Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (4): 574-9 (2009)

Original Article

Received: April 8, 2009
Accepted: April 29, 2009

Development of Gel With *Matricaria recutita* L. Extract for Topic Application and Evaluation of Physical-Chemical Stability and Toxicity

Maria B.R. QUEIROZ [1], Natália B. MARCELINO [2], Marcos V. RIBEIRO [1], Laila S. ESPINDOLA [1], Francisco R. CUNHA [2] & Mônica V. da SILVA [1]*

[1] *Departament of Pharmacy - Faculty of Health Sciences. University of Brasília, Darcy Ribeiro University Campus/ UnB - Asa Norte 70910-900, Brasília – DF, Brazil*

[2] *Department of Mechanical Engineering -Faculty of Technology. University of Brasília – UnB, Brazil*

SUMMARY. *Matricaria recutita* L. (Asteraceae), better known as chamomile, has been used due to its pharmacological properties. Laboratory-manufactured gels with chamomile extract were developed with the evaluation of the physical-chemical stability, as well as the study of its toxicity. The extractive solution was prepared by maceration with ethyl alcohol 95%. Part of the chamomile extractive ethanolic solution (CEES) was concentrated in rotoevaporator, obtaining a raw chamomile extract (RCE). For the preparation of gels, carbopol 940P, hydroxyethyl cellulose and hydroxypropyl methylcellulose were used with the addition of 3% and 5% chamomile extracts. The stability tests applied to the gels were as such: thermal stress, pH evaluation, viscosity and storage at different temperatures. In the end of the tests it was observed that the carbopol gel was the most stable. The Draize Test was employed as the toxicity test, with no irritation observed; however, on skin that underwent abrasion, some gels caused a little irritation.

INTRODUCTION

At the present, the employment of medicinal plants in dermatological and cosmetic products is increasing. Vegetal extracts must be standardized and studies that will guarantee the quality, safety and efficacy must be performed.

Research aimed at identifying, characterizing and relating pharmacological and toxic effects of products of vegetal origin constituents have been carried out, once a deep knowledge on their composition is a fundamental step towards the rational employment of those products.

Therefore, over the last decades, a gradual return of the interest in the employment of natural products has been observed. The collateral effects originated from their use are much smaller if compared to the effects caused by synthetic products, like the case of non-steroid anti-inflammatory drugs (NSAIDs), one of the most consumed therapeutic classes worldwide. Practically all NSAIDs available at the present can cause significant unwanted effects [1].

The vegetal species *Matricaria recutita* L. (Asteraceae), also known as chamomile, is one of the most representative medicinal plants [2-5]. The part used for therapeutic purposes is constituted of its desiccated and stabilized floral capitula [6,7] and they have been largely used in traditional medicine for centuries, due to their anti-inflammatory, spasmolytic, sedative, antibacterial and antifungal properties [8]. Camazulene, α-bisabolol, bisabolol oxid A and B terpenes as well as the flavonoids are some of the many substances responsible for those medicinal properties [8-12].

Among the important chemical substances of the chamomile, it is worth mentioning the apigenine, one of the most common flavones found in plants [13]. In chamomile, the apigenine is quantitatively the most abundant flavonoid found in its flowers and the responsible for its pharmacological properties [8,14].

According to different authors [6,5,16], the in vitro hydroalcoholic extract of chamomile has inhibited cyclooxigenase and 5-lipoxygenase enzymes and also the production of prostaglandins

KEY WORDS: Carbopol, Chamomile, Draize test, Gel, Stability test,Toxicity.

* Author to whom correspondence should be addressed. *E-mail:* mvalero2@unb.br

ISSN 0326-2383

and leukotrienes, which are important elements in the inflammation induction.

Chamomile extracts were obtained and incorporated into hydrosoluble gels, which have been largely used in cosmetic products and as a dermatologic base, as they are easily dispersed, non-oily and can carry hydrosoluble active principles. Usually, gel-former substances are polymers which when dispersed in aqueous medium, give viscosity to the preparation [17].

Some studies have demonstrated the toxic effects of chamomile. Serious allergic reactions have been associated with the plant [18,3].

The objective of this study was the development of semi-solid formulas for topic use containing chamomile extract, the evaluation of physical-chemical stability parameters and the study of its toxicity.

MATERIAL AND METHODS

Botanical Material

Matricaria recutita L. (Asteraceae) was donated by the Farmacotécnica Pharmacy, Institute of Pharmaceutical Manipulations Ltd., Brasília - DF. The floral capitula of the species were selected for the execution of the study and later on desiccated and stabilized in greenhouse with forced ventilation. Witness samples of the material can be found deposited in the Herbarium from University of Brasília, voucher number UB 45515.

Chemicals

For the Thin Layer Chromatography (TLC) analysis, p.a. methyl alcohol (Vetec® ,Brazil), p.a. dichloromethane (Dinâmica®, Brazil), p.a. ethyl acetate (Vetec® ,Brazil), p.a. cyclohexane (Dinâmica®, Brazil) were employed. The analytic standards used in TLC were luteoline (Sigma-Aldrich®, St Louis, MO, USA), apigenine (Sigma-Aldrich®, St Louis, MO, USA) and quercetine (Sigma- Aldrich®, St Louis, MO, USA). As developing reagents of TLC, NP/PEG (Diphenylboryloxyethylamine/Polyethylene glycol – 4 000) were used and Silicagel 60 F254 20x20 cm chromate-treated aluminum sheets (Merck®, Germany) were the plates used in TLC.

Collection of chamomile extracts and application in TLC

The extract was obtained by maceration of desiccated and stabilized chamomile floral capitula in the proportion of 20 g/100 mL ethyl alcohol (Synth®) during 12 days according Brazilian Pharmacopeia [19]. After the filtration method, CEES was obtained. One part of the extractive solution was concentrated through vacuum rotoevaporator, obtaining 59.75 g of RCE. All extracts were kept refrigerated at 5 ± 2 °C. At the moment of use, RCE was re-suspended in ethyl alcohol 95% (Synth®).

The TLC was prepared with the standards (luteoline, apigenine and quercetine) and with the chamomile extracts, using a mix of ethyl acetate, formic acid, acetic acid and distilled water (110:5:5:10) as the elution system. The development was made with NP/PEG and UV 365 nm [20].

Development of formulations and stability study

The gels were prepared by dispersion, in accordance with the physical characteristics of each polymer, employing a mechanical stirrer (Fisaton® 175) with constant agitation at 200 rpm until the achievement of the gel [21]. The ethanolic extractive solution was incorporated in the carbopol, HEC and HPMC gels and the raw extract only in the carbopol, at 3 % and 5 % concentrations, according to Table 1.

The pH of all formulations was verified at room temperature, right after the preparation and during the 90 days of the stability study, by employing a digital pH meter PG 1800 model (Gehaka®).

Twenty-four hours after the preparation, the gel samples were fractioned in duplicates of 25.0 g, being conditioned inside plastic pots. The samples were employed in the preliminary freeze/thaw and accelerate tests (storage temperature at 5 ± 2 °C, 37 ± 2 °C and 50 ± 2 °C, viscosity, organoleptic characteristics and pH) [22].

The freeze/thaw test was performed at 50 ± 2 °C and -10 ± 2 °C temperatures, intercalating the samples every 24 hduring 12 days [23]. The accelerated stability test was evaluated during days 1, 7, 15, 30, 60 and 90.

For the viscosity test, a rotational viscosimeter was employed (Brookfield Viscometer® DV-II, USA), being viscosity measured through the rotation speed of the spindle immersed in the sample [24]. The viscosity of jellifying formulations that were conditioned at 20 ± 2 °C temperatures was verified. An amount of 100 g of each sample was placed inside a glass becker [25] for viscosity reading. In each reading, the viscosities were checked every 2 min, during 20 min.

With CEES and RCE carbopol gels, a LV4 spindle at a 1.5 rpm rotation was used. For the reading of HPMC gels with CEES, a LV4 spindle at 0.1 rpm was used. For the HEC gel samples with CEES, a LV4 spindle at 1.5 rpm was used.

Components	Supplier	F1	F2	F3	F4	F5	F6	F7	F8
Carbopol 940P	Henrifarma	1	1	1	1	-	-	-	-
HEC	Vital Specialty	-	-	-	-	2	2	-	-
HPMC	Henrifarma	-	-	-	-	-	-	5	5
CEES	-	3	-	-	-	3	-	3	-
CEES	-	-	5	-	-	-	5	-	5
RCE	-	-	-	3	-	-	-	-	-
RCE	-	-	-	-	5	-	-	-	-
EDTA	Volp	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Methylparaben	Vital Specialty	0,20	0,20	0,20	0,20	0,20	0,20	0,20	0,20
Propylparaben	Vital Specialty	0,10	0,10	0,10	0,10	0,10	0,10	0,10	0,10
Imidazolinylurea	Vital Specialty	0,20	0,20	0,20	0,20	0,20	0,2	0,20	0,20
Glycerin	Merck	5	5	5	5	5	5	5	5
LSS	Vetec	1	1	1	1	1	1	1	1
Triethanolamine	Quimex	1	1	1	1	-	-	-	-
Ethyl Alcohol	Synth	5	5	5	5	-	-	-	-
Distilled Water qsp-	-	100	100	100	100	100	100	100	100

Table 1. Components and concentration percentages employed for the manufacturing of hydrogels. HEC = hydroxyethyl cellulose; HPMC = hydroxypropyl methylcellulose; CEES = chamomile ethanolic extractive solution; RCE = raw chamomile extract; EDTA = ethylenediaminetetraacetic acid; LSS = lauril sodium sulphate; F = formula; qsp = quantity sufficient.

Draize Test - primary skin irritation in rabbits

Male and female albino rabbits *(Oryctolagus cuniculus)*, of New Zealand lineage, were supplied by the Vivarium of the University of Brasília. The animals weighed between 2.8 and 3.8 kg. The rabbits were placed individually inside galvanized steel cages with 40 x 40 x 40 cm dimensions, accordingly identified. The animals were acclimatized under the Vivarium conditions for a period of 7 days and fed daily with a commercial rabbit food (Purina®) and water *ad libitum*. Each group had two rabbits totalized twelve animals, according literature [27].

The temperature of the room was kept at 22 ± 2 °C. An illumination system with a timer produced in the room a cycle of 12 h of light and 12 h of darkness. All the experiment with the animals was performed in accordance with the ethical norms for the care of laboratory animals and approved by the Animal Ethics Committee of the Biology Institute of the University of Brasília, number 16632/2006.

The animals were trichotomized on their dorsal side with an electrical shaver one day before the start of the test. The area used for the application of samples was of 6.0 cm^2. Samples of 0.5 g of the chamomile extract gel, from the positive control (Voltaren Emulgel®) and negative control (gel without the extract) were previously weighed separately, on surgical gauze and then applied, in duplicates, directly over the dorsal side. The gauze with the sample was fixed on the area between the inferior members during 4 hours before the beginning of the test readings [26].

After the exposition period, the patches were removed and then after waiting for 60 min, the first reading was made. The following readings were made after 24, 48 and 72 h. During the process, the occurrence of possible allergic manifestations, such as irritation, edema or scabs was observed following the Draize Table. During the entire period, the animals were individually kept inside their respective cages [26].

A week later, the animals underwent trichotomy again, on the dorsal side opposed to the previously used, following the same procedure described above; however, two parallel scratches were performed with the aid of a needle and the samples were applied once again. The evaluation followed the same procedure already described for unbroken skin [27].

RESULTS AND DISCUSSION

According to the TLC results, it was possible to observe that in some cases the chamomile extracts produced in laboratory contained apigenine, but no luteoline or quercetine, (Fig. 1). It was observed a higher concentration of apigenine in RCE and a similar profile was observed in CEES.

The fact the extracts did not have the three analyzed flavonoids can be explained according to Farias [28], who mentions that the quality of the raw materials alone does not guarantee the effi-

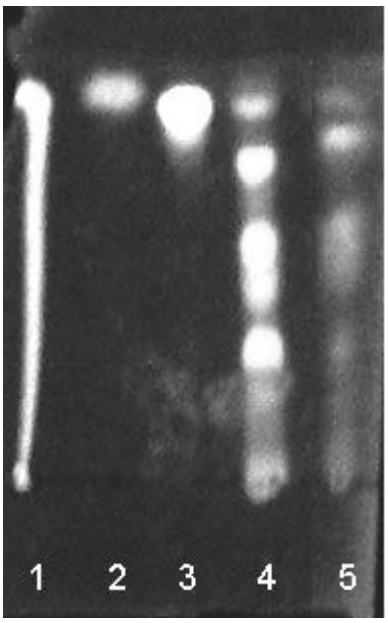

Figure 1. Thin layer chromatography: **1**: quercetine, **2**: luteoline, **3**: apigenine, **4**: RCE, **5**: CEES.

ciency, safety and quality of the final product. The safety and the efficiency of a phytotherapeutic drug must be defined for each product, because they are dependent on several factors, such as the methodology of collection of the extracts, the formulation and the pharmaceutical form of the final product, among other factors. Therefore, procedures for extract preparation must be standardized, in order to obtain the so called standardized products.

During the stability study, the formulations were macroscopically analyzed during the predetermined periods, totalizing 90 days of observation. The evaluation criteria were based on the literature, considering that certain physical and chemical alterations could not be tolerated, such as the presence of syneresis and expressive variations on the pH and viscosity values which could jeopardize the stability of the preparations [29,30].

During the freeze/thaw test (preliminary evaluation), all preparations were stable. During the accelerated study, at the end of 15 days, the HEC gel formulations (F5 and F6) displayed a change in the odor and beginning of syneresis at 37 ± 2 °C e 50 ± 2 °C, thus remaining until the end of 90 days.

Formulations prepared with HPMC (F7 and F8) at 30 days displayed at 37 ± 2 °C and 50 ± 2 °C a syneresis process. At 90 days, the same happened to the samples kept at 50 ± 2 °C, presenting a modified odor, in addition to syneresis.

As regarding pH evaluation, there was no significant alteration during the 90 days of observation in all analyzed gels, with pH values remaining between 5.0 and 6.0. The values of the initial pH of formulations carbopol gels remaining between 5.5 and 6.0; HEC gels remaining between 5.8 and 6.0 and HPMC gels 5.6 and 6.0.

According to Ansel *et al.* [31] and Ferreira [24], the pH determination is very important in the stability study as alterations on pH values may occur due to impurities, hydrolysis, decomposition and error in the process.

It is important to mention that homogeneity, color, odor, consistency and texture constitute the most simple verification method regarding the quality of the product. The color and odor modifications may provide indication of chemical and microbiological alterations [32]. For Barry [33], the homogenous appearance and nice odor are desirable characteristics.

The analysis of the rheological behavior of the formulations provided important data regarding physical characterization of the involved systems. The carbopol gels with both extracts of the study showed an absence of instability, whereas the HEC and HPMC gels showed relevant variations regarding viscosity during the 90 days of observation, according to Figures 2, 3 and 4, respectively.

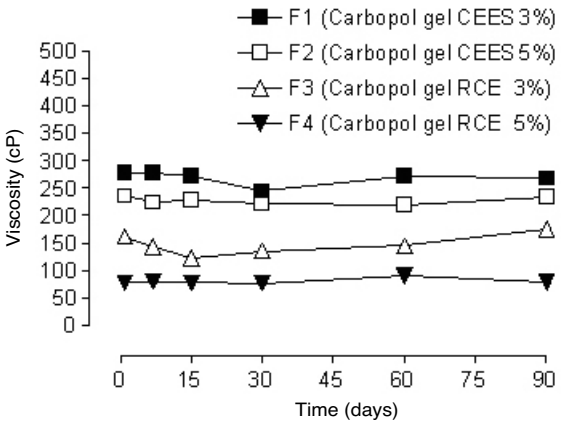

Figure 2. Viscosity values of carbopol gels (CEES e RCE), under storage conditions at temperatures of 20 ± 2 °C.

Figure 3 shows a considerable increase in the viscosity HEC gel CEES 3% and 5% for both formulations 5 and 6. After 60 days the materials display a more complex response to the shearing motion. The results indicates that beyond this critical time there is a structural change in the gel on shearing under constant shear rate,

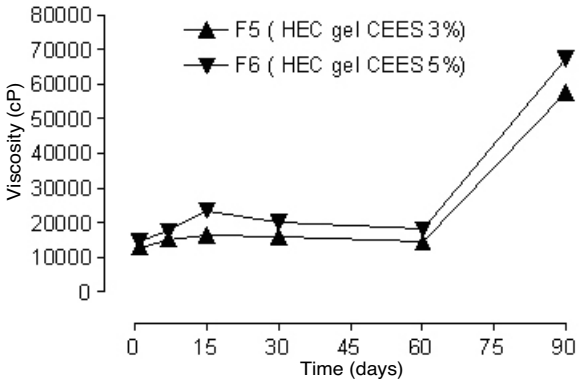

Figure 3. Viscosity values of HEC gels (CEES), under storage conditions at temperatures of 20 ± 2 °C.

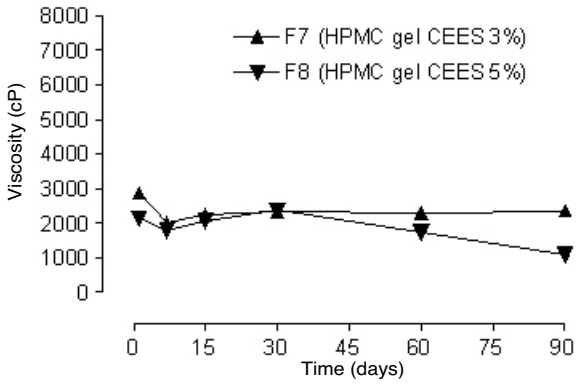

Figure 4. Viscosity values of HPMC gels (CEES), under storage conditions at temperatures of 20 ± 2 °C.

causing a time-dependence viscosity. We attribute this observed phenomenon to a kind of Rheopexy, in which unstable aggregate structures (or higher particle packing) have formed under moderate shear after a sufficient long time, due to Van-der-Waals forces. These unstable structures are usually non-homogeneous and anisotropic, characterized by the strong non-linear behavior (time-dependence viscosity) of the HEC gels. It is important to note that the observed structural changes here take place over a time scale which is much longer than the well-known linear viscoelastic response times.

According to Mambro & Fonseca [34], the decrease in the viscosity observed can be due to the extract incorporated in the formulations. A decrease of 20% in the viscosity of formulations may occur when plant extracts such as 2% *Salvia officinalis, Centella asiatica* or *Calendula* is added to them.

Based on the results displayed both on preliminary stability and accelerated stability of the gels being studied, we can draw the conclusion that carbopol gels, both with CEES and RCE in the studied concentrations displayed a higher stability when compared to the HEC and HPMC gels.

The toxicity test in albino rabbits was performed with carpobol gels chosen as the stable formulation within the 90 days of observation. When the gels were applied to unbroken skin, no relevant irritation was observed, according to the Draize table.

However, when abrasion to the skin of the animals was made with the aid of a needle, the F1, F3 and F4 formulations caused little irritation. All edema and erythema irritations caused by the formulations were mild and at the end of 72 h after the test, practically the skin from all rabbits was already free of any irritation. The toxicological tests performed on the negative (carbopol gel) and positive (Voltaren Emulgel®) control groups did not show any irritation.

Previous studies showed that the use of chamomile oil caused moderate irritant effect both on the unbroken skin and on the broken skin of rabbits, when observed 24 h after the application. On the other hand, when applied to the skin, chamomile oil compresses did not cause irritation in humans 48 h after the application. Chamomile contains allergen products and the most potent is represented by sesquiterpene lactones, present in small amounts such as anthecotulid, which has strong allergenic contact activity in the sensitization tests [15].

CONCLUSIONS

The results obtained with the development of semi-solid formulations containing chamomile extract, preliminary evaluation of stability and study of accelerated stability lead to the conclusion that it is possible to keep the stability of jellifying preparations using carbopol 940P during an observation period of 90 days under thermal stress conditions.

Another relevant aspect was the achievement, through the extractive process of maceration of the chamomile's floral capitula, of an ethanolic extractive solution and raw extract in the presence of apigenine, which is one of the most important chamomile flavonoids.

Carbopol gels with 3% and 5% CEES and RCE, applied to the unbroken skin of rabbits, did not show a cutaneous irritation potential. However, when the gels were applied to broken skin, the formulations containing CEES 3%, RCE 3% and RCE 5% caused a hardly perceptive irritation of no relevance.

Other tests will be necessary for the evaluation of the anti-inflammatory action of the obtained extracts in the carbopol gel. This will allow the confirmation of the efficacy of this plant for topical use as a phytotherapeutic agent of choice in cases of edema and inflammation, with the possibility of becoming an alternative formulation to diclophenac sodium, widely used in therapeutics.

Acknowledgements. To CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support and to Pharmacy Farmacotécnica, Institute of Pharmaceutical Manipulations Ltd., Brasília - DF, for the chamomile donation.

REFERENCES

1. Rang, H.P., M.M. Dale, J.M. Ritter & P.K. Moore (2003) *Farmacologia,* 5th ed., Elsevier Press, Rio de Janeiro.
2. Balazs, T. & R. Tisserand (1998) *Int. J. Aromather.* **9**: 15-21.
3. Paulsen, E. (2002) *Contact Dermatitis* **47**: 189-98.
4. Presibella, M.M., L.B. Villas-Bôas, K.M.S. Belletti, C.A.M. Santos & A.M.W. Santos (2006) *Braz. Arch. Biol. Technol.* **49**: 717-24.
5. Šarić, M.M., G. Stanić, Ž. Maleeš & S. Šarić (1997) *J. Chromatogr. A.* **776**: 355-60.
6. Carvalho, J.C.T. (2004) *Fitoterápicos antiinflamatórios – Aspectos químicos, farmacológicos e aplicações terapêuticas,* Tecmedd Press, Ribeirão Preto.
7. Souza, J.R.P., J.N. Rocha, J.M. Melo & S.L. Nixdorf (2006) *Hortic. Bras.* **24**: 233-6.
8. Mazokopakis, E.E., G.E. Vrentzos, J.A. Papadakis, D.E. Babalis & E.S. Ganotakis (2005) *Phytomedicine* **12**: 25-7.
9. Fonseca, N.F., M.F.M. Tavares & C. Horváth (2007) *J. Chromatogr. A* **1154**: 390-9.
10. Ramos, M.B.M., M.C. Vieira, N.A.H. Zárate, J.M. Siqueira & M.G. Ziminiani (2004) *Hortic. Bras.* **22**: 566-72.
11. Švehlíková, V., R.N. Bennett, F.A. Mellon, P.W. Needs, S. Piacente, P.A. Kroon & Y. Bao (2004) *Phytochemistry* **65**: 2323-32.
12. Svehlíková, V. & M. Repčák (2006) *Biochem. Syst. Ecol.* **34**: 654-7.
13. Sonaglio, D., G.G. Ortega, P.R. Petrovick & V.L. Bassani (1999) Desenvolvimento Tecnológico e Produção de Fitoterápicos, in: C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petronvick, *Farmacognosia: da planta ao medicamento,* UFRGS/UFSC: Porto Alegre/Florianópolis, pp. 238-49.
14. Wan, N.L., C. Guo, Q. Yu, Y. Li, X. Wang, X. Wang & C. Chen (2007) *J. Chromatogr. B.* **855**: 286-9.
15. Franke, R. & H. Schilcher (2005) *Chamomile Industrial Profiles,* Taylor & Francis.
16. Schulz, V., R. Hansel & V.E. Tyler (2002) *Fitoterapia Racional,* 4th. ed., Manole Press, pp 307-11.
17. Corrêa, N.M., J.F.B. Camargo, R.F. Ignácio & G.R. Leonardi (2005) *Rev. Bras. Cienc. Farm.* **41**: 73–8.
18. Mckay, D.L., & J.B. Blumberg (2006) *Phytother. Res.* **20**: 519-30.
19. Farmacopéia Brasileira, Ministério da Saúde – Agência Nacional de Vigilância Sanitária (1996) *Parte I – Métodos Gerais,* forth ed., Atheneu Press, São Paulo.
20. Wagner, H. & S. Bladt (1996) *Plant Drug Analysis: A Thin Layer Chromatography Atlas,* 2nd ed., Springer-Verlag Press, Berlin Heidelberg.
21. Thompson, J.E. (2006) *A prática farmacêutica na manipulação de medicamento,* Artmed Press, Porto Alegre.
22. Brazil, RE n° 1, (2005) *Guia para a realização de estudos de estabilidade.* Diário Oficial da União, Brasília DF. <www.anvisa.gov.br>.
23. Brazil (2004) *Guia de estabilidade de produtos cosméticos,* Brasília DF. <www.anvisa.gov.br>.
24. Ferreira, A.O. (2000) *Guia Prático da Farmácia Magistral. Boas Práticas de Manipulação,* Juiz de Fora.
25. Mura, P., M.T. Faucci, G. Bramanti, & P. Corti (2000) *Eur. J. Pharmac. Sci.* **9**: 365-72.
26. Oecd Guideline for the Testing of Chemicals (2002) N° 404: Acute Dermal Irritation/Corrosion.
27. Pinto, T.J.A., T.M. Kaneko, & M.T. Ohara (2003) *Controle Biológico de Qualidade de Produtos Farmacêuticos, Correlatos e Cosméticos,* second ed., Atheneu Press, São Paulo.
28. Farias, M.R. (2004) Avaliação da qualidade de matérias-primas vegetais, in: Simões, C.M.O., E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz, P.R. Petronvick, *Farmacognosia: da planta ao medicamento,* fifth ed., UFRGS/UFSC: Porto Alegre/Florianópolis, pp. 263-88.
29. Florence, A.T., & D. Attwood (2003) *Princípios físico químicos em farmácia,* Edusp Press, São Paulo.
30. Tadros, T. (2004) *Adv. Colloid. Interfac. Sci.* **108/109**: 227-58.
31. Ansel, H.C., N.G. Popovich, & L.V. Allen (2000) *Farmacotécnica: formas farmacêuticas e sistemas de liberação de fármacos,* 6th ed., Premier Press, São Paulo.
32. Prista, L.N., A.C. Alves, & R. Morgado (1995) *Tecnologia Farmacêutica,* 5th ed., Fundação Calouste Gulbenkian Press, Lisboa.
33. Barry, B.W. (2002) *Adv. Drug Deliv. Rev.* **54**: 31-40.
34. Mambro, M.V.D., & M.J.V. Fonseca (2005) *J. Pharm. Biomed. Anal.* **37**: 287-95.