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Extraction-Free, *In Situ* Analysis of Glucose in Cosmetic Formulations Based on Digital Image Colorimetry by Smartphone

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Synopsis

We are proposing an extraction-free methodology for *in situ* quantifying glucose in cosmetic formulations including oil-in-water (O/W) emulsions, shampoos, or gels. Quantification is based on the known glucose oxidase/peroxidase reaction which is employed directly into the cosmetic formulation to generate a colored product. Digital image colorimetry is then used to capture, calibrate, and quantify responses by the image-processing software ImageJ. We have demonstrated a linear relationship between smartphone camera-captured color intensity of the treated formulation and glucose concentration in all studied formulations. Upon methodology validation, favorable technical characteristics were obtained for glucose analysis in an O/W emulsion: the linearity range under the conditions employed was between 0 and $6.4*10^{-3}$% weight per weight concentration of glucose in the formulation. Additionally, analysis of two quality control samples yielded a coefficient of variation ≤11.6%, while bias was not higher than ±11.1%. Last, we successfully applied the proposed analysis setup in combination with a standard addition procedure to quantify spiked glucose in O/W emulsions, including colored emulsions, where bias was found between ±3.2% and ±15.9%. The above results indicate acceptable reproducibility and accuracy for the proposed methodology and accountable matrix effect. This study may open the route for routine glucose quantification in cosmetic or food emulsions and galenic ointments.

INTRODUCTION

Glucose is a hygroscopic molecule, component of the natural moisturizing factor (NMF) and is often used for its humectant/skin conditioning properties in skin care applications either added directly to the formulation or indirectly, as component of natural extracts. In addition, it has a role in certain formulations as a flavoring agent. Glucose is commonly present in bath products, cleansing products, skin care products, makeup, and hair care products. Moreover, it is also used in skin whitening products thanks to its indirect anti-melanogenic effect at 2% weight per weight concentration (w/w).[1] Glucose concentration in cosmetic products ranges widely, from 10^{-4}% to 91% in leave-on products and from 10^{-4}% to 84% in products intended for dermal contact.[2]

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Topical application of glucose may promote skin homeostasis by maintaining the skin microbiome i.e., functioning as a prebiotic.[3] It has not been clearly established, though, a topical glucose effect on the microflora involved in skin diseases such as *Propionibacterium acnes* (acne), or *Malassezia* spp. (folliculitis, dandruff and pityriasis) etc. Other prebiotics have been demonstrated to inhibit the inflammation-causing bacterium *Propionibacterium acnes*, in favor of beneficial species of the skin.[4,5] It has been manifested that excess glucose in hyperglycemic abscesses can significantly enhance *S. aureus* virulence potential, thus worsening infection.[6] Since glucose often enters cosmetic formulations through added extracts, it is not always mentioned in the product's list of ingredients. In such specific applications, the existence of an analytical method for glucose quantification in the frame of quality control may potentially be important.

In the area of topical pharmaceuticals, the availability of a direct method to inform on glucose content may be important for specific applications/patient groups: Although systemic hyperglycemia prevents wound healing, topical preparations of sugar-based compounds and honey have provided, in specific studies, promising results as a means toward wound repair.[7,8] No contribution to systemic hyperglycemia was observed.[8] Another study indicated that high glucose inhibits keratinocyte and fibroblast migration as well as wound healing *in vivo,* in a concentration-dependent manner.[9] Last, in the domain of nutrition, there is a need to quantify glucose in emulsions used for the purpose of parenteral nutrition.[10]

The glucose oxidase-based photometric method is commonly used for determining glucose in solution[11], either in biological fluids or food extracts, and presents the advantages of increased specificity and ease of analysis, since several commercial kits relying on this principle are available. A large number of alternative methods have been published including spectrophotometric, electrophoretic and chromatographic methods.[12] In general, chromatographic methods (usually HPLC or GC) and mass spectrometry are the gold standard methods of analysis of organic analytes in cosmetics and personal care products.[13] Although specific and sensitive, they present the drawback of requiring strong analytical expertise, expensive equipment, and typically extensive sample preparation.[14] A number of spectrophotometric methods are also commonly used such as Raman, IR, UV-Vis[13], which although simpler, still require sample preparation prior to analysis, which may often involve toxic waste generation. Additionally, immunological analytical techniques have been proposed for quantification of a number of analytes in cosmetics and personal care products[15], which are highly specific and sensitive, however they suffer from high analysis cost.

We here describe a low-cost and low-waste, extraction-free, smartphone-based methodology for *in situ* analysis of glucose in cosmetic formulations. The methodology relies on the glucose oxidase (GOD)-catalyzed specific glucose conversion to a colored product through peroxidase (POD)[11] inside the cosmetic preparation. Smartphone-based digital image colorimetry is then employed to capture, calibrate, and quantify responses. Smartphone-based image analysis is emerging as an increasingly popular technique, with applications over a range of domains.[16] The greatest advantage of the proposed method over existing analysis setups is the lack of a pretreatment step. The format's simplicity and the lack of need for analytical equipment also make the method very attractive.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Glucose monohydrate puriss was provided by Riedel-de Haën. The GOD/POD commercial kit was from Biosis (Athens, Greece). The working reagent (WR) provided therein contained a 0.2 M buffer pH 7.1, GOD > 18 IU/ml, POD > 2 U/ml, 0.25 mM 4-amino phenazone, 10 mM phenol derivative, and relevant cofactors. All cosmetics tested were commercial products available in the local market by Cosmetic S.A. (Athens, Greece).

APPARATUS

Pictures were taken with a 48-Megapixel camera of a Samsung Galaxy S10, in a portable white photo studio (PULUZ (China), cube of an edge of 23 cm) equipped with a single row of LED light (at 3 cm from the edge and parallel to it). An opening exists at the top center of the cube, where the smartphone camera is positioned. A Brookfield DV-E digital viscometer was used for viscosity measurements. Conductivity measurements were performed using a Cond 50 Violab conductivity meter equipped with a conductivity cell 2301T.

PREPARATION OF STANDARDS AND QUALITY CONTROL SAMPLES

Stocks and quality controls (QCs) of glucose in an O/W emulsion, a shampoo, or a gel, were freshly prepared by dissolving a suitable amount of glucose monohydrate powder or of a 1.0% weight per volume (w/v) glucose aqueous solution. Standards and QCs were then processed as described below.

PROCEDURE FOR QUANTITATIVE DETERMINATION OF GLUCOSE IN AN O/W EMULSION OR GEL

The color-generating reaction was run at 25°C. For this purpose, 1.0 g of a cosmetic product, containing varying concentrations of glucose, and 642 μL of the WR of the GOD/POD commercial kit were added into a vial and were thoroughly mixed, manually. Depending on the emulsion, the addition may have to be performed in aliquots to avoid phase separation. The mixture was loaded into the wells of a microstrip, and its surface was flattened with the flat end of a spatula. Around 5 minutes after reaction initiation and no longer than 20 minutes after WR addition, a picture of the colored formulation was taken with a smartphone camera. When dilution was required to lower emulsion glucose levels down within the linear range, emulsion B (composition in Table I) was used as the diluent.

PROCEDURE FOR PICTURE CAPTURING AND ANALYSIS

A 48-megapixel mobile phone camera (lens aperture: F2.0, 1× magnification factor, focal length (35mm): 25.9 mm, automatic adjustment of brightness) and a white photo studio, equipped with a single row of LEDs was used for capturing pictures of the cosmetic samples. The camera was positioned at the opening at the center of the top side of the

photo studio, lying parallel to, and at 23 cm above the microstrips. Apart from the stable lightning conditions, samples and standards had to be in the same photo for reliable quantification. It was also important that the microstrip wells were not placed right below the light source, to minimize glare. RGB image analysis was subsequently performed using the image editing software ImageJ.[17] The selected region of interest was typically around 1,500–2,200 square pixels, around the center of the well to avoid reflection from microstrip material in the edges. Any subregion within the selected central area having a bubble or glare was not taken into account upon picture analysis. The values of the Red, Green, Blue, Hue, Saturation, and Brightness channels were plotted against glucose concentration. The blue component color was optimum upon image analysis of the opaque emulsions and the transparent shampoo studied, while the green component color intensity was superior in the case of translucent gel analysis. Based on the group's experience, optimum monitoring channel might depend on cosmetic formula or even picture quality.

RESULTS AND DISCUSSION

METHOD PRINCIPLE AND OPTIMIZATION

We here present the application of a new analysis setup for glucose quantification in a broad range of cosmetic matrices. Quantification relied on exogenous addition to the emulsion/shampoo/gel of a commercially available, buffered mixture of GOD, POD and appropriate cofactors and chromogens (GOD/POD WR), to yield a colored end product, according to the widely established GOD/POD chromogenic reaction.[11] Indeed, in the presence of glucose, pink/red coloration of the cosmetic product was achieved, which was minimal at zero glucose concentration. To demonstrate the suitability of the approach for quantitative glucose analysis in cosmetic formulations, a glucose-free O/W emulsion was prepared ("emulsion B"). The emulsion composition (ingredients at concentrations above 0.001% w/w) and certain properties are available in Table I. The emulsion was free of any natural extracts, to avoid glucose presence. Aliquots of the emulsion were spiked with increasing glucose concentrations between 0.0 and $20*10^{-3}$% w/w to yield suitable standards. After the addition of the GOD/POD WR at various amounts per gram of emulsion (as shown in Table II) at 25°C, the resulting colored emulsions were loaded onto microstrip wells. A picture of the wells was captured by a smartphone camera, and different channel outputs were recorded. Table II data show that addition of 642 μL of WR/g emulsion is sufficient to provide a wide enough linear dynamic range without compromising goodness of fit. This relative amount of WR was used in all subsequent studies.

To select the optimum parameters for smartphone-based measurements, calibration graphs were constructed in two opaque O/W emulsions of different viscosity, oil content, and conductivity, emulsion B and emulsion A (composition in Table I). Parameters of the linearity of the response between measured signal and glucose concentration (equation, Pearson's coefficient of determination (R^2) and linear dynamic range) were determined in the RGB and HSB color spaces, by the ImageJ application.[17] The analytical parameters registered are provided in Table III. Based on Table III data, it appears that the blue channel output is the optimum to follow for both emulsions, since it presents better goodness of fit (higher R^2 value), in combination with a wide enough linear range and increased sensitivity

Table I
Composition and Properties of Tested Cosmetic Products (Source: Cosmetic S.A)

	Cream A	Cream B	Shampoo A	Gel A
Composition	Aqua, Urea, Paraffinum Liquidum, Glycerin, Cetearyl Alcohol, Glyceryl Stearate, Peg-100 Stearate, Petrolatum, Panthenol, Dimethicone, Triacetin, Phenoxyethanol, Sorbitan Sesquioleate, Sorbitan Stearate, Allantoin, Caprylyl Glycol, Ethylhexyl-glycerin, Hectorite, Hydroxy-ethylcellulose, Disodium EDTA, Bisabolol, Farnesol	Aqua, Panthenol, Cetearyl Alcohol, Glycerine, Octyl Stearate, Cyclomethicone, Paraffinum Liquidum, Ceteareth, Isononanoate, Dicetyl Phoshate, Ceteth-10 Phoshate, Cetearyl Octanoate, Isopropyl Myristate, Allantoin, Sodium Caproyl/Lauroyl Lactylate, Stearic Acid, Acrylamide/ Sodium Acrylate Copolymer, Linallol, Hydroxy Citronellal, Benzyl Alcohol, Eugenol, Imidazolidinyl Urea, Phenoxyethanol, Methyl-/Ethyl-/ Propyl-/Butyl-paraben, Perfume	Aqua, Sodium Laureth Sulfate, Tea-Lauryl Sulfate, Cocamidopropyl Betaine, Disodium Cocoamphodiacetate, Bis (C13-15 Alkoxy) Pg Amodimethicone, Salix Alba (Willow) Bark Water, Peg-6 Caprylic/Capric Glycerides, Peg-7 Glyceryl Cocoate, Peg-120 Methyl Glucose Dioleate, Sodium Chloride, Perfume Phenoxyethanol, Propylene Glycol, Citric Acid, Polyquaternium-11, Carbocysteine, Maris Sal (Sea Salt), Disodium EDTA, Methylparaben, Glycerin, Panthenol, Propylparaben, Pentylene Glycol, Butylene Glycol, Coumarin, Sodium Benzoate, Ethylparaben, Urtica Dioica (Nettle) Leaf Extract, Panax Ginseng Root Extract, Faex Extract, Santalum Album Seed Oil, Potassium Sorbate	Alcohol Denat., Aqua, Ammonium Acryloydimethyl-taurate/VP Copolymer, Glycerin, Aloe Barbadensis Leaf Juice powder
Oil % w/w	27.9	21.7	1.0	0.0
Individual components, % w/w	Surfactants: 8.0 Polymers: 0.35 Urea: 15	Surfactants: 3.1 Polymers: 1.0	Surfactants: 19.8 Polymers: 1.1	Ethanol: 62 Polymers: 0.6
Viscosity, mPa [shear rate]	~1,000 k [0.3 rpm]	~500 k [0.3 rpm]	~3.3 k [6.0 rpm]	~45 k [0.6 rpm]
Conductivity, mS/cm	2.92	4.70	14.56	4.00
pH	6.82 (prod.) 7.31(mix.)	6.51 (prod.) 6.82 (mix.)	5.40 (prod.) 6.61 (mix.)	6.37 (prod.) 7.97 (mix.)
Transparency	opaque	opaque	transparent	translucent

pH (prod.): pH of the product; pH (mix.): pH of the mixture (product + spiked glucose + WR). Viscosity, transparency, oil % w/w and individual components % w/w refer to untreated cosmetic product, while conductivity refers to the mixture (product + spiked glucose + WR). pH of both the product and the mixture is provided.

(provided by the slope of the calibration curve). In the following studies the blue channel output was registered upon image analysis of all emulsions.

A typical standard curve obtained in emulsion A is given in Figure 1, while in emulsion B, in Figure 2, left. The next step was to study the kinetics of the color-developing reaction, to establish the optimum time point for picture capturing. For this reason, the kinetics were studied for five preparations of emulsion B where glucose was present at a concentration between 0.0%, and 5.45% w/w. Color development was at 25°C, upon addition of 642 μL

Table II
Optimization Data in Terms of Amount of WR Added/g of Cosmetic Preparation

μL WR/g emulsion	R^2	Linear dynamic range (% w/w glucose)
100	0.993	$0.00–1.43*10^{-3}$
186	0.980	$0.00–2.29*10^{-3}$
445	0.998	$0.00–4.96*10^{-3}$
642	0.994	$0.00–7.50*10^{-3}$

Table III
Analytical Parameters Obtained for Various Channels Upon Digital Image Analysis of Glucose Standards in Emulsions A and B

Channel (emulsion)	Linear Equation	R^2	Linear Dynamic Range (% w/w glucose)
Red (A)	−2406x + 199.5	0.962	$0.00–6.37*10^{-3}$
Red (B)	Poor linear dependence		
Green (A)	−10990x + 193.8	0.987	$0.00–6.37*10^{-3}$
Green (B)	−8400x + 180.8	0.991	$0.00–7.08*10^{-3}$
Blue (A)	−8976x + 201.2	0.992	$0.00–6.37*10^{-3}$
Blue (B)	−7869x + 170.9	0.994	$0.00–7.50*10^{-3}$
RGB average (A)	−7410x + 198.0	0.992	$0.00–6.37*10^{-3}$
RGB average (B)	−5657x + 185.9	0.990	$0.00–7.08*10^{-3}$
Hue (A)	1602x + 230.9	0.892	$1.82–6.37*10^{-3}$
Hue (B)	Poor linear dependence		
Saturation (A)	11411x + 16.7	0.989	$0.00–6.37*10^{-3}$
Saturation (B)	5764x + 44.6	0.972	$0.14–7.50*10^{-3}$
Brightness (A)	−2479x + 200.4	0.986	$0.00–8.00*10^{-3}$
Brightness (B)	−1045x + 209.5	0.833	$0.63–7.50*10^{-3}$

R^2: correlation coefficient.

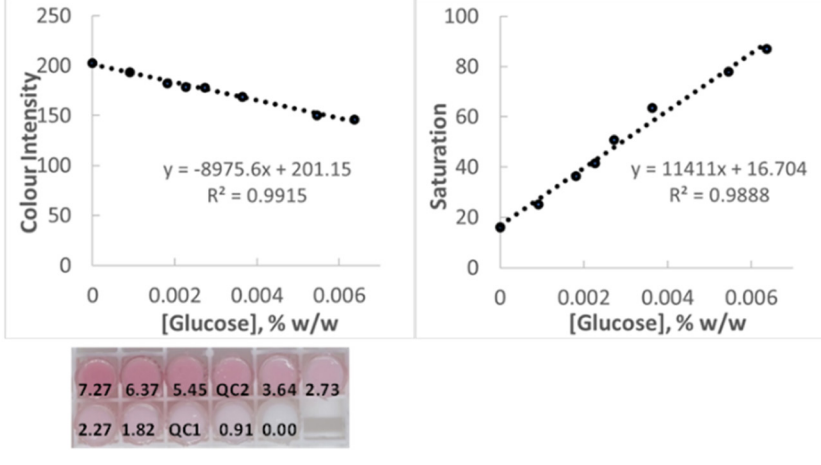

Figure 1. Left: Linear relationship between blue color component intensity and glucose concentration in emulsion A standards. Below, a picture of the corresponding wells is given, which was used to extract the graph data (where glucose concentration is indicated (in 10^{-3}% w/w)). Right: Saturation profile of the same emulsion A preparations, against glucose concentration.

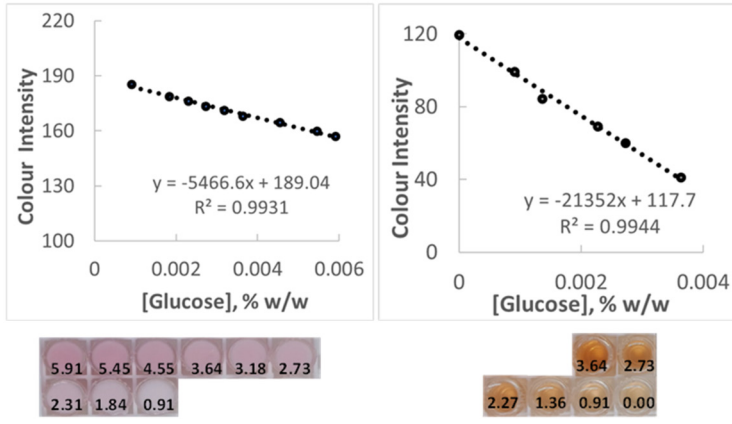

Figure 2. Linear relationship between color intensity and glucose concentration in emulsion B (left) or shampoo A (right) standards. Below each graph, a picture of the corresponding wells is given, which was used to extract the graph data (where glucose concentration is indicated (in 10^{-3}% w/w)).

WR/g emulsion to each preparation, in the order of decreasing glucose concentration. The blue intensity component of the pictures of the microstrips was monitored. Parameters of the linearity of the response between measured signal and glucose concentration (slope, Pearson's coefficient of determination (R^2)) are displayed in Table IV. From Table IV data it is evident that optimum time point for picture capturing is at five minutes (after reaction initiation in the last treated preparation). For a limited time after that (no longer than 20 minutes), the linearity of response remains satisfactory. Within this period, sensitivity increases with time (increase in negative slope of curve) at the compromise of the goodness of fit, however. In all experiments that follow, pictures were taken at around five minutes after processing initiation of the last sample/standard. In any case, pictures were taken no longer than 25 minutes from the beginning of processing of the entire series of standards/samples.

METHOD VALIDATION IN AN O/W EMULSION

The proposed analysis format was then validated with respect to the useful analytical range, and its reproducibility and accuracy were determined upon analysis of two QCs in emulsion A (Table V). The values of coefficient of variation and bias for the two QCs indicate acceptable technical characteristics for the specific analysis purpose in a cosmetic emulsion. For all standards (other than the zero standard), standard concentration was back-calculated within ±20.5% of their nominal concentration in emulsion A. These technical parameters support the reliability of the methodology for the extraction-free glucose quantification in emulsion A, with a lower limit of linearity of $0.91*10^{-3}$% w/w. When compared to chromatographic methods for the analysis of glucose extracted from various matrices, the proposed method is superior to certain such methods, or inferior to others in terms of lower limit of linearity[12], which in any case is very sufficient here, for the analytical needs of the cosmetic industry. The proposed method is however inferior in terms of precision or accuracy when compared to literature chromatographic methods.[18,19]

Table IV
Parameters of the Linearity of the Response Between Measured Signal and Glucose Concentration (Equation, Pearson's Coefficient of Determination (R^2)) Upon Monitoring Blue Channel Output Alteration with Time After Processing

Time After Initiation of Processing of Last Sample (Min)	Linear Equation	R^2
5	–8206 × +185.1	0.997
9	–9581 × +180.4	0.981
13	–10121 × +180.3	0.976
21	–10665 × +179.8	0.977
26	–10834 × +182.4	0.962
60	–10529 × +170.8	0.945

R^2: correlation coefficient.

Table V
Technical Parameters for the Quantitative Determination of Glucose With the Proposed Methodology in Emulsion A

Intermediate precision		***Accuracy***			***Linearity***	
Mean [Glu], 10^{-3}% w/w	**CV %**	**Measured [Glu], 10^{-3}% w/w**	**Spiked [Glu], 10^{-3}% w/w**	**Bias %**	**R^2**	**Linear range, 10^{-3}% w/w**
1.52	11.56 ($n = 3$)	1.52 ($n = 3$)	1.37	+11.06	≥0.991 ($n = 3$)	0.00 – 6.37
4.75	1.68 ($n = 3$)	4.75 ($n = 3$)	4.55	+4.37		

CV: coefficient of variation; [Glu]: Glucose concentration; n: number of replicates; R^2: correlation coefficient.

APPLICABILITY IN DIFFERENT MATRICES

Apart from emulsions A and B, additional glucose-free formulations of different viscosity, transparency, conductivity and pH, "shampoo A," and "gel A," were spiked with glucose between 0 and 7*10^{-3}% w/w. The ingredients present in the two formulations at concentrations above 0.001% w/w can be seen in Table I, together with certain properties of the formulations. Obtained calibration curves showed good linearity ($R^2 = 0.994$ for shampoo A, (Figure 2, right) and $R^2 = 0.989$ for gel A). Back-calculated glucose concentrations in the standards were acceptable (within ±14.1% of their nominal concentration for shampoo A standards) and marginally acceptable (within ±41.0% of their nominal concentration) for gel A standards.

Our findings altogether, support the applicability of the proposed quantification approach in different cosmetic matrices irrespective of their transparency, oil composition (up to 27.9% w/w oil), pH (5.4 – 6.8), viscosity (3 – 1,000k mPa), and conductivity (2.92 – 14.56 mS/cm). It is to be noted that the GOD/POD enzymatic system remains functional even at rather extreme conditions in cosmetic formulations (such as a surfactant concentration of ~20% w/w (shampoo A, Table I) and ethanol concentration of 62% w/w (gel A, Table I). Known protein denaturants (such as urea, surfactants) commonly present in cosmetic formulations have been shown to diminish the activity of at least one of the enzymes: For example, rather mediocre GOD activity inhibition was reported (by less than 10% to 25% at 25°C and pH 6.4) in the presence of urea and the anionic surfactant sodium n-dodecyl sulfate (at ≤2% w/w, each).[20] Much more significant was the inhibition in the presence at ≤2% w/w of the cationic surfactant Dodecyl Trimethyl Ammonium Bromide.[20] We did not observe a significant activity compromise under the conditions employed (total

surfactant concentration of up to ~20% w/w, urea concentration of 15% w/w, ethanol of 62% w/w (Table I)). It might be possible that the presence of polymers in the formulations offers a certain stability to the enzymatic system, despite the presence of denaturants.[21] The above provide an indication of the applicability potential of the assay to a good variety of cosmetic matrices.

ACCURACY UPON ANALYSIS OF COMMERCIAL PRODUCTS

Mock glucose-containing formulations were then prepared in glucose-free commercial creams (emulsion B, emulsion C (O/W, brown-colored hand and foot cream) and emulsion D (O/W, blue-colored body butter)). The concentration of spiked glucose in each matrix, as well as the color/transparency of each matrix, is indicated in Table VI. All mock glucose-containing formulations were then treated as "unknown" samples. The mock unknown samples were either analyzed directly, or were diluted appropriately, to lower glucose levels within the linear region of the method (dilution indicated in Table VI). As diluent, untreated emulsion B (glucose-free) was used. They were then submitted to the standard addition protocol adapted to the proposed analysis format. For spiked concentration calculation with the standard addition method, the blue channel output of emulsion B treated with the WR was taken as zero-analyte signal, in the case of the 1:1,000 and 1:10,000 dilutions.

For the 1:40 and 1:10 dilutions, emulsion B treated with the WR did not provide a suitable "zero signal" since there was a significant effect of the colored matrix. Instead, extrapolation to nonzero-analyte concentration (a standard containing 0.001 – 0.005% w/w glucose in the corresponding matrix) was used. A good linear relationship was obtained between light intensity and glucose concentration in emulsion, as indicated by the R^2 values displayed in Table VI. Our results also demonstrate an acceptable bias. An image of the colored wells upon application of the standard addition method is given in Figure 3, as well as of the initial emulsion.

These data provide an initial indication in favor of the validity of the approach for quantifying glucose content in commercial cosmetic matrices within the frame of QC. They also demonstrate that the proposed analysis format successfully takes into account

Table VI
Technical Parameters for the Quantitative Determination of Glucose in Commercial Products Using the Standard Addition Procedure

Matrix	Color Transparency	Dilution applied	Spiked [Glu], % w/w	R^2	Bias %
emulsion B (sensitive skin cream)	White opaque	Nondiluted	0.0091	0.993	−15.3
emulsion C (hand and foot cream)	Brown opaque	1:1,000	3.12	0.997	+4.9
emulsion C (hand and foot cream)	Brown opaque	1:40	0.08	0.991	+3.2
emulsion C (hand and foot cream)	Brown opaque	1:10	0.02	0.999	+13.12
emulsion D (body butter)	Blue opaque	1:10,000	8.26	0.995	+15.9

R^2: correlation coefficient.

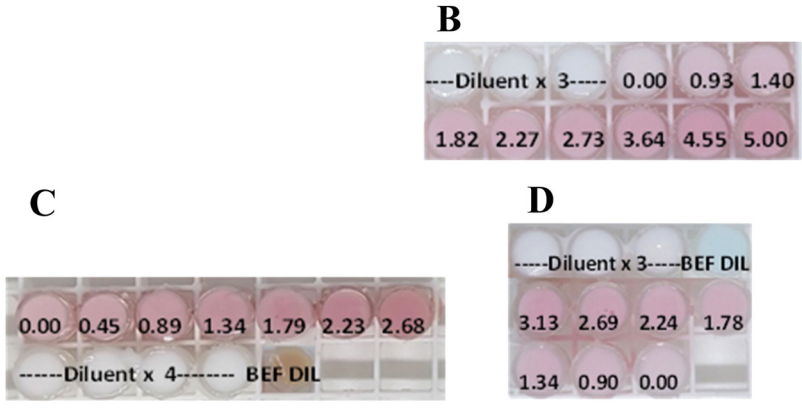

Figure 3. Images of microstrips loaded with each matrix of Table VI, upon application of the standard addition procedure in conjunction with the proposed assay format. Exogenously added glucose concentration is indicated (in 10^{-3} % w/w). B: emulsion B, C: emulsion C (1:1,000 dilution), D: emulsion D (1:10,000 dilution). BEF DIL indicates the cosmetic product before any dilution or other treatment.

any matrix effects in O/W emulsions, even in colored emulsions (commonly encountered in the cosmetics industry).

Glucose oxidase upon which this method relies, is a highly specific enzyme for glucose.[22] This is a prerequisite for the specificity of the proposed analysis method. Even if however, a matrix component is unspecifically generating color upon the proposed treatment, this is expected to be taken into account by the standard addition procedure.

The proposed analysis format inside the actual cosmetic formulation presents the advantage of no use of organic solvents or pretreatment procedures, or of analytical instrumentation. Those advantages may render it the method of choice in applications where the determined analytical figures of merit are sufficient for the analysis purpose.

CONCLUSION

We here provide evidence of the validity of a simple, extraction-free methodology for quantification of glucose in various cosmetic formulations. Quantification is based on enzymatic conversion of glucose by the POD/GOD system and is accomplished by digital image colorimetry. The favorable technical characteristics of the methodology in terms of intermediate precision and accuracy were demonstrated in an O/W emulsion. Glucose was moreover sufficiently accurately quantified upon application of the standard addition method on emulsions, even when colored, at concentrations addressing quantification needs of commercial glucose-containing cosmetic products. We demonstrated the sufficient robustness of the enzymatic system and the applicability of the proposed methodology in cosmetic matrices of different viscosity, color, transparency, pH and composition (including rather extreme concentrations of protein denaturants (ethanol, surfactants)). Abolishing the need for analyte extraction, for use of organic solvents and for instrumental-based quantification in emulsion analysis are great advantages, ensuring a straightforward, and low-cost/low-waste methodology. This study may open the route for routine glucose quantification in cosmetic, galenic, or food formulations.

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AUTHORS' CONTRIBUTIONS

Georgia Eleni Tsotsou: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing, review & editing, **Anastasia Kyriaki Tsara:** Investigation, Validation.

LIST OF ABBREVIATIONS

BEF DIL	before any dilution
k	thousand
[Glu]	Glucose concentration
GOD	Glucose Oxidase
mPa	milliPascal
n	number of replicates
NMF	Natural Moisturizing Factor
O/W	oil-in-water emulsion
POD	Peroxidase
QC	Quality Control
R^2	correlation coefficient
rpm	rounds per minute
WR	Working Reagent

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