

# Formulation, Preparation , Physico-Chemical, Microbiological Analysis and Clinical Uses of Capsaicin Microemulsions

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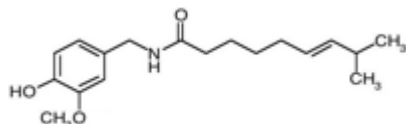
*The present paper aims at creating a pharmaceutical formulation, i.e. microemulsion with capsaicin as the active principle; the purpose was to solubilize capsaicin and draw up pseudoternary phase diagrams establishing the microemulsion domains. We created a pharmaceutical form: capsaicin ointment, aiming at releasing capsaicin in the microemulsion and ointment bases. Comparative microbiological determinations were performed between microemulsion and ointment. The novelty of the study is formulating microemulsions as a means of solving solubility issues and increasing the bioavailability of medicinal substances.*

**Keywords:** capsaicina, microemulsion, gram negative germs, gram positive germs

The purpose of the paper is the answer to an urgent need of today's scientific community, i.e. applying nanotechnologies to medicine functionalization. In this respect, we aimed at including capsaicin in stable nanostructured thermodynamic systems of the microemulsion (nanoemulsion) type, in order to obtain a pharmaceutical form with potential topical application. Last but not least, we can create retard disperse encapsulated systems applicable in various forms of cancer.

## Experimental part

### Materials and methods



**Capsaicin** C<sub>18</sub>H<sub>27</sub>O<sub>3</sub>N

Chemically speaking, capsaicin is 8-methyl-N-vanillyl-6-nonenamide.

Capsaicin, the active component in chilli pepper (*Capsicum annuum*) is an alkaloid containing 0.02-0.03% capsaicin, giving the pepper its spicy burning taste. It is an irritant to the mammalian epithelium, creating a burning sensation in the mouth, which may be considered a spicy taste element. Plants produce capsaicin in order to discourage their consumption by animals[1].

In medicine, internally used capsaicin intensifies the activity of the suprarenal cortex and the secretion of corticosteroid hormones, stimulating digestion. Intravenously administered capsaicin produces apnea and blood pressure decrease. Externally, capsaicin alcoholic tincture or various lotions, skin patches or creams are used in treating chronic pain (diabetic neuropathy, post-zoster algalias, other neuralgias, joint pains, lumbago, bladder hypersensitivity) and itching associated to certain dermatoses (anal itching, etc.) or terminal renal failure. [2,3]. The antalgic action mechanism is given by the inhibiting action upon substance P.

Applying capsaicin on the skin in moderate doses triggers a heating sensation, and in high doses a burning sensation. Certain Italian studies show the important role of orally-administered capsaicin in bladder cancer as it causes the death of cancer cells [4]. Capsaicin is a blood stream activator as well as an anti-inflammatory agent in rheumatismal processes, like ibuprofen [5-7], diclofenac, profenid, black pepper, curcumin, hot pepper etc. Capsaicin introduced in the blood stream through gel as a pharmaceutical formula plays a part in reducing internal bleeding, healing gastro-duodenal ulcers and protecting against haemorrhagic strokes[8]. Studies carried out at Harvard University indicate the use of capsaicin in treating neuropathic pain in post herpetic neuralgia. It may be used for atypical facial pain (caused by the irritation of the trigeminal nerve innervating the face area). Another role of capsaicin is stimulation of the immune system. Administering capsaicin attracts the cells of the immune system that help destroy bacteria making it useful in antibiotic-resistant infections, and inactivating toxic substances issued from cellular destruction and adverse allergic and non-allergic reactions, the potential adverse effects of common drugs being well-known in medical practice of various specialties [9-21]. A study performed in India showed that a diet rich in spices and chilli peppers (with high capsaicin levels) and low in proteins leads to good results in decreasing body weight and normalizing triglycerides in the body.

The most commonly used capsaicin doses that were clinically tested in treating neuropathic pains range between 0.025%, 0.075% and 0.1% and under gel form [22]

We used capsaicin in concentrations of 1 and 5% that we introduced in the microemulsion and the ointment bases (1.5 and 10%)

The microemulsion contains: Oil phase: Flaxseed oil + capsaicin (3:1); Aqueous phase: distilled water + glycerine (4:1); Emulsifier: Tween 80 ;Coemulsifier: ethyl alcohol

The ointment bases: I(1%), II(5%), III (10%) contain (tab.1) the following:

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All authors had an equal contribution to this work.

**Table 1**  
CONTENT OF OINTMENT BASES

| Components<br>(g) | Formula |      |      |
|-------------------|---------|------|------|
|                   | I       | II   | III  |
| Lanolin           | 2       | 2.5  | 5    |
| Vaseline          | 17.8    | 16.3 | -    |
| Cholesterol       | -       | 0.2  | 1.25 |
| Cetaceum          | -       | -    | 4    |
| Paraffin oil      | -       | -    | 7.75 |
| Capsaicin         | 0.2     | 1    | 2    |

Other materials: distilled water, glycerol, flaxseed oil, ethyl alcohol, tween, coemulsifiers.

The lipophilic phase is made up of aliphatic, aromatic hydrocarbons, esters, triglycerides, vegetable oils, lanolin, fatty alcohols, mineral fats, as well as various lipophilic substances.

Emulsifiers are selected according to the administering manner. Ionic, non-ionic and amphoteric derivatives are used, such as: sorbitan esters, ethylated fatty alcohols, saturated C<sub>8</sub>-C<sub>10</sub> polyglycolized glycerides, saccharose esters, PEG ethers and esters, betains and lecithins.

Coemulsifiers: alcohols, glycols, propylene glycol derivatives, polyglycerol derivatives, aromatic alcohols like benzyl alcohol, aliphatic polyols like ethyl-2-hexandiol 1,3 and glycol esters.

#### Ointment bases

Ointment bases are made up of excipient mixtures, and rarely of just one. Quantitatively speaking, they account for the most part of an ointment and give the preparation the desired consistency [23]

The most precise and modern method of microemulsion preparation is based on establishing pseudoternary diagram, that had the emulsifier (surfactant - S)/coemulsifier (cosurfactant - CoS) (S/CoS) ratio fixed [24].

These pseudoternary phase diagrams vary the ratio of water, oil, emulsifier, coemulsifier, determining the area where the formation of microemulsion takes place.

Drawing up a phase diagram, to the purpose of localising the microemulsion areas, is practically achieved through titration. Starting from a definite S/CoS ratio, various mixtures are made between the lipophilic phase and S/CoS. In this system the aqueous phase is added drop by drop. After each addition, the mixture is gently stirred, for homogenization purposes, then examined between cross-polarized filters. The optical aspect is noted: transparency, opalescence, isotropy and phase number. Thus we obtain a definition of borders that may achieve mixtures, point by point, starting from 4 components.

Pseudoternary diagrams are used, in which two components are grouped in order to form a pure pseudo constituent. A constant emulsifier/coemulsifier ratio is commonly used as a pseudoternary constituent. Tracing the specific concentrations water/oil/surfactant may be used by means of figure 1.

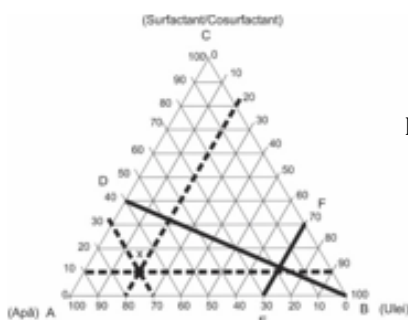


Fig.1. Tracing pseudoternary phase diagrams

## Results and discussions

In order to prepare the capsaicin microemulsion we drew up the phase pseudoternary diagrams by means of which the mixture ratios were found. The phase diagrams varied the ratio of water, oil, emulsifier and coemulsifier and determined the area where the microemulsion was formed.

It was found to be stable in the mixing ratio oil (O), water (W), emulsifier (surfactant-S) and coemulsifier (cosurfactant - CoS) of 1:1:2:1 (mass ratio) (fig.2).

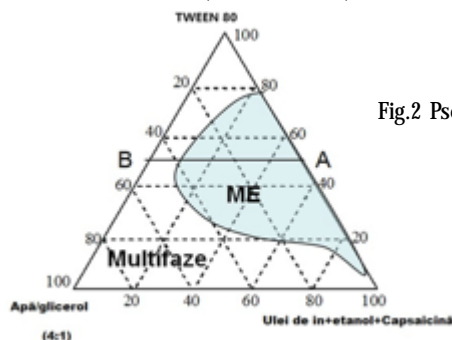


Fig.2 Pseudoternary phase diagram

Viscosity was measured (fig.3). The existence of the two peaks in the viscosity curve with the increase of water content explains the passage of the system through the three states: microemulsion W/O, bicontinuous structure, and microemulsion O/W.

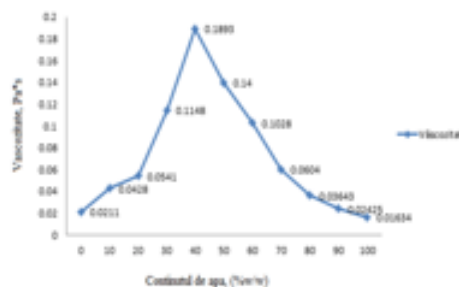


Fig.3. Viscosity measurement

Electrical conductivity is measured (fig.4). These variations are the expression of the transition from W/O microemulsions with low conductivity, to O/W microemulsions, where the continuous aqueous phase increases conductivity.

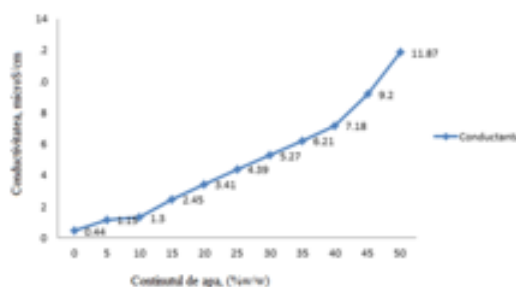


Fig.4. Electrical conductivity measurement

The emulsion type we determined was W/O.

The stability of the microemulsion was determined as compared to ointments. In order to shorten the time necessary to study the stability of pharmaceutical preparations, the emulsion and ointment bases were subjected to unfavourable conditions, and extreme stress conditions respectively, by means of high temperatures (boiling), and low temperatures (freezing).

A temperature stability study was initiated for the capsaicin microemulsions and ointment bases. The capsaicin microemulsions maintained their characteristics for 6 months after preparation, without alterations in colour, homogeneity and transparency. The capsaicin ointment bases remained stable for 6 weeks.

As a result of the studies, it was found that their stability was also preserved in case of temperature variations.

After freezing and bringing back to room temperature, both the microemulsion and the ointment bases containing capsaicin kept their homogeneous structure without phase separation.

#### Releasing capsaicin from microemulsion and ointment bases

*In vitro* experiments regarding the release and permeation of capsaicin from the samples analysed were carried out by means of a Franz diffusion cell, in stationary mode, using a cellulose membrane as a penetration barrier. The cell has a diffusion area of 2 cm<sup>2</sup> and a receptor volume of 5 mL. As a receptor environment we used a mixture 1:1 (v/v) ethanol/buffer citrate-phosphate pH 7 (Wang). In the donor compartment we added 1.755 g formula III capsaicin sample, and 5 mL microemulsion respectively, covering them with a paraffin film. The liquid in the receptor was thermostated at 37°C and continuously stirred with an electromagnetic agitator. Every 10 min a 2 mL sample was taken from the receptor liquid, and the same volume of fresh solution was added in the same place. The spectrometry samples UV-Vis were examined, calculating the cumulative capsaicin amount per area unit of the cellulose membrane (µg.cm<sup>-2</sup>).

The Franz cell (fig.5) adapted and used in our experiment consists of a donor space, where the sample to analyse is administered, and a receptor space where the tampon solution phosphate- citric acid pH 7.4 is introduced and the active substance (capsaicin) concentration released in time is determined.

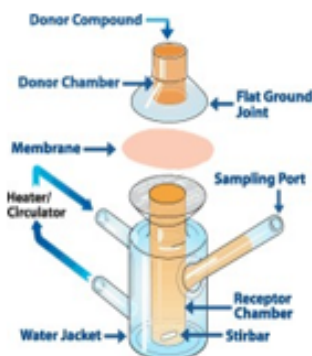


Fig.5. Franz cell

It may be concluded that during the interval 0-60 min the release takes place according order 1 kinetics (fig.6).

Similarly, it was noted that capsaicin release is better in microemulsion as compared to ointment bases (table 2).

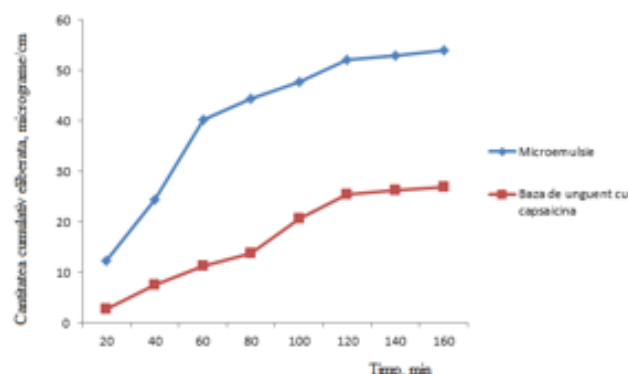


Fig.6.Release of capsaicin microemulsion and ointment

**Table 2**  
VARIOUS FORMULAS OF CAPSAICIN RELEASE

| Time, min | Microemulsion (µg/cm <sup>2</sup> ) | Capsaicin ointment base (µg/cm <sup>2</sup> ) |
|-----------|-------------------------------------|---|
| 20        | 12.346                              | 2.739   |
| 40        | 24.427                              | 7.485   |
| 60        | 40.233                              | 11.249  |
| 80        | 44.367                              | 13.873  |
| 100       | 47.862                              | 20.722  |
| 120       | 52.219                              | 25.361  |

#### Determination of antimicrobial activity

In order to assess the antimicrobial activity of capsaicin by the diffusimetric method we measured the diameter of the inhibition areas in the microemulsions and ointment bases under analysis.

The results obtained upon analysing the antimicrobial activity of capsaicin microemulsions and ointment bases on Gram negative bacteria are seen in table 3, figure 7 and figure 8.

**Table 3**  
ANTIMICROBIAL ACTIVITY OF MICROEMULSIONS, ALCOHOL, OINTMENT BASES AND GENTAMICIN ON SOME GRAM NEGATIVE BACTERIA

| Gram negative bacteria                 | Micro-emulsion                   |    | Alcohol 96° | Gentamicin | Ointment bases |    |     |
|--|----------------------------------|----|-------------|------------|----------------|----|-----|
|  | 1%                               | 5% |             |            | I              | II | III |
|  | Diameter of inhibition area (mm) |    |             |            |                |    |     |
| <i>Enterobacter hormaechei</i> (EnteB) | 10                               | 12 | 6           | 20         | 0              | 0  | 0   |
| <i>Escherichia coli</i> (EC)           | 0                                | 12 | 6           | 17         | 8              | 0  | 0   |
| <i>Pseudomonas aeruginosa</i> (PA)     | 12                               | 14 | 8           | 30         | 11             | 10 | 12  |

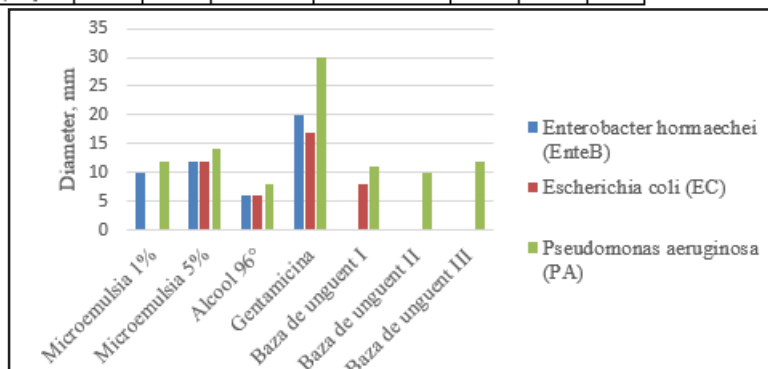


Fig.7.Testing inhibition capacity on gram negative bacteria



Fig.8. Activity on gram negative germs

**Table 4**  
ANTIMICROBIAL ACTIVITY OF MICROEMULSIONS, ALCOHOLS, OINTMENT BASES AND GENTAMICIN ON SOME GRAM POSITIVE BACTERIA

| Microbial strains                         | Microemulsion                    |    | Alcohol 96° | Gentamicin | Ointment bases |    |     |
|---|----------------------------------|----|-------------|------------|----------------|----|-----|
|   | 1%                               | 5% |             |            | I              | II | III |
|   | Diameter of inhibition area (mm) |    |             |            |                |    |     |
| <i>Staphylococcus aureus</i> (SA)         | 0                                | 8  | 10          | 16         | 0              | 0  | 0   |
| <i>Streptococcus pyogenes</i> (Spy)       | 22                               | 25 | 14          | 20         | 8              | 11 | 14  |
| <i>Streptococcus pneumoniae</i> (Spn)     | 17                               | 19 | 10          | 16         | 7              | 9  | 12  |
| <i>Enterococcus casseliflavus</i> (EnteC) | 0                                | 0  | 0           | 12         | 0              | 0  | 0   |

The highest antimicrobial activity on some Gram negative bacteria was registered for the 5% capsaicin microemulsion -bacteria *Enterobacter hormaechei* and *Pseudomonas aeruginosa*, and the lowest antimicrobial activity was seen in ointment bases I, II and III upon EntE and EC.

The antimicrobial activity of microemulsions and ointment bases upon some Gram positive bacteria are shown in table 4, figure 9 and figure 10.

The 5% microemulsion had better antimicrobial activity on the *Streptococcus pyogenes* bacteria (diameter of the inhibition area 25 mm) as compared to the 1% microemulsion (diameter of the inhibition area 22 mm)

When using ointment bases, the activity of formula III (inhibition diameter 14 mm) was slightly superior to the efficiency of formulas I and II (diameters 8 mm, and 11 mm respectively).

Table 5 and figure 11 show data on the antimicrobial activity of capsaicin microemulsions and ointment bases, alcohol 96° and fluconazole on the *Candida albicans* fungus.

According to the data in figure 11, in case of the *Candida albicans* fungus, fluconazole had a remarkable antimicrobial potential (inhibition area diameter 20 mm), unlike the microemulsion and ointment bases, which had smaller inhibition diameters (inhibition area diameter 12 mm, and 0 mm respect

The results of the study show that the 5% microemulsion had a higher antimicrobial activity than ointment bases, but inferior to gentamicin and fluconazole.

Ethyl alcohol 96° showed a low antimicrobial effect, but in association to the microemulsion under study it led to favourable results.

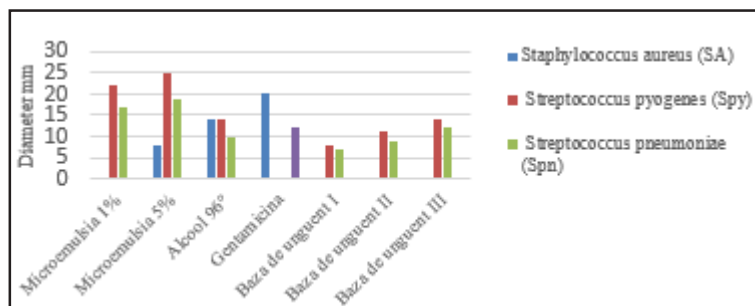


Fig.9. Inhibition capacity on Gram positive germs



Fig.10. Activity on gram positive germs

| Fungi                   | Microemulsion                    |    | Alcohol<br>96° | Fluconazole | Ointment bases |    |     |
|-------------------------|----------------------------------|----|----------------|-------------|----------------|----|-----|
|                         | 1%                               | 5% |                |             | I              | II | III |
|                         | Diameter of inhibition area (mm) |    |                |             |                |    |     |
| <i>Candida albicans</i> | 0                                | 12 | 5              | 20          | 0              | 0  | 0   |

**Table 5**  
ANTIFUNGAL ACTIVITY OF MICROEMULSIONS, OINTMENT BASES, ETHYL ALCOHOL AND FLUCONAZOLE

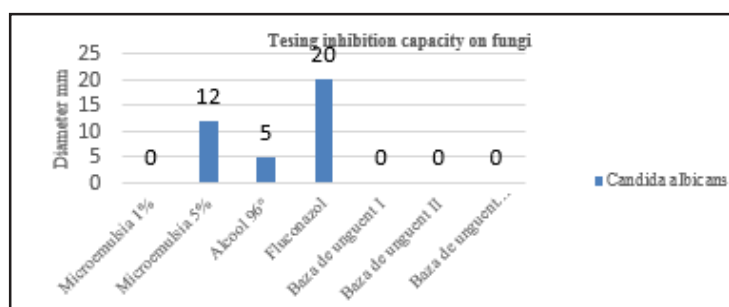


Fig. 11. Diameters of inhibition areas for fungi



## Conclusions

It was found that the microemulsion under study is of the water in oil type (W/O), proving that the capsaicin microemulsion scored higher in temperature variation as compared to ointment bases, thus it may be considered that the microemulsion has better stability in time without property alteration.

It was evinced that there is better capsaicin in microemulsion as compared to ointment bases by means of the Franz diffusion cell, using a cellulose membrane as a penetration barrier.

The study proved that the microemulsion has a much higher antimicrobial activity than the ointment bases, but lower than gentamicin and fluconazole in case of reference microorganisms. Taking these results into account, it may be concluded that the microemulsion pharmaceutical form may include other active substances or natural oils like lavender, which may be a future method of destroying the bacteria in nosocomial infection, as well as a possible modulation of endosymbiont proliferation in various skin conditions like acne or rosacea (but taking into consideration the potential for local irritative action and only after the informed patient's consent for testing) [25,28]. Another research direction could be the use in pilar conditions like alopecia areata, or diffuse alopecia, on their own or associated to other conditions, including autoimmune or pregnancy-induced, observing the ethics of medical research [27, 28] Also, microemulsions could be obtained using natural extractive Kombucha solutions which are also remarkably effective in dealing with this type of microorganisms. Formulating microemulsions is a means to solve solubilisation problems and increase the bioavailability of medicinal substances.

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