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A HPLC method to evaluate the influence of photostabilizers on cosmetic formulations containing UV-filters and vitamins A and E

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ABSTRACT

This paper reports a simple and reliable HPLC method to evaluate the influence of two currently available photostabilizers on cosmetic formulations containing combined UV-filters and vitamins A and E. Vitamins and UV-filters, widely encountered in products of daily use have to be routinely evaluated since photoinstability can lead to reductions in their efficacy and safety. UV-irradiated formulation samples were submitted to a procedure that included a reliable, precise and specific HPLC method employing a C18 column and detection at 325 and 235 nm. Methanol, isopropanol and water were the mobile phases in gradient elution. The method precision was between 0.28 and 5.07. The photostabilizers studied [diethylhexyl 2,6-naphthalate (DEHN) and benzotriazolyl dodecyl p-cresol (BTDC)], influenced the stability of octyl methoxycinnamate (OMC) associated with vitamins A and E. BTDC was considered the best photostabilizer to vitamins and OMC when the UV-filters were combined with both vitamins A and E.

1. Introduction

Sunscreen lotions are meant to prevent skin damage by absorption of UVB and UVA sunlight radiation. Unfortunately, it is difficult to achieve broad-spectrum photostable organic sunscreen formulations since most chemical filters exhibit some photoreactivity that can lead to formation of photoproducts [1]. These photoproducts may still act as filters but also lead to reactive intermediates behaving as photo-oxidants or may also promote phototoxic or photoallergic contact dermatitis [2,3].

On the other hand, there are many products of daily use containing not only active substances for anti-aging treatment but also UV-filters. Among the frequently used antioxidants in anti-aging products are vitamin A, C and E derivatives. Vitamin A palmitate acts on the epithelization in dry and rough skin, as well as on keratinization considered as being abnormal [4] and it also absorbs UV radiation between 300 and 350 nm, with a maximum at 325 nm [5], which suggests it may have a biologically relevant filter activity as well. Vitamin E acetate is a free radical scavenger. It can reduce DNA damage and keratinocyte death (sunburn cell formation) [6], enhance stratum corneum hydration and reduce skin roughness [3].

However, combinations of UV-filters and anti-aging substances such as vitamins A and E also form reactive intermediates that may react with the active substances, leading to reduction of efficacy and safety [3,7]. Consequently, cosmetic formulators are trying very hard to stabilize these types of formulation by searching for new UV-filters or using photostabilizers.

Several groups have reported studies using UV-filters in solution, in polymer films, in liquid films, on glass or stratum corneum and even on humans *in vivo* [8], but in most of them the photochemistry of sunscreen agents was examined in dilute solutions, which may not be particularly relevant. In thin films and in the skin, the photochemistry of photounstable sunscreens is more complex than in dilute solutions. As a consequence, photochemistry studies based on thin films are more similar and relevant to practical applications than the ones that are done in dilute solutions. In 1995, the European Cosmetic Toiletry and Perfumery Association (COLIPA) task group published a collaborative initial test for the photostability of four sunscreen agents used in products tested in liquid films on glass surfaces [8]. The behavior of sunscreens is not predictable from the photostability of its individual filter. Thus, it is also important to evaluate the combinations of filters used in the formulation [9,10].

The aim of this study was to develop a simple and reliable HPLC method to evaluate the influence of two currently available photostabilizers on photostability of cosmetic formulations containing

Abbreviations: OMC, octyl methoxycinnamate; BP-3, benzophenone-3; OC, octocrylene; DEHN, diethylhexyl 2,6-naphthalate; BTDC, benzotriazolyl dodecyl p-cresol; Vit A, vitamin A palmitate; Vit E, vitamin E acetate.

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Table 1
Formulation components.

Components	Percentage in each formulation (w/w)		
	F1	F2	F3
Hydroxyethyl cellulose	0.50	0.50	0.50
Glycerin 86%	3.00	3.00	3.00
Distilled water	61.15	56.15	61.1
BHT	0.05	0.05	0.05
Phosphate-based self-emulsifying wax (cetearyl alcohol, dicetyl phosphate, ceteth-10 phosphate)	3.50	3.50	3.50
C12–C15 alkyl benzoate	6.00	6.00	6.00
Octyl methoxycinnamate	7.00	7.00	7.00
Benzophenone-3	4.00	4.00	4.00
Octocrylene	5.00	5.00	5.00
Diethylhexyl 2,6-naphtalate (DEHN)	–	5.00	–
Benzotriazolyl dodecyl p-cresol (BTDC)	–	–	0.05
Propyleneglycol	3.00	3.00	3.00
Polyglyceryl-10 myristate, diphenyl methicone, trietilexanoine	4.00	4.00	4.00
Cyclopentasiloxane	2.00	2.00	2.00
Phenoxyethanol and methylparaben, ethylparaben, propylparaben and buthylparaben	0.80	0.80	0.80

combined UV-filters and vitamins A and E, which are used in many products and need to be routinely evaluated since photoinstability can lead to efficacy and safety reduction.

2. Materials and methods

2.1. Formulations under study

The formulations contained a phosphate-based self-emulsifying wax (cetearyl alcohol, dicetyl phosphate, ceteth-10 phosphate) and hydroxyethyl cellulose, and were supplemented by the UV-filter combination, octyl methoxycinnamate (OMC), benzophenone-3 (BP-3) and octocrylene (OC) (formulation 1). This formulation could be further supplemented with two different photostabilizers: diethylhexyl 2,6-naphthalate (DEHN) (formulation 2) and benzotriazolyl dodecyl p-cresol (BTDC) (formulation 3), as described in Table 1. Formulations 1–3 also contained 0.6% (w/w) vitamin A palmitate (1,000,000 UI g^{-1}) (formulations 1A, 2A, 3A) or a combination of 0.6% of vitamin A palmitate (Vit A) and 2% (w/w) vitamin E acetate (VitE) (formulations 1AE, 2AE, 3AE).

2.2. Photostability studies

In photostability studies, 40 mg of each formulation (F1–F3) containing vitamin A or the combination of vitamins A and E were spread onto an area of 10 cm^2 (approximately 4 mg cm^{-2}) of a glass plate and left to dry for 15 min before exposure to 30 min UVA/UVB irradiation (280–400 nm) from a 300 W Xenon solar arc simulator (Oriel Corporation, Stratford, CT). The radiation was filtered through a dichroic mirror (280–400 nm) and a WG 305 long pass filter, which does not allow passage of light of less than 280 nm. Irradiance, which was approximately 0.186 mW cm^{-2} was measured at 290 nm and resulted in a cumulative UVB dose of approximately 334.8 mJ cm^{-2} or 15.9 MED cm^{-2} [3], as calculated with a 70260 Oriel Radiant Power Meter equipped with a silicon probe and U-340 filter.

For each exposed plate, a duplicate plate serving as a negative (non-irradiated) control was kept in a dark place at 30 °C. UV-exposed samples were immersed in 25 mL of isopropanol and the dried film was dissolved ultrasonically. This solution was quantified by HPLC analysis (Shimadzu) on a C-18 column and absorption of eluates at 325 nm (for BP-3, OC, OMC, DEHN and Vit A analysis) and 235 nm (for Vit E analysis). BTDC did not absorb at these selected wavelengths. Gradient elution was employed using 84% of methanol:isopropanol (55:45, v/v) as solvent A and 16% water as solvent B for 1 min, with a linear gradient decreasing from 16 to 0% B, followed by an isocratic elution (0% B). The method described was based on previous studies of this research group [3].

2.3. Linearity and specificity

Standard stock solutions of 2000 μg mL^{-1} of OMC, BP-3, OC, DEHN, Vit A and Vit E were prepared separately and aliquots diluted to the required concentrations ranging from 6.25 to 400 μg mL^{-1}. The working standard solutions were prepared in replicates of three and the standard curve was constructed plotting peak areas versus concentrations described previously. The least-squares fit method was employed to statistically evaluate the results for linearity by a regression line and the corresponding slope, *y*-intercept and coefficient of linear correlation (r^2) [11,12].

To assess the separation, UV-filters, vitamins and possible interferents from the excipients in the cosmetic formulations were evaluated. As a control, approximately 40 mg of a formulation containing no UV-filter or vitamin (placebo) was spread on a glass plate, left to dry for 15 min and then immersed in 25 mL of isopropanol and the dried film dissolved ultrasonically. This solution was quantified by HPLC analysis.

2.4. Accuracy/recovery and precision

Accuracy/recovery was determined by a comparison between the theoretical concentrations of standard substances added to topical formulations analyte-free and those obtained from the chromatographic analysis.

Precision, calculated as relative standard deviation (R.S.D., %), was obtained in intra-day (10 injections/day) determinations [11,12]. These studies were performed with formulations 1–3 containing the combination of vitamins A and E.

2.5. Statistical analysis

Results obtained were statistically analyzed using Kruskal–Wallis, a non-parametric test.

3. Results and discussion

3.1. Linearity, specificity, accuracy/recovery and precision

The chromatographic separation of UV-filters on C18 columns was optimized by adaptations of the mobile phase. Isocratic elution with a methanol:water (88:12, v/v) mobile phase gave good results in separating the UV-filters under study in a relatively short

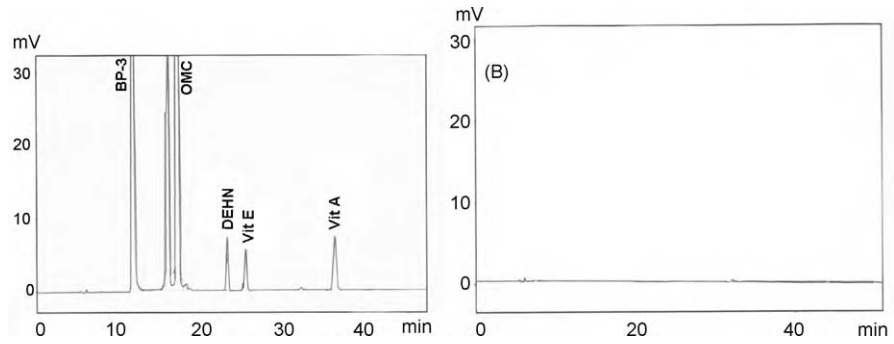

Fig. 1. Chromatographic profile (A) of an isopropanol solution of the sunscreen agents and vitamins in the study. UV-filter and vitamin peaks: benzophenone-3 (BP-3), octocrylene (OC), octyl methoxycinnamate (OMC), diethylhexyl naphtalate (DEHN), vitamin E acetate (VitE) and vitamin A palmitate (Vit A); BTDC did not absorb at the wave lengths used, and (B) of placebo. Detection at 325 and 235 nm; C18 column (250 mm × 4 mm); gradient elution with methanol:isopropanol:water; flow rate 1.0 mL min^{-1}.

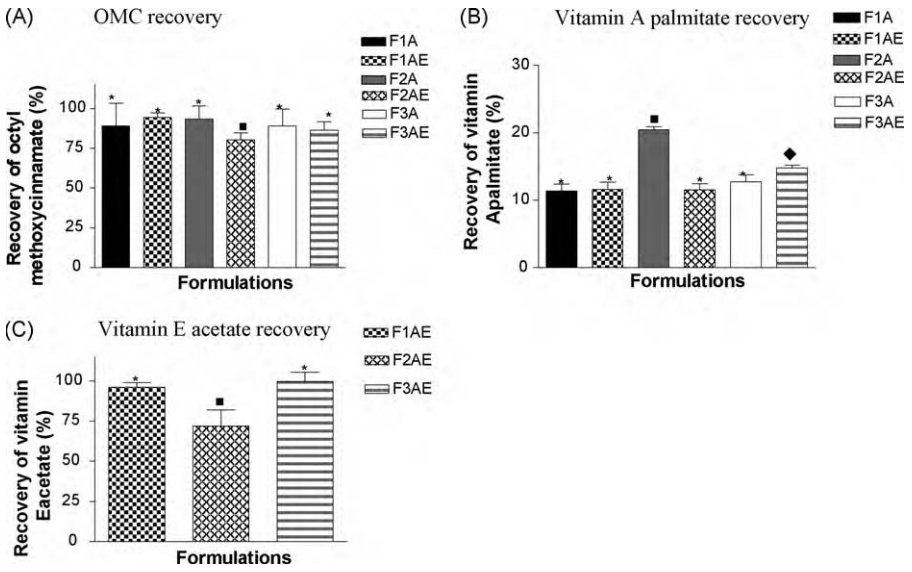

Fig. 2. Recovery of UV-filter OMC (A) and vitamins A (B) and E (C) contained in formulations F1, F2 and F3, after 30 min. UVA/UVB irradiation, expressed as percentages of the initial amounts (negative control). *Different symbols indicate statistically different values ($p < 0.05$).

Table 2
Accuracy/recovery and precision in determinations of UV-filters, octyl methoxycinnamate (OMC), benzophenone-3 (BP-3), octocrylene (OC) and stabilizer diethylhexyl naphtalate (DEHN) in formulations 1–3 containing the combination of vitamins A and E.

UV-filter	Formulation	Theoretical concentration (μg mL^{-1})	Obtained concentration ± S.D.[a] (μg mL^{-1})	Accuracy /recovery (%)	Precision
BP-3	F1	64.00	54.46 ± 1.95	85.09	3.58
	F2		59.07 ± 2.24	92.29	3.80
	F3		6.89 ± 1.06	95.15	1.74
OC	F1	80.00	66.33 ± 1.92	82.91	2.89
	F2		72.2 ± 3.66	90.26	5.07
	F3		72.43 ± 2.82	90.53	3.90
OMC	F1	112.00	97.37 ± 2.44	86.93	2.51
	F2		107.69 ± 3.09	96.15	2.87
	F3		107.75 ± 2.89	96.20	2.68
DEHN	F2	90.00	76.11 ± 2.11	95.14	2.78

[a] Standard deviation ($n = 10$).

Table 3
Accuracy/recovery and precision of vitamin E acetate and vitamin A palmitate determinations in formulations 1–3 containing the combined vitamins.

Vitamins	Formulation	Theoretical concentration (μg mL^{-1})	Obtained concentration ± S.D.[a] (μg mL^{-1})	Accuracy /recovery (%)	Precision
Vit E	F1	320.00	320.11 ± 1.09	100.03	0.34
	F2		318.19 ± 0.89	99.43	0.28
	F3		317.77 ± 3.02	99.30	0.95
Vit A	F1	56.00	55.23 ± 1.63	98.62	2.95
	F2		55.92 ± 1.07	99.86	1.92
	F3		55.21 ± 1.83	98.58	3.32

[a] Standard deviation ($n = 10$).

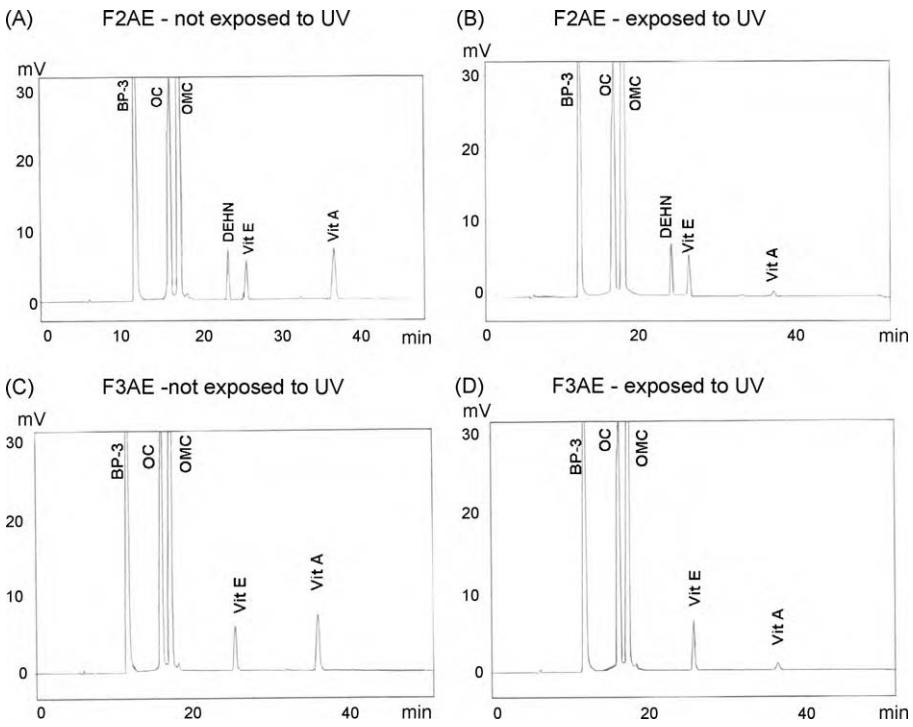

Fig. 3. Chromatographic profiles (HPLC) of formulations 2AE (A and B) and 3AE (C and D) exposed or not to UVA/UVB radiation. UV-filter and vitamin peaks in formulation 2AE (BP-3, OC, OMC, DEHN, Vit E and Vit A) and formulation 3AE (BP-3, OC, OMC, Vit E and Vit A). Detection at 325 and 235 nm, C18 column, gradient elution. Conditions similar to Fig. 1.

time (27 min) [10]; however gradient elution was necessary when vitamins were present due to polarity. Good results in separating the UV-filters and vitamins were obtained by gradient elution as follows: 84% of methanol:isopropanol (55:45, v/v) as solvent A and 16% water as solvent B for 1 min, with a linear gradient decreasing from 16 to 0% B, followed by an isocratic elution (0% B) [3] (Fig. 1A).

HPLC results were validated in terms of linearity, precision and accuracy. The correlation coefficients were all above 0.999 and the method showed linearity over the concentration interval studied. The precision (R.S.D., %) was between 0.28 and 5.07 (Tables 2 and 3). The accuracy/recovery values were low (82.91–100.03) (Tables 2 and 3) because a small amount of the formulation was lost when spread onto the glass plate, but the procedure was kept because it is currently used for the photostability evaluation of sunscreens.

3.2. Photostability studies

To detect the alterations that occurred in the formulations under study after UVA/UVB irradiation, and to choose the most photostable one, the recovery of UV-filters, photostabilizers and vitamins present in the formulations was analyzed. All substances peak areas were in the linear range of the dose–response curve. None of the photostabilizers used altered OMC recovery in the presence of vitamin A (Fig. 2A); however, when these photostabilizers were combined with both vitamins, F2AE was shown to be more photounstable than F1AE and F3AE, in terms of OMC (Fig. 2A) and vitamin E recovery ($p < 0.05$) (Fig. 2C). On the other hand, when all formulations were analyzed in terms of vitamin A only, formulation F2A was the most photostable, followed by F3A and F1A ($p < 0.05$) (Fig. 2B). But, when the photostabilizer was combined with both vitamins, F3AE was considered the most photostable ($p < 0.05$) (Fig. 2B).

The chromatographic profiles of formulations, both exposed and not exposed to UVA/UVB irradiation, are shown in Fig. 3.

Previous studies of this research group showed that UV exposure led to a reduction in the content of some UV-filters and also to a lower protection of vitamin A palmitate. In contrast, a photostable UV-filter combination promoted a higher vitamin A protection than a photounstable one [3]. Carlotti et al. [13] suggested that the degradation process of vitamin A palmitate is through an oxidative mechanism. Thus, the use of an antioxidant such as BHT (which was used in all formulations under study) is necessary for proper storage over time.

When a molecule absorbs energy from UV radiation, it is promoted to a singlet excited state. In this state it can lose the energy by emission of a photon or heat and return to a triplet excited state or directly to the ground stage. In the triplet stage, the molecule may take on a diradical character that undergoes chemical processes such as photoaddition/substitution reactions including hydrogen and electron abstractions, cycloadditons, isomerizations, and fragmentation. Many molecules are considered acceptors (or quenchers) and operate on the singlet and triplet excited state of donors, although quenching of the singlet excited state is relatively rare [14].

Some patents and scientific papers show the importance of UV-filter stabilizers in sunscreens containing avobenzone and OMC, among them DEHN, which is an acceptor of triplet excited state energy [14,15].

Octocrylene and 4-methylbenzilidene camphor can stabilize avobenzone as they have triplet energy similar to avobenzone (55–59 kcal mol^{-1}) [16]. DEHN present in formulations 2A and 2AE has estimated triplet energy between 2 and 59 kcal mol^{-1} [17] and can also stabilize UV-filters. BTDC, the other photostabilizer under study, present in formulations 3A and 3AE is a free radical scavenger and an avobenzone protector since it reduces the potential for degradation reactions by minimizing the lifetime of excited states and inhibiting side reactions of excited state intermediates [17]. In addition, in the present experimental

conditions it can be suggested that BTDC was a better UV-filter stabilizer for formulations containing UV-filters, and vitamin A and E derivatives.

There are some studies suggesting that photo-excitation of vitamin E results in the formation of a triplet state, which can sensitize singlet oxygen formation and may, therefore, be capable of inducing the formation of other reactive oxygen species as well as the degradation of other molecules [18]. The formation of photodecomposition products is mainly observed in retinyl palmitate containing formulations since it is very photounstable, due to photooxidation and to free radicals production [19].

Under the present experimental conditions, it is suggested that DEHN is not a good stabilizer for formulations containing UV-filters, and vitamin A and E derivatives (F2AE), since photodegradation of vitamin E and octyl methoxycinnamate was enhanced probably through the production of free radicals and to an interaction between vitamin and sunscreen.

On the other hand, formulations supplemented with BTDC (F3AE) showed higher photostability when compared to other ones, probably due to its free radical scavenging properties [17].

By validating methods to guarantee the correct analysis of substances of fundamental importance, this report is a contribution to the many studies on new substances that increase the photostability of sunscreen formulations containing different anti-aging substances.

4. Conclusions

In the present experimental conditions, it can be concluded that the proposed HPLC method was adequate for the simultaneous determination of the combined sunscreen UV-filters and vitamins. The extraction procedure was specific and efficient, showing good precision. Photostabilizers influenced the stability of OMC when combined with vitamins A and E. One of them, BTDC (present in formulation 3) was considered the best photostabilizer to vitamins and OMC when UV-filters were combined with both vitamins A and E.

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References

[1] R.K. Chaudhuri, Z. Lascu, G. Puccetti, A.A. Deshpande, S.K. Paknikar, Photochem. Photobiol. 82 (2006) 823.
[2] V.A. DeLeo, S.M. Suarez, M.J. Maso, Arch. Dermatol. 128 (1992) 113.
[3] L.R. Gaspar, P.M.B.G. Maia Campos, Int. J. Pharm. 343 (2007) 181.
[4] P.M.B.G. Maia Campos, G. Ricci, M. Semprini, R.A. Lopes, J. Cosmet. Sci. 50 (1999) 159.
[5] C. Antille, C. Tran, O. Sorg, P. Carraux, L. Didierjean, J.H. Saurat, J. Invest. Dermatol. 121 (2003) 1163.
[6] L.R. Gaspar, P.M.B.G. Maia Campos, IFSCC Mag. 6 (2003) 213.
[7] S. Seite, D. Moyal, M.P. Verdier, C. Hourseau, A. Fourtanier, Photodermatol. Photoimmunol. Photomed. 16 (2000) 3.
[8] G. Berset, H. Gozenbach, R. Christ, R. Martin, A. Deflandre, R.E. Mascotto, J.D.R. Jolley, W. Lowell, R. Pelzer, T. Stiehm, Int. J. Cosmet. Sci. 18 (1996) 167.
[9] V. Vanquerp, C. Rodriguez, C. Coiffard, L.J. Coiffard, Y. De Roeck-Holtzhauer, J. Chromatogr. A 832 (1999) 273.
[10] L.R. Gaspar, P.M.B.G. Maia Campos, Int. J. Pharm. 13 (2006) 123.
[11] Guide for Validation of Analytical and Bioanalytical Methods, Resolution R.E. no. 899, Brazilian Sanitary Surveillance Agency, Brasilia, DF, 2003.
[12] A.M. Maia, A.R. Baby, W.J. Yasaka, E. Suenaga, T.M. Kaneko, M.V. Velasco, Talanta 71 (2007) 639.
[13] M.E. Carlotti, V. Rossatto, M. Gallarate, Int. J. Pharm. 240 (2002) 85.
[14] C. Bonda, Cosmet. Toiletries 123 (2008) 49.
[15] E. Damiani, W. Baschong, L. Greci, Photochem. Photobiol. B: Biol. 87 (2007) 95.
[16] E. Chatelain, B. Gabard, Photochem. Photobiol. 74 (2001) 401.
[17] N. Shaath, Happi (October) (2007).
[18] S. Dad, R.H. Bisby, I.P. Clark, A.W. Parker, Free Radic. Res. 40 (2006) 333.
[19] Q. Xia, J.J. Yin, W.G. Wamer, S.H. Cherng, M.D. Boudreau, P.C. Howard, H. Yu, P.P. Fu, Int. J. Environ. Res. Publ. Health 3 (2006) 185.