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IMMOBILIZATION OF PARTICULATE SYSTEMS ON THE SKIN BY THE MEAN OF EMULSIONS

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ABSTRACT

For controlled delivery purposes, it can be interesting to immobilize polymeric particles onto the surface of the skin for a prolonged periode of time, either for pharmaceutical or for cosmetic applications. This paper shows that this result can be achieved by incorporating particles in emulsions specifically designed for this purpose.

In order to evaluate the extent and duration of adhesion of such systems, an artificial substrate has been prepared and its surface energy and hydration degree have been adjusted to reproduce those of human skin. A

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hydrodynamic method has been used to study the removal either of crude particles or of H/L emulsion embedded particles after deposit on either artificial substrate or human skin. Immobilization of the particles on the skin for prolonged periods of times (up to 330 minutes) under immersion conditions, has been proved to be feasible. It has been shown to be dependent on formulation since particle retention was increased from 40% up to 98% when embedding the particles into the emulsion tested. Additionnaly, good correlations between detachment from the artificial substrate and those from the human skin have been obtained. These results confirm the ability of the artificial substrate to mimic properties of the skin which are of importance for adhesion measurements.

INTRODUCTION

Over the last decades, the ability to control the delivery rate of active drugs to a predetermined site in the human body has become a big challenge for the pharmaceutical industry. Application of the controlled release concept has been proved to be efficient in pharmacy, leading to an increase of therapeutic efficiency and reduction of unwanted side effects of drugs.

The skin has been considered recently as an alternative path for controlled systemic administrations of drugs by means of transdermal systems or iontophoresis (1). Alternatively, for pharmaceutical or cosmetic applications, skin can represent a specific target by itself. In many cases, it would be highly desirable to ensure that the drug remains localized in the epidermis for a prologed period of time and that drug access to the systemic circulation is restrained. In the case of mucosal administration of drugs, for local effect, it has been shown that many advantages could be obtained by associating the controlled release concept to an immobilization of the active ingredient in front of the site of action (2). In a similar path, localization and a controlled delivery of the drug restrained to the skin on the epidermis is highly desirable

for drugs such as corticosteroids and antifungals in the therapy of skin deseases. For cosmetic applications, the system must prolong the presence of an active ingredient either on the skin surface or in the epidermis (sunscreens, antidandruff products, antiacne products, delivery of perfumes...).

Conventionnal topical dosage forms are often unable to be fully effective for specific skin targeting because of unwanted side effects or of unaesthetic aspect following application. Recently, particulate systems dispersed into a suitable vehicule have been designed as new controlled delivery systems (3). Particles can either be the controlling element of the system or the active ingredient itself while the vehicule ensures a regular deposit of the particles and their adhesion on the skin surface after application. Huc et al. (4) have shown the efficiency of collagen microcapsules dispersed in an L/H emulsion in controlling the systemic delivery of hydrophilic drugs through the skin. Different porous polymeric microparticles based on polyacrylamide (Orgasol®, Atochem technical bulletin), polymethyl-methacrylate (Micropearl®, Seppic technical bulletin) or other polymers (3) loaded with the active ingredient, have been proposed either for systemic administration of drugs or cosmetic applications. Efficiency of particulate systems in controlled delivery depends on their ability to be maintained in full contact with skin as long as the controlled release effect is necessary for a given application.

Hence, the present research consists in an evaluation of the extent and the duration of the adhesion of such formulations on the skin. In this purpose, inert spherical particles have been selected as model particles and incorporated in an H/L emulsion. This type of preparation has been chosen preferentially to pure lipophilic preparation which are generally not well accepted by utilizators because of the greasy sensation. Furthermore hydrophilic bases were not studied because of too easy dilution or detachment from the skin in normal life conditions: transpiration or washing.

In order to evaluate the persistence of such formulations after deposition on excised human skin, or on an artificial substrate designed to reproduce some of the human skin physico-chemical characteristics, a hydrodynamic method has been used.

MATERIALS AND METHODS

Particles

Two types (A and B) of polyamide based round shaped particles (Orgasol, Atochem, Paris, France) hybridized with 30% titanium dioxyde, differing only by titanium dioxyde crystalline habit, have been used (18). Particles diameter has been determined by Coulter counter standard technique, using sodium chloride solution 0,9% (Isoton II, Coultronics, Margency, France) as an electrolyte. The volume surface diameter (d.v.s.) was 4.7 mm for each type of particles. Additionnaly, the surface tension of the type A particles has been determined to be 52,5 $mJ.m^{-2}$ by measuring contact angles of water and formamide on compressed particles discs and by using the above described calculation procedure.

Particle-Loaded Emulsions

Particle-loaded emulsions have been prepared using a two-step technique. First, particles were incorporated in the lipophilic phase allowing a complete wetting of particles. Then, the emulsification was achieved leading to an uniform 4% particle dispersion in the emulsion vehicule. Qualitative optical microscopy observations have confirmed that particles are mainly located inside lipidic phase. No particles were observed in the aqueous phase and only few of them were at the lipidic aqueous interface. Additionaly, a non loaded emulsion has been prepared following the same procedure as a blank.

A H/L highly viscous emulsion has been used as an adhesive base. Its composition is as follows:

Cetyl alcohol	6
Stearyl octanoate	22
Beeswax	7
PEG-7-hydrogenated castor oil	7
Phenoxyethanol	0.5
Methyl paraben	0.25
Propyl paraben	0.25
Bentone 38®	1
Caprilic capric triglycerides	4
Sodium borate	0.48
Panthenol	0.5
Water	51.02

Substrate Preparation

A synthetic substrate has been used as a skin surface model in order to mimic some of the skin surface properties. It is very similar to that of Charkoudian used in testing of adhesion to the skin (5),

This substrate is composed of 9,2% 150 Bloom gelatin, 3,1% 275 Bloom degree (Sanofi Bioindustries, Angoulème, France), water (73,6%) and 3,7% maleated soybean oil (Ceraphyl GA, Van Dyk and Co, Mallinckrodt, Le Vésinet, France). This mixture has been prepared at 50°C under drastic stirring conditions. The pH of this mixture has been adjusted between 7,5 and 8 by adding 1N sodium hydroxyde solution. Prior to casting, a definite quantity of a 3% formaldehyde solution (Prolabo, Paris, France) was added in order to achieve a complete crosslinking of gelatin. Furthermore, the synthetic substrate has been protected against bacterial contamination by addition of methyl and propyl hydroxybenzoate sodium salt

(Cooper, Melun, France) to a final concentration of 0,05%. Casting is made immediately either on smooth surfaces or on forearm skin replicas in order to reproduce the topography of skin.

The hydration degree, H, of the prepared substrate has been calculated by weight determinations after immersion in 200 ml of water and in an isotonic sodium chloride solution according to equation (1).

$$H = \frac{Wh - Wd \times 100}{Wh} \qquad (1)$$

where Wh is the hydrated substrate weight, Wd is the dried substrate weight obtained after constant weight dessication (40°C).

An initial hydration degree, Ho, of substrate has been determined after a curing time of one hour according to equation (1) where Wd has been quoted from the substrate composition.

Substrate Surface Energy

The surface energy has been determined on a smooth surface substrate by contact angles measurements at room temperature (6), using a goniometer (Ramé Hart model 100, U.S.A.).

The data concerning the different wetting liquids are tabulated in table 1 where γlv stands for liquid superficial tension and γl^d and γl^p stand respectively for the dispersion and polar component of γlv. Contact angles reproductibility was inferior or equal to two degrees. All liquids were highly purified grade. The sessile drop technique has been selected with a one microliter deposition. Surface energy, γs, has been estimated by two techniques.

TABLE 1

Surface Tension and Corresponding Polar and Dispersive Components of Wetting Liquids and Contact Angles (Mean of 10 Determinations) Obtained on Artificial Substrate (H = 42%).

Wetting liquids	Origin	γlv	γld	γlp	mean θ
bidistilled water	Millipore	72.8	21;8	51.0	67°95
castor oil	Prolabo	31.9	30.8	1.1	23°10
bidistilled glycerol	Prolabo	63.4	37.0	26.4	47°20
benzyl alcoohol	Prolabo	39.2	34.9	4.3	18°40
methylene iodide	Merck	50.8	50.8	0	37°94

The Zisman's plot method (7) allows the determination of γ_c, the critical surface tension and gives an empirical representation of surface tension. Cosinus of contact angles, θ, for different wetting liquids are plotted against the corresponding surface tension of the liquid. Generally, this representation gives a linear relationship. The intercept of the slope for $\cos \theta = 1$ corresponds to the γ_c value. Zisman's plot equation is given by (2) :

$$\cos \theta = 1 + b\,(\gamma lv - \gamma_c) \qquad (2)$$

where b is the slope.

Surface tension has been equally determined (8) by the harmonic mean equation:

$$\gamma lv_1\,(1 + \cos\theta_1) = \frac{4\,\gamma s^d\,\gamma l_1^d}{\gamma s^d + \gamma l_1^d} + \frac{4\,\gamma s^p\,\gamma l_1^p}{\gamma s^p + \gamma l_1^p} \qquad (3)$$

$$\gamma lv_2\,(1 + \cos\theta_2) = \frac{4\,\gamma s^d\,\gamma l_2^d}{\gamma s^d + \gamma l_2^d} + \frac{4\,\gamma s^p\,\gamma l_2^p}{\gamma s^p + \gamma l_2^p} \qquad (4)$$

where $\gamma = \gamma^d + \gamma^p$ and the subscripts 1 and 2 refer to the testing liquids 1 and 2, respectively. If γ^d and γ^p of two testing liquids (1 and 2) are known, the dispersion and polar components of solid surface tension (γs^d and γs^p) can be obtained from the contact angles θ_1 and θ_2 by solving the equation system (3) (4). Water and methylene iodide are convenient. However, other liquid pairs can be used (9).

Biological Substrate

Abdominal human excised skin has been selected as a biological comparative substrate.

S.E.M. Microphotography

S.E.M. microphotography (Jeol JSM T330 A scanning microscope, Japon) have been carried out according to a standard procedure.

Adhesion Measurements

The different formulations have been deposited uniformly on a circular substrate surface (25 mm in diameter). The thickness of the layer has been calculated to be in the range of 20-50 mm by a weight determination. These systems are immersed in a 150 ml sodium chloride solution (0,9%) and are submitted to a defined hydrodynamic flux produced by a rotating turbine (320 rpm) during 330 minutes. A well defined distance between the substrate and the paddle (1 cm) has been maintained constant.

At defined periods of time, the whole solution has been removed for particle concentration determination and replaced by an identical quantity of fresh liquid. Each particle suspension has been sonicated for 30 minutes prior to particle concentration determination by Coulter counter®. Results are expressed as a cumulated percentage of detached particles.

TABLE 2

Physicochemical Properties of Artificial Substrate and Human Skin *in vivo*.

Physicochemical Properties	Artificial Substrate	Human Skin	References
Superficial tension	γc = 32,5 mN/m	γc = 37 mN/m	(13)
		γc = 27,5 mN/m	(16)
		γc = 26,8 mN/m	(17)
	γs^d = 32.0 mN/m	γs^d = 23.1 mN/m	(10)
	γs^p = 12,3 mN/m	γs^p = 15,1 mN/m	
	$\gamma s^d/gd^p$ = 2.6	$\gamma s^d/gd^p$ = 1.5	
	γs = 44.3 mN/m	γs = 38.2 mN/m	
Hydration (H)	H = 75% after 330' immersion in NaCl 0.9%	H = 17 to 41% in *stratum corneum* H = 70% in *dermis*	(14)

At the end of the experience, the amount of formulation retained on the substrate has been estimated by counting the residue removed by gentle scrapping. Finally, the 100% value has been determined by cumulating the different times counts.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Artificial Substrate

Adhesion of liquids or semi-solid systems on solid substrates is mainly influenced by their physicochemical characteristics and especially by their wetting properties (7,8). Consequently, the characteristics of the

synthetized substrate have to be well defined prior to adhesion measurements. Table 2 summarizes the physicochemical characteristics determined on a smooth artificial substrate. As a comparison, the corresponding human skin values obtained from literature have been tabulated as well.

The surface energy of the artificial substrate determined by the harmonic mean equation, was γs = 44.3 mN/m. The corresponding ratio of dispersive and polar components of the surface energy, $\gamma s^d / \gamma s^p$, is found to be 2.6. These values are close to the human skin data considering El Shimi and Goddard (10) have found γs = 38.2 mN/m and a ratio, $\gamma s^d / \gamma s^p$, equal to 1.5. Thus, a mainly hydrophobic substrate ($\gamma s^d / \gamma s^p$ ratio > 1) of low energy surface is obtained which compares well to skin surface. These results are confirmed by the determination of the critical superficial tension γc according to Zisman (7). Indeed, γc, has been shown to mainly correspond to the dispersive component of the surface energy of the solid γs (11,12). The critical superficial tension γc = 32.5 mN/m of the artificial substrate obtained from Zisman's plot method is in agreement with the previous results where γs^d = 32,0 mN/m. The γc values for the skin reported in the literature show a large scatter, due to the heterogeneity in the skin sample origin and to the varying nature of the different wetting liquids used (13). Consequently, the determination of surface energy parameters by the harmonic mean equation gives more representative data and is preferable.

In order to mimic skin properties it is also necessary to obtain a similar hydration degree. According to Scheuplein and Blank (14), an hydration gradient exists in the skin. The hydration degree of the stratum corneum ranges from 17% to 41% was due to the skin integrity and to the external conditions. Hydration increases to 70% in the dermal portion. Consequently, the hydration of the artificial membrane has been set up at 42% at the beginning of the adhesion studies, since it roughly corresponds to the hydratation values of the stratum corneum.

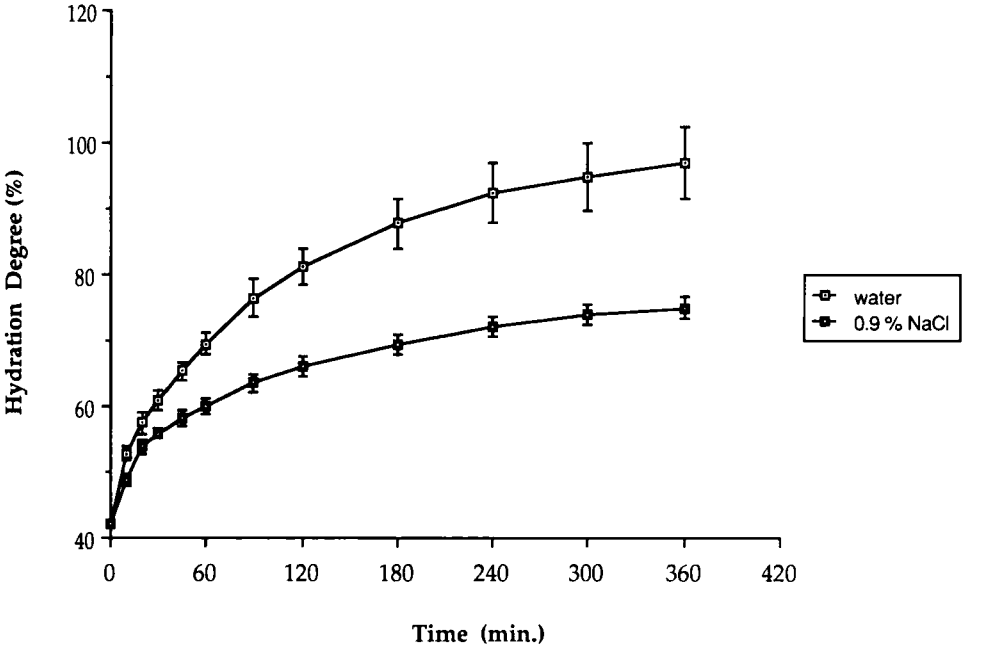

FIGURE 1

Hydration kinetics for artificial substrate (initial hydration = 42%) under immersion conditions.

Additionaly, since adhesion studies are to be performed in a liquid medium, it has been verified that swelling was moderate all through the experiments. Figure 1 shows that swelling is rapidly stabilized in the isotonic solution compared to water. Swelling increases up to 75% under immersion conditions, resulting from the hydration process, is moderate and reasonnable. This value is quite acceptable because in normal use of lipophilic preparation, an increase of the hydration content of the *stratum corneum* is is likely to be observed due to a decrease in the evaporation process.

Finally, S.E.M. microphotography of the substrate (figure 2) shows that a topography similar to that of skin is obtained by the replica technique.

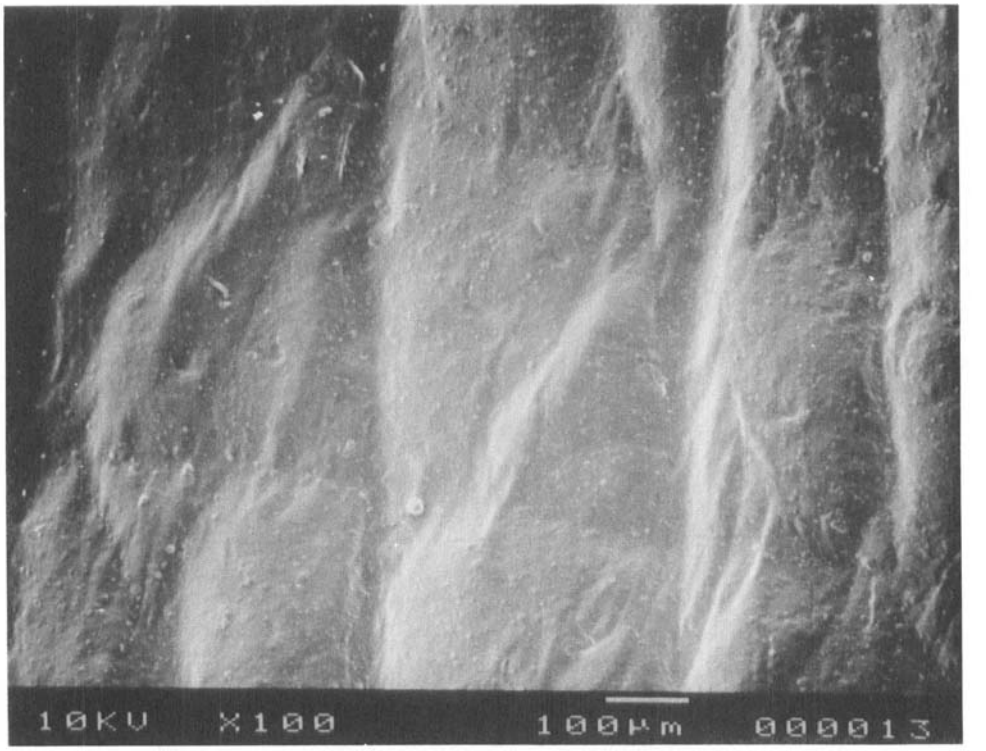

FIGURE 2

S.E.M. microphotography of the artificial substrate casted on skin replica.

These results show that an artificial substrate exhibiting some surface properties close to those of the skin can be obtained. This artificial substrate can be used as an alternative to *ex vivo* skin adhesion experiments.

Adhesion Measurements

Figure 3 shows a S.E.M. microphotography of crude particles (type A). Type B particles are of similar shape. Studies of the crude particles deposit reproductibility on the different substrates have been performed by S.E.M.

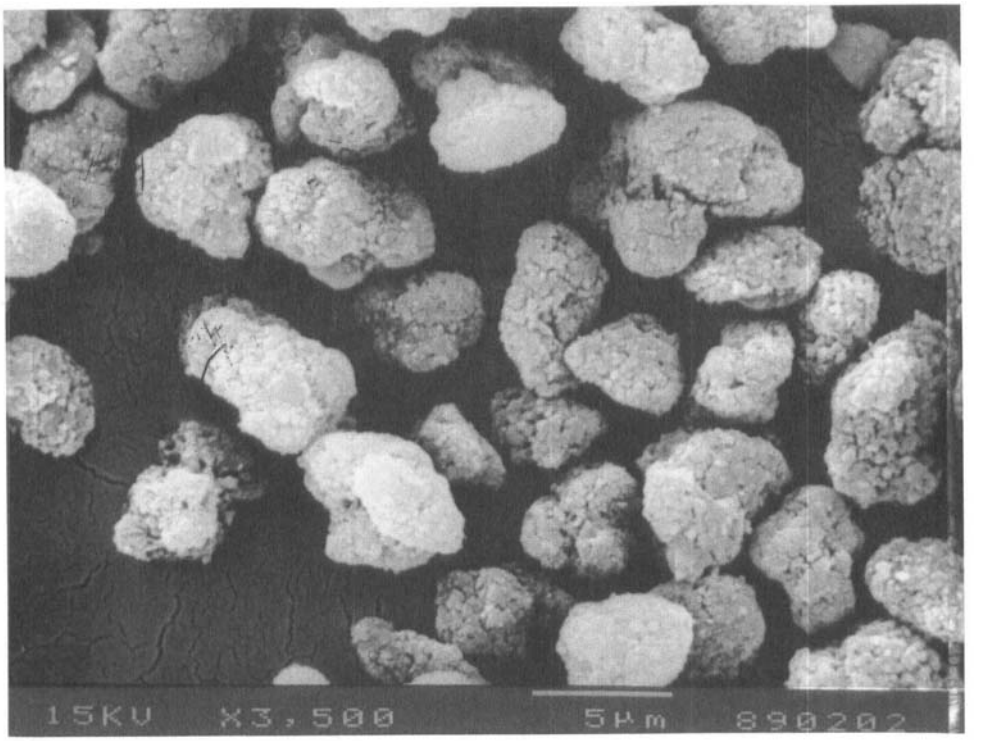

FIGURE 3

S.E.M. microphotography of crude particles (type A).

Figure 4 shows a typical aspect of A particles deposit on the artificial substrate prior to adhesion measurement. The particle deposition seems to be quite regular. Adhesion measurements of the crude particles on the artificial substrate are expressed as the percentage of detached particles as a function of time (figure 5). Whatever the type of particles (A or B), at first, an important detachment occurs during the first twenty minutes but then rapidly levels off. Finally, after 330 minutes of immersion, 30% of the particles initially spread remains onto the surface. These particles are possibly sheltered from the water flux in the valleys of the skin relief.

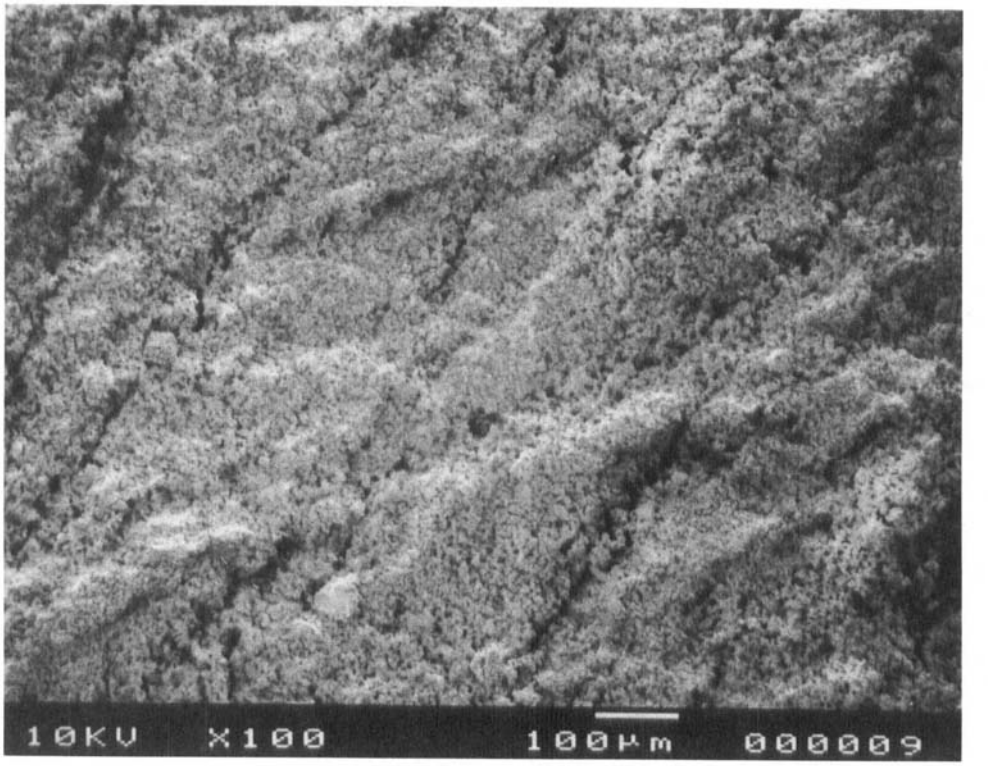

FIGURE 4

S.E.M. microphotography of aspect of deposit of particles on the surface of artificial substrate.

Figures 6 and 7 show the results obtained for particle-containing emulsions and base emulsion spread respectively on artificial substrate and human skin. Results are expressed as percentages of total counts as a function of time. The counted percentages are very low and relatively different regardless of the particles nature in the emulsion or substrate Indeed, in these experiments, not only particles were counted but also emulsion or oil droplets which are detached under the liquid flux influence. This phenomenon was confirmed by the examination of the size distributions determined on Coulter counter® which were very different from those corresponding to the unformulated particles. Consequently, a direct

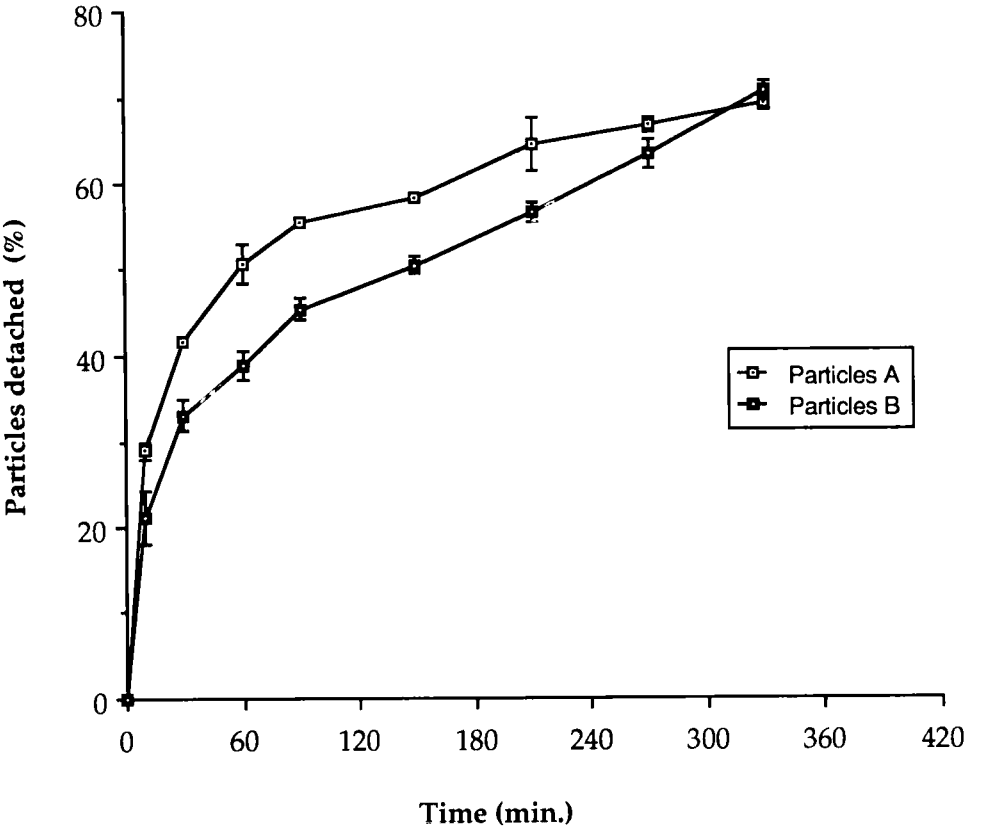

FIGURE 5

Percentage of detached particles from the artificial substrate as a function of time (mean of 3 determinations).

determination of the detached particles percentage is not possible. However, if the base emulsion curves are considered as a blank for the experiment, the percentage of detached particles can be estimated to be very low and always inferior to 2%. Therefore, retention of particles on the skin surface, or on the artificial substrate, is closely dependent on retention of the emulsion and is always superior to 98% after 330 min of immersion. Considering the 30% retention obtained in the same conditions for the crude particles, the H/L emulsion is very efficient in retaining particles on the experimental substrates.

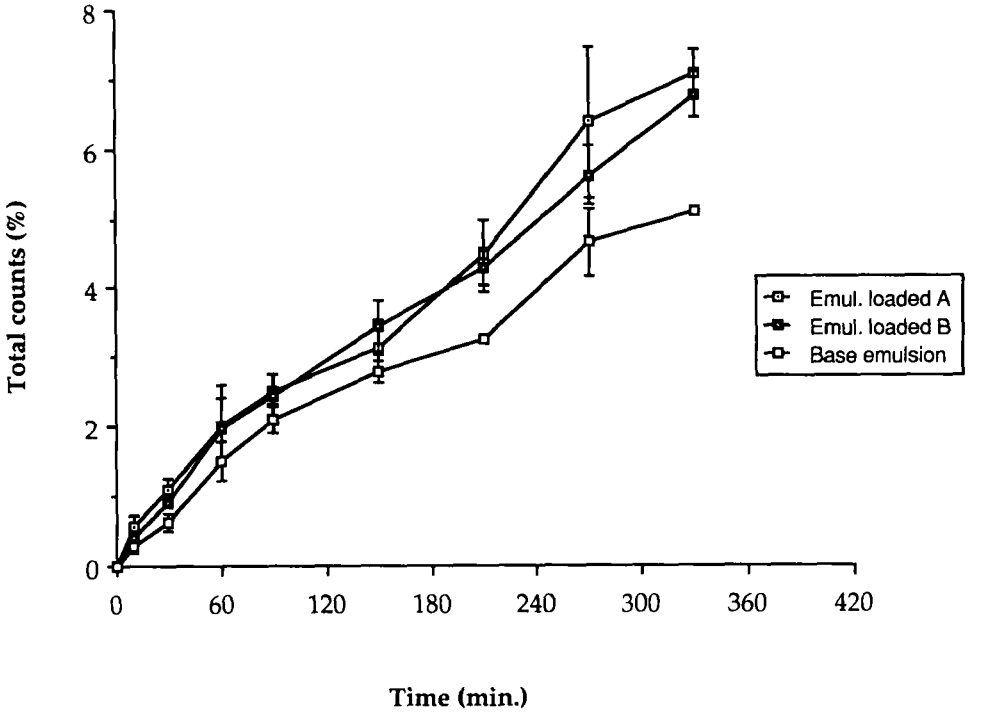

FIGURE 6

Percentage of total counts during detachment experiments for particles loaded emulsions and non loaded emulsion, deposited on artificial substrate as a function of time (mean of 3 determinations).

A qualitative description of the mechanism of retention of the particles on the skin surface can be given. As shown precedently for self cohesion of oil coated particles (15), it is likely that wetting and viscosity are two properties which cooperate to achieve particle retention on the skin. As stated before, particles are embedded into the continuous lipidic phase of the emulsion. At first, the lipidic phase ensure a complete wetting of skin, giving the system part of its adhesive properties. Secondly, viscosity of the emulsion allows a sufficient rigidity of the 20-50 mm thick emulsion layer

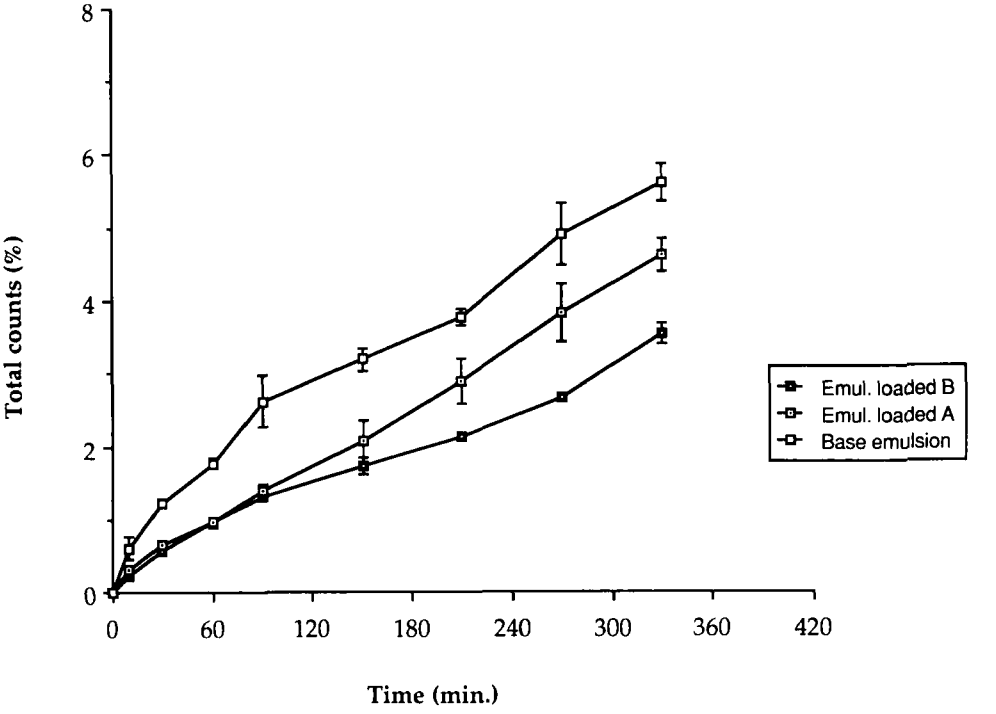

FIGURE 7

Percentage of total counts during detachment experiments for particles loaded emulsions and non loaded emulsion, deposited on human skin as a function of time (mean of 3 determinations).

when it is submitted to shearing by the hydrodynamic flux. Consequently, by varying wetting and viscosity of the emulsified system, there is a mean for the formulator to modulate the adhesion degree of the system.

From a practical point of view, it is likely that under less drastic conditions, i.e., non saline immersion, or no immersion, detachment will be reduced, thus resulting in a prolongation in the contact duration. Moreover, combination of data from figures 6 and 7 leads to an excellent correlation between the results obtained on the artificial substrate and on the excised

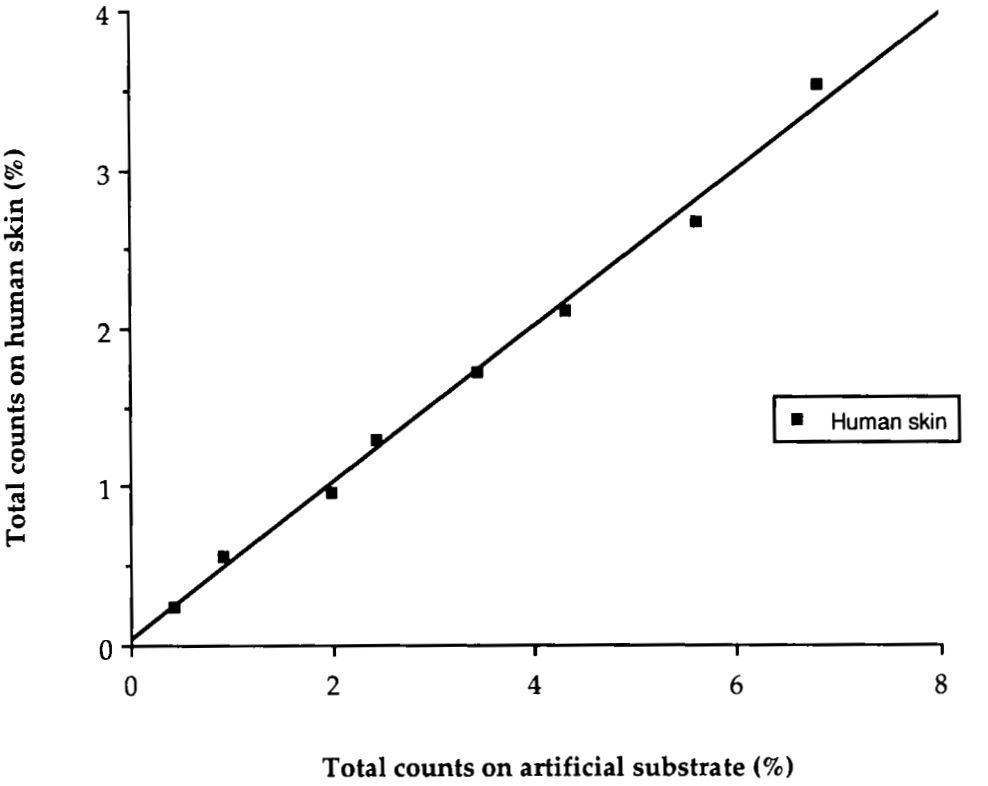

FIGURE 8

Percentage of total counts for the human skin and for the artificial substrate, in the case of particles B loaded emulsion (mean of 3 determinations).

human skin (figure 8). This result confirms the possibility of using the artificial substrate as a skin substitute for such adhesion measurements.

CONCLUSION

The synthetized artificial substrate has been shown to possess physico-chemical characteristics close to the skin. It is well suited for particle adhesion measurements by flux methodologies, as shown by the correlations obtained between the detachment determinations compared to human skin.

Dispersing particles into an emulsion allows a very high level of particle retention (up to 98%). Therefore, immobilization of particles on the skin for prolonged periods of time seems possible even under immersion conditions. Therefore, design of controlled release forms for skin protection, skin targeting or transdermal systemic delivery from such particulate systems becomes a realistic objective.

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