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A test for antioxidant activity in cosmetic formulations

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Synopsis

The aim of this study was to develop a technique to assay for the activity of antioxidants in a finished cosmetic product. This was accomplished by adapting the Randox Assay for Total Antioxidant Status kit so that diluted samples could be evaluated by kinetic as well as end-point determinations. Using this technique, we found that a finished product had an IC_{50} of 0.07 gm of product and a relative antioxidant activity concentration of 52.7 nmoles/mg.

INTRODUCTION

Environmental insult to human skin by ultraviolet (UV) radiation, as well as by cigarette smoke and air pollution, generates reactive oxygen intermediates that contribute to both acute and chronic skin damage (1,2). For example, immediately after overexposure to sunlight, an erythemal response is induced that is associated with epidermal inflammatory oxidative reactions. Moreover, in terms of chronic exposure, the involvement of oxygen free radicals has also been implicated in actinic skin damage that manifests itself in elastosis, collagen disorganization, and most notably in the appearance of wrinkles (3).

Due to increased outdoor leisure activities, these visible signs of photodamage and premature aging have become widespread in our society. To address this problem, the cosmetics industry has devoted much research toward the development of various skin care products. Although protective sunscreen products that absorb UV and diffuse photonic energy are widely used, cosmetic products that contain antioxidants, which scavenge deleterious reactive oxygen species produced in skin after environmental trauma, have also become standard for a healthy skin care regimen.

Although analytical techniques are available to measure the level of antioxidants in cosmetic products, in general, they do not provide any information regarding their potential activity. Further, due to the complex nature of cosmetic formulations, extracting and determining biochemical activity in a finished product can be a challenging task. Previously, we evaluated the antioxidant potential of certain cosmetic ingredients (4) and also the antioxidant efficacy of finished products on skin (5). In this study, we

now report on a novel technique to measure antioxidant activity directly in a complete cosmetic product.

MATERIALS AND METHODS

SAMPLE PREPARATION

A typical cosmetic formulation containing a blend of emulsifiers was prepared as either a control with no antioxidants or as a complete formula with a mixture of antioxidants. The following antioxidants were used in the formulation: 2.0% tocopheryl acetate (Hoffman-LaRoche, Parsippany, NJ), 0.1% butylated hydroxy toluene (Rhone-Poulenc, Cranbury, NJ), 1.0% magnesium ascorbyl phosphate (Barnet, Englewood, NJ), 0.1% ubiquinone 50 and 0.5% N-acetyl-L-cysteine (Selzer, Carlsbad, CA), 0.1% rosemary (Robertet, Oakland, CA), and 0.1% tocopherol cysteamine (Mercier, S. Plainfield, NJ).

ASSAY

The Randox Assay for Total Antioxidant Status kit (Randox, Antrim, UK) was adapted for use in cosmetic products by diluting the formulations to be tested to 1% in isopropyl alcohol. At 1% in isopropyl alcohol, the samples are sufficiently clarified and the antioxidants solubilized to allow the reaction to proceed without interference. Briefly, 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) is reacted with a peroxidase and H_2O_2 to convert ATBS into a radical cation. In this state, ATBS forms a chromogen that can be measured spectrophotometrically at 600 nm. In the presence of antioxidants, this color formation is inhibited. Typically, 50–100 µl of the 1% sample is diluted in water up to 250 µl. Then, 1.5 ml of chromogen solution is added, followed by the addition of 0.3 ml of substrate solution. The absorbance (A) of the samples is then measured immediately in a Beckman DU-7500 spectrophotometer using the kinetics/time program.

CALCULATIONS

Percent inhibition was calculated as $(dA_{vehicle}-dA_{product}/dA_{vehicle}) \times 100$ and used to quantitate an IC_{50} value. Also, a range of 15 to 85 nanomoles of an antioxidant standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used to determine the relative activity of a product.

RESULTS

One hundred microliters (1 mg) of a 1% cosmetic sample dilution was assayed, and a typical three-minute kinetic plot of the data is shown in Figure 1. The sample without antioxidants had a dA/min of 0.126 whereas the sample containing antioxidants had a dA/min of 0.011. Thus, there was an 86.8% decrease from the control sample. From similar kinetic plots, the average value that would inhibit the reaction by 50% was then determined to be 0.7 mg of the sample. Since the sample was a 1% dilution of the

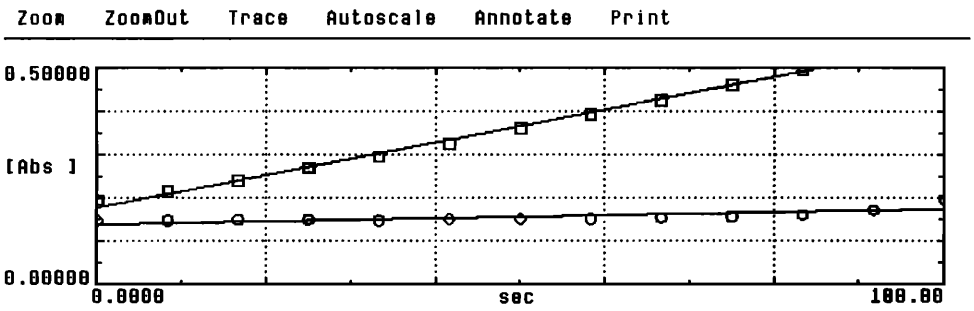

Figure 1. Kinetic plot of the increase in absorbance at 600 nm over time. (–□–): Vehicle formulation was 0.126 dA/min. (–○–): Antioxidant formulation was 0.011 dA/min.

product, 0.7 mg is multiplied by 100 in order to determine that 0.07 gm of the cosmetic product is equivalent to 50% inhibition in this assay. In this way, relative measurements of effectiveness can be calculated and used for comparison to other products.

Based on regression analysis from the standard curve and end-point assay analysis, the relative antioxidant activity concentration that was determined for this product was 52.7 nmoles/mg (± SD 1.7) of material. In contrast the placebo contained only 11.4 nmoles/mg (± SD 1.2), although when compared to a blank, there appeared to be some background antioxidant activity by the formulation itself. These data are summarized in Figure 2.

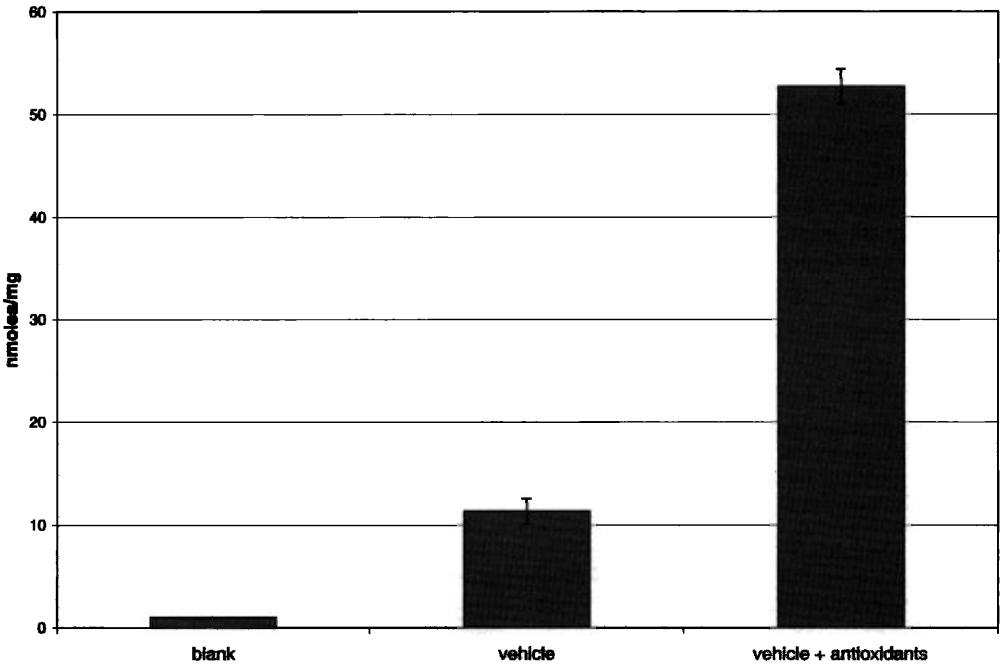

Figure 2. Increase in the amount of nmoles/mg of antioxidant activity in cosmetic formulations as determined by standard curve and end-point assay measurements.

DISCUSSION

Antioxidants in skin care products have been found to be effective protectants against free radical-mediated oxidative damage in skin. Also, due to the rise in photodamage in an aging population, the topical application of cosmetic products that contain antioxidants has become an important area of research in skin care products. Thus, the need to measure the activity of antioxidants in a finished product is of critical importance. In this report we describe a novel and simple technique to quickly assess the relative antioxidant potential of whole product formulations. Additionally, it can be utilized for stability studies and, since the chromogen develops in the visible region, perhaps even a qualitative result can be obtained by workers in the field who lack spectrophotometers but need to assay the antioxidant activity of a product.

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