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The "bubble point" for validation of drug release or simulated absorption tests for ointments

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The aim of the present study was to design a test to ascertain the behaviour and reliability of a membrane used in drug release and simulated absorption tests in order to arrive at useful indications for simulating topical as well as gastro-intestinal absorption. The membrane can be used in two different conditions: a) as a simple porous membrane placed between the ointment and an accepting liquid phase, generally water phase; b) as a membrane soaked in a lipophilic liquid phase to simulate the horny layer between the ointment and accepting water phase. In this study the "bubble point test" was used to test the integrity of the soaking film as well as the membrane, during and after drug release and simulated absorption tests with different types of ointment. In the case of a drug release test from an ointment, the bubble point test may determine the test conditions, that is the ointment applied to either a dry or hydrated membrane. Only the use of a previously hydrated membrane can guarantee constant conditions in the *in vitro* model. Use of a dry membrane may lead to infiltration of liquid components of the ointment base, thus altering the contact conditions between the two phases of the cutaneous compartment model (lipogel and W/O creams). The use of a hydrated membrane may also lead to interactions between the two phases of the compartment, with osmotic exchanges between the acceptor phase and ointment sample (hydrogel, PEG gel, O/W creams). The hydrated membrane is therefore reliable only for comparison between lipophilic base ointments. In a simulated absorption test, determination of the bubble point makes it possible to ascertain the physical integrity of the lipoid liquid film immobilized by capillary action in the inner microporous structure of the membrane during the test. This condition is essential to maintain a balance between the parameters regulating the diffusion process between the different compartments of the system. The use of a lipoid-soaked membrane makes it possible to avoid interactions between the ointment sample and an aqueous acceptor phase, such as hydrosoluble bases. Since the diffusion across a lipoid film immobilised within a porous membrane depends on the drug release rate from the ointment base, the test allows a contextual evaluation of the release kinetics as well as an indication of the drug absorption possibilities through an *in vitro* model of the cutaneous compartment.

1. Introduction

In recent years, there has been a widely felt need to develop a system to measure drug availability from topical dosage forms. The two main aims are: first, to design a test which would indicate the most appropriate excipient for the therapeutic goal in the selection and orientation of the ointment formulation process; second, to set up a quality control system to establish the release profile of drugs from different manufacture batches to ascertain both homogeneity and continuity of the production process (Martin et al. 1989). A polymeric porous membrane is currently used in almost all drug availability tests for topical dosage forms.
According to the cutaneous compartment model adopted, the membrane can be used under two conditions: a) as a simple porous membrane placed between the ointment and a acceptor liquid phase, usually water phase; b) as a membrane soaked in a lipophilic liquid phase in order to simulate the horny layer between the ointment and accepting water phase.
In the first case, the polymeric porous membrane acts as a simple mechanical barrier separating the ointment from the water phase, thus preventing direct contact between them. At the same time, contact between the phases is assured through the membrane pores. This model and test type indicates the ointment's drug release capacity, but not absorption, as there is no barrier simulating the cutaneous compartment.
The second case uses a simple cutaneous compartment model. A lipophilic liquid saturating the membrane constitutes a real membrane which is in contact with the ointment on one side and the accepting phase on the other.

This model simulates the cutaneous barrier through which the drug diffuses to reach the water-phase simulating the plasmatic compartment. It is therefore possible to simulate cutaneous absorption that is comparable to *in vivo* tests.
In both cases, the membrane is soaked in a liquid that fills the capillary network in the membrane's thickness. For this reason, the liquid, either water or lipophilic liquid, forms a membrane supported by the porous structure of the polymeric membrane. During the test this thin liquid phase comes into contact, and can interact, with the ointment on one side and the acceptor phase on the other. The test, which takes into account the conditions of both soaking and use, is reliable only if the soaking conditions remain unaffected in the membrane model.
The aim of this study was to individuate and design a test to study the behaviour and reliability of a membrane used in release and absorption tests, and therefore to obtain useful data for simulating gastro-intestinal and topical absorption. The "bubble point test" was used in this study, that is a test based on the principle according to which a liquid soaking a porous membrane is retained in the weave of the capillary canals as long as the pressure applied by another fluid, such as a gas, to the retained liquid is greater than the capillary force of cohesion to the pore walls. The test is frequently used to ascertain the integrity conditions of microporous membranes used in the sterilizing filtration process. It has been adopted here to verify the integrity, of the soaked film and membrane, during and after release tests and tests simulating drug absorption from different ointment types.

2. Investigations, results and discussion

2.1. Release test through microporous membranes

In various ointment release tests, the polymeric porous membrane is used to mechanically separate the ointment sample, applied to one side of the membrane, from the accepting water phase in contact with the other side. The two phases come into contact through the thin capillary pores of the membrane (usual diameter < 1 μm). The phenomenon of capillarity and the extremely limited contact surface between the two phases at the pores immobilizes the liquid in the membrane and prevents dispersion of the ointment sample in the acceptor phase. However, the process of molecular diffusion of the drug toward the acceptor phase is not hindered.
Polymeric membranes of various types and porosities have been used in the different models. In many cases, the membrane was previously hydrated (Guy and Hadgraft 1990); in others it was applied directly to the ointment sample and then placed in contact with the accepting water phase (Kundu et al. 1993).
As is well known, capillarity causes a liquid, placed in contact with a microporous membrane, to be absorbed through the pores, before spreading through the net-like structure of the membrane. This leads to the production of a real liquid membrane, which is supported by mechanical structure of the polymeric membrane.
Having been previously soaked in water, the pores of the membrane are full. This water comes into contact with the ointment surface through the pores. If the ointment is applied directly to the dry membrane, the liquid components will permeate the membrane so that ointment comes into contact with the accepting phase on the opposite side of the membrane, through the capillary canals. These will no longer be permeated with water, but with the ointment components that have seeped through.

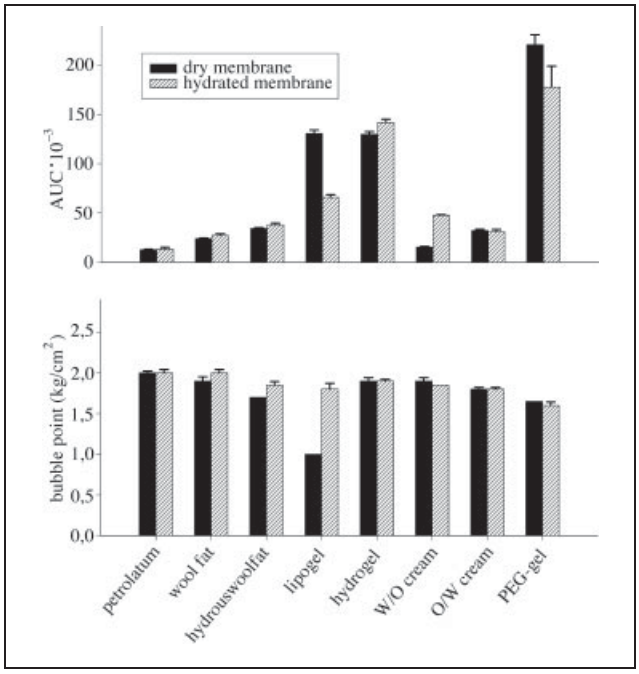

Fig. 1: Comparison between AUC values (μg/cm² released amount · time (0 ÷ 180 min)) calculated from the release curves of benzocaine from different ointments, in the two operational conditions (data expressed as mean ± standard deviation from 6 replicates), and the bubble point values of the different membranes at test conclusion

To ascertain the influence of the different applications of the membrane in the release process, the ointment series were tested under two conditions. The membrane was either applied directly to the dry state or was previously soaked in water. The diffusion cell and operational conditions remained the same. The bubble point of each membrane was measured after each test.
Figure 1 compares the AUC values of benzocaine in the ointments in the two above-mentioned conditions, with the bubble point values of the different membranes at test conclusion. The results confirm the difference in drug release capacity from the ointments due to their different type and chemical-physical characteristics. The drug release rate was also influenced by the method of membrane application. In most cases the difference was limited (e.g. for the two classical excipients lanolin and petrolatum, or the O/W ointment), while in other cases (e.g. lipogel) the difference was considerable.
Despite a different drug release rate, in most cases the bubble point remained the same as for membranes soaked in water, demonstrating that the membrane remained saturated. This indicates that although a dry membrane was applied, the excipient, given its nature and viscosity, did not seep through the thin membrane pores after ointment sample application. After immersion of the cell in acceptor fluid, the water seeped through the membrane to the other side, constituting an interface necessary for drug diffusion between the ointment and water-phase. The slight difference could be attributed to a discontinuity in the interface, given that the dry membrane was permeated with air on application, leading to a reduced diffusion surface. This may be the case for petrolatum, lanolin, and hydrogel, where the release value of the dry membrane was lower than the hydrated membrane.
An important difference in release rate was observed for lipogel based on Miglyol 812; it was almost double for the dry membrane. At the end of the test the bubble point val-

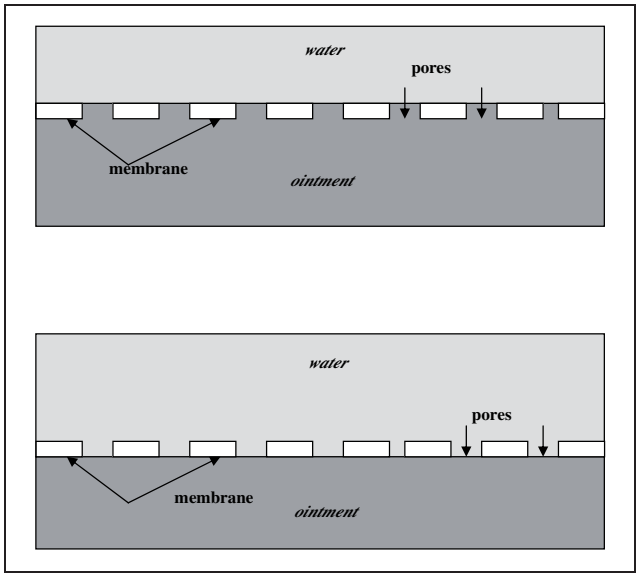

Fig. 2: Mechanism of communication between ointment sample and aqueous acceptor phase in the cutaneous compartment model:
A) hydrated membrane: communication between ointment sample and aqueous acceptor phase through water immobilised in the membrane.
B) dry membrane: the communication between ointment sample and aqueous acceptor phase through lipogel oily liquid phase diffused in the membrane network

ue was the same as for water with a previously hydrated membrane. Instead, when a dry membrane was used, the bubble point value was the same as for a membrane soaked in Miglyol 812, used in the preparation of lipogel. This indicates how the lipogel can spread within the membrane pores when the ointment sample is applied to the dry membrane. Instead, after applying the hydrated membrane, the water force of cohesion to the pores prevents the oily liquid from seeping through (Fig. 2). The different release rates may be due to the interfaces created by the conditions. This behaviour in the two operational conditions was confirmed by deferring testing time between 30 and 180 min and determining the bubble point for each group at the end of the test. The results are compared in Fig. 3, which shows how the two release courses are accompanied by a different course for the bubble point. When using a dry membrane, after 30 min it was already possible to reach a value of 0.98–1.00 kg/cm^2, from a value of 0.3 kg/cm^2 of membrane. That is the same value as a membrane soaked in Miglyol 812, which remained constant for up to 3 h. When a hydrated membrane was used, the bubble point values, initially 2.10 kg/cm^2, were found to be about 1.8 kg/cm^2 until the end of the test. This demonstrates a continuing permanence of the drug-containing aqueous phase throughout the test. This behaviour was confirmed with other lipogels with differing concentrations of gelling component, as can be observed in Fig. 4. The use of liquid paraffin and olive oil with three different concentrations of bees-wax, as well as Miglyol 812, led to different release rates for the same concentration levels, depending on the nature of the oily phase. In accordance with well known general behaviour, for each group the greater the gel concentration and viscosity, the lower the release rate. The bubble points, however, were different under the two above-mentioned conditions of membrane application. When the hydrated membrane was used, the bubble points at the end of the 3 h test remained at a constant value as the soaking in water. For the dry membrane the bubble points remained at the value of the membrane soaked in the oily phase of each gel. For this reason, the results of a release test from a lipogel may be significantly different depending on the use of the polymeric microporous membrane.

A particular case was observed with the polyethylenglycol ointment. The release rate was very high (see Fig. 1), with little difference between the two means of membrane application. The bubble points were the same in both cases, even though lower than those for water. In both cases a swelling and fluidification of the ointment sample were observed, indicating the diffusion of water from the acceptor phase. The bubble point values at different testing times, as for lipogels, were the same for the application of dry and hydrated membrane. These values are lower than those of the membrane soaked in polyethylenglycol, and remain constant over the test period, as can be observed in Fig. 5. This demonstrates the complex composition of the soaking liquid, resulting from osmotic interaction be-

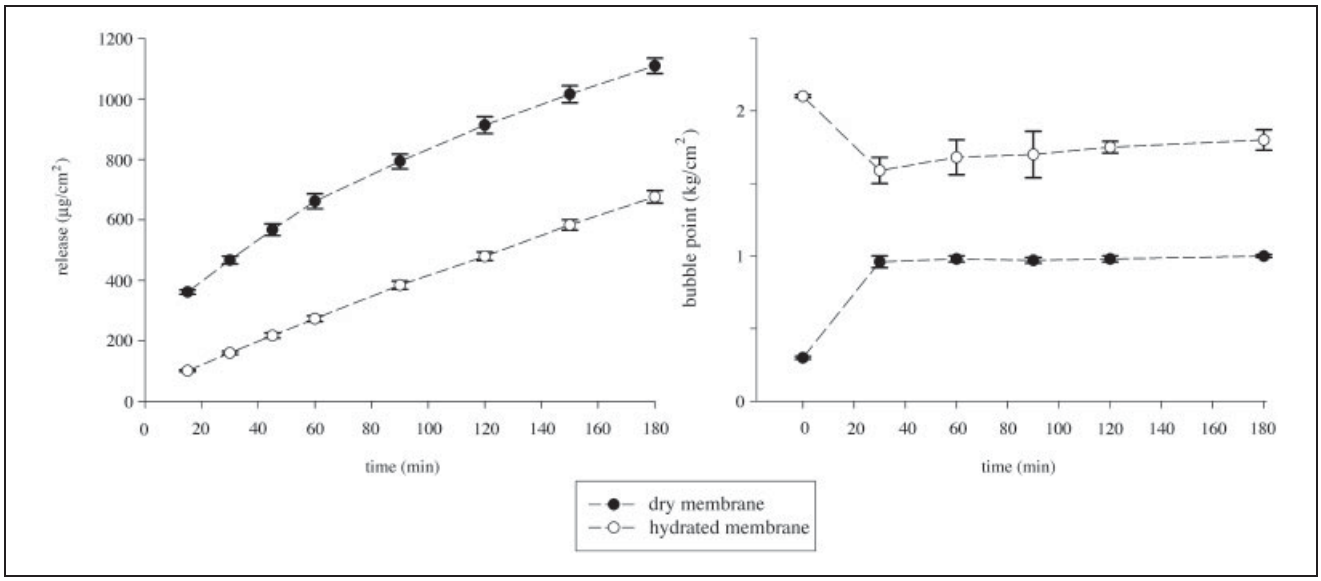

Fig. 3: Comparison between release courses of benzocaine from lipogel under the two conditions, and bubble point values of the membranes at test conclusion

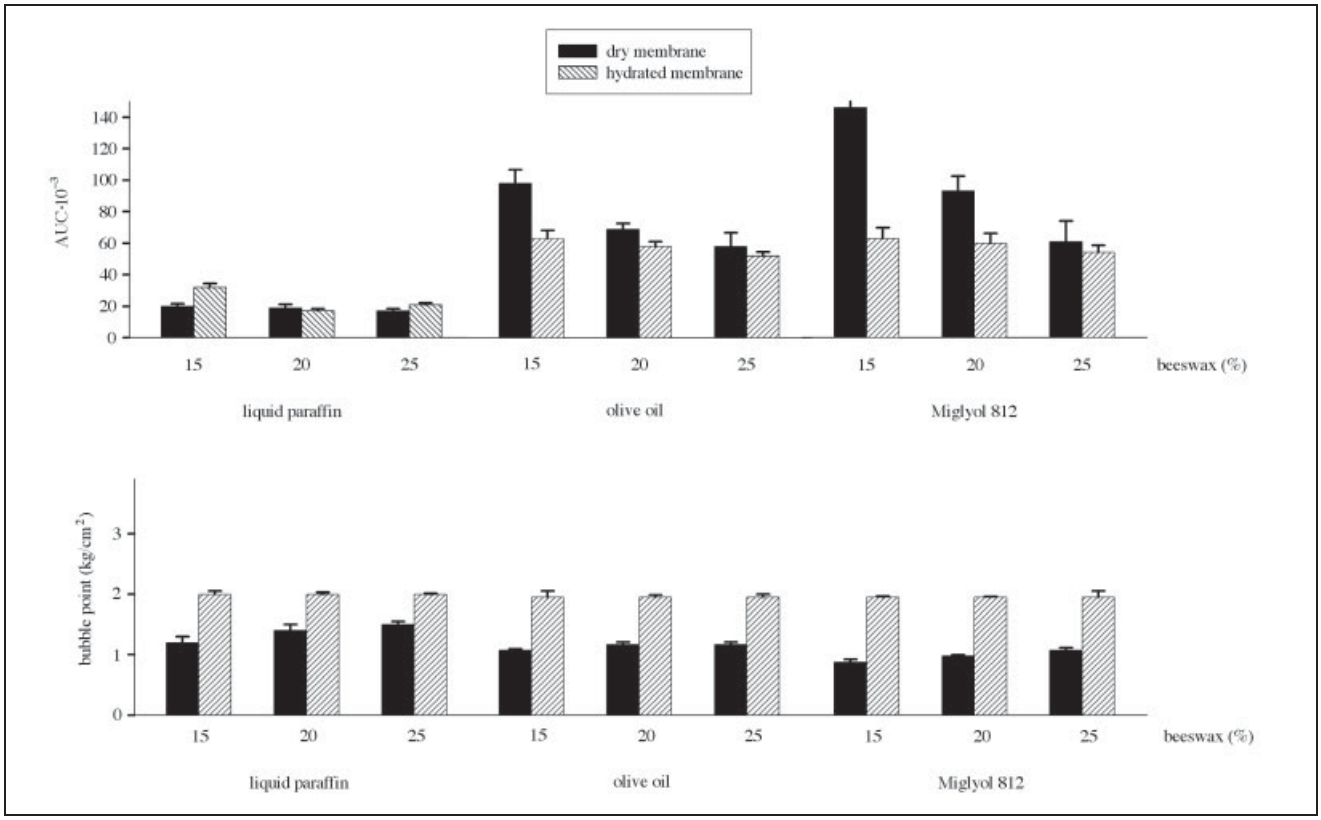

Fig. 4: Comparison between AUC values (μg/cm^2 released amount time (0 ÷ 180 min)) calculated from the release curves of benzocaine from different ointments, obtained by gelling three different oily phases with increasing concentrations of bees wax, under the two operational conditions (data are expressed as mean ± standard deviation from 6 replicates), and bubble point values of the membranes at test conclusion

tween the water-phase and polyethylenglycols of the excipient. A release test through a simple porous membrane is thus unreliable for this type of ointment because of the osmotic effects, which alter drug diffusion.

2.2. Simulated absorption test

An *in vitro* model of a cutaneous compartment to test drug absorption capacity through a cutaneous barrier uses a sample of explanted skin. An ointment sample is applied to the skin sample and the other side is placed in contact with a water phase simulating the plasmatic compartment (Franz 1975). Simplified models of an *in vitro* cutaneous compartment simulating percutaneous drug absorption use a polymeric porous membrane soaked in a lipophilic liquid. This reproduces the cutaneous barrier (Striker 1971, 1973; Loth and Holla-Benninger 1978; Shah et al. 1991) between the ointment sample and an aqueous plasmatic

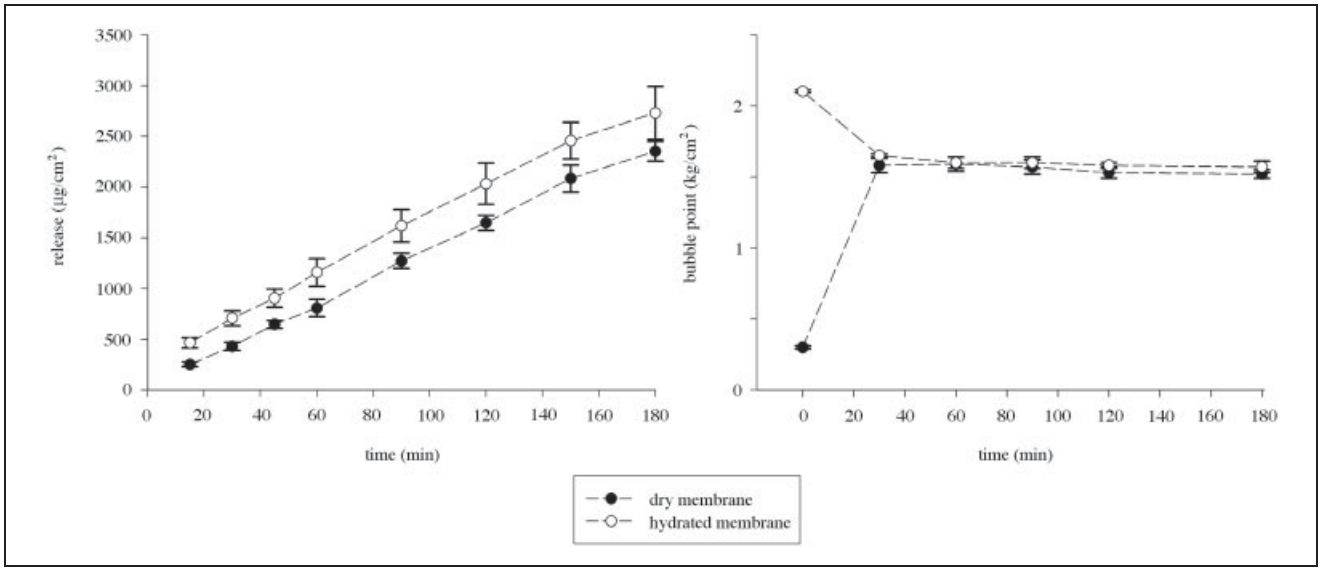

Fig. 5: Comparison between the release kinetics of benzocaine from polyethylenglycol ointment in the two operational conditions, and bubble point values of the membranes at test conclusion

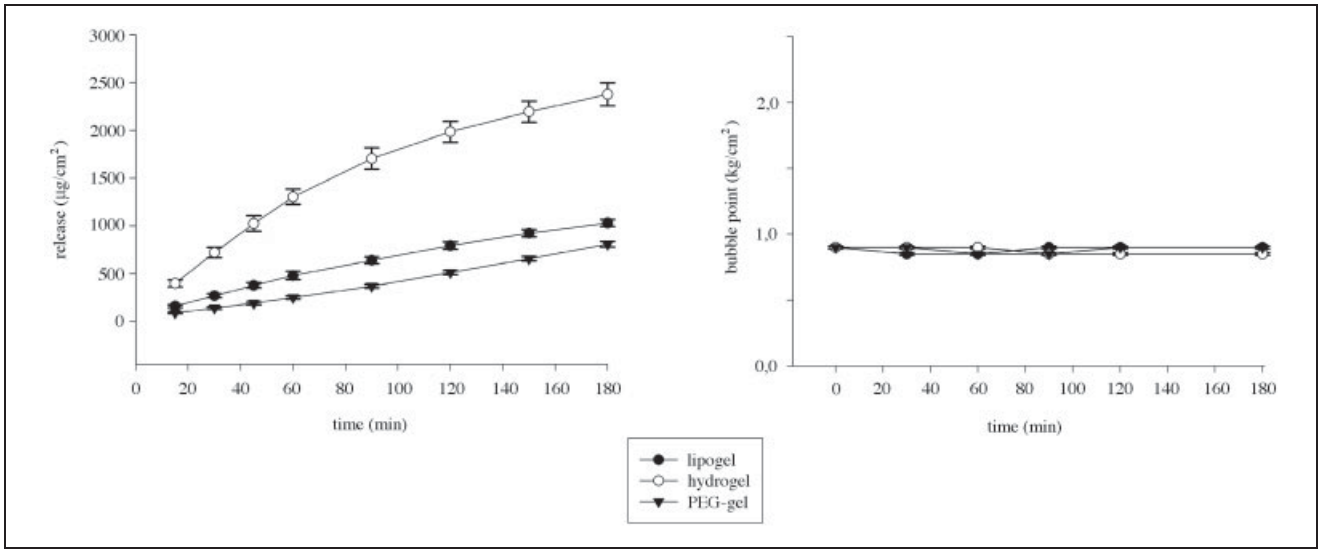

Fig. 6: Comparison between simulated absorption curves of benzocaine from lipogel, hydrogel, and PEG gel ointments through a membrane soaked in *n*-octanol, and its bubble point values at test conclusion

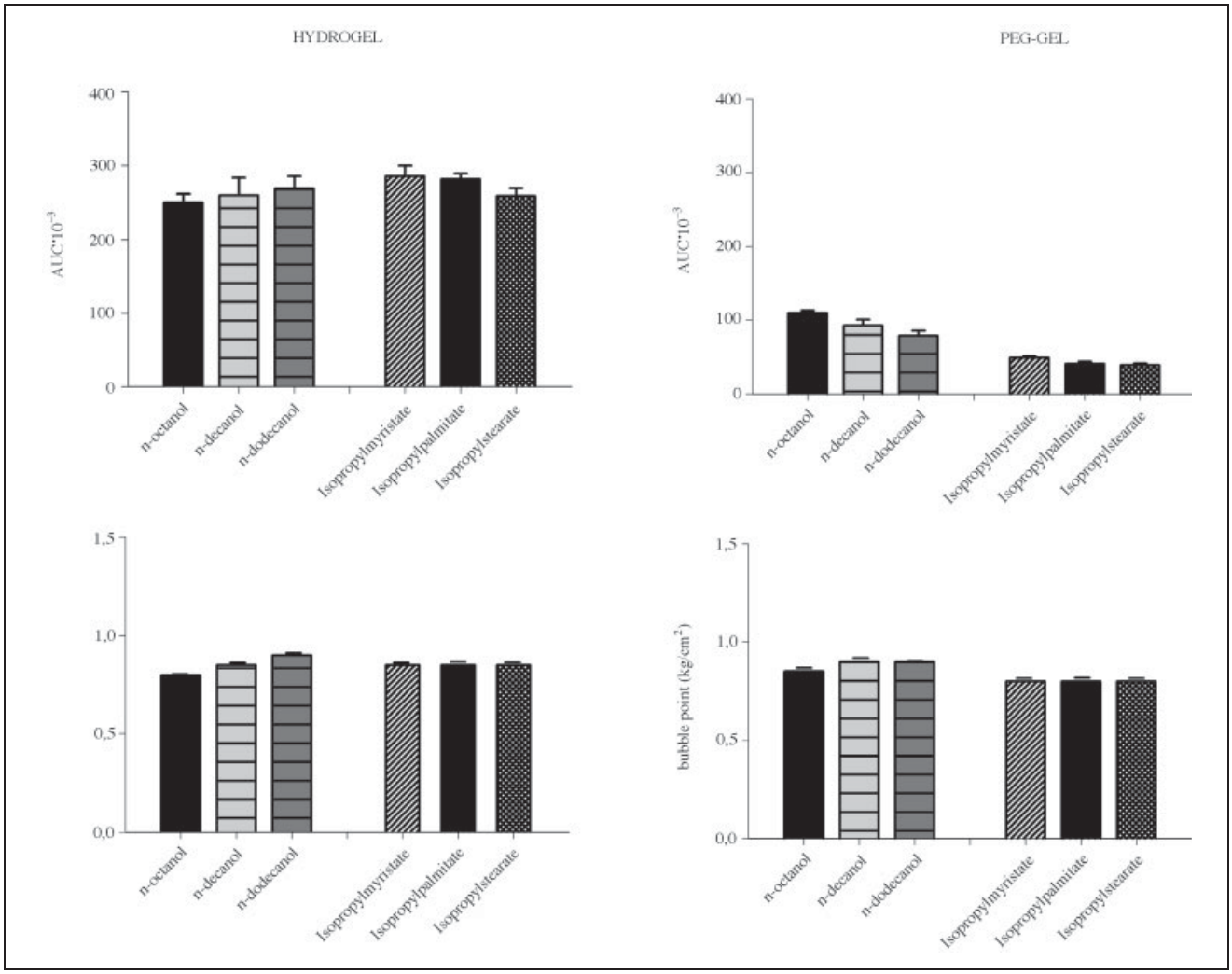

Fig. 7: Comparison between AUC values (μg/cm² released amount · time (0 ÷ 180 min)) calculated from the simulated absorption curves of benzocaine from hydrogel and PEG gel, through membranes soaked in *n*-octanol, *n*-decanol, *n*-dodecanol, isopropylmiristate (IPM), isopropylpalmitate (IPP), isopropylstearate (IPS) (data expressed as mean ± standard deviation from 6 replicates), and bubble point values of the membranes at test conclusion

phase. In this system, the drug is diffused first from the ointment to the lipophilic phase immobilized in the membrane, and then to the plasmatic phase. The model is effective only if the membrane conditions remain unaffected throughout the test, that is as long as the lipoid film does not mix with either the ointment or aqueous phase.
Determination of the bubble point is useful for verifying that this condition is respected. If the composition of the soaking liquid changes during the test, the altered chemical-physical conditions of the membrane lead to a variation in the membrane bubble point value with respect to the inchoate soaking conditions.
Fig. 6 shows the simulated absorption curves for benzocaine from three different ointments through a *n*-octanol soaked membrane. The bubble point value measured at the beginning of the test, which remained unchanged at 30 min measurements, demonstrated that the soaking rate was constant over the test period, whichever ointment was applied. Many simulated absorption tests which use lipoid saturated porous membranes propose different lipophilic liquids and their mixes to simulate the cutaneous barrier. In this study, we tested various liquids among those normally used, to confirm the validity of the bubble point test in verifying the reliability of a simulated absorption test. The persistence of membrane impregnation with the lipoid substance throughout the test was ascertained. Two series of soaking liquids were tested, the first with the three alcohols, *n*-octanol, *n*-decanol and *n*-dodecanol (Striker 1971, 1973; Loth and Holla-Benninger 1978), and the second with isopropylic esters of myristc, stearic and palmitic acids (Shah et al. 1991; Pirotte and Jaminet 1984; Hadgraft and Ridout 1987; Gummer et al. 1987; Hadgraft and Ridout 1988; Green et al. 1989).
Fig. 7 shows the AUC values of benzocaine after three hours of simulated absorption from two different ointments, using membranes soaked in the two above-mentioned series of lipophilic liquids, and comparing their respective bubble points.
The simulated absorption test showed no significant changes in the series of soaking liquids. Significant differences were found in the ointment bases, even though the drug concentration in each ointment was constant. The fact that the bubble points remained unaltered at the end of the test demonstrates that the film of lipoid soaking liquid remained constant throughout the test, guaranteeing constant operational conditions.

2.3. Conclusions

Determination of the bubble point for microporous membranes used in drug release and simulated absorption tests is useful for ascertaining membrane integrity throughout the test.
The bubble point helps establish test conditions in the case of drug release tests from an ointment, where the ointment is applied to a microporus membrane. Test results, however, vary according to the ointment used. Only the use of a pre-hydrated membrane guarantees the constant conditions in the *in vitro* model which are necessary for a correct comparison of results.
Use of a dry membrane may result in infiltration of liquid components of the ointment base which form a continuous film within the membrane, altering the contact conditions between the two phases of the cutaneous compartment model (lipogels and W/O creams). Interactions between the two phases of the compartment may also result from the hydrated membrane. Osmotic exchange between acceptor phase and ointment sample may lead to misleading test results (as in the case of hydrogel, PEG gel, O/W cream). Interaction between the ointment sample and water acceptor phase can be avoided by use of a lipoid impregnated membrane. Direct contact between the oint-

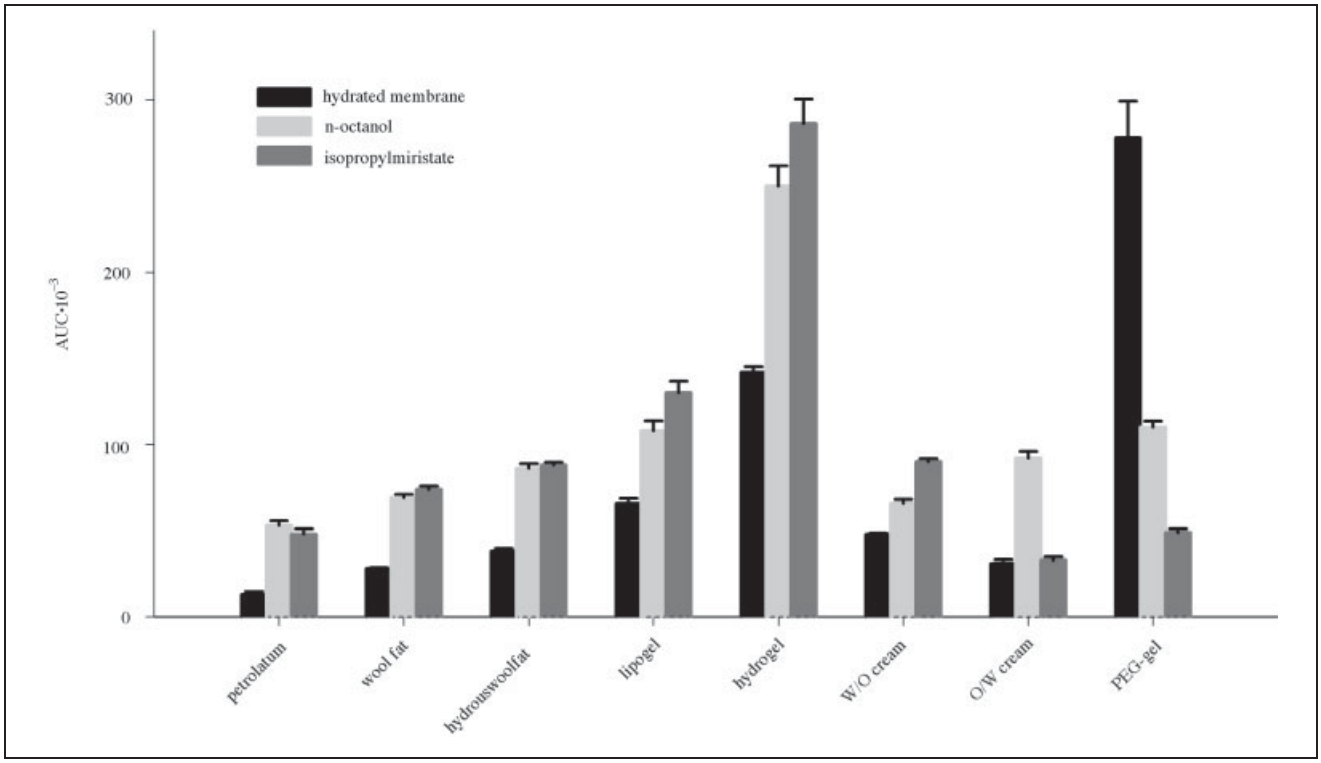

Fig. 8: Comparison between AUC values (μg/cm^2 released amount · time (0 ÷ 180 min)) calculated from the simulated absorption curves of benzocaine from different ointments tested at the same concentration but with different liquid soaking membranes

ment sample and acceptor phase is avoided by the use of a membrane impregnated with lipoids, as used in the simulated absorption test (Realdon et al. 1996, 2002). The hydrated membrane is therefore only reliable for comparing lipophilic based ointments (as in the case of petrolatum, lanolin, hydrous wool fat, lipogel, W/O cream).

In the second case of the simulated absorption test, determination of the bubble point makes it possible to ascertain the physical integrity of the lipoid liquid film immobilized by capillarity in the inner microporous structure of the membrane throughout the test. This condition is essential to maintain a balance between the parameters that regulate the diffusion process between the system compartments.

The test permits a contextual evaluation of the release kinetics, and an indication of the absorption potential of a drug using an *in vitro* model of the cutaneous compartment. As the complexity of the cutaneous barrier *in vivo* cannot be reproduced using a simple physical *in vitro* model, confirmation *in vivo* is required.

Figure 8 compares the AUC values of benzocaine availability from different ointments tested at the same concentrations but with different test types. The release test through a hydrated membrane expresses the drug release capacity of the ointment according to its dispersion state (solution, suspension, emulsion), and is only due to the partition relationship between the ointment and water.

Drug release is always conditioned by the ointment. The simulated absorption test indicates how the drug is able to overcome a lipoid membrane, which simulates the natural one, and diffuse into the plasmatic compartment representing blood circulation.

Fig. 8 shows that the results depend greatly on the nature of the lipoid soaking liquid. A study of the correlation with *in vivo* conditions would help establish the best composition of a soaking liquid.

4. Experimental

4.1. Materials

Petrolatum, lanolin, beeswax, polyethylenglycol 400 and 4000, cetostearyl alcohol, wool alcohols, polysorbate 60, and glycerol were of pharmaceutical grade. Hydroxyethylcellulose (Natrosol HHBR, Clariant International Ltd, Muttenz, Switzerland), Miglyol 812 (Contensio Chemicals, Witten, Germany); 1-octanol, 1-decanol (Aldrich, U.S.A.); 1-dodecanol (Acros Organic, NJ, U.S.A.); isopropylmyristate, isopropylpalmitate, isopropylstearate (Henkel Chimica, Fino Mornasco, Como, Italy); and benzocaine (Hoechst AG, Frankfurt, Germany) were also used. MF-Millipore membranes, HA type, pore size 0.45 μm (Millipore Corp., Bedford, MA, U.S.A.), 25 mm diameter, were used for release and simulated absorption tests.

4.2. Bases

Petrolatum, lanolin, hydrous wool fat, a lipogel (Miglyol 812 85%, beeswax 5%), a hydrogel (Natrosol HHBR 3%, ethanol 20%, glycerol 5%, water 72%), an W/O cream (petrolatum 46.75%, wool alcohols 3%, cetostearyl alcohol 0.25%, water 50%), an O/W cream (petrolatum 25%, cetostearyl alcohol 10%, polysorbate 60 5%, glycerol 10%, water 50%), a polyethylenglycol gel (PEG 400 60%, PEG 4000 40%) were used. Benzocaine 3% was dispersed in each of the above-mentioned bases.

4.3. Drug release test

Ointment samples (3.0 ± 0.2g) were placed in cells (diameter 20 mm, depth 10 mm) in the centre of 45 mm diameter Perspex dishes. A 25 mm diameter Millipore membrane was placed on the surface of the samples and fixed with a Perspex ring. Cells were placed in a 400 ml beaker containing 250 ml phosphate buffer 1/15 M, pH 7.4, thermostated at 32 ± 0.5 °C under constant stirring at 60 r.p.m. Aliquots of diffusion solution were collected at fixed time intervals for benzocaine determination at 254 nm. The assay was performed simultaneously on 6 replicates for each ointment.

4.4. Simulated drug absorption

The procedure described above was used, except for the use of Millipore HA membrane impregnated with isopropylmyristate, isopropylpalmitate and isopropylstearate or *n*-octanol, *n*-decanol and *n*-dodecanol.

4.5. Bubble point test

This is the force used to disperse liquid retained in the membrane given the tension between liquid and solid within the pores (Emory 1989a, 1989b). When increased gas pressure is applied to the saturated membrane surface, it produces a bubble flow in the water of the collector when the surface tension is balanced after ejection of the liquid (Main Catalog, Millipore Corp., Bedford, MA, U.S.A.). This pressure, called "bubble point", is proportional to pore size s and surface tension of the liquid, and can be expressed as:

$$P_{bp} = K4\sigma\cos(\theta)/d$$

where P_{bp} is the bubble point pressure, K is a correction factor, σ is the surface tension of the liquid, θ is the contact angle of the liquid against the solid, and d is the pore diameter. At constant pore size, the bubble point for a given polymeric membrane is different for each liquid in relation to its surface tension.

The membrane was retrieved after the release or simulated absorption test, cleaned with filter paper, and bubble point was measured using the above-mentioned apparatus. Air was introduced under increased pressure to measure the pressure at which the first air bubbles appeared in the end bottle.

4.6. Mathematical analysis

To provide a summary measure for evaluating the experimental data, the area under the curve (AUC) was calculated for the time-course of μg amount of drug released from ointments, from time 0 to 180 min. The AUC was obtained by the trapezium rule, i.e.:

$$AUC = \frac{1}{2}\,\Sigma(t_{i+1} - t_i)\,(a_i + a_{i+1})$$

where t_i ($i = 0, 15, 30, \ldots, 180$) is the time (minutes) of measurement and a is the amount ($\mu g/cm^2$) of drug released at those times.

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