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Effect of concentration on skin permeation of caffeine from gel formulations

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Abstract

Various studies have considered the impact of different physico-chemical drug characteristics, skin thickness, or formulation on the absorption from the skin surface to underlying tissues or to the systemic circulation, but they rarely discuss the influence of drug concentration on the permeation flux of molecules. This research therefore aimed at studying the influence of drug concentration in a caffeine formulation. For this study, three same base gels were used at 1, 3 and 5% of caffeine to evaluate the effect of concentration on *in vitro* release through synthetic membrane and on *ex vivo* permeation of caffeine through human skin. No correlation was found between transfer through synthetic membrane and that observed through the skin. This shows that the diffusion flux of caffeine permeation does not depend on the concentration of the formulation but rather on the quantity of formulation applied. This is evidenced by the fact that the lowest lag time (T_{lag}) and higher absorption rates were obtained with gel at 1% of caffeine applied at 1mg/cm^2.

Keywords: Absorption rate; Bioavailability; Caffeine; Diffusion flux; Lag time.

Introduction

The therapeutic efficacy of any drug depends on its bioavailability. Bioavailability is the rate and extent to which the active ingredient or therapeutic moiety is absorbed from the drug product and becomes available at the site of action (Luong *et al*., 2000). Thus, the two considerations – the extent of absorption of the drug from its formulation, and the rate at which it is absorbed – form the basis of bioavailability and bioequivalence testing and are the predictors of therapeutic performance and therapeutic equivalence. Application of the term bioavailability to topical dosage forms requires, first, a careful definition of the term topical dosage form and, second, a specific adaptation of the general definition of bioavailability to the special case of topical dosage form (Surber and Davis, 2002). The motive for topical or local delivery is the direct application of drug to the target site to maximize efficacy, while minimizing systemic absorption, to improve safety. The targeted therapeutic action by the drugs applied to the skin can be a local or systemic action. In the first case, they can act either on the surface of the skin (antiseptics, keratolytics) or in the deep part of the skin

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(corticosteroids, analgesics), therefore the active drug should penetrate into the deep structures and should remain there at an effective concentration. In the case of a systemic action (estrogen, piroxicam), the drug absorption is essential for reaching the action area (Barry, 1987a; Barry, 1987b; Lippold, 1992; Parikh *et al*., 1984). Consequently, the optimization of the drugs applied through the skin occupies an important place in modern therapy where transdermal application constitutes an alternative to the oral route (Barry, 2001).

The simple concept of measuring the bioavailability of oral formulations can be applied to transdermal systems designed for systemic action. As defined above, the bioavailability should be correlated to the process of drug concentration in the internal environment which includes the site of action of the latter within a reasonable time. Thus, two transdermal delivery systems are considered bioequivalent if they have a comparable bioavailability based on plasma concentration profile over time, when they are administered to the same individuals at the same dose. Application of the term bioavailability for dosage forms intended for topical and regional dermatological treatment is more complex. While the determination of drug concentration in blood is a standard procedure for oral and transdermal forms, it can hardly be used consistently with the topical formulations because the rate circulating in the blood is usually too low to be analyzed by conventional methods. In addition, it can be argued that the relevance of any serum concentration-time curve of a topical agent is questionable, because the curve reflects the amount of drug after the active moiety has left the site of action. Series of methods exist to quantify or locate the drug in the skin and underlying tissue (Bronaugh *et al*., 1982). Development of new, and improvement of existing bioanalytical techniques to measure drugs in the skin and muscle is an active area of research that, similar to the effect of the introduction of techniques to measure drug levels in blood in the 1960-1970s, will transform understanding and lead to improvements in biopharmaceutical and therapeutic quality of topical agents. The difficulty of determining the relative bioavailability and bioequivalence of topical forms has led to the definition of three equivalence types for topical forms (Surber and Davis, 2002). Bioequivalence may be determined by several means, specifically, pharmaceutical, biopharmaceutical or therapeutic parameters, or a combination of these. Pharmaceutical equivalence applies to pharmaceutical products which contain the same amount of drug in the same pharmaceutical form or similar forms and meet to identical or comparable pharmaceutical standards like drug stability or release rate. The dilemma is, for example, that two transdermal delivery systems, containing different amounts of the same drug that are delivered to the body with the same kinetics (and which are bioequivalent) under identical conditions are by definition, pharmaceutically inequivalent. This issue becomes even more complex when semisolid dosage forms, in all their diversity, are compared (Verbeeck *et al*., 2006). Some regulatory authorities consider a pharmaceutical product therapeutically equivalent to another product if it contains the same drug and which when administered to the same individuals, shows the same efficacy and toxicity than the same product for which the effectiveness and safety have been established.

Thus, evaluation of drug percutaneous absorption is one of the main steps in the initial design and later in the evaluation of any skin delivery drug system. It is important to know the fate of chemicals applied to the skin: first assess the fraction of applied dose actually absorbed and available to exert pharmacological or toxic effects; and

secondly to determine the local concentrations of the drug in skin structures or in the deeper tissues. Percutaneous pharmacokinetic studies can clarify the fate of the drug product during its distribution in the skin strata: retention in the stratum corneum, binding to skin proteins, epidermal and/or dermal metabolism, skin clearance, accumulation in the deeper skin structures. These data are useful in assessing local and / or systemic toxicity. The literature contains many examples of how the composition and manufacture of the finished dosage form can alter the effectiveness of the drug (Amidon *et al*., 1995; Leuner and Dressman, 2000; Contreras-Solis *et al*., 2008). This is particularly true with topical therapy, for which vehicles have profound effects on percutaneous absorption.

This study focused particularly on the effect of the drug (caffeine) concentration on its release and skin permeation; other factors influencing skin penetration (vehicles, absorption promoters, pH, saturation) being widely discussed. The parameters of skin absorption of caffeine from three gels containing 1, 3 and 5% of caffeine were compared. Caffeine (1,3,7-trimethylxanthine; $C_8H_{10}N_4O_2$; solubility = 21.88 mg/ml; Log $K_{oct/water}$ = 0.07) is a substance commonly used in cosmetics. A comparison of drug release has been made previously through polysulfone membranes.

Experimental

The following chemicals and reagents were employed: Carbomer Carbopol 934® (BF Goodrich); Triethanolamin-TEA (Cooper); Glycerin (Cooper); Propylenglycol-PG (BASF); Dimethylenecopolyol-DC 193 (Dow Corning). Chromatographic analysis was carried out using HPLC (Interface, Merck Hitachi D-7000; UV- Detector, Merck Hitachi L-7400; Autosampler, Merck Hitachi L-7200; Pump, Merck Hitachi L-7100).

Caffeine gels. Three gels containing 1, 3 and 5% of caffeine were prepared according to the formula:

Water=59.5; Carbopol=0.5; TEA=1.2; Glycerin=34.2; PG=2.0; DC193=2.3 (% $^{w}/_{w}$)

After the dissolution of caffeine in water, Carbopol® was dispersed in the solution with rapid stirring, then neutralized with half of T.E.A. Mixing DC193, PG and glycerin were added with continuous stirring and neutralization was performed with the second half of TEA to obtain pH 7.

Franz™ diffusion cells. The diffusion cells used were made of glass, static type, and included three separate parts: First-a supplier donor compartment containing the drug, with application surface of 3.14 cm^2 and occlusion provided by Parafilm® ; Second-a lower receiver compartment containing a receptor medium consisting to isotonic phosphate buffer (PBS, pH = 7.4) in which the substance released through the membrane is determined with the receiver volume being about 10ml; Third-a diffusion membrane of Polysulfone membrane or skin. A thermostated bath surrounding the lower part of the cell was used to maintain the temperature at 37 °C. The homogeneity of the temperature and, of course, the content of the lower compartment was maintained by a magnetic stirrer. These cells are called static since the receiver compartment is periodically renewed during the tests by sampling liquid and replaced with new fluid (Fig. 1).

In vitro release of caffeine. The applied method is in compliance with the American Association of Pharmaceutical Scientists (AAPS) and the Food and Drug Administration (FDA) recommendations. This study was performed using Franz™ cells described above. The membranes used were polysulfone membranes which had been soaked in a mixture ethomeen 15% isopropyl myristate. The membranes were installed on the Franz cells (6 cells per gel), the lower

compartment was filled with liquid receiver (10 ml of PBS pH = 7.4), the system having been stabilized for two hours. A 300 mg sample of each formulation was applied to the donor compartment using a micro-spatula. The cells, maintained at 37° C with stirring, were closed with Parafilm ® to prevent evaporation. Samples of receiver liquid were taken at 30, 60 and 360 minutes, and were immediately tested. The survival liquid (remaining after taking a sample) was completely renewed each time a sample is taken.

Ex vivo skin absorption of caffeine: The method of American Association of Pharmaceutical Scientists (AAPS) and Food and Drug Administration (FDA) was used. Skin penetration *ex vivo* was studied in biopsies of human skin placed in Franz™ cells. The biopsies were obtained during abdominal plastic surgeries. The skin was allowed to defreeze one day before its use. Just before use, the skin was freed of subcutaneous fat by the means of a scalpel. The whole skin was then dermatomed at more or less constant thickness of 390 to 400 µm using a Brown™ dermatome (Emergence, 94573 Rungis). The dermatomed skin was cut into pieces of about 4 cm^2 each. A control of precise thickness was made for each biopsy using a specific device. The skin samples were then mounted in diffusion cells without further processing. Skin surface temperature was maintained at 32 ± 1°C, measured using a mini-thermometer (TESTO 0900.0519). Once the skin was set, the cells were stabilized in a water bath at 37 °C overnight. The gel was applied on the epidermal surface of the skin biopsy using a micro spatula. Specific amounts of gel (1, 2 and 5 mg/cm^2) were weighed for gels containing 1, 3 and 5% $^{w}/_{w}$. caffeine respectively. Sampling of receiver liquid is done at 30, 60, 90, 180 and 360 minutes for analysis.

Quantitative analysis of caffeine. Samples were analyzed for caffeine using HPLC. A reverse phase column was used (Merck Lichrospher® 100RP 18, 125× 4mm, 5µm). The mobile phase consisting of methanol: aqua (30:70). The elution parameters were a flow a flow rate of 1 ml/min and an injection volume of 20µl. UV detection was at 272 nm. The analytical parameters for this assay were as follows: retention time = 3.5 min; limit of detection = 0.5 µg/ ml; reproducibility relative standard deviation = 2%. For each sample, three successive tests were made. For the quantitative determination of caffeine a standard range of seven points covering concentrations of 0.5 to 2000 mg/ml, prepared from caffeine identical to that used for the preparation of gels was employed.

Data treatment. Caffeine flux through membranes and skin were calculated using Fick's second law of diffusion. According to this law, the total amount of drug (*Q*) appearing in the receptor solution in time *t* at the steady state is expressed as follows:

$$Q / A = KLC_O (D t / L^2 - 1/6) \qquad (1)$$

Where A = effective diffusion area,
C_0 = initial drug concentration in the vehicle,
D = diffusion coefficient and corresponds to the diffusivity of the drug in the membrane,
L = the thickness of the membrane
K = partition coefficient of drug between membrane and vehicle.

The flux, *J*, was determined from the slope of steady state portion of the amount of the drug permeated divided by *A* versus time. The lag time values were determined from the x-intercept of the slope at steady state. From Eq. 1 the flux is expressed as:

$$J = C_0KD / L = C_0 K_p \qquad (2)$$

Caffeine release rates were calculated using the Higuchi equation (Higuchi, 1962):

$$Q/A = 2C_0 (Dt / \pi)^{\sqrt{2}} \qquad (3)$$

Where Q = amount of the drug released
D = apparent diffusion coefficient, denoting diffusivity of the drug in the vehicle
t = time, A = the area of the diffusion membrane,
C_0 = the initial concentration of the drug in the vehicle and π a constant.

Equation 3 may be simplified to:

$$Q / A = k t^{½} \qquad (4)$$

Where k = release rate constant and was determined from the slope of the amount of the drug released per unit area versus square root of time.

Results

Caffeine extracted from the supernatant of the gels (aqueous part) corresponds to the amount of caffeine dissolved in the gel (Fig. 2). For the three gels, the cumulated quantity per unit area according to time is displayed in Fig. 3. The flow was estimated by the regression curve. Cumulative caffeine amounts were plotted against square-root of time (Fig. 4). The slope of the plot indicates the release rate ($\mu g/cm^2/min^{-0.5}$). Caffeine release characteristics are summarized in Table 1. Absorption rates of caffeine through the membrane (compared to caffeine amount applied) were found to be 55%, 42.63% and 39.13% for gels 1, 3 and 5% respectively. Figure 5 presents progressing cumulative drug amount during the experience time. The diffusion flux (J) corresponds to the slope of the line ($\mu g/cm^2/min$) and the lag time T_{lag} corresponds to the point of intersection of the line with the x-axis (minutes). Diffusion flux is the amount of substance absorbed per unit area and time. This is presented in Fig. 6 and Fig. 7. The values of the diffusion flux *(J)* are compiled in Table 2. Lag time is the time required for the diffusion flow to become stable. During these experiments, T_{lag} varied from 2 to 4 hours. (Fig. 8). The absorption rate was calculated from the amount of caffeine initially deposited and the cumulative amount in the receiver compartment after 6 hours. The results are summarized in Table 3 and Figures 9 and 10. The intersection of the line with the x-axis is given by:

$$h^2 / 6D = T\ lag \qquad (4)$$

From this equation, diffusion coefficient for each of the gels and for each of the deposited amounts were calculated. The results are shown in Table 4 and Figures 11 and 12.

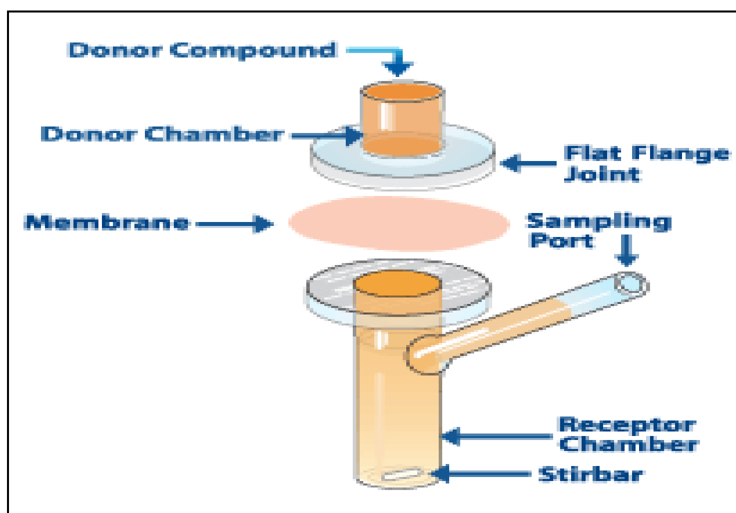

Figure 1: Schematic diagram of a Franz™ cell.

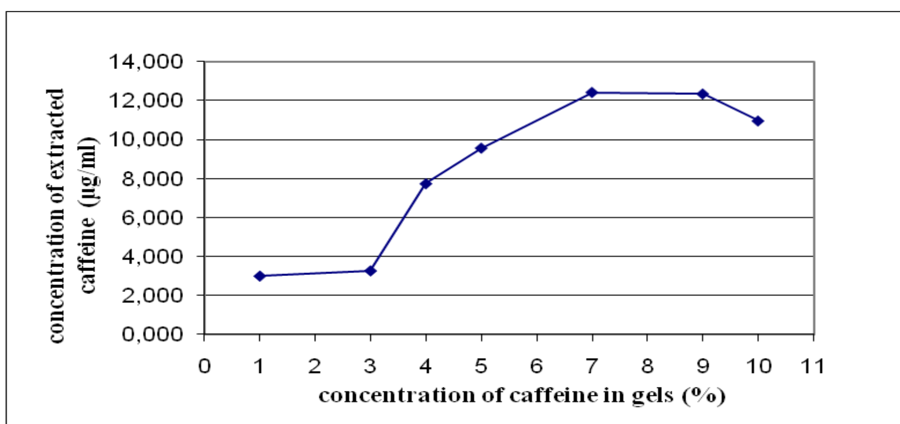

Figure 2: Dissolved caffeine concentration of the gels.

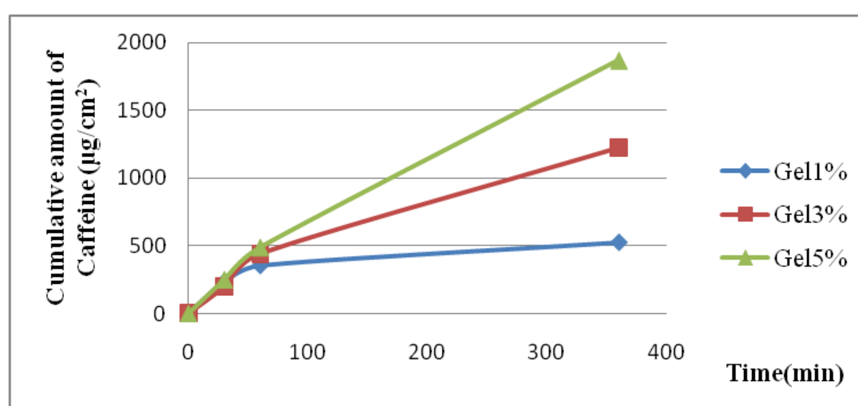

Figure 3: Profile of *in vitro* release of caffeine against time

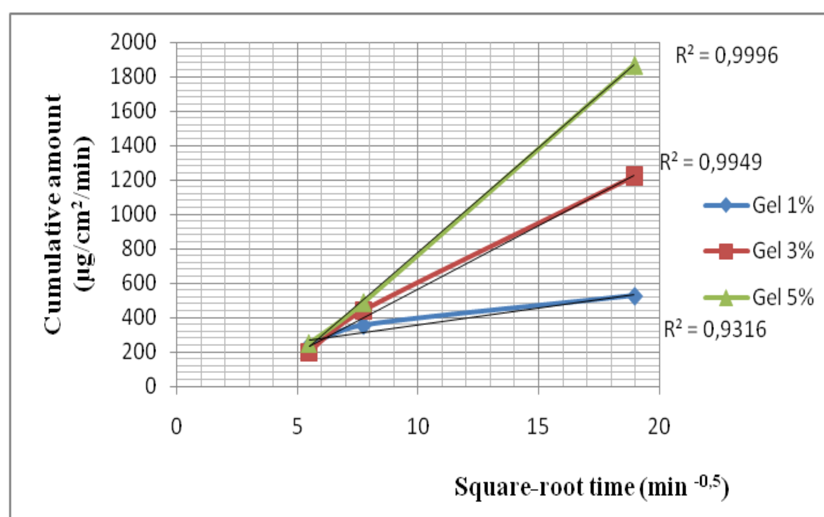

Figure 4: Profile of *in vitro* release of caffeine against square root of time

Table 1: Characteristics of caffeine release through the polysulfone membrane. N =6 (±SD)

	Diffusion flux (µg/cm^2/min)	Release rate (µg/cm^2/min^{0,5})	Cumulative amount after 6h (µg)
Gel 1%	0.57 (±0.14)	19.51 (±1.09)	1650 (±77.5)
Gel 3%	2.68 (±1.93)	73.89 (±17.33)	3837 (±67.82)
Gel 5%	4.87 (±0.84)	121 (±19.34)	5870 (±86.29)

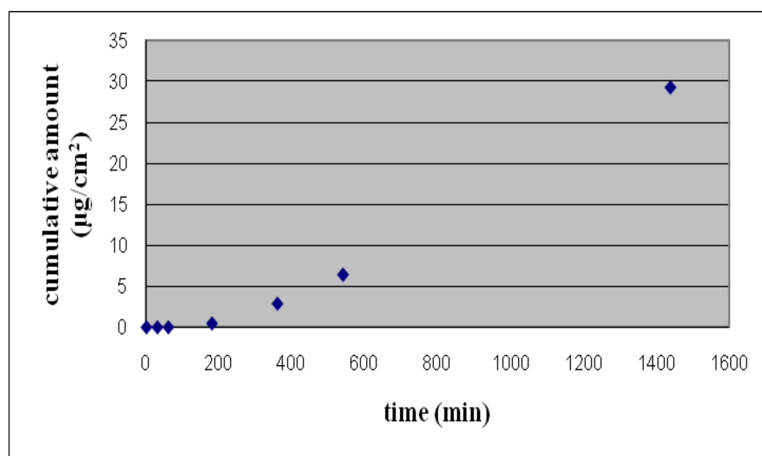

Figure 5: Permeation curve: Cumulative amounts (µg/cm^2) as a function of time (min)

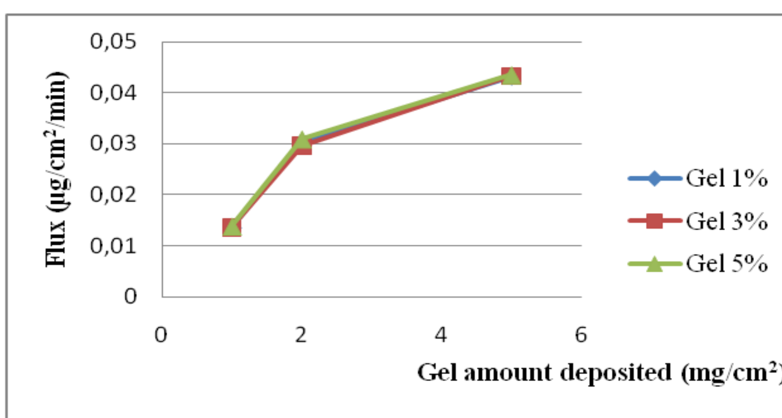

Figure 6: Evolution of permeation flux from the three gels

Table 2: Average values of J (µg/cm2/min) N = 6 (±SD)

	GEL 1% of caffeine	GEL 3% of caffeine	GEL 5% of caffeine
1 mg/cm^2	0.0136 (±0.0008)	0.0135 (± 0.002)	0.01388 (± 0.001)
2 mg/cm^2	0.0304 (± 0.002)	0.0296 (± 0.001)	0.0309 (± 0.003)
5 mg/cm^2	0.0432 (± 0.006)	0.0333 (± 0.003)	0.0435 (± 0.005)

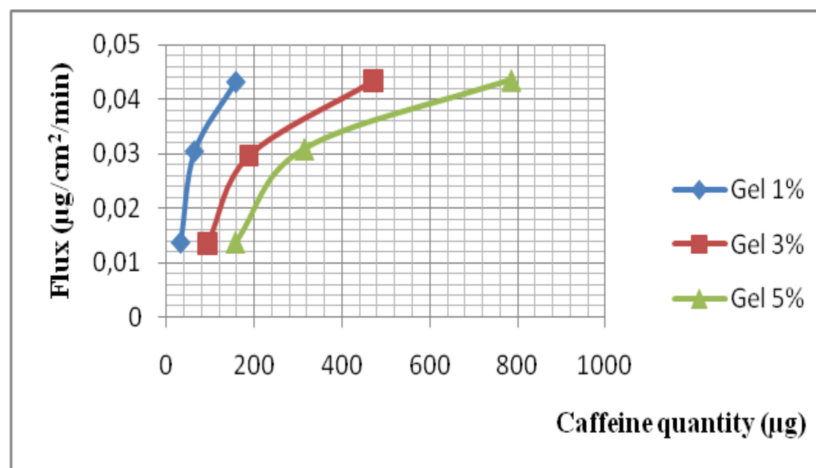

Figure 7: Diffusion flux (*J*) as a function of quantity of caffeine applied (µg) for each gel.

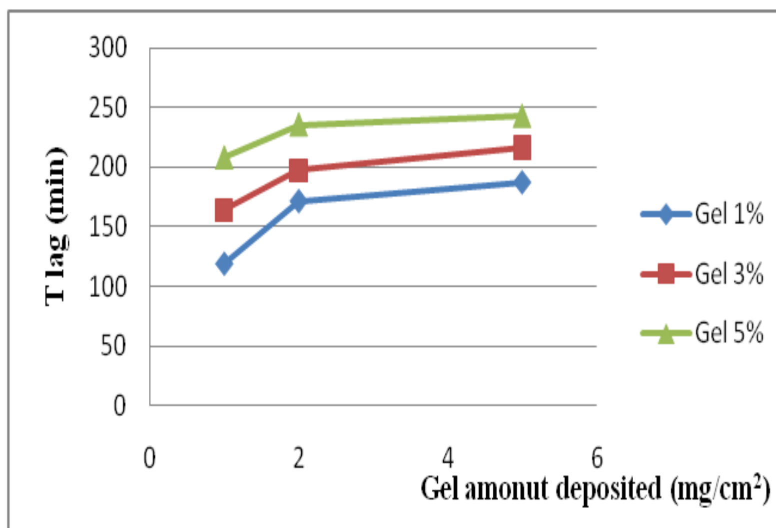

Figure 8: Variation of the lag time (T_{lag}) with amount of gel amount deposited .

Table 3: Quantities of Caffeine deposited (µg), accumulated amounts after 6 hours (µg) and absorption rate (%)

	Gel 1 %			Gel 3%			Gel 5%		
	Caffeine amount deposited (µg)	Cumulative amount 6h (µg)	Absorption rate (%)	Caffeine amount deposited (µg)	Cumulative amount 6h (µg)	Absorption rate (%)	Caffeine amount deposited (µg)	Cumulative amount 6h (µg)	Absorption rate (%)
1mg/cm2	31,4	3.52 (±1.31)	11,21	94,2	4.18 (±1.35)	4.43	157	6.88 (±0.53)	4.38
2mg/cm2	62,8	5.41 (±1.43)	8.61	188,4	7.14 (±1.11)	3.78	314	11.14 (±1.4)	3.54
5mg/cm2	157	6.99 (±1.75)	4.45	471	10.19 (±2.01)	2.16	785	12.75 (±2.23)	1.62

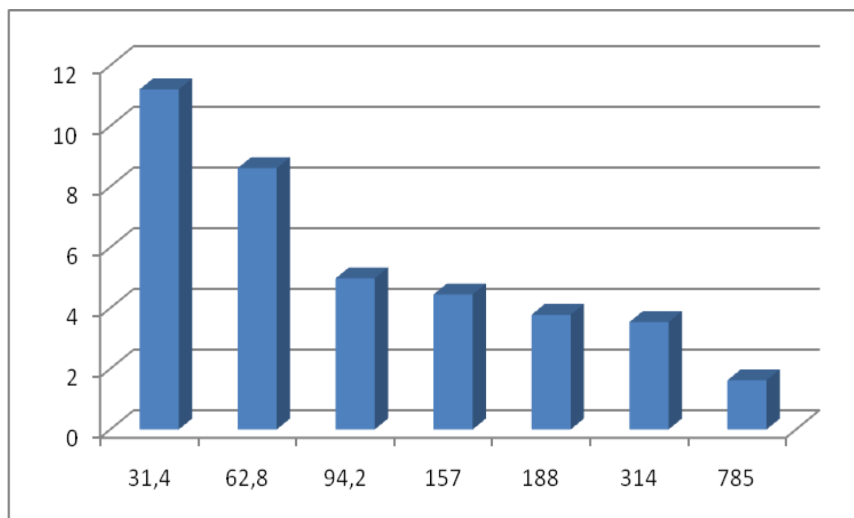

Figure 9: Absorption rate (%) vs. caffeine amounts applied (µg)

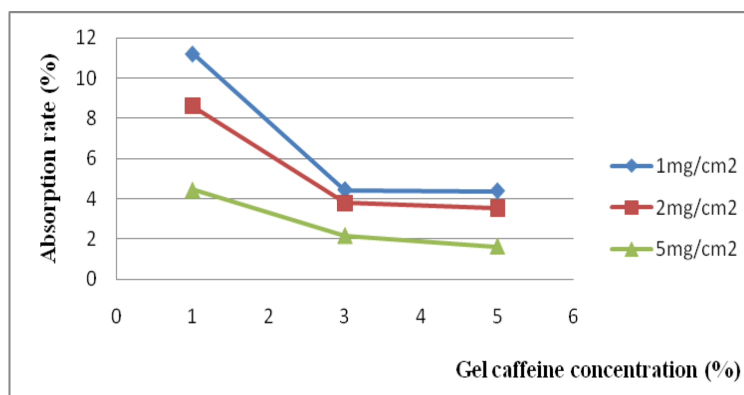

Figure10: Variation in the absorption rate (%) vs. concentrations of caffeine in the gel (%).

Table 4: Values of diffusion coefficients D_m .10^{-6} ($cm^2.min^{-1}$)

	Gel 1% of caffeine	Gel 3% of caffeine	Gel 5% of caffeine
1 mg/cm^2	1.99	1.61	0.856
2 mg/cm^2	1.38	1.33	0.756
5 mg/cm^2	1.27	1.22	0.731

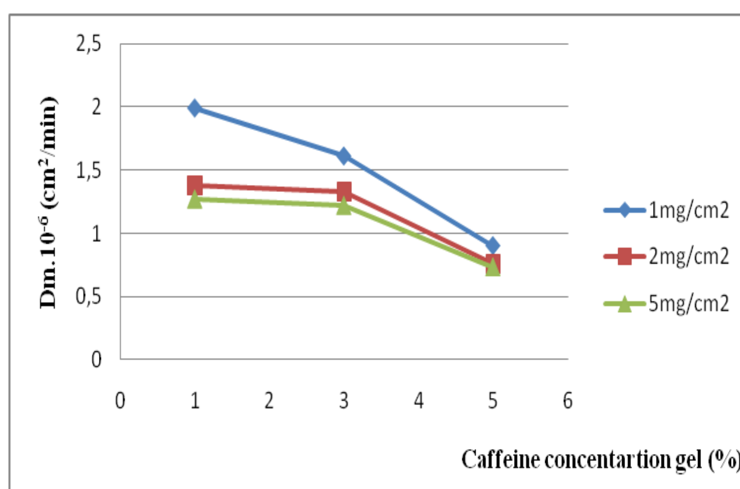

Figure 11: Diffusion coefficient D_m (cm^2/min) as a function of caffeine concentration in gel (%)

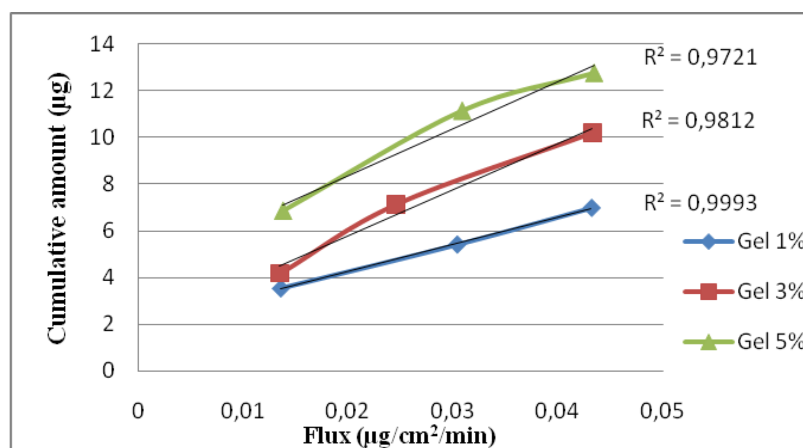

Figure 12: Correlation between the diffusion flux, *J* (µg/cm2/min) and the amount accumulated after 6 h (µg).

Discussion

Caffeine solubility study shows that the amount of caffeine extracted from different gels increases according to the percentage of caffeine in the gel until it reaches a threshold limit (7% of caffeine) at which the concentration of caffeine becomes stable, and then decreases (Figure 2). The plateau corresponds to the limit of solubility of caffeine in the gel. This could correspond to a saturation of the vehicle by caffeine. The decrease of the dissolved caffeine concentration could be explained by a phenomenon of crystallization of the molecule which is preventing its diffusion in the vehicle. It is accepted that the concentration governs the thermodynamic activity and influences the distribution of a molecule in his vehicle. A maximum diffusion is generally obtained when the concentration in its vehicle reaches the limit of solubility. Only the portion of drug dissolved in the vehicle can be released at the surface of the skin and spread. 1, 3 and 5% of caffeine gels are located in the ascending and linear curve, and are therefore valid for our comparative study.

The *in vitro* release of caffeine has been studied on polysulfone membrane. According to one study (Clement *et al*., 2000), the polysulfone membrane is not a limiting factor for the release of caffeine and this enabled to study the *in vitro* release of this molecule. As shown in Figure 3, the release flux of caffeine through the membrane becomes stable from 60 minutes. The steady state flux can be estimated by linear regression through the data obtained from 1 and 6 hours. The values produced are, respectively: 0.57± 0.4, 2.68± 1.93 and 4.87± 0.84 µg/ cm^2/min for gels 1, 3 and 5%. Flux increased jointly with increasing concentration of caffeine in the gel.

Plots of cumulative caffeine amounts versus square-root of time (Figure 4) showed very good regression coefficients (R^2> 0.93). The slope of the plot indicated the release rate (µg/cm2/$min^{-0.5}$). It is more important in case of higher concentration of caffeine. Similarly, the increase in caffeine accumulated after 6 hours is proportional to the increase of gel concentration (Table 1). Higher diffusion flux and higher release rate of caffeine are obtained with most concentrated gels. Similarly, cumulative amount of caffeine increases proportionally to drug concentration increase. Despite the increase in the release rate and the cumulative amount of caffeine for the same amount of gel, the absorption rate of caffeine decreases while the concentration of drug in gel increases. Absorption rate was higher with gel 1% (55% of the applied caffeine found lodged in receiver middle); a negligible difference in this rate was recorded for gels between 3 and 5 % (42.63% for the gel 3% and 39.13% for the gel 5%).

In vitro release study of caffeine showed a satisfactory release rate (19.51, 73.89 and 121 µg/cm^2/$min^{-0.5}$ for gel 1, 3 and 5% respectively) within a relatively short time convenient for the comparative study of the skin absorption from theses three gels. The study of caffeine diffusion through the skin showed that, for the same gel (then same caffeine concentration) the diffusion flux increases with increase in the amount of applied gel (Figure 6). Contrary to what was expected, the diffusion flow did not depend on the gel concentration of caffeine; almost the same diffusion flow values were obtained with the three gels at the same amount applied. Furthermore, when the same quantity of caffeine was deposited, the higher flux value (*J*) was obtained with the less concentrated gel. For example, when 157 µg of caffeine was applied (as 5 mg/cm^2 from gel 1% or 1mg/ cm^2 from gel 5%), the flux was more pronounced in gel 1%. Similarly, as shown in Figure 7, it was observed that for 200 µg of caffeine, the flux (*J*) from gel 3%

(0.03 µg/ cm^2/min) was higher than that obtained from gel 5% (0.02 µg/cm^2/min).

It was immediately apparent that the diffusion through epidermal tissue is significantly slower than through the synthetic membrane. During our experiments, Lag time varied from 2 to 4 hours. As shown in Figure 8, the T_{lag} increases with the amount of gel deposited but also with increasing caffeine concentration in gels. The shorter T_{lag} was obtained with gel 1% applied at 1mg/cm^2. Absorption rates through the skin are clearly lower than those noted through membranes. Cumulative amounts of caffeine after 6hours increased slightly with increasing amounts of caffeine applied on the skin. Consequently, absorption rate decreased when concentration or quantity of gel applied increased (Figure 9). Higher absorption rates were obtained with gel 1% (figure 10). These results suggest that a quantity of caffeine used was retained by the skin structures for the more concentrated gels. However one cannot express the penetration of a molecule in terms of ratio of the applied doses except in the case of defined dose situation, where everything that has been deposited on the skin has been used, and when the flow absorption has been reduced to zero. The higher diffusion coefficient (D_m) is obtained with gel 1%, then decrease with more concentrated gels but also when quantity gel deposited increases (Figure 11). At the end of the study, a good correlation has been set up between the diffusion flows (J) and the cumulative amount of caffeine (R^2 >0.97). This correlation proved to be stronger with less concentrated gel (Figure 12).

Conclusion

This type of studies may be useful in case the active drug were distributed under various dosages, therefore the key question is to know whether it is preferable to prescribe the most concentrated formulation or to adapt a more intensive treatment with a less concentrated dosage. As an example, in the case of caffeine with this formulation, it seems that the gel 1% at 5 mg/cm^2 is more efficient in terms of flow, T_{lag} and absorption rate than the more concentrated gels applied for an equivalent quantity of caffeine.

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