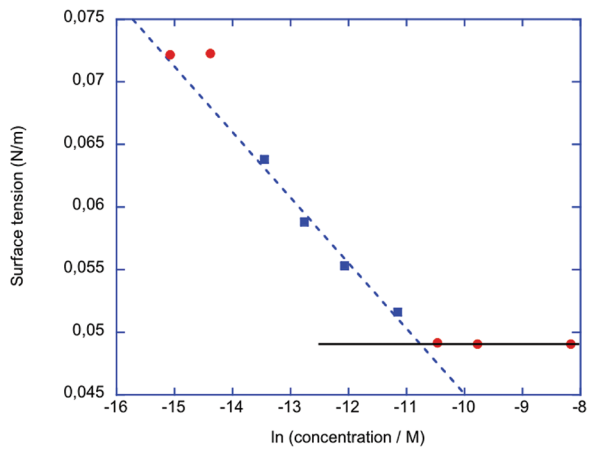

Figure 2. Tensiometric data on $C_{16}G_8$ dissolved in neat water. The critical micelle concentration (cmc) is given by the intersection of the two linear ranges. The data points represented by squares were used in the calculation of the molecular cross-sectional area by means of the Gibbs adsorption isotherm.

Ellipsometric data on the adsorption of $C_{16}G_8$ to hydrophobised silica is shown in Figure 3. The adsorption at each concentration is rapid and levels out at stable values. The maximum adsorbed amount of 2.9 mg/m^2 (1.7 mmol/m^2) is obtained at concentrations close to the cmc, and corresponds to an area of 100 Å^2/molecule in the adsorbed layer.

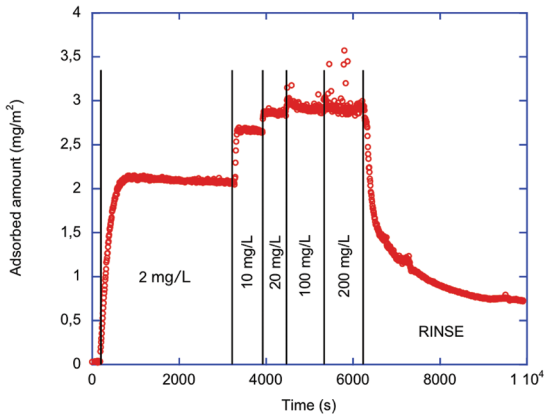

Figure 3. Ellipsometric data on the adsorbed amount of $C_{16}G_8$ on hydrophobised silica in the concentration range 2-200 mg/L.

It is interesting to compare the tensiometric and ellipsometric data on $C_{16}G_8$ with corresponding data for ethoxylated C_{16} surfactants, polysorbate and conventional APGs. Comparison of the cmc of $C_{16}G_8$ with that of the ethoxylated analogues $C_{16}E_9$, $C_{16}E_{12}$ and $C_{16}E_{21}$ (2.1, 2.3 and 3.1 µM, respectively) (9) shows that the cmc of $C_{16}G_8$ is one order of magnitude higher, which may tentatively be attributed to a more pronounced hydrophilicity of the sugar-based headgroup. This assumption is substantiated by the fact that Polysorbate 80, which has a larger and more hydrophilic headgroup than the simple $C_{16}E_n$ surfactants, has a cmc close to that of $C_{16}G_8$, namely 13 µM (8), in spite of having a more hydrophobic $C_{18:1}$ tail. Direct comparison with the cmc of existing C_{16} APGs is not possible, since these surfactants are insoluble at room temperature (see introduction). However, the cmc of tetradecyl-β-maltoside ($C_{14}G_2$) has previously been determined by tensiometry and found to be 14 µM (10). Again, we thus find evidence that the increased solubility caused by the long hydrophilic headgroup of $C_{16}G_8$ offsets the effect of the more hydrophobic alkyl chain in terms of cmc. For the $C_{16}E_n$ series, the surface tension above the cmc increases monotonously with increasing hydrophilicity of the headgroup, from 32 mN/m for n=6 to 45 mN/m for n=21 (11, 12). Solubility arguments can thus be used also to rationalise the finding that the surface tension of $C_{16}G_8$ at concentrations above the cmc (49 mN/m) is considerably higher than for $C_{14}G_2$ (38 mN/m) (10) and polysorbate 80 (43 mN/m) (8). However, the effect of headgroup size and solubility on cmc seems to be more pronounced for alkylglycosides than for ethoxylated surfactants, considering that the cmc of $C_{14}G_2$ (14 µM) is lower than that of $C_{16}G_8$ (22 µM), whereas the opposite trend holds true for the corresponding ethoxylated pair $C_{14}E_6$ (6.0 µM) (13) and $C_{16}E_{21}$ (3.1 µM). Comparisons of surface excess and molecular size in the adsorbed layers reveal further interesting differences between alkylglycosides and ethoxylated surfactants. The adsorbed amount of polysorbate 80 has been reported to equal 1.4 mg/m^2 (1.1 mmol/m^2) at a concentration of 0.028 g/L (14), which is to be compared with 2.9 mg/m^2 (1.7 mmol/m^2) for $C_{16}G_8$, as determined by us. Consequently, it can be concluded that $C_{16}G_8$ adsorbs twice as efficiently as polysorbate 80 at the hydrophobic surface. The explanation for the difference may, at least in part, be that the headgroup of polysorbate is branched and flexible. In contrast, the headgroup of $C_{16}G_8$ is linear and quite rigid, which would serve to favour packing. The possibility to form intermolecular hydrogen bonds between adjacent molecules in the adsorbed layer may also contribute to a

denser molecular packing in monolayers of $C_{16}G_8$. When $C_{16}G_8$ is compared to simple, single-chain ethoxylated surfactants, we find that it adsorbs equally efficient in terms of mass, but less efficiently in molar terms. The maximum adsorbed amount of $C_{16}E_8$ on a hydrophobic surface has been reported to be 5.5 mmol/m^2, which corresponds to 2.8 mg/m^2 and a cross-sectional area of 30 Å^2 (15). Quite surprisingly, there seem to be no systematic studies of the effect of headgroup size on the adsorbed amount of $C_{16}E_n$ surfactants at hydrophobic surfaces, and it is therefore impossible to compare the behaviour of these surfactants with that of alkylglycosides in a more general sense.

Wetting and Dispersion of Hydrophobic Particles

Interestingly, $C_{16}G_2$, $C_{16}G_8$ and polysorbate 80 behave completely different when used as dispersants in the preparation of dispersions of micronised BDP. $C_{16}G_2$ proved entirely useless and produced a notably inhomogenous dispersion containing macroscopic particle aggregates. $C_{16}G_8$ and polysorbate 80 both produced dispersions that appeared homogenous to the naked eye. However, laser diffraction analysis reveals very significant differences. When prepared with high-shear mixing, the dispersion with $C_{16}G_8$ has a particle size distribution that shows virtually perfect symmetry in a lin-log plot (Figure 4a), which is often found for primary particles in micronised powders. When polysorbate 80 is used, on the other hand, the same preparation protocol gives a size distribution that is markedly skew-symmetric, with a shoulder on the large-size side (Figure 4b). This is a tell-tale sign of incomplete dispersion and presence of composite particles, and thus strongly suggests that $C_{16}G_8$ is a more efficient dispersant for BDP than polysorbate. This conclusion is corroborated in a quite dramatic fashion by the results from the experiments that utilised low-shear mixing. When $C_{16}G_8$ is used as dispersant in this case, we again obtain a symmetric size distribution, consistent with efficient wetting and dispersion (Figure 4c). However, the system prepared with polysorbate 80 is bimodal, with a prominent second maximum that stems from a very significant amount of undispersed particles residing in aggregates (Figure 4d). The possibility to prepare homogenous dispersions of hydrophobic particles without having to resort to powerful agitation is extremely important from a process and manufacturing point of view. For obvious reasons, surfactant solutions foam during high-shear mixing, which is problematic in terms of both containment and floatation of particles. Decreasing shear translates as less foam, which adds to the robustness of any unit operation involving dispersion or emulsification.

It is, in itself, a quite remarkable finding that $C_{16}G_8$ allows for preparation of good dispersions of the extremely challenging material BDP, using only the low shear of a magnetic stirring bar. However, this nice surprise is probably directly related to the equally surprising efficiency by which it adsorbs to hydrophobic surfaces, as revealed by ellipsometry. In any case, the results clearly indicate that elongation of the headgroup of alkylglycosides has the potential to produce materials that add new and valuable functionality and versatility to the toolbox of the formulator.

CONCLUSION

The results presented in this paper show that elongation of the headgroup of alkylglycosides conveys new surfactant functionality that addresses significant unmet needs in the formulation community. As a case in point, we have shown that $C_{16}G_8$ allows for preparation of homogenous particulate suspensions of exceedingly cohesive materials, without the use of violent agitation (and thus without inducing concomitant, problematic foaming). The fundamental physicochemical properties of $C_{16}G_8$ that provide the backdrop to its functionality are unexpected in the sense that they are not readily predicted from the properties of conventional non-ionic surfactants (either ethoxylated or sugar-based ones). Therefore, our results also showcase the critical importance of fundamental physicochemical characterisation as a part of successful, rational development of tomorrow's ingredients and products.

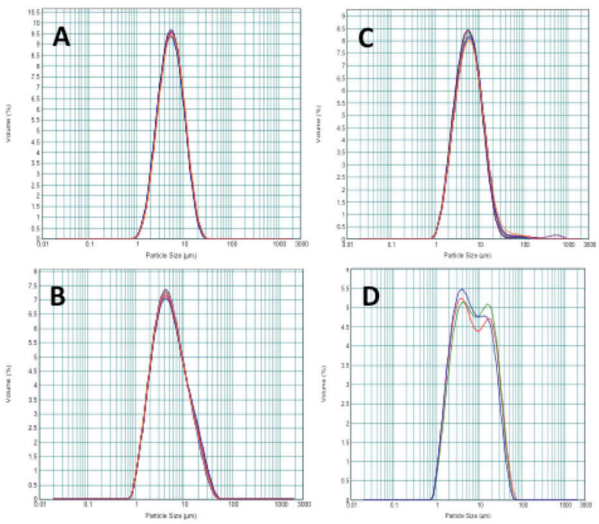

Figure 4. Laser diffraction data on dispersions of micronised BDP prepared using either low- or high-shear mixing, with either $C_{16}G_8$ or polysorbate 80 as dispersant. (A) $C_{16}G_8$ with high-shear mixing; (B) polysorbate 80 with high-shear mixing; (C) $C_{16}G_8$ with low-shear mixing; (D) polysorbate 80 with low-shear mixing. Each panel contains the results from three independent measurements.

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Sessions

Tuesday 7 June

Session 1: Skin ageing processes and causes of premature skin ageing

KEYNOTE - Skin ageing beyond UV: basic mechanisms and clinical implications
Prof Jean Krutmann, Leibniz Research Institute for Environmental Medicine, Germany

Oxidative stress and ageing - the effects of environmental pollution, sunlight and diet
Prof Mark Birch-Machin, Newcastle University, UK

The unique microenvironmment of the ageing human adult scalp
Prof Des Tobin, Centre for Skin Sciences, UK

Premature skin ageing induced by air pollution: new in vitro insights approaching realistic conditions
Prof Imke Meyer, Symrise, France

Session 2: New technologies and treatments for premature skin ageing

KEYNOTE - Understanding ageing, its limits and possibilities
Prof Suresh Rattan, Aarhus University, Denmark

Benefits of topical Q10 treatment on human skin
Dr Anja Knott, Beiersdorf, Germany

Microarray analysis of skin ageing
Marta Hlobilová, Contipro, Czech Republic

A novel mechanism to prevent solar-light induced oxidative stress
Varun Mathur, The HallStar Company, USA

A novel active ingredient induces a beneficial dermal remodelling, leading to clinical improvement on photoaged skin
Alain Mavon, Oriflame, Sweden

Wednesday 8 June

Session 3: Advertising and claim support for anti-ageing skin care products

Smoothing the way from script to screen - getting your skin care ads to air
Niamh McGuiness, Clearcast, UK

Supporting breakthrough anti-ageing skin care claims
Dr Jack Ferguson, Skinnovation Ltd, UK

Session 4: Assessment and delivery of anti-ageing skin care benefits

Anti-ageing skin care strategies
Prof Paul Matts, Procter & Gamble, UK

The influence of age, ethnicity and anatomical site on skin mechanics and composition
Dr Michael Sherratt, University of Manchester, UK

In vivo methods to evaluate anti-photo ageing and anti-pollution efficacy claims of cosmetic products
Dr Stephan Bielfeldt, proDERM, Germany

Optical Coherence Tomography (OCT) - a new tool for quantification of skin ageing
Jon Holmes, Michelson Diagnostics Ltd, UK

Ex vivo evaluation of efficacy of topical skin formulations
Ardeshir Bayat, Science of Skin, UK

Programme co-ordinator: Dr Jack Ferguson, Skinnovation Ltd, UK

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