PaO (period after opening)

Luigi Campanella and Cecilia Costanza
Department of Chemistry, University of Rome "La Sapienza", Rome - Italy

Received: *December 2009. Presented at: The IX ISCD International Multidisciplinary Congress "Wellness and Beauty Outside In: East & West working together", Rome, 21-23 October 2009.*

Key words: *PaO; Photosensor; Cosmetics; Ecopersistence;*

Summary

The fundamental aim of the 2003/15/EC Directive is actually to guarantee the safety of the cosmetic products on the basis of new scientific progress in safety matters pertaining to the life-time of cosmetic products. In order to enhance consumer information PaO (Period after Opening) indication on the label aims to inform the consumer about the stability of the cosmetic, as it indicates the period of time after opening during which the product can be used without harmful effects.
For PaO (period after opening) determination of cosmetic products, no specific validated scientific methods exist, nor any standardized protocols (characterizing the most suitable analysis for the different products), or references that accurately document protocols of this kind (there are no recognized bibliographical references describing the effective correspondence between the residence time in thermostated rooms and the effective aging of the product). The evaluation must reasonably take into account the physical-chemical characteristics of the products and the normal or expected conditions of use. The environmental persistence test is proposed here for the determination of PaO of two commercial cosmetic products, a face powder and a daytime cream at the time of opening, and after accelerated artificial ageing in the artificial aging device in order to estimate the possible degradation.

Riassunto

La Direttiva 2003/15/CE del 27-2-03 che regolamenta i prodotti cosmetici, più nota come VII Modifica, sancisce l'obbligo per tali prodotti di indicarne nella confezione il PaO (Period after Opening) cioè il tempo di stabilità e quindi di sicurezza d'uso a partire dal momento di apertura della confezione. Per la determinazione del PAO dei prodotti cosmetici non esistono metodi scientifici specifici e validati, né protocolli standardizzati (che individuino la tipologia di analisi più idonea per i diversi prodotti), né riferimenti bibliografici che documentino con precisione protocolli di questo genere. La valutazione deve tener conto delle caratteristiche fisico-chimiche dei prodotti e delle normali o ragionevolmente prevedibili condizioni d'uso. In questo lavoro si propone un metodo per la

determinazione del PAO nei prodotti cosmetici; si applica il test di persistenza ambientale a due cosmetici commerciali (cipria e crema da giorno) all'atto dell'apertura, e dopo l'invecchiamento artificiale accelerato in veterometro, pervenendo alla conclusione che tale risposta può essere diagnostica ai fini della sicurezza del consumatore.

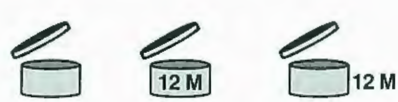
INTRODUCTION

Stability and state of preservation of cosmetic products

The European Union (EU) has set up a rapid alarm system for non food products, including cosmetics, which poses a serious risk to public health (RAPEX), together with provisions for withdrawal from the market of products representing a threat to consumers' health and safety.
In Italy the cosmetics sector is regulated by law no. 713 of 11 October 1986 and subsequent modifications (Decree law no. 300 of 10 September 1991, Decree law no. 126 of 24 April 1997, Decree law no. 87 of 15 February 2005).
In the early 1970s the Member States of the European Union decided to harmonize their legislation governing cosmetic products in order to avoid the circulation inside the Community of non controlled products.
Following a wide-ranging discussion among experts from all the Member States a Directive (76/768/EEC) was adopted on 27 July 1976. The principles underlying this Directive are related to consumer's rights, the encouragement of trade exchanges and the elimination of the trade barriers.
For example, a product that is to circulate freely in Europe must have the same type of packaging, label and safety rules.
The fundamental aim of the Directive is actually to guarantee the safety of the cosmetic products. On the 27 February 2003 this Directive was further completed and modified (2003/15/EC) on the basis of new scientific progress in safety matters.
As a first consequence of this Directive, on the 5 September 2003, the European Commission issued important safety regulations (Directive 2003/80/EC) pertaining to the life-time of cosmetic products in order to enhance consumer information.

Directive 2003/15/EC of 27 February 2003, incorporated into Italian legislation by DLgs no.50 of 15 February 2005 (1) provided for the introduction of two new labelling regulations and laid down that, as from the 11 March 2005, any cosmetic product that does not comply with the new regulations cannot be manufactured in or imported into the European Community. However, no provision was made to limit transfer to the final customer to whom cosmetics complying with the pre-existing regulations may legitimately be sold provided they were marketed prior to 10 March 2005:

1. For production with a minimal duration greater than 30 months after opening, better known as PaO (Period after Opening), the indication consists of a small open-jar symbol accompanied by a figure indicating the number of months and the letter "M" inside or close to the jar symbol. It was adopted throughout the European Union with the Directive 2003/80/EC of 5 September 2003:



This symbol is particularly important for products that, once opened and coming into contact with the environment, could be subjected to degradation and are likely to become dangerous (for example, due to microbiological contamination).
The PaO must be indicated on the primary and secondary packaging of the cosmetic (primary packaging is the container in direct contact with the product, while secondary packaging may be, for example, the envelope containing it). On some products, for example, single-use disposable products or those for which, owing to their composition or method of manufacture, display practically zero risk of alteration (for example, products that do not allow direct contact betwe-

en the contents and the outside environment, snch as spray products nnder pressure), the symbol will not appear as it is not necessary.

2. the inclusion in the list of ingredients of the presence of one or more of the 26 moleculles identified by the Scientific Committee for Products destined to Consumers (SCCP) as an important cause of allergic reactions due to contact among consumers allergic to perfumes.

PaO indication on the label aims to inform the consumer about the stability of the cosmetic, as it indicates the period of time after opening during which the product can be used without harmful effects.

The PaO symbol must be present on the labels of all the cosmetic products, with the exception of:

- products with a durability of less than 30 months, as these products already carry a "best use before" date;
- single-dose products (for example, free samples);
- products manufactured in a such way as to avoid contact between the cosmetic and the surrounding environment (for example, aerosols);
- products for which the producer certifies that the formula is such as to prevent any risk of deterioration having a negative effect on the product's safety over time.

The new PaO symbol have been progressively introduced: all the products affected by the Directive are labelled with this symbol since 11th March 2005.

Products without PaO, already marketed before this date, can be continued to be sold. In order to clarify the meaning and interpretation of the post-opening period, it is necessary to specify that:

- PaO is an indicative period of time established on the basis of the knowledge acquired from the manufacturers concerning their products;
- a cosmetic is considered "opened" when it is used for the first time. The post-opening period must therefore be computed from this first use;
- the information justifying the presence or absence of PaO is accessible to the control authorities.

Toxicity

Art. 3 of the Directive 92/32/EEC of 30 April 1992 regulating the classification, packaging and labelling of dangerous substances marketed in European Union countries provides for the "determination and the evaluation of the property of the substances" through toxicological tests involving experiments on animals. According to the results of the experiments, a substance will be classified in one of the following categories:

- very toxic
- toxic
- injurious
- not dangerous.

A standard protocol provides for the study of:

1) Short-term toxicity, subdivided into the study of:
   - acute toxicity, normally carried out on mouse or rat, using LD_{50}.
   - irritation of the eyes, skin and mucous membrane, usually carried out on albino rabbits by means of "Draize tests" in cosmetics and aimed at assessing the tolerability of the skin or mucous membrane to contact with the substance under investigation.
   - sensitization, normally carried out on guinea pigs to assess the potential of the chemical substance to induce allergic or immune responses as a result of multiple administrations.
2) Repeated toxicities, the assessment of which is carried out through the study of:
   - sub-acute, sub-chronic and chronic toxicity, usually performed on two species, one of which a rodent (normally rat), and mon-

key or dog. The duration of the studies varies from two to four years; the administration method is that of exposure.

- oncogenesis.

3) Reproductive toxicity and teratology in order to detect any interference by the new substance in the reproductive sphere and on offspring; the studies are subdivided into three groups:
   - Fertility and reproduction
   - Teratology.
   - Peri-post natal toxicity studies

In short-term toxicity (acute effect) the toxicity level is defined on the basis of the lethal amount of the chemical compound and the method of exposure; the Lethal Dose, 50% and Lethal Concentration, 50% used to classify a product as very toxic, toxic or injurious are set out in the following (Table I) (2):

LD_{50} is the amount of substance that, administered in a single dose, causes the death of 50% of the experimental animals; it only indicates the short term toxicity (acute toxicity) of the substance not the long term toxicity (which is the result of contact with small amounts of a given substance over long periods of time); it is usually expressed as the amount of substance administered versus the weight of the sample animal (for instance, mg of substance per 100 grams for small animals or per kg for larger animals); the administration method must be also defined (oral, cutaneous, etc.). An LD_{50} greater than 2000 mg/kg means that the substance tested may be considered as not particularly dangerous.

For oral LD_{50} EU regulations prescribe rat as the experimental animal, while for cutaneous LD_{50} the use of rabbit is also allowed.

LC50 denotes the concentration in the atmosphere that causes the death of 50% of the experimental animals when inhaled for a given period of time. For LC50 EU regulations prescribe the use of rat as experimental animal with an exposure of 4 hours.

The methods or procedures leading to the substitution of an animal experiment or the reduction of the number of animals required, and to the optimization of the experimental procedures in order to reduce animal suffering are alternative methods to experimentation in vivo. This concept corresponds to Russel and Burch's "3Rs" (3): replacement, refinement, reduction:

1) replacement identifies the substitution, where possible, of higher animals with biological materials of lesser complexity (cellular bacteria, cultivations, isolated organs, cultivations in vitro), computerized models, videos, films;
2) refinement refers to the search for increasingly specific experimental procedures that can at least reduce the suffering and stress of the animals used
3) reduction is aimed at limiting the number of animals used for a particular experiment that achieves given study results. This can be achieved by standardizing the animal population, the main factor involved in result variability.

TABLE I

Category	Oral LD_{50} mg/kg	Cutaneous LD_{50} mg/kg	Inhaled LC_{50} mg/L/4 hours
very toxic	<25	<50	<0.5
toxic	25-200	50-400	0.5-2
injurious	200-2000	400-2000	2-20

Methods of the first type allow certain experimental information to be obtained without using animals; methods of the second type are suitable for obtaining comparable levels of information using a smaller number of animals and allow maximum information to be obtained from a single test on animals; methods of the third type all allow suffering and damage caused by the experimental practices to be reduced.

Methods of the first type include "biological substitutive methods" and "non biological substitutive methods"; the former ones consist of "in vitro methods", and make use of various types of biological material (of human or animal origin); the latter ones take advantage of the contributions of sciences such as mathematics, computer science and statistics, etc.

A new experimental approach, in order to be considered alternative to conventional animal experimentation, must be reproducible, reliable, rapid and not more expensive than the one it is intended to replace.

The European centre responsible for enforcing the above parameters by the new method ("validation") is the ECVAM (European Centre for the Validation of Alternative Methods), established by the European Commission in 1991 at the proposal of the European Parliament as part of the "Joint Research Centre" in Ispra (Varese/Italy). ECVAM coordinates the validation of the alternative methods at Community level, and constitutes a point of reference for the exchange of information on the development of these methods by means of a database of available methods (either already validated or in the process of validation) managed by the same centre. The reliability and importance of a method are established through the validation process. Reliability refers to the reproducibility of results in time and space, that is, both in the same laboratory and in different laboratories (so-called "standardization"); significance measures the usefulness and importance of the method with regard to a given aim.

Validation tests are extremely long (they can even last years) and are designed to verify if, for given substances, a new method produces results similar to those previously obtained by means of animal experimentation.

The ultimate destination of a new method is its inclusion in the international regulations, and the introduction of alternative tests among the guidelines of the OECD (Organization for Economic Cooperation and Development).

The OECD not only gathers together the member countries of the European Union but also the United States, Japan and others; it has the task of harmonizing the different experimental protocols in the form of guidelines. OECD guidelines are periodically modified in order to adapt them to new scientific knowledge and to any legislative changes.

A EU White Book (4) invites the scientific community to make the utmost effort to develop chemical tests that do away with the need for animal experimentation, and that can provide information – particularly danger warnings concerning the toxicity of a compound – in real or quasi real time.

AIM OF THE WORK

For PaO (period after opening) determination of cosmetic products, no specific validated scientific methods exist, nor any standardized protocols (characterizing the most suitable analysis for the different products), or references that accurately document protocols of this kind (there are no recognized bibliographical references describing the effective correspondence between the residence time in thermostated rooms and the effective aging of the product).

The evaluation must reasonably take into account the physical-chemical characteristics of the products and the normal or expected conditions of use.

The environmental persistence test is proposed here (5) for the determination of PaO of two commercial cosmetic products, a face powder (the PaO on the label is 24 months) and a daytime cream (the PaO on the label is 12 months) (both opened and unopened cosmetics) at the time of opening, and after accelerated artificial ageing in the artificial aging device in order to estimate the possible degradation.

Persistence

Persistence is related to the way a substance is used, its toxicity and its life cycle; it is an important index, both positive, regarding the assessment of durability or longevity during the use of a product, and negative, regarding the assessment of chemical pollutant risk, related to the prolonged time of interaction with human organism and ecosystem.

Environmental persistence, in the positive sense of durability, is the ability of a material to maintain its physical – mechanical characteristics over time and its original appearance. Environmental persistence, in the negative sense of environmental risk assessment of chemical substances, is related to the absence of degradability in the environment and indicates that a substance is not easily biodegradable by bacteria, fungi or other natural agents; is the result of either the absence or inefficiency of sinks or of the inability of a substance to reach potential sinks: a substance will only disappear from the environment if it is degradable, the rate of disappearance depending on the kinetics of the process involved.

Ecopersistence is not directly measurable, but can only be inferred from the continuous presence of a substance in the environment or from a lack of laboratory degradation data.

The European Commission (6) defines organic compound persistence in terms of half-life of the single medium or on the lack of any half-life as the result of the studies and biodegradation tests carried out. While some of these tests are suitable for identifying the substances as non persistent (that is substances subjected to rapid and complete biodegradation in all environmental compartments), they could be not utilized to classify a chemical substance as "persistent"; generally speaking, a substance with a half life greater than one year in all environmental compartments is considered "persistent" (7).

Principles of the ecopersistence test method

In the present work, an innovative method is proposed for evaluating environmental permanence or ecopersistence (5); this both positive and negative index is determined using a titanium dioxide photosensor.

Titanium dioxide, TiO_2, is the most important Ti compound with an oxidation degree of +4; it represents one of the more efficient photocatalysts of oxidative degradation of many organic compounds (8-10); it is the most frequently used semiconductor among those available as it is photostable, non toxic (LD_{50} > 10000 mg/kg) and insoluble in water. When TiO_2 absorbs light having an energy ≥ 3.2 eV (band gap energy of titanium anatase), electrons and holes are formed in the conduction and valence bands, respectively (e^-_{cb} and h^+_{vb}).

$$TiO_2 + h\nu \rightarrow TiO_2(e^- + h^+) \rightarrow TiO_2(e^-_{cb} + h^+_{vb})$$

$$e^-_{cb} + O_2 \rightarrow O_2^{\bullet -}$$

$$h^+_{vb} + H_2O \rightarrow OH^\bullet$$

$$O_2^{\bullet -} + OH^\bullet + \text{organic substance} \rightarrow \text{organic substance degradation } (CO_2 + H_2O + \text{mineral acids})$$

Hydroxyl radicals are powerful oxidizing agents and readily oxidize organic pollutants and subsequent intermediates formed during the mineralization process to carbon dioxide.

The superoxide anion radical, formed as a result

of oxygen reduction from photogenerated electrons (eq. 4), itself having a relatively low oxidant capacity, can combine with organic peroxy radicals (ROO.) to generate an unstable tetroxide (ROOOOH) which decomposes into reaction products (10-11).
Species such as $HO_2^{\bullet}$, $O_2^{\bullet}$, H_2O_2, are present either at the interface or in solution and can be involved in the complex degradation scheme leading to the final mineralization of organic substrates.
TiO_2 due to its structural composition can also act as pH indicator (5, 12).
Several tests were performed to evaluate the performance of TiO_2 as a pH indicator in terms of calibration curve slope (mV/pH), potential (pH) jump on acid and base additions and end-point potentiometric acid-base titrations (5).
The 350 nm UV photoactivated TiO_2 acts as a photocatalyst of organic substance degradation and as a pH material indicator. It therefore allows measurement of the time needed to reach the acidification corresponding to the production of CO_2 due to the decomposition of the considered compound.
The beginning of the process of acidification can be considered as the conclusion of the period of induction; the rate of pH variation over time can be considered as to be calculated starting from this time: higher it is, greater the concentration of carbonic dioxide and carbonic acid in environment and of mineral acids produced by mineralization in unit time.
Before acidification TiO_2 potential (pH) versus irradiation time generally decreases (the pH increases) to a minimum (maximum); after the induction time, it changes slope and begins to increase (the pH decreases).
The time needed to observe the change of slope (induction or delay time) is related to the beginning of the degradation of molecules leading to CO_2, H_2O and mineral acid production; after this time, TiO_2 potential (pH) increases (decreases) as rate of mineralization (Figure 1).

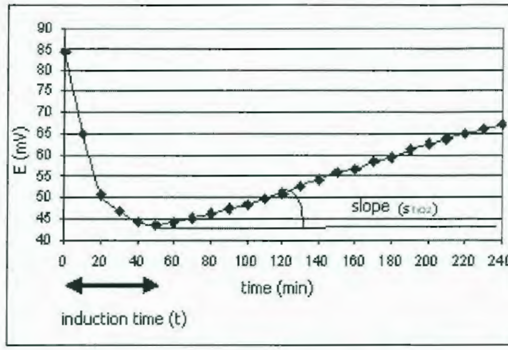


Fig. 1 Typical trend of titanium dioxide potential versus UV irradiation time (350 nm) for an organic compound.

We reasonably assume that the index of environmental persistence, P_{env}, or ecopersistence, is assumed to be the ratio between the induction or delay or activation time t, needed to induce the degradation, and the curve (straight line point) slope s of the increasing TiO_2 potential versus irradiation time $sTiO_2$ (the increase of TiO_2 potential with time corresponds to the increase of acidity over time):

$$P_{env} = \frac{t}{s_{TiO_2}}$$

In some cases degradation takes place without any activation time, t=0, therefore P_{env} =0, and $sTiO_2$ is taken as an index of degradability assessment.

Experimental section
Apparatus

UV source: Polilight®, supplied by Rofin (Dingley, Victoria, Australia), equipped with high quality interference filters, at central UV wavelength of 350 nm.
For ecopersistence test: Orion pH Meter (model 420) for the measurement of titanium dioxide potential (uncertainty was ± 0.1 mV).
For artificial weathering of real matrices: Weatherometer QUV Accelerated Weathering

Tester (Q - Panel Company, Cleveland, OH USA) - Model QUV/spray Q-Panel LAB-Products in the following conditions of UV exposure: spectral irradiance 0.6 $W/m^2/nm$, relative humidity 58 %, T = 45 °C, λ = 310 nm.
This apparatus was equipped with 8 mercury lamps (40 W each), perfectly simulating sunlight up to 370 nm and relatively well up to 400 nm.
Commercial cosmetics: face powder and daytime cream.

Ecopersistence measurement

Titanium dioxide phototocatalyst was used in suspension with the suspension acting as electrode. The photoreactor was a 150 mL Pyrex glass beaker. The 350 nm *Polilight®* was placed inside the photoreactor and 0.5 cm away from the free surface of the suspension.
For each analysis the suspension consisted of 50 mg of TiO_2 in 105 mL of
- cosmetic product as is: 5 mL of daytime cream or 1.5 g of face powder;
- aqueous extract of the cosmetic product (just opened vase, artificially aged in a weatherometer in an opened vase, artificially aged in a weatherometer in a closet)
  - 1.5 g of face powder in 300 mL of water,
  - 4 g of daytime cream in 800 mL of water, after two filtrations;

RESULTS

The results of the measurements on just opened cosmetics did not allow the P_{env} value to be estimated because TiO_2 potential continues to decrease for the full duration of the analyses (up to 8h) so lacking the value of the induction time. The difficulties encountered in applying the test could be due to the cosmetics' composition; the presence of the fat component probably renders impermeable the suspension/emulsion of cosmetic in TiO_2/water and prevents the passage of ultraviolet radiation and thus does not allow catalyst activation.
The cosmetic product was then extracted. The results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged, artificially aged in closed pots and in an opened pot up to 200 h, are reported in Table II.

TABLE II
Results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged, artificially aged in closed pot and in opened pot up to 200 h.

Aqueous extracts of cosmetic products	P_{env} (min^2/mV)		
	Just opened and unaged	**Artificially aged in closed pot up to 200 h**	**Artificially aged in opened pot up to 200 h**
Face powder	293±10	290±10	75±5
Cream	114±10	112±10	30±3

The results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged and in an opened pot up to 400 days, are reported in Figure 2 for face powder and in Figure 3 for cream of labelled commercial products.
The values of the time for which P_{env} falls down abruptly are perfectly corresponding with PaO value if to 1 week of artificial ageing in the described conditions we let correspond 1 year of natural ageing.

CONCLUSIONS

PaO values for cosmetics, generally determined by chemical and biological long tests of stability can be rapidly and reliably determined by the proposed photosensor and artificial ageing of the cosmetic samples basing on a correspondance factor of natural and artificial ageing in controlled conditions and on the behaviour of P_{env} during artificial ageing.

References

1) **Direttiva 2003/15/CE del 27 febbraio 2003**, recepita in Italia con DLgs n.50 del 15 febbraio 2005, Gazzetta ufficiale dell'Unione europea 11/03/2003 (riportare in inglese)
2) **Curini R, Bacaloni A, Tentolini U, Curini S. (2006)** Università Degli Studi Di Roma "La Sapienza" - "Laboratorio chimico per la sicurezza. Valutazione del Rischio Chimico".
3) **Russel WMS, Burch RL. (1959)** The Principle of Human Experimental Technique, Meuthen, London.
4) Commissione delle Comunità Europee, LIBRO BIANCO *Strategia per un politica futura in materia di sostanze chimiche,* Bruxelles, 27 febbraio 2001 COM (2001) 88.
5) **Campanella L, Costanza C. (2009)** Ecotoxic. *Environ. Saf.* **72:** 261- 272.
6) European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels, Technical Report - ECETOC 1-195, (2003).
7) **Klopffer W. (1994)**Atmospheric Degradation of Organic Substances ESPR. *Environ. Sci. & Pollut. Res* **1 (2):** 108 -116
8) **Mills A, Le Huute S. (1997)** An overview of semiconductor photocatalysis, *J. Photochem. Photobiol. A: Chem.* **108:** 1-35.
9) **Hidakaa H, Shimuraa T, Ajisakaa K, Horikoshia S, Zhaob J, Serpone N. (1997)** Photoelectrochemical decomposition of amino acids on a TiO2/OTE particulate film electrode, *J. Photochem. Photobiol. A: Chem.* **109:** 165-170.
10) **Soja GR, Watson DF. (2009)** "TiO2-Catalyzed Photodegradation of Porphyrins: Mechanistic Studies and Application in Monolayer Photolithography." *Langmuir* **25:** 5398-5403.
11) **Vamathevan V, Amal R, Beydoun D, Low G. and Mcevoy S. (2002)** Photocatalytic oxidation of organics in water using pure and silver-modified titanium dioxide particles, *J. Photochem. Photobiol. A,* **148:** 233–245.
12) **Campanella L, Battilotti M, Costanza C. (2005)** Fotodegradazione e degradazione elettrochimica di coloranti. *INQUINAMENTO* vol. 47, pp. 22-28, ISSN: 0001-4982.

Author Address:

Luigi Campanella
Department of Chemistry
University of Rome "La Sapienza"
Piazzale Aldo Moro 5
00185, Rome - Italy
Email: luigi.campanella@uniroma1.it