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# O/W microemulsion and hydrogel formulation of methotrexate and comparison of releasing studies.

**Abdullah Levent ALPARSLAN[1*], Ercument KARASULU[2], Gulbeyaz Yildiz TÜRKYILMAZ[3], Dogan UVEY[4]**

1 Department of Pharmaceutical Technology, Faculty of Pharmacy, Istinye University, Istanbul, Turkey.
* Corresponding Author. E-mail: levent.alparslan@istinye.edu.trTel. +90-542-3134516.
2 Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University, Izmir, Turkey
3 Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University, Izmir, Turkey
4 Department of History of Pharmacy and Ethics, Faculty of Pharmacy, Istinye University, Istanbul, Turkey.

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**ABSTRACT**: Microemulsions are ideal carriers for poorly soluble substances. They increase the absorption of drugs with low bioavailability. Methotrexate (MTX), which is used internally in cancer and psoriasis and has many side effects and it is less soluble in water and its passage through the skin is problematic due to its high molecular weight. An O/W microemulsion formulation containing MTX has been developed in order to reduce the side effects of the drug, not to have a first pass effect on the liver, to increase its bioavailability in topical use and to provide ease of use to patients. The releasing profiles of the gel formulation, prepared by reducing the fluidity of these formulations by polymers, were investigated through the nylon membrane. Mostly W/O microemulsion systems are available due to design of preparations but in this study O/W microemulsions and gel forms containing MTX were designed for topical use. Innovation side of trials will inspire in vivo studies and clinical studies that may cause less harm to patients dermatologically and provide optimal effect.

**KEYWORDS**: Microemulsion; O/W; hydrogel; metotrexate; franzcell; topical, release

## 1.INTRODUCTION

Methotrexate (MTX) is a folic acid antagonist with antineoplastic activity. It has been applied parenterally and orally for years in the treatment of different dermatological indications like psoriasis, rheumatoid arthritis (RA), atopic dermatitis (1,2). Low-dose methotrexate has been preferred for the treatment dermatological and RA disorders due to several side effects like gastrointestinal disorders, hepatic dysregulations, pneumonitis, hematologic disorders, infections, and nephrotoxicity(3,4). When MTX is used topically, its benefit decreases along with its systemic side effects (5,6). Topical MTX has limited passive diffusion through the skin due to the thickness of the psoriatic plaque. Poor cutaneous absorption and penetration of topical MTX depends on high molecular weight and dissociative form at physiological pH (7-10).

Microemulsions are transparent and thermodynamically stable quaternary systems, composed of oil, water, surfactant and cosurfactant (11,12). They have higher absorption potential as transdermal drug delivery systems in comparison to emulsions and gels, owing to their very small droplet size, which is between 5 – 200 nm (12,13). Microemulsions are ideal delivery systems for steroids, hormones, diuretics, antivirals and peptides. Microemulsions are excellent candidates as potential drug delivery systems because of their improved drug solubilization, long shelf life, and ease of preparation. They increase the absorption of hydrophobic drugs with very low solubility. Thanks to the surfactant in formulation, it increases membrane fluidity and therefore permeability. Thus, drug absorption can be increased (14,15). The combination of cosurfactant and surfactant in the formulation provides very low surface tension. Due to the decrease in surface tension, the microemulsion is formed spontaneously. The biological compatibility of the surfactants to be used as pharmaceuticals and the molecular structure of the alcohol used as cosurfactant are also important. The ratio of surfactant to co-surfactant varies between 1:1 and 1:9. Surfactants in the

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microemulsion formulation reduce droplet size too (16-18). Microemulsion has the advantage of high moisturizing and permeability, and forms a drug depot in the skin after transdermal administration so as to realize the effective penetration of the drug. Therefore, microemulsions have great potential for topical and transdermal drug delivery especially for low soluble and low permeable drugs. Besides they are useful for skin disorders (19,20).

Since MTX is an acidic compound with low solubility lipophilic bases, it was considered microemulsion and its hydrogel form will be an ideal carrier for topical delivery (21,22). Many studies have demonstrated that microemulsion formulations improved transdermal and dermal delivery properties (23-25). Transdermal permeation of drugs are related with mobility of drug in carrier, release of drug from formulation and permeation of drug into skin. Mobility of active ingredients in microemulsions are facile (26,27). Oleic acid is a fatty acid and used as oil phase in microemulsions. Oleic acid perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multi lamellar stratum corneum. Hence it may induce highly permeable pathways in the stratum corneum (28-30).

The primary aim of our present study was to develop a stable O/W microemulsion formulation in order to apply topically by hydrogel and compare microemulsion and gel formulations in releasing studies. Mostly W/O microemulsion systems are available due to design of preparations. In this study O/W microemulsion formulation were evaluated to suggest a topical formulation with improved activity of MTX and gel form were experienced to compare as potential alternative for application.

## 2.RESULTS and DISCUSSION

### 2.1. Formulation studies of microemulsion and gels

Formulation codes were given according to the ratio of Surfactant/Cosurfactant.Tween 80 and Span 80 were used as surfactants. They were mixed easily and transparently. Propanol was cosurfactant and it supported the mixture of oil and water spontaneously as emulsion. Surfactant/Cosurfactant ratios were evaluated as 1, 1.5, 2, 2.5, 3, 3.5 and 4. While surfactants, cosurfactant and oil were mixed, water amount was evaluated thanks to adding to the system drop by drop. Water intake capacity of microemulsion was observed. In each ratio, while oil amount is 2, volume of water was listed from top to bottom from most to least (Table 1).

In formulation codes; surfactant /cosurfactant ratios were given. The sum of surfactants and cosurfactants was 8. According to the ratio of surfactants Tween 80, Span 80 and Propanol amounts were calculated. Water amount was observed after titration drop by drop. Internal phase was oil if the amount of water was higher.

For the water rich microemulsion formulation HLB value was designed depending on the amounts of surfactants. In order to establish O/W type microemulsion, ratios of oil were studied as 0.5, 1, 1.5 and 2 (Table 2) due to their higher water intake capacity. By the way the amounts of water intake capacity, percentages and optimum formulation were determined with the help of pseudoternary phase diagram (Figure 1). HLB value was 10,72 [0,6 x 15 (*60 % Tween80*) + 0,4 x 4,3 (*40 % Span 80*)]

**Table 1** Fixed oil ratios and higher water amounts in different Surfactant/Cosurfactant ratios

| FormulationCode | Oil | S+ CoS | Tween 80 | Span 80 | Propanol | Water |
|---|---|---|---|---|---|---|
| **LA1-1** | 2 | 8 | 2,4 | 1,6 | 4 | 15,1 |
| **LA1,5-1** | 2 | 8 | 2,88 | 1,92 | 3,2 | 10,5 |
| **LA2-1** | 2 | 8 | 3,2 | 2,13 | 2,66 | 7,6 |
| **LA2,5-1** | 2 | 8 | 3,43 | 2,29 | 2,29 | 6,5 |
| **LA3-1** | 2 | 8 | 3,6 | 2,4 | 2 | 5,4 |
| **LA3,5-1** | 2 | 8 | 3,73 | 2,49 | 1,77 | 2,6 |
| **LA4-1** | 2 | 8 | 3,84 | 2,56 | 1,6 | 2,2 |

**Table 2** Fixed S/CoS ratio and researching of increased water amount accoring to decreased oil ratio.

| Formulationcode | S/CoS | Oil | S+CoS | Tween80 | Span80 | Propanol | Water |
|---|---|---|---|---|---|---|---|
| **LA1-1 (a)** | 1 | 0.5 | 9.5 | 2.85 | 1.9 | 4.75 | 23.0 |
| **LA1-1 (b)** | 1 | 1.0 | 9.0 | 2.70 | 1.8 | 4.50 | 18.5 |
| **LA1-1 (c)** | 1 | 1.5 | 8.5 | 2.55 | 1.7 | 4.25 | 17.2 |
| **LA1-1 (d)** | 1 | 2.0 | 8.0 | 2.40 | 1.6 | 40.0 | 15.3 |

Phase studies were studied to investigate the effect of surfactant to cosurfactant ratio on the extent of stable O/W microemulsion region. O/W microemulsion would transform gel due to the higher amount of water. While the ratio of oil was decreasing under 2 as 1.5, 1 and 0,5 respectively amounts of water were increased inversely proportional. Optimum formulation was determined thanks to microemulsion point in pseudoternary phase diagram. External phase was water.

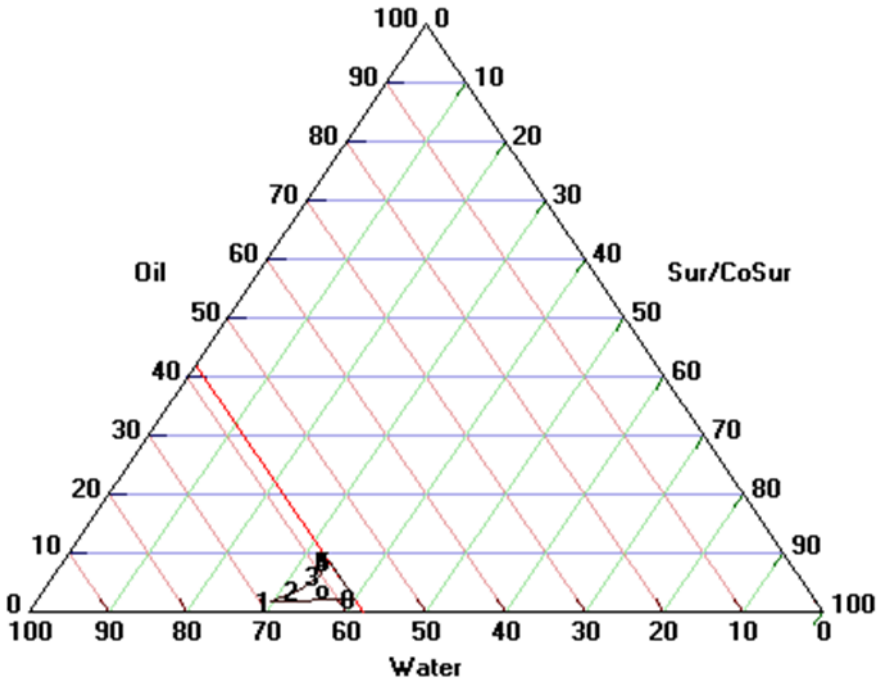


**Figure 1** Optimum point of O/W triangle phase diagram

Here oil percentage was 3,9 % and water percentage is 61,3 %. Values are available for O/W system.

**Table 3** Composition of microemulsion formulation

| Formulation | % |
|---|---|
| Oil: Oleicacid | 3.9 |
| Surfactant: Span 80 (%40) + Tween 80 (%60) | 7 + 10,4 |
| Cosurfactant: Isopropanol | 17,4 |
| Water | 61,3 |

The viscosity of the O/W microemulsion was increased with Carbopol 980. Topical administration would be easier. Since the amount of water in the W/O microemulsion type was low, gelation could not be

achieved by sufficient carbopol amount. 0.5% w/w diethanolamine was added to the formulation as hardener. The hydrogel containing 1% by weight of carbopol was white in appearance and had little consistency. More additions of Carbopol caused to turbidity.

### 2.2. Quantification of methotrexate

The peaks of the mobile phase were observed in 40 seconds. Calibration curve was drawn according to five concentrations as the minumum measurable value 0.1 µg/mL and 0.5, 1, 5 and 10 µg/mL respectively (Fig 3). Correlation coefficient was calculated asr²: 0,9996 and standard deviation: 1.68. Hence it has been determined that the line is completely linear.

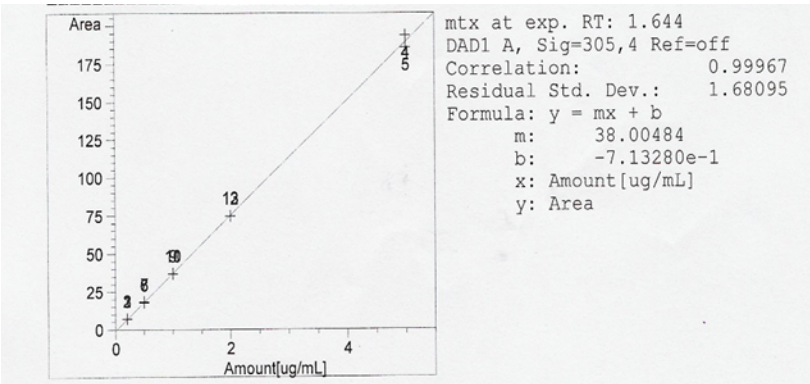


**Figure 2** Calibration curve, equation, standard deviation and correlation of MTX determination

MTX peak was clearly determined in 2 minutes and there was no interference. Injection volume was 20 µl. -

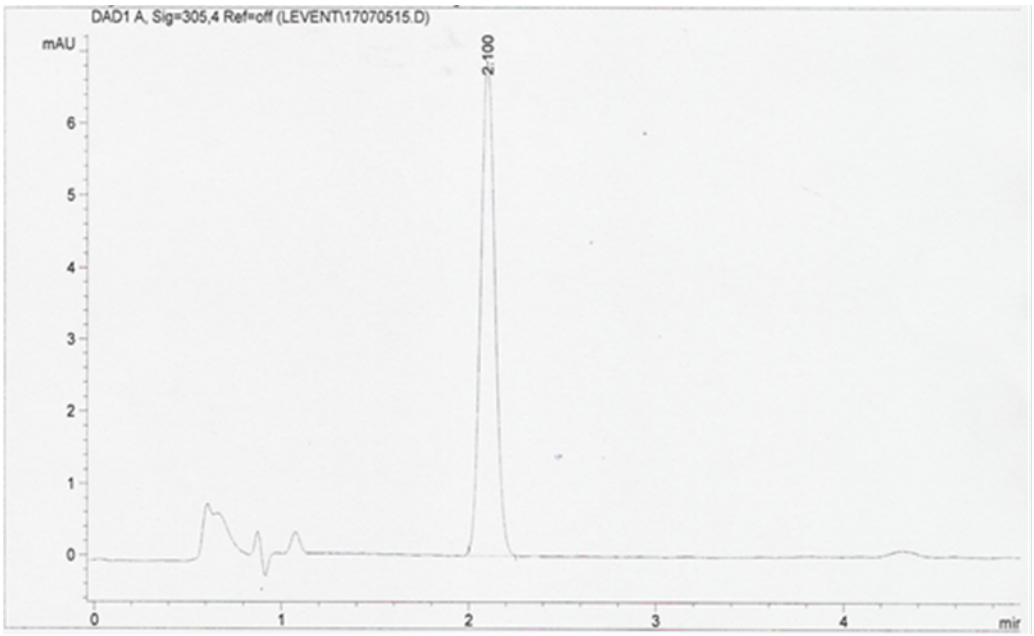


**Figure 3** Peak of MTX in HPLC

Short-term and room temperature stability tests and repeatability tests were evaluated according to the same determination method.

### 2.3. Recovery studies

MTX was determined from microemulsion and gel formulations containing 1 ppm MTX dissolved in pH 7.0 phosphate buffer. Recovery percentage rates were calculated based on the 1 ppm area on the calibration curve.

It had been observed that there would be no problems with MTX concentration detection in the releasing studies.

**Table 4** Recovery percentages of formulations

| Formulation | Area | Recovery % |
|---|---|---|
| Microemulsion in phosphatebuffer pH 7.0 | 37.65 | 99.9% |
| Gel in phosphatebuffer pH 7.0 | 35.94 | 97.3% |

### 2.4. In vitro releasestudies of MTX

In vitro release behaviors of MTX from microemulsion and gel are shown in Figure 4-5. Releasing concentration values of microemulsion and gels against time are shown in Table 5-6.

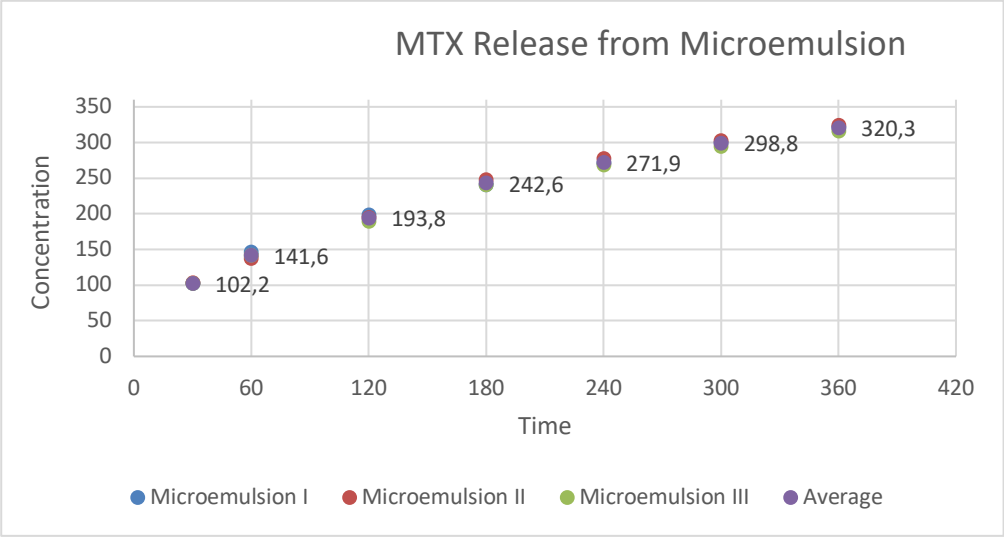


**Figure 4** Relasing of MTX fromMicroemulsion Series

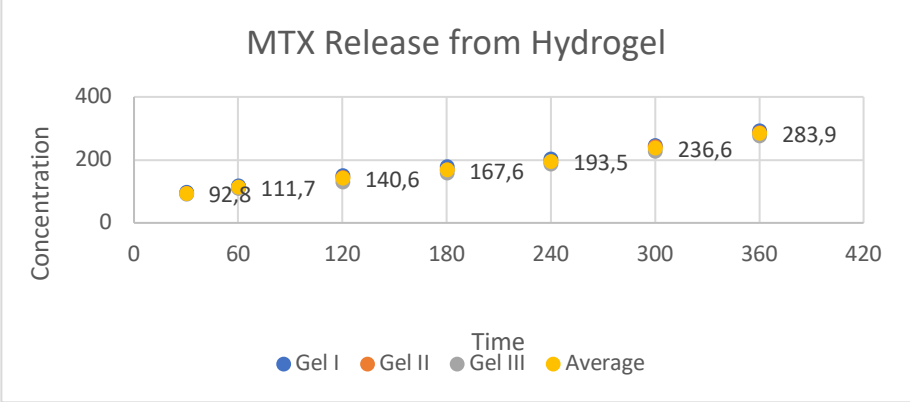


**Figure 5** Relasing of MTX from Hydrogel Series

**Table 5 and 6** Releasing amounts of MTX from hydrogel series, standart deviations and relative standart deviation

| Time | Microemulsion I | Microemulsion II | Microemulsion III | Average | SD | RSD |
|---|---|---|---|---|---|---|
| 30 | 101,9 | 102,8 | 102,0 | 102,2 | 0,49 | 0,48 |
| 60 | 146,2 | 137,3 | 141,2 | 141,6 | 4,47 | 3,15 |
| 120 | 198,2 | 194,6 | 188,7 | 193,8 | 4,81 | 2,48 |
| 180 | 240,1 | 247,8 | 239,9 | 242,6 | 4,50 | 1,85 |
| 240 | 270,4 | 276,9 | 268,5 | 271,9 | 4,40 | 1,62 |
| 300 | 299,8 | 302,6 | 293,9 | 298,8 | 4,43 | 1,48 |
| 360 | 321,1 | 324,0 | 315,8 | 320,3 | 4,15 | 1,30 |

| Time | Hydrogel I | Hydrogel II | HydrogelIII | Average | SD |
|---|---|---|---|---|---|
| 30 | 96.0 | 92.5 | 90.0 | 92.8 | 3.01 |
| 60 | 117.6 | 109.8 | 107.8 | 111.7 | 5.18 |
| 120 | 148.4 | 143.7 | 129.8 | 140.6 | 9.67 |
| 180 | 176.7 | 168.5 | 157.7 | 167.6 | 9.53 |
| 240 | 201.2 | 192.7 | 186.6 | 193.5 | 7.33 |
| 300 | 244.5 | 239.7 | 225.6 | 236.6 | 9.82 |
| 360 | 291.5 | 286.0 | 274.3 | 283.9 | 8.78 |

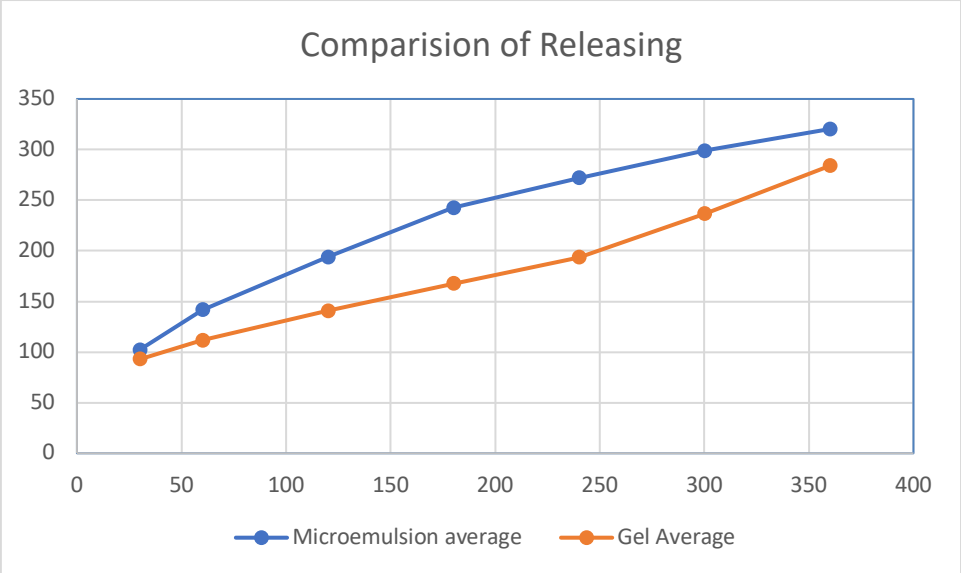


**Figure 6** Comparision of average MTX amounts from microemulsions and hydrogels

Releasing of MTX from microemulsions were better than hydrogels in during 6 hours. In each sampling time releasing of MTX from microemulsions was at least 10 %higher then from hydrogels.

This was an expected result, that some of the active ingredient in the gel was retained by the polymer structure.

### 2.5. Characterization of microemulsions

There fractive indexes of microemulsions were measured as 1,375 and 1,372 and close to that of water as the external phase (1.334).

These values indicated that microemulsion formulations were transparent systems. pH values of the formulations ranged from 6.5 to 7.1. Therefore, pH of formulations are convenient for topical applications. The electrical conductivity values ranged from 1058 to 1260 µs/cm. Electrical conductivity measurement is a

useful tool to differentiate W/O droplets from O/W-type droplets and bicontinuous structures. Conductivity is actually a measure of the ionic activity of a solution in term of its capacity to transmit current. The measurements provide determining whether microemulsion is oil contionous or water continous. Conductivity of internal phase was only 7,24 µs/cm. It is obviously clear that microemulsion type is O/W.

The viscosity value of microemulsion and gel were measured as 22 cps, 1100 cps in 25 °C respectively.

### 2.6. Particle size distribution

Particle size distribution is an important characteristics of emulsion for the evaluation. In this study average particle size of microemulsion containing MTX was measured 64,5 nm and zeta potential was -535 mV. Any visible oil droplets and turbidy were not observed on the surface of the microemulsion samples. Polydispersity index (PdI) was measured as 0,188. Diameters of microemulsion droplets were in nano size and they it demonstrated that this formulation would be a good vehicle for drug delivery (31).

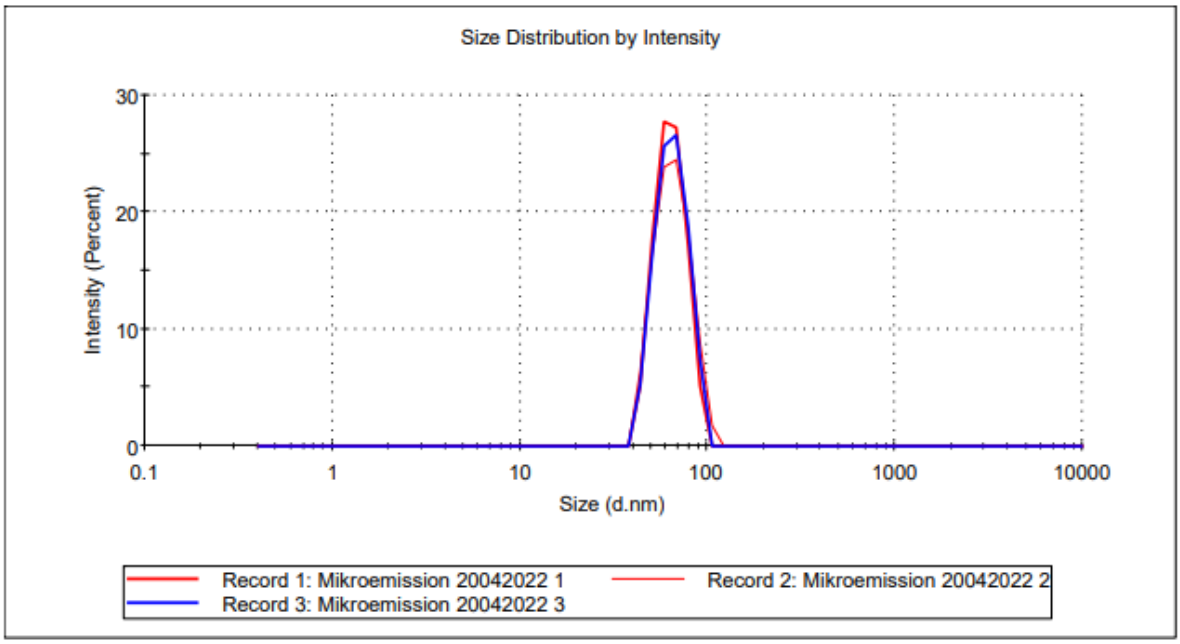


**Figure 7** Particle size distribution of microemulsion

## 3. CONCLUSION

Formulations studies have done in a labor intensive work for balanced and precisely trials to create phase diagrams. While creating the microemulsion formulation, surfactants were studied in the selection of >10HLB value. MTX loading capacities were different from W/O to O/W; while 5 mg MTX could be loaded into the W/O microemulsion, 60 mg MTX could be loaded into the O/W microemulsion. O/W type microemulsion should be prepared to load MTX more.

Determination assay of MTX was quick, practical and did not need much more chemical consumption. Controlled releasing could be arranged by using different quantities of polymers.

## 4. MATERIALS AND METHODS

Materials were obtained from different corporations. Methotrexate hydrate (Sigma Aldrich, St.Louis), Carbopol 980 (Parkoteks Chem.Co. Istanbul), Propanol, Tween 80, Oleic acid (Riedel de Haen, Seize), Span 80 (Fluka, Steinham), Pre-Cut Naylon membranes 0,2µm (Altech, Izmir). Water was deionized and filtered in laboratory. All chemicals and solvents were analytical grade.

### 4.1. Preparation of formulations

HLB value of surfactans was designed for O/W type microemulsion according to the ratios of Tween 80 and Span 80. 1-propanol was used as cosurfactant. Oleic acid was the oil phase of formulation. Surfactants, cosurfactant and oil phase was mixed over magnetic stirrer in 200 rpm (Jeio Tech, Republic of Korea). Mixture was titrated by slowly water drops in 25 °C. In order to reach the higher water amounts

different Surfactant/Cosurfactant ratios were experienced as 1, 1.5, 2, 2.5, 3, 3.5 and 4 respectively while oil phase and surfactant + cosurfactant was 2 : 8.

Pseudo-ternary phase diagrams of oil, water, surfactant + cosurfactants were constructed. Phase diagram were drawn by visual inspection of mixtures of the ingredients which were titrated with waterdrop by drop and stirred well at room temperature. According to the percentages of weights optimum microemulsion region and related point were determined by using pseudo-ternary phase diagram programme (32).

*4.1.1 Preparation of hydrogels*

While the sum of surfactants were equal to cosurfactant ratio (oil 2: surfactants 4: cosurfactant: 4,) the amount of oil phase was decreased as the ratios of 0.5, 1, 1.5. These oil phase ratios were studied in order to determine higher water amounts. 20 mg MTX was dissolved in determined water amount before titration. Hydrogels were prepared by 1% (w/w) Carbopol 980 powder while pouring in to the system slowly and well mixed with a glass rod. After the mixture had been kept at ambient temperature for 24 h, 0,5 % (w/w) triethanolamine was added and well mixed until the gel was formed.

**4.2. Quantification of MTX**

MTX was determined by HPLC (agilent 1100) using Quat.Pumpand DAD detector. The mobil phase was Acetonitrile (ACN) :Phosphate/ citrate buffer (pH: 6.0) (10:90). Column was ACE $C_{18}$ and flow rate was 2 ml/min. System temperature was maintained constant at 35°C and MTX was quantified on the basis of absorption at 305 nm. Injection volume was 2 µl.

Calibration points MTX were chosen as 0.1, 0.5, 1, 5, 10 µg/ml. Analytical validation of the parameters were examined. Short-term temperature stability was analyzed from 4 h to 24 h. The interval value of calibration points was 1 µg/ml and this concentration was repeated ten times successively as three parallels.

**4.3. Recovery studies**

Phosphate buffer was prepared in respect of Pharmacopea (33). 9,07g $KH_2PO_4$ was dissolved by 1000 ml distilled water kept in ultasonic mixture for 30 minutes. 11,87g $Na_2HPO_4..2H_2O$ was again dissolved by distilled water. 413 ml from the first solution and 587 ml from the other solution were taken. Then solutions were mixed well in ultrasonic again.

Each microemulsion and gel formulations were prepared as three series. 0,1g of sample were placed into calibrated 10 ml volume tricflask and 10 ml volume was completed by prepared pH 7.0 phosphate buffer. The mixtures were kept in ultrasonic water bath 10 min. and passed from 0,45 µm filters into eppendorfs. Ependorfs were stirred for two minutes. The avarage results of recoveries were calculated.

**4.4. In vitro release studies of MTX**

Naylon membranes with 0,2 µm pore diameter were kept in the phosphate buffer during 2 hours. 0,5 g of donor formulations were placed over the membranes in 37 °C. Releasing of MTX from microemulsions and gels to receiver phosphate buffer was measured initially in 30th min. by taking 1 ml sample. Phosphate buffer solution was added to the receiving phase as much as the sample volumes taken. Sampling continued every hour till the 6th h.

**4.5. Characterization of microemulsions**

Refractive indexes (Opti Duo Bellingham Stanley, UK), pH and electrical conductivity (Hanna Edge, USA), and viscosities (Vivro viscometer SV10) of optimum microemulsion formulations with and without MTX were measured respectively. Each instrument were calibrated before measuring of triplicate samples in room temperature. Viscosities of microemulsions and gels were measured in Vibro viscometer.

**4.6. Particle size distribution**

Conductivity and viscosity of microemulsions were loaded as initial parameters of measuring. Particle size distribution and average droplet size of triplicate microemulsions were measured (MalvernNano ZS, UK). Polydispersity index and zeta potential of formulations were determined by instrument.

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**Conflict of interest statement:** Fill in this section according to the signed conflict of interest statement when submitting your article. If there is no conflict of interest to be declared by any of the authors, write "The authors declared no conflict of interest" in the manuscript.

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