

Cosméto Scientifique

1340, Saint-Germain
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Souper & Conférence / Dinner & Conference

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3 avril 2007 April 3rd

Conférence

OPTIMISATION DE LA CONSERVATION & ANALYSE DES FACTEURS DE RISQUE

(voir questions page centrale)

PRESERVATIVE OPTIMIZATION & RISK FACTOR ANALYSIS

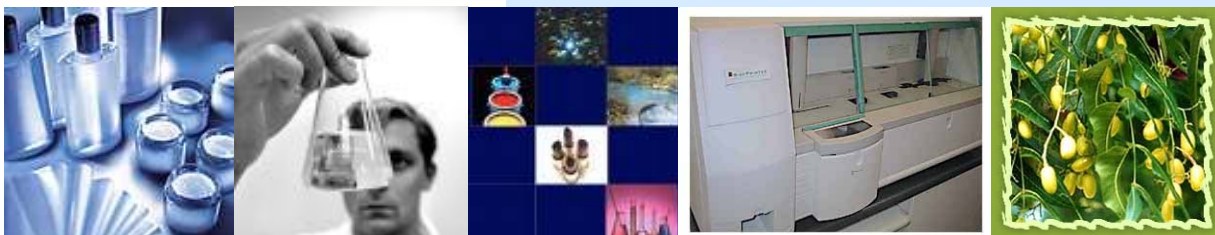
(see questions in central page)

Par / By

Steven Schnittger

Executive Director—Microbiology R&D

Estée Lauder Inc.



Mot de l'Éditeur...

Installée depuis cinq ans à Montréal et titulaire d'un doctorat en Chimie Organique, j'ai d'abord travaillé dans des laboratoires d'analyses pharmaceutiques avant de rejoindre les Laboratoires Dermo-cosmetik / GM Collin en qualité de Directrice



de laboratoire en charge des départements de AQ/CQ, Évaluations Cliniques et améliorations des formules en R&D.

Depuis mon arrivée au Québec, j'ai assisté à plusieurs conférences organisées par la SCC, une jeune association dynamique à fort potentiel de développement.

Cette année, j'ai accepté de m'impliquer dans le Comité en tant que secrétaire—éditrice.

Mon engagement est de m'assurer que les informations arrivent à temps auprès de tous nos membres et supporteurs. Avant chaque conférence, vous recevrez le bulletin listant notamment les dates importantes à réserver pour les événements de la SCC.

La prochaine conférence sur les Conservateurs sera un must absolu. Dans les **pages centrales** du bulletin sont listées des questions spécifiques auxquelles répondra volontiers notre conférencier.

Merci,
Éditeur, SCC Québec

"The first step towards knowledge is to know that we are ignorant"
Prime Minister Benjamin Disraeli (1804-81)

Thank you,
Nathalie Rakotondrazafy
Éditeur, SCC Québec

Editor's note...

Installed in Montreal for five years and having a *Ph.D.* in Organic Chemistry, I have worked first in pharmaceutical analytical Laboratories before joining Laboratoires Dermo-Cosmetik / GM Collin as Lab Director in charge of QA/QC, Clinical Evaluation and formulas improvement

in R&D.

During this time I have attended several conferences organized by the SCC Quebec chapter and have found it to be a young and dynamic association with endless potential for growth.

I have agreed to participate in the Committee and for this year I will be the Secretary—Editor.

I will be committed to ensure that all the members and our supporters get the information on time. Before each conference, you will receive a bulletin, explaining the important dates that you should reserve to attend SCC programs.

The upcoming conference on Preservatives is an absolute must. This time we have asked specific questions in the **center page** of this bulletin. The speaker will clear all of these issues.

COMITÉ EXÉCUTIF 2007

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Mot du Président...

Dear members,

It was really pleasant to see the large turnout at the last conference on Natural Products. Since the year 2007 started with great promise we hope that your continued support will propagate us to newer heights. Since the SCC calendar is measured by our Social events, we all realize that time flies and we are on the threshold of our 2nd conference and our semi annual golf tournament.

Please read the center page of the bulletin to see details of the upcoming conference, which seems to be a must for everyone. The formulating chemist in the role of optimization of preservatives, the QC chemist, to learn how to read Microbiology reports. We all know that microbes are a fact of life and at any given moment there are at least 6 million on our skin. But as a chemist and decision maker, when should we panic. Steven Schnittger VP/R&D Microbiology for Estee Lauder will be here on the 3rd of April to clear this for us.

Here in Montreal the week of 26th March are the IFSCC Praesidium members, here to decide the various issues concerning the International Federation of the Societies of Cosmetic Chemists including the upcoming IFSCC conference, to be hosted in Amsterdam in September. The Federation was created to give each society the

Chair's note...



opportunity to communicate to the international community of Federation members information on its activities, seminars, education courses and

news. For our part the SCC Quebec comity will entertain the Praesidium members with a tour of Montreal followed by dinner and show at the Montreal Casino. The event is sponsored by SCC/New York.

Wishing you the best,

Eric Ittah

Président, SCC Québec

Chair, Quebec SCC



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Makeup Powders Preservation and Water Activity

Valerie Vignier, Anne-Marie Mounier and Veronique Heidet-Hommeau
 Laboratoire de Biologie Vegetale Yves Rocher, Issy les Moulineaux cedex, France
 Bernard Cahagnier
 Expert en Microbiologie, Nantes, France

One of the problems still facing the cosmetic industry is microbiological pollution and its various consequences. For the manufacturer, contamination alters a product's organoleptic and physicochemical characteristics and degrades its active ingredients, which leads to a refusal of the product. For the consumer, the application of a product contaminated by a pathogenic microorganism could result in infections, or at least could alter the balance of the skin/mucous membrane, our first barrier against infection.

Contamination may derive from a variety of sources: the raw materials, the manufacturing process, storage, packaging or use. To ensure that the microbiological quality remains good from manufacture to utilization, most products are preserved by the addition of antimicrobial agents.

Regulations and changes in safety requirements in cosmetology promote optimization of preservatives added to formulas, but for very specific formulations such as makeup powders the principal difficulties are encountered when evaluating the efficacy of these preservatives. In order to optimize the preservation system, the aims of this study were to evaluate the microbiological risk of makeup powders in terms of specific forms of microorganisms adapted to makeup powders. Thus for test microorganisms we chose *Bacillus* spores and mold spores, and not *Staphylococci* and Gram negative bacteria that rarely or practically never are found in powders.

The concept of Water Activity

In the analysis of microbiological risk, we considered water to be a fundamental parameter. Moisture content quantitatively characterizes the hydration in a product, but alone is not enough to account for the availability of the water. Therefore we use the concept of thermodynamic water activity to express the availability of water for microbial growth in a product.

This concept was introduced by Lewis¹, who seems to have been the first to speak of the "activity of water", universally abbreviated as A_w (or a_w). To illustrate the concept, consider a heterogeneous product containing several parts. In the presence of an atmosphere, water migrates, possibly with endo- or exothermic effects: some parts hydrate, others dry; water vapor diffuses to the atmosphere or inversely gives up water to the product. After a certain time (sometimes several hours) a thermodynamic equilibrium is established in the system, characterized by three conditions:

- The temperature is identical at all points.
- The "chemical potential" of any constituent, and notably of water, is identical in each part. In the case of water, this chemical potential in fact represents the change in enthalpy needed to transform one mole of water from free-vapor state to the bound state in the substance.
- There is a certain partial pressure (p) of water vapor in atmosphere in equilibrium with the product. By definition, above pure water (the reference state) this pressure is equal to the saturation vapor pressure (p') where the relative humidity is equal to 100% and the A_w of

the atmosphere above pure water is 1.0

Thermodynamics allows us to establish ratios between absolute values (which we can't measure) and reference values (which we can measure). Thus, we can obtain the "relative activity" as a ratio of the actual chemical potential of any ingredient to the reference chemical potential of that ingredient. Similarly, the "relative pressure" is a ratio of the actual pressure of any ingredient to the reference pressure of that ingredient. In the case of water, the first ratio is called the "water activity" (A_w) and the second ratio is called the "relative humidity" (RH).

Although vapor is not a perfect gas, we can with only a small error (on the order of 0.2%) state the following equivalence:

$$A_w = \text{RH}\% \div 100 = p \div p'$$

Confusion has therefore often arisen because although there is a numerical equivalence, the two concepts are physically different. A_w is in fact the chemical potential of the water in the substance (i.e., the free energy). RH is a ratio of two pressures in a gas.

It is vital to understand that the A_w equivalence only holds if there is thermodynamic equilibrium between the product and the atmosphere. Without this equilibrium (the product is drying, for instance), this equality is meaningless. Also, the term A_w is meaningful only if the product is aqueous or has a water-continuous phase (i.e., an o/w emulsion). For powders, this condition is not initially fulfilled but if products pick up moisture from air during storage and use, a water-continuous system

may appear at the surface.

Microbiological risks are directly related to A_w . Microbial behavior differs greatly depending on the water's availability (Table 1)^{2,3}. In this kind of product, risks may be different over time, because A_w measurements could change during storage or use (as a result of drying or picking up moisture).

Water Activity and Water Content

The molecular interactions between water and the product constituents (principally hydrogen bonds between water molecules and polar radicals of proteins, carbohydrates and lipids) are expressed macroscopically by a relation of thermodynamic equilibrium that graphically reflects the sorption-desorption isotherm (Figure 1).

The generally sigmoid sorption isotherms depict relations obtaining at thermodynamic equilibrium between moisture content and the equilibrium relative humidity (ERH) of the surrounding air, or the A_w of this product.

The nonlinearity of the sorption isotherm reflects the successive phases through which the product gradually passes as it is hydrated. For low A_w values (A in Figure 1), the first water fractions are strongly bound to the macromolecules of substrate and are unavailable for reactions that use water as the reagent or diffusion medium. In the median part of the curve (A to C in Figure 1), the A_w and the moisture content change in parallel. Some scientists consider that in this area, successive layers of water molecules (hydrogen bonds) pile up on the monolayer formed at the start of hydration.

However, moisture content increases faster than A_w in the area (C to B in Figure 1) where the sorption isotherm straightens. In this part of the curve the water is progressively bound weakly to the substrate (osmotic phenomena, capillarity and other factors) and is therefore more available for certain biochemical or microbiological reactions. Such "solvent" water is absolutely necessary for the growth of microorganisms.

Considering the shape of the sorption isotherm, it is therefore relatively easy to predict at which water content values a given product will be host to microbial growth. Following work by Scott⁴, the thermodynamic concept of water activity has been used by microbiologists to describe microbial behavior as a function of hydration.

.../...

Table 1. Limiting Aw for growth of microorganisms at optimal temperature

Species	Minimum at growth
Most bacteria	>0.95
<i>Bacillus cereus</i>	0.93
<i>Bacillus subtilis</i>	0.99
<i>Clostridium botulinum</i>	0.94
<i>Pseudomonas spp.</i>	0.95
<i>Lactobacillus spp.</i>	0.88
Most yeasts	>0.85
<i>Debaryomyces hansenii</i>	0.83
<i>Saccharomyces bailii</i>	0.80
<i>Saccharomyces rouxii</i>	0.62
Most molds	>0.78
<i>Aspergillus flavus</i>	0.78
<i>Aspergillus niger</i>	0.75
<i>Penicillium aurentiogriseum</i>	0.81
<i>Penicillium veridicatum</i>	0.81
<i>Monascus bisporus.</i>	0.61

Table 2. Theoretical ERH values at 25°C for the saturated salt solutions used in this study

Salt	ERH (%)
(NH ₄) ₂ SO ₄	80
KCl	85
BaCl ₂	90
K ₂ SO ₄	98

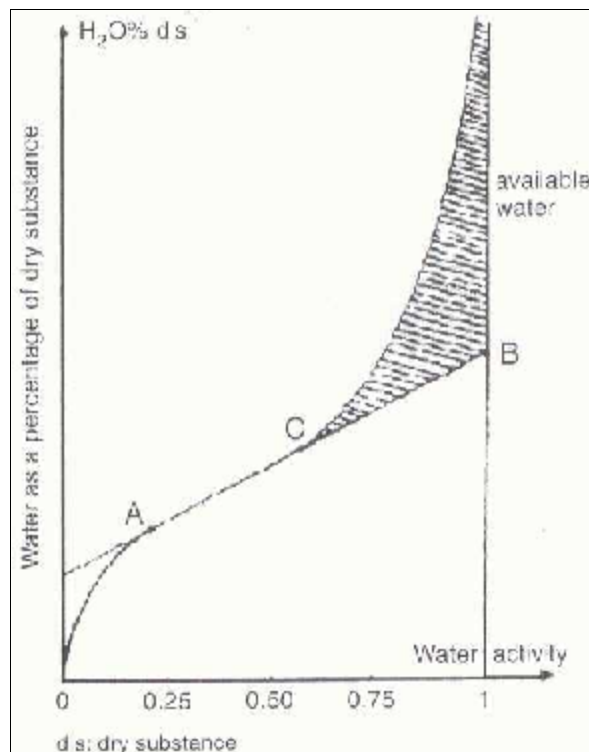


Figure 1. Sorption-desorption isotherm for a generic material. With increasing water activity and water as a percentage of dry substance, water becomes weakly bound and available as a diffusion medium. A = strongly bound water amount B = Non-solvent water maximum amount C = Critical point of solvent water appearance

Formula 1. A general formula and specific raw materials for the powders used in this study

General formula	Raw materials used in this study	Amount % (w/w)
Talc and/or mineral	Hydrous magnesium silicate (3MgO,4SiO ₂ ,H ₂ O)	50-80
Pigment	<ul style="list-style-type: none"> Yellow iron oxide (CI77492); hydrated ferric oxide FeO(OH)nH₂O Red iron oxide (CI77481); ferric oxide Fe₂O₃ Black iron oxide (CI77499); mixture of ferrous and ferric oxides Titanium oxide (CI77891); titanium dioxide (TiO₂) 	5-30
Fat binder		2-10
Preservatives		0-0.8

Materials

Substrate: The production of the makeup powders used in this study was subcontracted, and the powder composition cannot be detailed for reasons of confidentiality. The powder referenced as “FA” is a formulation without preservative manufactured at our request and is not available commercially. The powder referenced as “F2” contains preservative and is commercially available.

Nonetheless, we consider it is important to give the general formula of these powders (Formula 1). This formula shows the specific main ingredients of the makeup powders tested in the study⁵. We chose conventional formulas that represent most of our references. Powders with a high oil (binders) or wax content were excluded from the study.

Culture media: Tryptic soy agar^a was used to preserve, grow and enumerate bacterial strains. Sabouraud dextrose agar^b was used to preserve, grow and enumerate molds. Yeast glucose chloramphenicol agar^c was used to enumerate mold strains by inclusion.

Malt extract agar was used to harvest mold spores. One liter of this agar medium contains 40g of malt agar^d and 3g of soytone^e. One liter of the broth used to obtain *Bacillus* spores contained 5g of tryptone^f and 2g of glucose^g.

We used neutralizing polyvalent diluent for microbial enumeration of products after inoculation. One liter of the neutralizing polyvalent diluent contains 30g of polysorbate 80^h added to 28g of lethin polysorbate thiosulfate (LPT) foundation broth^k.

Microorganisms

The microorganisms detectable in this type of product are molds or sporulating bacteria. We decided to work with mold spores and *Bacillus* spores. We chose xerophilic molds known to be sporulating:

Penicillium aurentiogriseum IP 1231.80

Aspergillus niger IP 1431.83

Aspergillus ochraceus no.933677

The first two were from the national collection of cultured microorganisms of the Institut Pasteur de Paris and were kept frozen at -80°C , according to the laboratory's procedures (established following AFNOR standards). The third (*A. ochraceus*) came from the mold collection of the Museum d'Histoire Naturelle de Paris and was kept cold ($+5^{\circ}\text{C}\pm 2^{\circ}\text{C}$) by successive subcultures on agar medium.

For the bacteria, we chose to work with spores of *Bacillus subtilis* CIP52.62, a strain also coming from the collection of the Institut Pasteur de Paris.

Equipment: Water activity was measured using an instrument^l that was designed for the determination of the ERH above the product, at a constant adjustable temperature. The instrument consists of a controlled temperature chamber and a measurement device fitted with a probe using a mixture of electrolytes of several salts. The probe is used to measure products samples such as powders, granulates, pastes and liquids. The instrument is calibrated using six saturated salt solutions of ERH between 11% and 98%.

For some tests at 85% ERH, we used a climatic chamber^m designed to study the influence of temperature and humidity. The control panel displays the recommended and actual values of the temperature and the percentage relative humidity.

Surface inoculations were done with a spiral platerⁿ to be used with pre-poured Petri plates. A microprocessor drives a syringe, with logarithmically distributes a volume of 50 μl . A distribution tip applies the sample in decreasing amounts as it moves from the center to the periphery of the plate. This application system enables enumerations of suspensions of microorganisms containing between 4×10^2 and 4×10^5 CFU/ml.

.../...



Methods

Equilibrium Relative Humidity: The different ERH chosen were maintained using the saturated salt solutions in Table 2 (see pp.6)⁶. The solutions were prepared by adding sterile distilled water. The mixture was stabilized at 30°C. Surplus water was discarded to leave just the “slurry”.

Preparation of inocula of *Bacillus* spores: We deemed it very important to preserve the integrity of the product during the evaluation of the efficacy of the preservatives. For this reason, we used anhydrous inocula in the form of spores to contaminate products during the study.

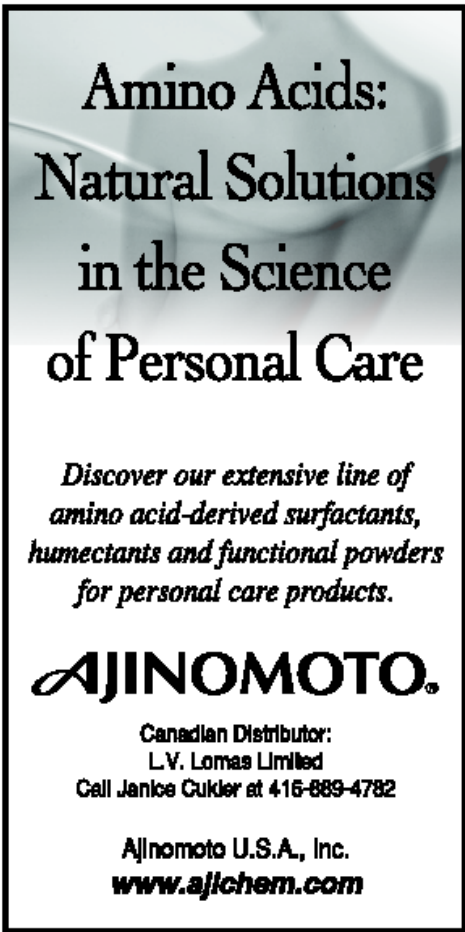
After 7 to 10 days of incubation at 30°C±2°C, the broths containing the *Bacillus* spores were centrifuged twice at 3500rpm for 3 minutes, with a distilled water washing between each centrifugation.

The centrifuge tubes were immersed in a 100°C water bath for 8 minutes and then in melting ice for 2 minutes. Two centrifugations at 3500rpm for 3 minutes were then performed, with a wash in distilled water in between. The pellets were collected in sterile talc and dried overnight at 40°C±2°C, then mixed with decontaminated talc. After enumeration and adjustment with sterile talc to obtain an inoculum of 10⁴ to 10⁵ spores, this contaminated talc was used as inoculum for the tests.

Preparation of inocula of mold spores: Again, to preserve the integrity of the product during evaluation of the preservative efficacy, we used anhydrous inocula in the form of spores.

The molds were inoculated on the surface of a Petri plate containing malt agar and were incubated for six days at 25°C±2°C. Sterile glass beads 3mm in diameter were placed on the surface of the subcultures. Depending on the mold tested, a bead represented an inoculum of 10⁴ to 10⁵ spores. These spore-coated beads were used as inoculum for the tests.

Protocol: After seeding, the powders were added to the sterile Petri plates, which were then stored either in sealed chambers containing saturating salt solutions or left in the ambient atmosphere whose ERH was measured as about 50%. All tests were performed at room temperature (approximately 20-25°C).



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^a T.C.S. Biomerieux reference, Craponne, France

^b S.D.A. Difco reference 210950, Difco, Becton Dickinson France, Le pont de Claix, France

^c Y.G.C. Biomerieux reference 42600

^d Difco reference 0024-17-3

^e Difco reference 0436-175

^f Difco reference 0123-17

^g Merck reference 24 370.294. Merck, Briare le canal, France

^h Merck reference 8.22187.2500

^k AES reference AEB 140562, AES, Combours, France

^l Thermoconstanter TH200; model RTD200, Novacina, Pfaffikon, Switzerland

^m VC0020 climatic chamber, Votsch, Balinger, Germany

ⁿ WASP spiral plater, AES, Combours, France

Sampling was scheduled at times t0, t+7days, t+14d, t+28d and t+60d (for certain tests, samples were also collected after 6 months and 1 year of contact). At each sampling, 1g of product was diluted tenfold in a validated neutralizing diluent. After 30 minutes of contact, the microorganisms were enumerated by inclusion in trypticase soy agar or yeast glucose chloramphenicol agar and by surface seeding of pre-poured Petri plates. The incubation was done at 30°C±2°C for the bacteria for 72 hours for the enumeration by inclusion and for 24 to 48 hours for the surface enumerations. For the mold spores, the incubation at 25°C±2°C was conducted for 5 days for the enumerations by inclusion and for 48 hours for the surface enumerations. The results were expressed as X (log10 CFU/g).

Results and Discussion

Aw of powders: We first examined some commercial products (free or compact powders) whose Aw ranged from 0.25 to 0.35. These values are well below the values shown in table 1 as the threshold of growth for various microorganisms.

Evolution of Aw values during storage of powders and different constituents at 98% ERH:

After 1 month-and for some products after 4 or 6 months-we determined the Aw values of free powders, compact powders and their principal components (shown in Table 3, see pp.14) when stored at high ERH (98%). The aim of the test was not to precisely define the minimal time to reach fixed values (may be shorter) but only to establish the maximal values reached after an overtime storage, and also check stability of data by new measurements after 4 or 6 months.

After 60 days of contact, the Aw values of the powders were essentially identical irrespective of their texture. The increased surface contact in the free powders versus compact powders did not affect the fact that maximal value of Aw is reached in approximately 1 month.

.../...pp14

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POUR LA FABRICATION DE MEILLEURS PRODUITS^{MD}

BASF

1. La méthode USP pour l'évaluation de l'efficacité antimicrobienne (**challenge-test**) est souvent perçue comme un test facile à passer. Quel est votre point de vue?
2. La Direction des produits thérapeutiques (DPT) a-t-elle statué sur les méthodes alternatives et plus rapides pour l'évaluation de la **qualité microbiologique** des produits. Quelles sont les méthodes acceptées et/ou reconnues par la DPT et/ou la FDA?
3. Comment expliquer une contamination en production (analyses microbiologiques régulières hors normes) alors que le **challenge-test** fait sur le produit est conforme (compte < 10 à 7 jours pour les 3 pathogènes *P. aeruginosa*, *S. aureus*, *E. coli* et levures & moisissures), les BPF étant respectées?
4. Quelle différence entre un **challenge-test** USP qui coute 160\$ et un à 400 ou 600\$, bien que ces laboratoires soient tous certifiés par Santé Canada?
5. Pourquoi refaire un **challenge-test** après 6, 12 ou 18 mois? Et si le challenge-test à ces intervalles de temps ne passe pas, que faire alors que le produit est déjà sur le marché?
6. Que faire si après 6 mois l'analyse microbiologique régulière passe et non le **challenge-test**?
7. Si le budget est limité a un seul **test microbiologique** par produit, quelle serait l'analyse la plus représentative du lot de production: celle du vrac ou celle du produit fini après remplissage?
8. **Analyse de l'air**: comment la faire et quelles sont les normes (USP / CTFA)? Que peut-on faire en cas de décompte de levures, moisissures ou pathogènes? Quelle méthode de désinfection utiliser pour l'air en zone de production (méthode de fumigation au formaldéhyde ou glutaraldehyde interdite)?
9. **Hygiène—Non hygiène**: quelles sont les normes en zone de production: salles de pesée, de fabrication, de conditionnement, ...
10. **Analyse de l'eau**: comment faire l'analyse de l'eau purifiée: compte total de bactéries ou bactéries plus coliformes ou autres?
11. Quelles méthodes utiliser pour **l'analyse de l'eau purifiée** USP « 1231 » ou USP « 61 » (différence)?
12. Que suggèreriez-vous lorsque les résultats d'**analyse microbiologique de l'eau** déjà utilisée en fabrication sortent hors norme?
13. Du point de vue d'un chimiste, comment valider un **laboratoire de microbiologie**?
14. Quelles sont les **responsabilités** respectives des fabricants, des formulateurs et des laboratoires **d'analyse**, tous étant inspectés par Santé Canada?

1. The USP method for **Antimicrobial effectiveness** is often perceived as a easy test to pass. What is your point of view?
2. Are there other alternative and faster methods used to evaluate **microbiological quality** of products that are accepted and recognized by Health Canada and FDA?
3. If a product passes a **challenge-test** (T7 <10 count for the 3 pathogens *P. aeruginosa*, *S. aureus*, *E. coli* and Yeast & Molds), how do you explain a contamination in production (fails micro count), GMP standards used?
4. What is the difference between **challenge-test** USP which cost 160\$, 400\$ or 600\$, all labs are certified by Health Canada?
5. Why redo a **challenge-test** at 6, 12 or 18 months (half-life)? What should I do if the later challenge-tests do not pass, my product is already on the market?
6. What to do if the regular micro test passes at 6 months and not the **challenge-test**
7. If financial restrictions permit you only one micro test, what would be the most representative of the batch: that of the bulk product or that of the finished product?
8. **Air analysis**: how do you analyze air. What are the standards (USP / CTFA)? If there is a count of yeast or molds or pathogens, what can you do about this? What disinfection methods for air can you use in production or packaging rooms (usual method of fumigation with formaldehyde or glutaraldehyde are no longer permitted)?
9. **Hygiène—Non hygiène**: what should be the specifications for production room, packaging room...
10. **Water analysis**: when you analyze water, what do you ask for: Total bacterial count plus coliforms or other pathogens as well?
11. Which USP method should be used to **analyze purified water** USP « 1231 » or USP « 61 » (difference)?
12. If the micro count of **purified water** is out of spec and you have already used this water for production, what should you do?
13. From the point of view of a chemist, how do we validate a **Microbiology lab**.?
14. Who is liable (responsibilities), the manufacturer, the formulator chemist, the external laboratory? All are inspected by Health Canada

Événements à retenir / Dates to remember

March 31 Mars April 1 Avril	IFSCC Praesidium, Montreal
April 3 Avril	Souper Conférence / Dinner Conference Preservatives / Conservateurs <i>Par / By Steven Schnittger—Estee Lauder Companies</i>
June 4 juin	Journée du Golf / Golf Day
September 4 septembre	Souper Conférence / Dinner Conference Hormones—Mature Skin
October 9 octobre	Journée d'Éducation / Education Day Fragrances
November 27 novembre	Party de Noël / Christmas Party

Date de tombée des publications Publications deadlines Bulletin / Newsletter

Vol8 # 3	May 1 st —1 ^{er} mai
Vol8 # 4	Aug. 1 st —1 ^{er} août
Vol8 # 5	Nov. 1 st —1 ^{er} nov.
Vol9 # 1	Jan. 1 st —1 ^{er} janv.



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Failure to reach an Aw value greater than 0.90 after one month or more of contact denoted a low affinity of the tested products for water. Lower values obtained for the compacted powder (0.64) and free powder (0.72) do not favor the growth of most organisms.

Table 3. Evolution of Aw values during storage of powders and different constituents at 98% ERH

	Aw At T0	Aw at T+30 days	Aw at T+51 days	Aw At Extended time
Titanium dioxide	0.36	0.82		0.89(after 6 months)
Yellow iron oxide	0.38	0.90		0.91(after 6 months)
Red iron oxide	0.30	0.88		-
Black iron oxide	0.38	0.75		-
Free powder	0.24	0.72	0.71	0.68(after 4 months)
Compacted powder	0.25	0.64	0.72	0.63(after 4 months)

Another test confirmed the low affinity of this type of product for water. Several pots of compact eyeshadow were placed in a chamber at 98% ERH. After one month of contact, Aw was measured as 0.85. Then we took a second pot and crumbled its powder before measuring Aw, which was 0.51. This showed that crumbling of the powder allowed a rapid and significant drop in Aw.

Number of *Bacillus* spores: We compared the numbers of *Bacillus subtilis* spores in two eyeshadows at two different relative humidities. The first eyeshadow (FA) contained no preservative; the second (F2) was a commercial product containing 0.4% (w/w) of a mixture of three preservatives. We monitored spore numbers for two months at 85% ERH and at approximately 50% ERH. The results are presented in Figure 2.

No decrease in *Bacillus subtilis* spore numbers was observed at either ERH and regardless of the time of contact. Other tests not presented here showed a significant regression in the case of an inoculum with the vegetative form of *Bacillus subtilis*. Anagnostopoulos and Didhu⁷ observed that the percentage germination of *Bacillus stearothermophilus* dropped when Aw decreased.

The tests presented in Figure 3 were done in sealed chambers containing a saturated salt solution. To validate our results, we did the same tests in parallel in an oven in an atmosphere of 85% ERH. All the results were in full

agreement.

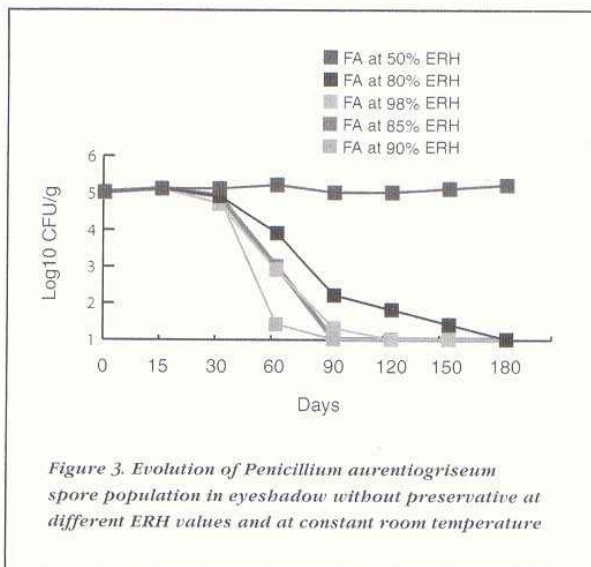
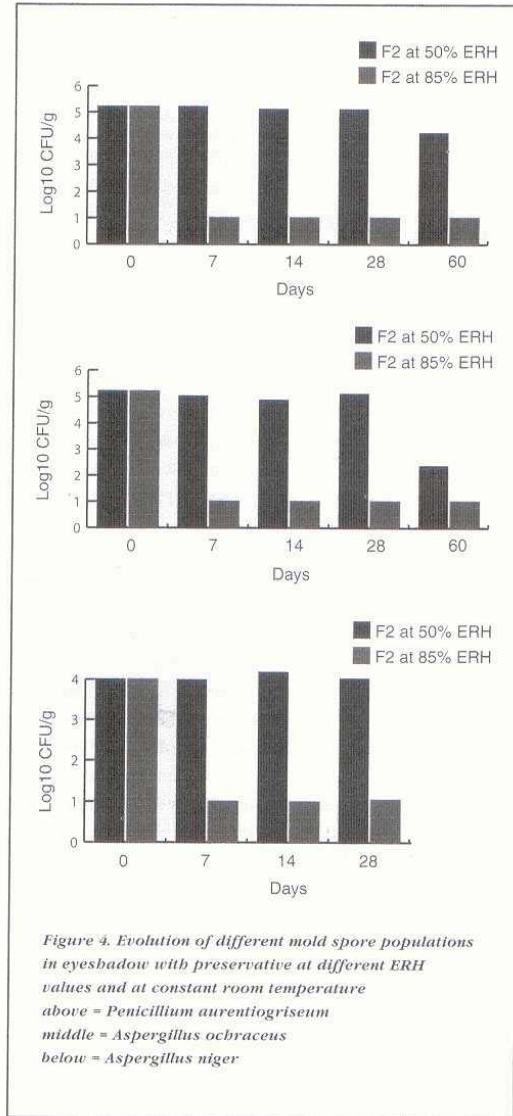
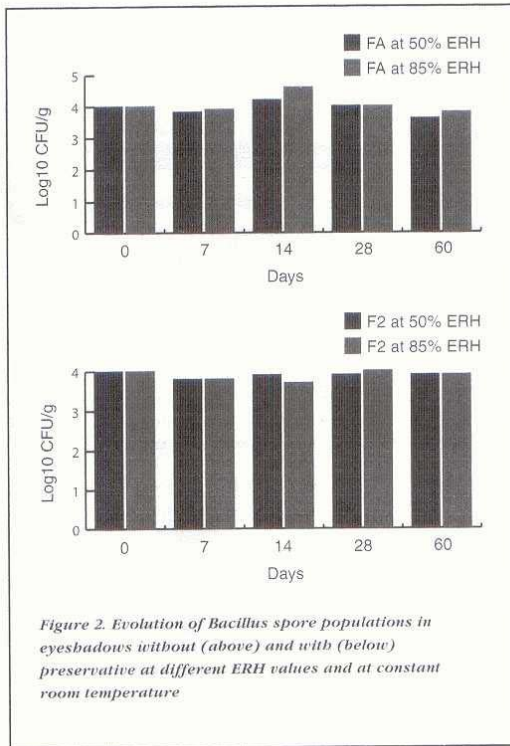
Mold spore population: impact of different relative humidities on a product without preservative: We measured the mold spore population in specifically prepared eyeshadow without preservative at five equilibrium relative humidities. The results are presented in Figure 3.

At an ERH of about 50%, no decrease in spore population was measured after 6 months of contact. At higher ERH values, after 2 months of contact there was a significant decrease in viable spores, even without preservative, which were no longer numerable after six months of contact.

In the next test we determined the number of mold spores in an eyeshadow with preservative at two different relative humidities. Three molds were tested: *Penicillium aurentiogriseum*, *Aspergillus niger* and *Aspergillus ochraceus*. The results are presented in Figure 4.

At an ERH of about 50%, the three test strains behaved similarly during the first thirty days, with a measurable decrease after two months for *Aspergillus ochraceus*.

At an ERH of 85%, viable mold spores were undetectable after 7 days, regardless of the strain studied. At 0.85 Aw, it can be assumed that the xerophilic spores exhibit incipient metabolic activity. During this phase, they may be more vulnerable. At this ERH, the increasing mobility can then favor the action of the preservative. The tests done on eyeshadow F2 are presented in Figure 4. Two other formulations were tested under the same conditions (ERH and strains), and the results (not presented here) were similar to those presented in this article. .../...



Conclusions

The following conclusions can be drawn from this study:

1. The tested commercial products and their main constituents have very low intrinsic Aw values.
2. As can be seen in Table 3, these same products have little affinity for water, indicated by initial Aw values between 0.24 and 0.38. The Aw values reached a maximum of 0.91 for an equilibrium value of 98% ERH.
3. For *Bacillus subtilis*, we observed no increase in the number of viable spores in any test. The powders studied have little affinity for water and the risk of bacterial growth is remote, even nonexistent.
4. Regarding the results obtained from different conditions and for different products, the behavior of the molds differed markedly from that of the *Bacillus spores*.

• *In a powder without preservative*, for the higher ERH (85%), at a period of 30 days there is no modification of the mold-viable spores number. Beyond this period, the number of mold-viable spores decreases. In contrast, the same spores were stable throughout the study at 50% ERH.

• *In a powder with preservative*, at 85% ERH, spores are undetectable after 7 days. For the lower ERH, the preservatives efficiency is not demonstrated.

This shows that even without preservative and with a water supply, these products that interact poorly with water do not favor the survival, germination and growth of mold spores. More over, preservative efficacy is higher against mold spores when ERH increases. Maybe we can argue that water is useful for the transfer of the preservatives to the cells and that, at the same time, the activation of mold spores by an increase of humidity has a weakening effect.

5. This study shows that these eyeshadows have little affinity for water and are a very poor substrate for the growth of microorganisms, irrespective of the test conditions. Furthermore, under extreme conditions (high ERH), the preservative was effective against the mold tested.

At low relative humidity, one of the identified risks is that microorganism counts do not decrease even in products with preservative. This risk implies actions upstream in the manufacturing process and hence excellent microbiological quality of the ingredients and during their handling. The action of the preservatives in this type of product is enhanced at high equilibrium relative humidity.

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