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RESEARCH PAPER

Effect of Gelling Conditions and Mechanical Treatment on Drug Availability from a Lipogel

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ABSTRACT

This study has been conducted to determine whether the rheological differences depending on gelling and treatment conditions could have an influence on drug availability. Lipogels with constant composition were obtained by gelling olive oil with monodiglycerides at rest, under stirring, and milled after gelling. The considerable differences in rheological characteristics produced significant differences on in vitro drug release tests, whereas a lesser influence was observed on in vitro simulated absorption test. The rheological differences appeared not to influence in vivo drug availability. Also, rheological differences owing to the concentration of the gelling agent showed no significant influence on in vivo availability.

INTRODUCTION

Ointments can be obtained by gelling an oleaginous phase with various types of lipophilic substances. The type and concentration of gelling agent can affect the structure on which the rheological characteristics of the preparation depend and consequently on the requirements of physical stability, consistency, and spreadability (1).In turn, the operative conditions in which gelling takes place can have a significant influence on the formation of the internal reticulated structure and therefore on the above-mentioned characteristics (2–4).

In a previous study (5), we observed how the rheological characteristics of the same formulation of lipogels showed significant differences according to whether the product was gelled at rest or under stirring or if, after

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gelling, it was milled. Differences in drug release rate corresponded to the rheological differences.

This study was aimed at determining whether rheological differences in a lipogel resulting from various gelling conditions and mechanical treatment could also influence in vivo drug availability. Methylnicotinate was used as a test drug. The results of the in vivo absorption test based on the intensity and duration of the erythema produced by the drug according to our method (6,7) were compared with those of the conventional in vitro release test through a porous membrane and through an in vitro simulated absorption test.

MATERIALS AND METHODS

Materials

Olive oil (Eur. Pharm. III) from a single batch (Olio Carli, Imperia, Italy) was used to prepare the gels. Glyceryl stearate (Tegin 4100 containing 45% monoester) as gelling agent was purchased from Goldschmidt Italia (Pandino, Italy), and the test drug (methylnicotinate) was obtained from Aldrich-Chemie (Steinheim, Germany).

Preparation of Lipogels

The calculated amount (15, 20, and 25% w/w) of glyceryl stearate was heated at 70°C with olive oil to complete melting. Just before gelling, methylnicotinate (in 20 m*M* concentration) was dissolved into the mass. A series of six 2-kg batches were gelled in two different conditions: cooling under stirring (100 rpm) to 50°C up to clouding of the melted mass, then allowing to gel at rest; and cooling to 25°C with continuous stirring (100 rpm) under vacuum to avoid inclusion of air bubbles at a rate of 2°C/min. The lipogels were then maintained at room temperature (18–20°C) for 7 days before use in the different tests. Portions of lipogels were milled in a roller mill (Erweka, Frankfurt, Germany) before testing.

Determination of Rheological Characteristics

A Rotovisco RV 20 viscometer (Haake, Karlsruhe, Germany) and a rheocontroller RC 20 with M5 on a PK 100 sensor system were used. Measurements were taken at shear rates from 0 to 0.5 s^{-1} at 20°C by using plate/plate PQ2 equipment.

Determination of In Vitro Drug Release (7)

Lipogel samples (approximately 35 g) were placed in six Perspex cells (90-mm diameter; 10-mm depth). A cellulose acetate dialysis membrane (Visking Tubing, London, UK), soaked in water for 20 h, was placed on the surface of the lipogel samples. The cells were then placed with the membrane upward in 2-L beakers containing 1 L of phosphate buffer 1/15 *M*, pH 7.4, thermostated at 37°C under continuous stirring (100 rpm). At 15 min intervals, 2 mL of diffusion fluid was drawn and the concentration of methylnicotinate was spectrophotometrically determined at 263 nm.

In Vitro Simulated Drug Absorption (7)

The procedure for the release test described above was used, except that the membrane was formed of two different coupled membranes (8): one was soaked in isopropylmiristate (9–11), and one was soaked in water for 20 h in direct contact with the ointment sample.

In Vivo Drug Absorption

In vivo drug absorption was determined as described previously by Realdon et al. (6). In brief, the absorption of methylnicotinate was estimated by calculating the intensity and duration of the erythema produced on volunteers with a X-Rite 918 tristimulus reflection colorimeter (X-Rite Inc., Grandville, MI). The erythema induced was expressed as parameter a^*, indicating relative red/green chromaticity. Measurements of a^* before lipogel application were considered baseline values.To evaluate the experimental data, the area under the curve (AUC) was calculated for erythema persistence from time 0 to 180 min after lipogel application.

Statistical Analysis

Data were expressed as mean ± SE. Significance of differences was determined by using ANOVA, followed, when appropriate, by the Newman-Keuls multiple comparison test. Differences were considered significant when $p < 0.05$ (two-tailed).

RESULT AND DISCUSSION

An ointment consisting in a lipogel may be prepared under various conditions according to type and dimension of equipment used and packaging conditions. The molten

mass is prepared so that when the mass is sufficiently fluid to be dosed volumetrically, it is poured into the definitive containers in which the gelling process will be completed. Alternatively, the molten mass may be gelled under stirring and later poured into containers. The lipogel could also be prepared by following one of these conditions and then by incorporating the drug by mechanical action followed by homogenisation or refining with a roller mill.

The rheological characteristics of the ointment are inevitably different according to the conditions followed. The molten mass left to gel at rest has high viscosity levels and equally high yield values owing to its well-organized structure produced by potentially coarse crystals of gelling agent.

On the other hand, if gelling takes place under continuous stirring, the gelling agent forms prevalently small crystals that remain oriented in the direction of flow. When stirring stops, they tend to take on an easily deformable random structure.

If the gelled mass is then agitated mechanically or refined, the reticulated structure is destroyed, causing a significant drop in viscosity. Even if the same formulation is adopted, the rheological differences, which depend on the structure of the system, lead to differences in drug molecule route when diffusing from ointment and therefore produce quite significant differences in drug release rate (5). Destruction of the structure after milling, similar to the friction in applying ointment to the skin, eliminated these differences. Differences in rheological parameters can also be produced when adopting the same conditions. Batches of ointment with the same composition can have different viscosity values as a consequence of the random nature of structure formation and the inevitable small differences in production of different batches, which could also affect drug availability.

We attempted to determine how rheological characteristics influence drug availability by keeping constant the formulation of ointment made of olive oil lipogels with 20wt.-% monodiglyceride at a concentration of 20 m*M* of methylnicotinate. The use of methylnicotinate made it possible to compare results of availability in vivo [evaluated on the basis of the intensity and duration of the cutaneous erythema produced (6,7) after application of the ointment for a set time] with results of in vitro tests according to the criteria currently applied: a drug release test versus aqueous phase through a porous membrane and an absorption simulating test based on drug diffusion versus the simulated plasmatic phase through a porous membrane impregnated with a lipid phase simulating the cutaneous barrier (7).

Influence of Gelling Conditions on Rheological Characteristics of Ointment Batches

Figure 1 compares the flow curves from the same batch in the two above-mentioned conditions: by allowing to gel at rest and after milling. In the first case, the mass showed clear and high yield values, indicating a rigid and organized structure and high shearing stress, verifying the structure's resistance to deformation and a large area of hysteresis. After milling, which led to destruction of the structure and random disposition of gelling agent microcrystals, yield point was negligible and a slow peak of shearing stress orienting crystals in the direction of flow was observed.

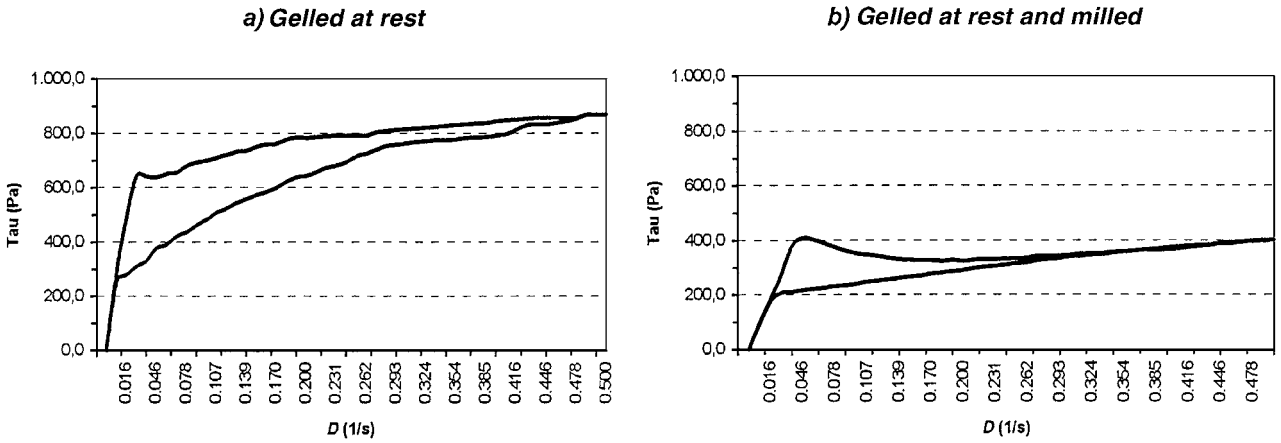

Figure 1. Rheograms of ointments prepared by gelling a) at rest and b) after milling.

Figure 2. Apparent viscosity ($D = 0.5\,s^{-1}$) at 20°C of ointment batches prepared by gelling at rest (●) and by gelling at rest and milling (■).

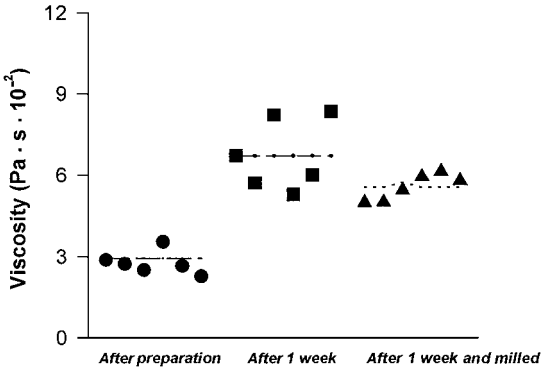

Figure 4. Apparent viscosity ($D = 0.5\,s^{-1}$) at 20°C of ointment batches gelled under stirring immediately after gelling (●); after 1 week (■); and after 1 week and milled (▲).

Figure 2 shows the relative viscosity levels of the different batches of lipogels obtained at rest and after milling. It can be seen that masses with different viscosity levels were obtained. However, the difference between average viscosity of lipogels obtained at rest and those milled was quite high (1771 and 682 Pa, respectively).

The batches of ointments gelled under stirring resulted in well spreadable masses of low consistency, which increased after 1 week at room temperature, as can be seen by the flow curves reported in Figure 3. It can be inferred that when gelled under continuous stirring, the microcrystals are orientated in the direction of flow. After 1 week at rest, microcrystal formation was complete. The same lipogels were milled, and the structure demonstrated some deformation.

The viscosity levels of the six batches of lipogels in the abovementioned three conditions are reported in Figure 4. Low levels of viscosity found immediately after preparation revealed slight differences among batches with the microcrystals still being orientated in the direction of the stirring flow, as a consequence of a lack of a reticulated structure. After 1 week at rest, the crystals built a network consolidated by the completion of gelling crystal formation with a consequent sharp increase in viscosity. The random arrangement led to different conformation of the structure, as shown by the dispersion of the values within the band of ±25% of the average. Refinement caused destruction of the structure and a leveling of viscosity values.

Influence of Gelling Conditions on Drug Availability

The different structure conformation of the gelled system produced by the different conditions forces the drug

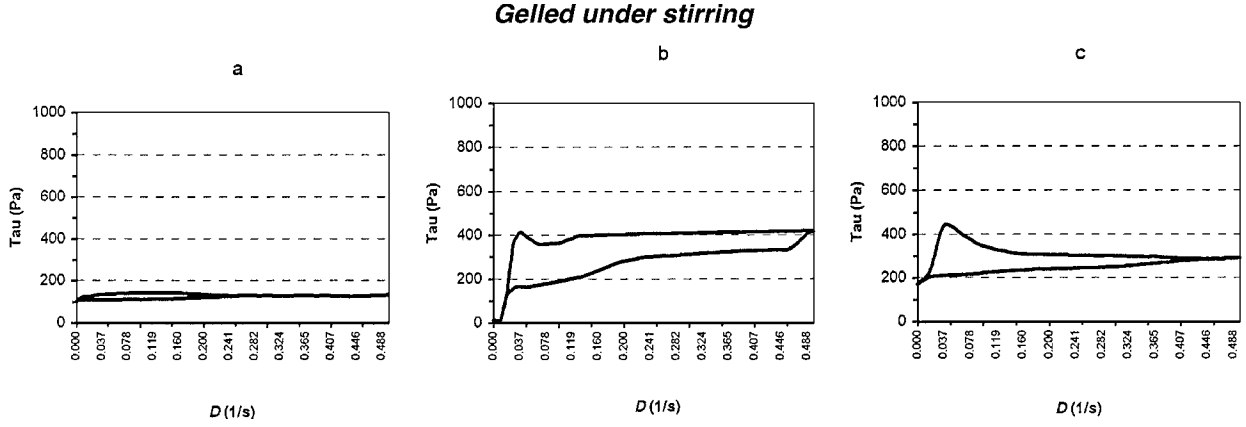

Figure 3. Rheograms of an ointment prepared under stirring a) immediately after gelling, b) after 1 week, and c) after 1 week and milled.

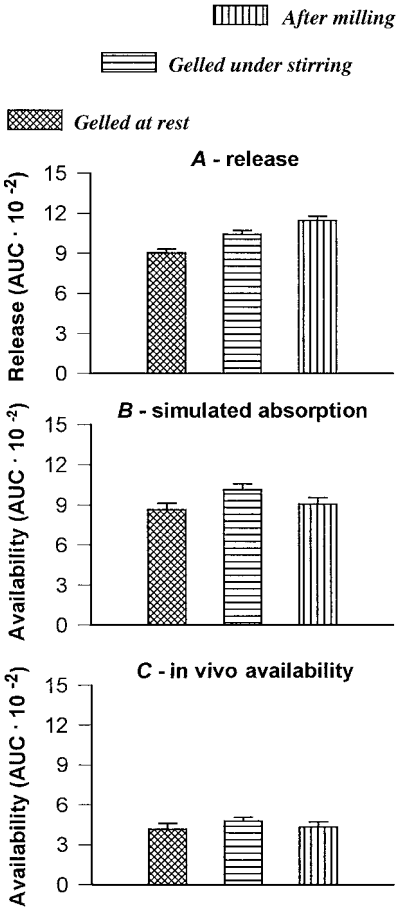

Figure 5. AUC of A) release curves of, B) simulated absorption curves of, and C) time-course of intensity of methyl nicotinate skin erythema produced by lipogels prepared in different conditions: gelled at rest, gelled under stirring and milling.

molecules to take tortuous routes to diffuse from the ointment to the cutaneous compartment. At the usual release test with a simplified model of the cutaneous compartment, drug diffusion rate may be indicative of the complexity of the route taken when diffusing from the ointment. Figure 5A compares the mean of values of the area under the methylnicotinate release curves from the six different batches of lipogel for each of the different treatment conditions adopted. The release changed within a range of ±4% from the average within batches obtained in each different treatment condition adopted. On the other hand, differences in relation to modality of gelling and mechanical treatment were significant ($p < 0.01$). Such differences appeared strictly in relation to the average viscosity of gels. In fact, although this appeared to vary in the sense of gelling at rest > gelling under stirring > milled, release varied inversely. Hence, it appears that the higher the lipogel viscosity and therefore the more complex its structure, the lesser the drug release.

An in vitro model frequently used to evaluate the capacity of ointments to absorb a drug through the skin is a porous membrane impregnated with a lipid phase to simulate the cutaneous barrier. In this system,drug transfer into the plasmatic phase occurs by means of the crossing by diffusion of the impregnating lipid phase (7–11). Compared with the previous test, the two partition relationships (ointment/lipid phase and lipid phase/plasmatic phase) detremine how the drug reaches the plasmatic phase. In a previous study (7) that also used methylnicotinate in different excipients, the results obtained were more correlated with absorption in vivo than shown using the simple release test.

The results, expressed as values of AUC of drug concentration in the plasmatic phase, show significant differences ($p < 0.01$) between samples of ointment gelled at rest and those under stirring and between samples of ointments gelled under stirring and those gelled at rest and milled (Fig. 5B). These differences can be attributed to the different internal structures of the systems.

In vivo drug absorption rate was obtained by measuring the intensity and duration of the superficial vasodilation effect produced by the application of ointments containing methylnicotinate on the skin. The mean of AUC values of erythema intensity from the six different batches in the different conditions are shown in Figure 5C. The differences observed did not appear to be significant. It must therefore be concluded that the different physical characteristics of a lipogel as a consequence of different preparation conditions and mechanical treatment have no influence, despite the differences in availability observed in in vitro tests.

Influence of Quantity of Gelling Agent on Drug Availability

The consistency and therefore the rheological characteristics of a lipogel can be modulated by varying the concentration of gelling agent, as confirmed by the relative viscosity levels of the gels based on olive oil in three concentrations of 15, 20, and 25wt.-% monodiglyceride, prepared under the constant condition of gelling at rest [6.75 (±1.16), 17.71 (±1.08), 41.80 (±1.72) Pa s, respectively], or refined further with a roller mill [4.48 (±0.14), 6.82 (±0.81), 9.27 (±0.76) Pa s, respectively]. In fact, the greater the number of crystals involved in the formation of a reticulated structure, the more compact and resistant the structure is to the forces that cause deformation.

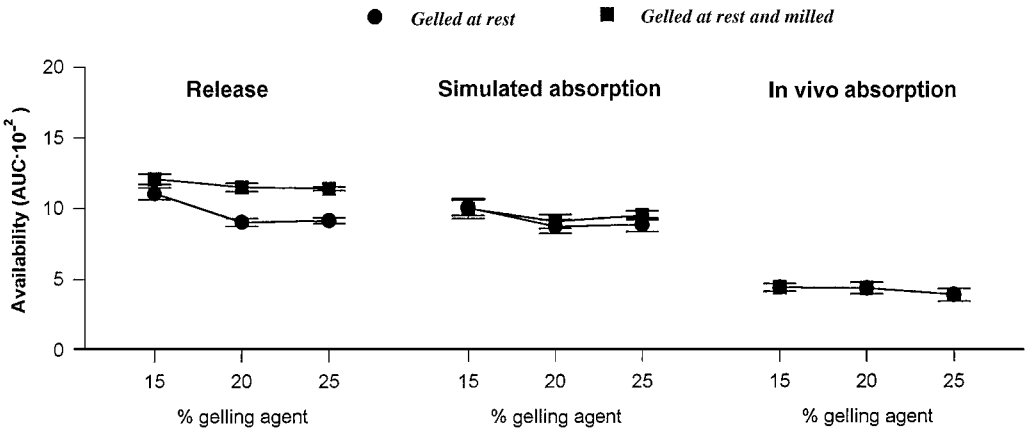

Figure 6. AUC of release rates, simulated absorption rates, and in vivo absorption of lipogels prepared in different conditions with three concentrations of gelling agent.

The different samples of lipogels underwent release, simulated absorption, and absorption in vivo tests to evaluate drug availability. The results, expressed as AUC values of availability, are compared in Figure 6. The results of the release test appeared to be highly influenced by viscosity, with highly significant ($p < 0.01$) differences between the batches gelled at rest and those milled. No significant difference was observed in the simulated absorption test between batches gelled at rest and those milled; significant differences ($p < 0.01$) were observed between the batches at lowest concentrations (15% of gelling agent) and those at highest (20 to 25% of gelling agent). Instead, results of absorption in vivo did not show significant differences, in relation to both gelling agent concentration and to the different types of lipogel treatment. The abovementioned formulation conditions and technical treatment had no influence on the practical effects of a therapeutic response.

CONCLUSIONS

Given the qualitative composition, the rheological characteristics of lipogels, and therefore of aspect and consistency, can be varied by modifying the concentration of gelling agent and the technical and mechanical means of preparation. Despite the differences in drug availability in relation to the viscosity of the gels as highlighted in the in vitro release test, and to a much lesser degree by the in vitro simulated absorption test, the biological response produced by the drug absorption in vivo appeared to be influenced neither by the rheological characteristics of lipogels nor by the concentration of the gelling agent. Therefore, alternative operating conditions, related to production requirements and packaging, can be applied in the preparation of ointments as lipogels without compromising drug availability in vivo. These include preparation of a molten mass that is poured, while still fluid, into definitive containers and left to gel, by complete gelling of the mass under stirring and later transferred into containers, or by the refinement of the gelled mass before packaging.

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