CAPSAICIN MICROEMULSIONS: PREPARATION, CHARACTERIZATION AND *IN VITRO* RELEASE STUDY

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Abstract

In this study, pseudoternare phase diagrams were made and oil: water: surfactant: cosurfactant (O:W:S:CoS) weight ratios corresponding to microemulsion states were highlighted. Soybean oil was used as the oily phase (O) and a mixture of water and glycerol in a ratio of 4:1 (wt / wt) was used as the aqueous phase (W). As a surfactant (S) was used sorbitan monooleate (Span 80) and cosurfactants (CoS) were ethanol and 1-butanol in the mass ratio S: CoS = 2:1. Viscosimetric and conductometric analyses revealed the transition state of the O/W, W/O microemulsions and bicontinuous structures. *In vitro* study of capsaicin release and permeability was performed using a Franz cell, with a cellulose membrane. As a liquid receiver was used a liquid mixture 1:1 (v/v) of ethanol and citric acid - disodium phosphate buffer, with pH = 7.4 at a temperature of 37^{o}C. The drug content of capsaicin was analysed by a HPLC technique. It was determined capsaicin optimal concentration of 0.12% (wt/wt). The microemulsions behaviour as a better vehicle than gel behaviour and ethanol action as a better permeation enhancer than 1-butanol were demonstrated. A clinical trial on capsaicin microemulsions use as improving method of blood collecting for obstructive pulmonary disease (OPD) diagnostic was conducted. The results confirm the high potential of microemulsions as topical drug vehicles.

Rezumat

În această lucrare s-a realizat un studiu privind prepararea şi caracterizarea unor microemulsii cu capsaicină. Microemulsiile s-au preparat folosind uleiul de soia, un amestec de apă și glicerol în raport masic de 4:1 și un amestec de Span 80 și etanol sau 1-butanol în raport masic de 2:1. Pentru stabilirea rapoartelor de amestecare corespunzătoare stării de microemulsie s-au construit diagramele pseudoternare de fază. Structura microemulsiilor s-a determinat prin măsurători conductometrice și vîscozimetrice. S-a realizat un studiu *in vitro* de eliberare a capsaicinei din microemulsii și hidrogel.

S-a studiat posibilitatea folosirii microemulsiilor cu capsaicină la îmbunătăţirea metodelor de recoltare de sânge în vederea diagnosticării bolilor pulmonare obstructive cronice. Rezultatele obţinute arată faptul că microemulsiile cu capsaicină, ca forme farmaceutice vasodilatatoare, favorizează prelevarea intracapilară a probelor de sânge.

Keywords: capsaicin, microemulsions, capilar blood gas measurements

Introduction

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is an active component, which together with other compounds from capsaicinoide class as dihydrocapsaicin, nordihydrocapsaicin, is found in chili peppers, a plant which belongs to *Capsicum* species. Because of burning sensation, capsaicin is used in food as spicy seasoning. In medicine, capsaicin is used in rheumatoid arthritis, osteoarthritis, diabetic neuropathy treatment and post herpetic neuralgia. Repeatedly applied to the skin, capsaicin causes desensitization of nociceptive fibers, reduction of nerve conduction and blocking neuromediators. By a regular use of capsaicin, the burning effect reduces the amount of substance P, which acts as a chemical mediator of pain. Side effects of capsaicin are mainly burning, irritation, redness at the application site etc. Due to its irritant action, common treatment with capsaicin is applied on skin areas. Capsaicin topical action mechanism involves its binding at cutaneous nociceptors, initially causing the neurons excitation and a period of increased sensitivity, followed by a susceptibility reduction to nociceptors desensitization [7].

Due to its vasodilatory action, capsaicin is also used in pulmonary disease diagnosis [6]. The most commonly used doses of capsaicin, clinically tested for neuropathy pain treatment are between 0.025%(wt/wt) and 0.075%(wt/wt). Although most commonly used formulations are gels, creams and emulsions, lately more and more studies assessed the topical capsaicin administration in the form of microemulsion [1]. Microemulsions are thermodynamically stable, transparent, optically isotropic systems, having low viscosity and droplet size between 5-100 nm. It is prepared by mixing the hydrophilic with lipophilic phase in the presence of a mixture of surfactant and cosurfactant. Cosurfactants are generally short-chain saturated alcohols (C2-C6). Generally, microemulsions comprise three types of microstructures: water-in-oil microemulsion, bicontinuous structures and oil-in-water microemulsion [5].

Researches have highlighted microemulsions use in parenteral and oral drug administration, and especially in topical administration way. Microemulsions use as vehicles for drug delivery presents many advantages, like: stability, bioavailability, absorption, release and transdermal permeation etc. [10].

The aim of this study is represented by preparation, characterization of capsaicin microemulsions and capsaicin *in vitro* permeability evaluation related to other formulations. It was accomplished a study on capsaicin microemulsions use for improving the technique of capillary blood collecting for chronic obstructive pulmonary disease diagnosis.

Materials and Methods

Materials

Soybean oil, sorbitan monooleate, ethanol and 1-butanol were obtained from Sigma Chemical Co (St Louis, MO). Oleoresin, natural extract from *Capsicum frutescens L* with capsaicin content of 6% and pure capsaicin were supplied by Inres (France). Propylene glycol, glycerol, sodium carboxymethylcellulose (CMCNa), methanol, acetonitrile (*p.c.*) were supplied by Merck KgaA Co. Germany. Cellulose membrane: Spectra-por 2, molecular weight from 12.000 to 14.000 was obtained from Spectrum Co., USA. NaOH (0.1 M), distilled water (ppi QSP), the buffer citric acid / disodium phosphate, pH = 7.4. All reagents and solvents used were of pharmaceutical grade, not purified and that corresponded to FR X protocols.

Determination of capsaicin

Determination of capsaicin from oleoresin or the receiver fluid in Franz cell was performed by HPLC method.

In a stopper vial 10 mg of capsaicin were dissolved in 50 mL ethanol. A dilution of 1 / 3, using a syringe this made and the solution obtained was filtered through a microporous filter. In another stopper vial, 150 mg of oleoresin were taken and dissolved in 100 mL ethanol. The solution was filtered through a microporous filter.

There were taken 3.5 g of sample and were dissolved in 100 mL ethanol. It was shaken by ultrasound technique for 10 minutes, and then the solution obtained was filtered using a microporous filter. Samples were analyzed using high performance liquid chromatography. It was used a Waters HPLC chromatographic system 27027.

Chromatographic conditions: Waters 515 pumps; column: Symmetry C18 (259x4, 6x5µm100 Å); mobile phase: water (0.4% H_3PO_4) / acetonitrile (30:70 v / v); flow: 1 mL / min; UV detector: 225 nm; injection volume: 10 mL.; temp. 40 °C in the column; retention time 4.9 min. for capsaicin and 6 min. for dihydrocapsaicin; analysis time: 10 min.

A calibration curve (peak area *versus* drug concentration) was constructed by running standard capsaicin solutions in ethanol for each series of analysed samples.

Preparation of Water –in-Oil (W/O) capsaicin microemulsions

Oleoresin was dissolved in cosurfactant (ethanol, 1-butanol). The capsaicin content in microemulsions was: 0.08%, 0.12% and 0.24% (w/w). Surfactant : Cosurfactant mass ratio each sample was 2:1 (wt/wt).

The oily phase, represented by a mixture of soybean oil and alcohol in 1:1 mass ratio, was mixed with the surfactant (Span 80, HLB = 4.3) in the following mass ratios: 1:9, 2:8, 3:7, 4:6 , 5:5, 6:4, 7:3, 8:2, 9:1. These

mixtures were titrated with the aqueous phase containing water and glycerol in 4:1 (wt/wt), at temperature of 25°C.

Samples were gently shook and left to stand for 6 hours. At equilibrium obtaining, the number of phases is observed and their structures were microscopically studied [11]. Microemulsions formed were stable and remained clear and transparent for 12 months.

Preparation of hydrogel capsaicin

In a mortar 7 g of CMCNa were finely triturated. The fraction was separated by sieving fine white powder and 2.5 g from this powder were placed in a stainless steel bowl. 20 g propylene glycol were added under vigorous stirring, followed by portions of oleoresin ethanol solution (1.43 g) and the mixture was stirred until homogenization. Then, 57 g of pure water and 3.5 g of NaOH 0.1 M were successively added. The tank was placed in a warm water bath and mechanically mixed for 45 minutes. A consistent gel was obtained using a storage period of two months.

Viscosity measurement

Samples viscosity was measured at 25°C with Brookfield DV-III rheometer, using a cone-plate system with 60 mm diameter and 1-degree angle. Shear speed was 10-20 s^{-1}.

Electrical conductivity measurement

The electrical conductivity was measured at a temperature of 25°C using Consort 868 multiparameter device, with SK10B conductivity electrode type, characterized by a cell constant of 1.0 cm^{-1}. Conductivity measurement was performed in triplicate at oil-surfactant-cosurfactant-capsaicin mixture titration with aqueous phase along dilution line AB. The limit of conductivity measurement error was ± 0.02 μS cm^{-1}.

In vitro capsaicin release study

In vitro experiments concerning capsaicin release and permeation from the analyzed samples were made using a Franz diffusion cell in steady state, using the cellulose membrane as a penetration barrier. The diffusion cell has an area of 2.15 cm^2 and a receiver volume of 5 mL. As a receiver medium it was used a mixture of 1:1 (v/v) ethanol / phosphate buffer solution pH = 7.4.

1 g of sample with capsaicin was added into donor compartment, being covered with paraffin film. The liquid from receiver was thermostated at 37°C and mixed continuously with an electromagnetic stirrer. At intervals of 20 minutes were taken 200 μL of liquid sample from the receiver, and instead it was added the same volume of fresh solution.

Samples were analyzed by HPLC method, by calculating the cumulative quantity of capsaicin per unit surface area cellulose membrane ($\mu g \cdot cm^{-2}$).

Permeation rate in steady state conditions (steady-state), J ($\mu g \cdot cm^{-2} \cdot h^{-1}$) of capsaicin through cellulose membrane was calculated from the function slope $Q_t = f(t)$, where Q_t represents capsaicin cumulative quantity passed through the membrane.

The cumulative drug permeation (Q_t) was calculated from the following equation [4]:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \qquad \text{Eq (1)}$$

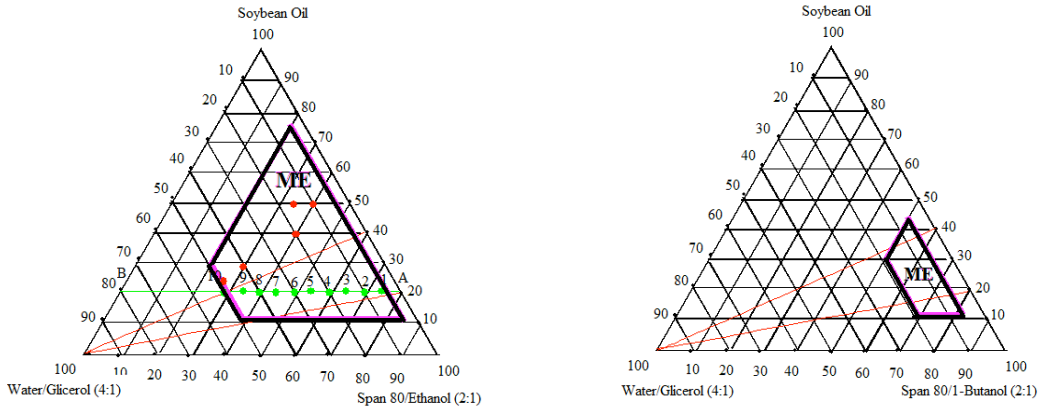
where: C_t is the drug concentration of the receiver solution at each sampling time, C_i the drug concentration of the ith sample, and V_r and V_s are the volumes of the receiver solution and the sample, respectively. Data were expressed as the cumulative capsaicin per unit of membrane surface area Q_t/S (S = 2.15cm^2). The steady-state fluxes (J_{SS}) were calculated by linear regression interpolation of the experimental data at a steady state:

$$J_{SS} = \frac{\Delta Q_t}{\Delta t \cdot S} \qquad \text{Eq (2)}$$

Results and discussion

Pseudo-ternary phase diagrams

In order to find the corresponding components mixing ratio of the microemulsion state, pseudo-ternary phase diagrams were plotted (Figure 1).



(a) (b)

Figure 1

Pseudo-ternary phase diagram of microemulsion system made of soybean oil/span 80/water/glycerol/ethanol/capsaicin (a) and soybean oil/span 80/water/glycerol/1-butanol/capsaicin (b)

In the layout phase diagram, two groups of microemulsions with capsaicin were prepared and studied, one in which ethanol was used as cosurfactant, and another one that used 1-butanol as cosurfactant (Table I).

Table I

Composition of microemulsions with capsaicin and the steady-state flux penetration through cellulose membrane

Formula	Soybean Oil %(wt/wt)	Aqueous phase (water/glycerol 4:1) %(wt/wt)	Span 80 %(wt/wt)	Ethanol %(wt/wt)	1Butanol %(wt/wt)	Oleoresin % (wt/wt)	J_{SS} ($\mu g \cdot cm^{-2} \cdot h^{-1}$)
M1	20	18.67	40	-	20	1.33	5.529 ± 0.75
M2	20	18	40	-	20	2.00	8.864 ± 0.16
M3	20	16	40	-	20	4.00	4.306 ± 0.40
M4	20	18.67	40	20	-	1.33	11.129 ± 0.59
M5	20	18	40	20	-	2.00	12.400 ± 0.34
M6	20	16	40	20	-	4.00	6.390 ± 0.23
G	-	-	-	-	-	2.00	2.494 ± 0.32

Each value represents the mean ± standard deviation (S.D), (n = 3).

Phase diagrams were used to determine the components concentration in order to establish the microemulsions appropriate field. In microemulsions preparation, cosurfactants are designed to minimize, in addition to surfactant, the interfacial tension. They are adsorbed at O/W interface and they modify the interfacial membrane flexibility, favoring microemulsions formation. Considering the aqueous phase absence conditions, alcohol molecules interact with surfactant polar groups through hydrogen bonds, affecting critical packing parameter. At water addition, the surfactant polar groups are targeted toward the water droplet center, while the hydrophobic part is immersed in the continuous oily phase. In this way, W/O microemulsions are obtained.

The alcohols with short chains, like ethyl alcohol, determine surfactant film flexibility increasing and destabilize the liquid crystal phase, favorable to microemulsions formation [12]. Considering ethyl alcohol case, the area corresponding to the monophasic surface represents 60% of the phase diagram. When using 1-butanol the corresponding microemulsion domain presented a surface of 15%, due its increased solubility in oily phase [2].

In accordance with dilution line AB (Figure 1), the first ten samples obtained by water titration at 5% (wt/wt) difference are homogenous, transparent and slightly yellow-orange colour. These samples correspond to the microemulsion state. With increasing water content above 55% (wt/wt), at lower surfactant concentrations, the samples start to get turbidity or the phase separation occurs.

Viscosity and electrical conductivity

Viscosity depends largely on microemulsions structure, the type and micelle aggregates shape, the dispersed particles concentration and interactions. Therefore, viscosity measurements provide useful information

about phases alternation and structural changes in a microemulsion. All the samples showed a Newtonian flow.

If the viscosity of appropriate line AB samples is analyzed, it appears that for a concentration of 0%-30% in water (wt/wt), viscosity values are relatively small and slightly increasing from 8.77 to 11.65 mPa·s, while for a concentration of 30%-45% in water (wt/wt), the viscosity decreases from 11.65 mPa ·s to 10.55 mPa·s, then increases again to 11.21 mPa·s.

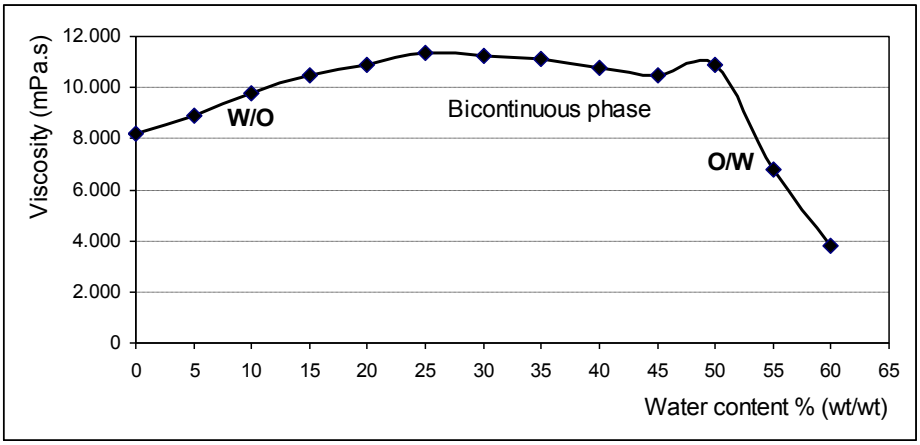


Figure 2

Viscosity as a function of water percentage solubilized in the microemulsion system. The system contains soybean oil/monooleat sorbitan (1:4) with CoS/S ratio of 0.5.

The existence of two peaks on the viscosity curve with increasing water content explains the system passing through three states: W/O microemulsion, bicontinuous structure and O/W microemulsions. The viscosity increase for the samples with 0%-30% in water (wt/wt) is explained by water droplets organisation in clusters (Figure 2). The slight decrease of viscosity with increasing water content indicates a transition from W/O microemulsions to bicontinuous structure.

The sharp viscosity decrease with increasing water content from 55% (wt/wt), explains the system transition from the bicontinuous structure to O/W microemulsion, where water, the external phase presents a lower viscosity than oil. These viscosity changes explain the percolation phenomenon, also highlighted by electrical conductivity (Figure 3).

Ions absence led to very low values of microemulsions electrical conductivity. To highlight the structural changes, an aqueous solution of 0.01M NaCl was used [3]. In these conditions, changes in electrical conductivity were revealed due to increased water content. At low water

content 0-10% (wt/wt), conductivity presents low values of about 10.15 μS/cm, followed by a slight increase till 125.76 μS/cm, corresponding to a water content of 35-45% (wt/wt), then faster growth after this value (Figure 3). Conductivity increasing above percolation limit is due to ions transfer from one drop to another along the water channels that appear in oily phase as a result of attractive interaction between water droplets. These variations, which approximately correspond to viscosity variations, are due to microemulsions W/O transition, with low conductivity in O/W microemulsions where the continuous aqueous phase contributes to conductivity increase. High electrical conductivity values above percolation limit are due to Na^+ and Cl^- ions presented in the external phase.

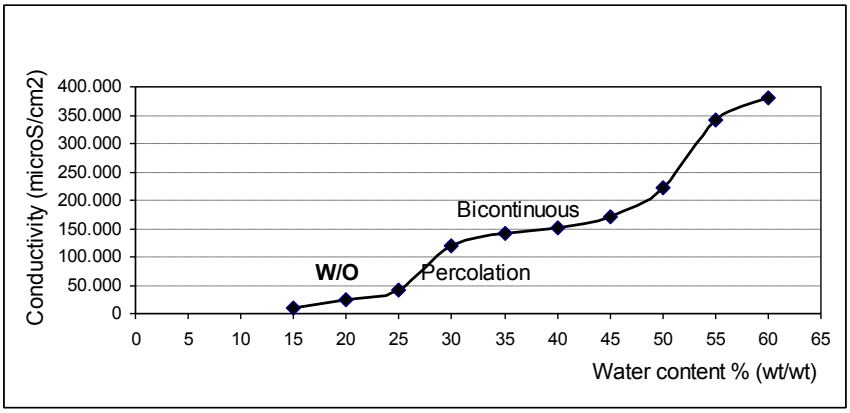


Figure 3

Electrical conductivity as a function of the water content percentage solubilized in the microemulsion system M5. The system contains soybean oil/monooleat sorbitan (1:4) with CoS/S ratio of 0.5.

In vitro release of capsaicin from microemulsions and CMCNa hydrogel

The aim of this paper was a comparison between the capsaicin release from microemulsions and CMCNa hydrogel. The cumulative amount variation of capsaicin released from microemulsions and hydrogel was comparatively represented.

The two mixing reports O/W/S and S/CoS are the same in all the microemulsions. The only difference between them is represented by cosurfactant nature and capsaicin concentration.

Graphical representation of $Q_t = f(t)$ function in figure 4, shows the cumulative capsaicin release from microemulsions M1, M2, M3, M4, M5, M6 and the CMCNa hydrogel, in the range of 10-360 minutes.

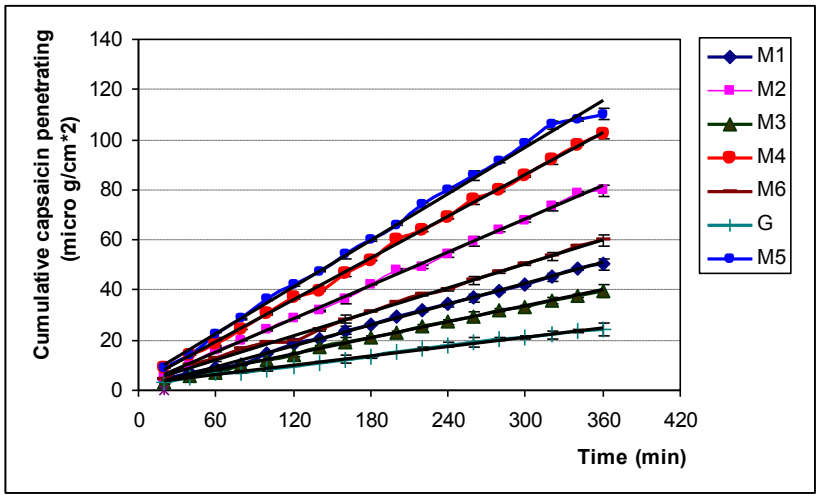


Figure 4

In vitro release profiles of capsaicin through cellulose membrane from microemulsions M1- M6 and CMCNa hydrogel with capsaicin 0.12% (G)

Cumulative intake values determined 360 minutes after the beginning of the experiment, for capsaicin microemulsions were: Q_{M1} = 50.52±0.56µg·cm^{-1}; Q_{M2} = 79.74±0.75µg·cm^{-1}; Q_{M3} = 39.72±0.43µg·cm^{-1}; Q_{M4} = 102.48±1.06µg·cm^{-1}; Q_{M5} = 110.23±0.82µg·cm^{-1}; Q_{M6} = 59.87±0.31µg·cm^{-1} and for hydrogel with 0.12% wt/wt capsaicin , Q_G = 24.38±0.18.

Furthermore, the determined flux values flow values (t-test, $p<0.05$) show better microemulsions permeation in relation with hydrogel, and the permeation optimal concentration of capsaicin, which is 0.12% wt/wt, both in ethanol and butanol microemulsions.

Higher values of ethanol microemulsions flux is due to higher capsaicin solubility in ethanol and its hydrophilic action on cellulose membrane, favoring the diffusion phenomenon. Studies have also shown that an increasing amount of alcohol leads to decreased diffusion of active substances [9].

Also, in this study it was used a mixture of water/glycerol as the aqueous phase, whose optimal ratio was set at 4:1 (wt/wt). Glycerol can play both the role of cosurfactant and a viscosity modifier agent. Glycerol content increasing determined an increased viscosity, leading to a decreased diffusion process and capsaicin permeation through cellulose membrane.

Applications of capsaicin microemulsions in obstructive pulmonary disease diagnostic

One of the objectives of this study was the use of capsaicin microemulsions to the intracapillary of blood sampling for the diagnosis of chronic obstructive pulmonary disease. The study was based on the vasodilator action of capsaicin [6]. In this way, the results concerning O_2 and CO_2 arterial pressures $P_{(a)_{O_2}}$, $P_{(a)_{CO_2}}$ (standard method) were compared from O_2 and CO_2 capillary pressures $P_{(c)_{O_2}}$, $P_{(c)_{CO_2}}$, after using capsaicin microemulsion with ethanol (0.12% capsaicin) and CMCNa hydrogel with 0.12% (wt/wt) capsaicin.

Tests were conducted on a total of 50 patients of different age and sex, from North Hospital in Marseille. The study was approved by the Hospital's Ethics Committee. Capsaicin microemulsion was topically administered on the ear lobe, and the blood samples were taken from this area after 60 min.

There were statistically analyzed the differences between $P_{(a)_{O_2}}$ and $P_{(a)_{CO_2}}$ obtained using standard arterial blood gas measurements and the same value obtained using capillary blood gas measurements ($P_{(c)_{O_2}}, P_{(c)_{CO_2}}$) [8]. A linear distribution of values was obtained after a critical study of the diagrams. The correlation coefficients obtained were $r^2 = 0.9837$ ($p = 0.05$) for CO_2 and $r^2 = 0.9841$ ($p = 0.05$) for O_2.

For 95% confidence level, the statistical deviation is below 1.5% for both gases, which supports the accuracy of the proposed method.

Note that were tested other microemulsions with capsaicin and it was showed that at 0.08% concentration, the results are inconclusive due to low penetration ($p<0.05$) and at a concentration of 0.24% occurred skin irritation. Regarding the use of capsaicin gel, results were poor, resulting in errors of over 0.05 ($p = 0.021$).

Conclusions

In this study we analyzed five microemulsions W/O as potential vehicles for the transdermal release of capsaicin. Pseudoternare phase diagrams were plotted highlighting the appropriate areas of microemulsion state. 1- Butanol determines a total one-phase area smaller than in the case of ethanol.

Phase transitions at water content increasing were highlighted by viscosimetric and conductometric measurements.

In vitro permeation study of capsaicin through cellulosic membrane on strictly sink conditions showed that the highest values of the steady-state flux are obtained for a microemulsion containing 0.12% (wt/wt) capsaicin and ethanol, compared with 1-butanol microemulsion, respectively of hydrogel CMCNa.

The microemulsion with 0.12% (w/w) capsaicin and ethanol was used in capillary blood gas measurements. CO_2 and O_2 pressure values are comparable with those obtained by standard arterial blood measurement. It was demonstrated that microemulsions can be used as vehicles for capsaicin in transdermal delivery, having superior properties in comparison with gels.

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