



# Preparation, characterization and *in vitro* evaluation of theophylline loaded microemulsion formulations

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## ABSTRACT

Theophylline is widely used as a bronchodilator drug in asthma and chronic obstructive lung disease because it is effective and inexpensive. Theophylline blood concentrations should be monitored since its therapeutic index is narrow, its optimum blood levels range from 10 to 20 µg/mL; its serious side effects take place over 20 µg/mL; its efficacy falls below 10 µg/mL blood concentrations. The aim of the present study is to formulate and characterize oil-in-water microemulsion systems for oral delivery of theophylline. Microemulsion formulations represent an interesting delivery vehicle for poorly water-soluble drugs, allowing for improving their solubility and dissolution properties. A simple and reliable UV-Spectrophotometric method was developed and validated for the determination of theophylline in the concentration range of 25-150 µg/mL. *In vitro* drug release profile showed that after 1 hour, 40% of the drug released through the formulation in Simulated Intestinal Fluid at pH 6.8 as a release medium and the microemulsion formulations released more than 75% of the drug during the 5 hours study period. The globule size of drug-loaded formulations was in the range of 203.6 ± 0.2 nm. Results confirmed that the proposed microemulsion formulation containing theophylline could improve and control the drug release profile in comparison to the conventional dosage form.

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## 1. INTRODUCTION

Theophylline, which is a methylxanthine derivative, is widely used in the treatment of asthma because it is effective and cheap all over the world, including our country [1]. There is evidence of the anti-inflammatory and immunomodulating effects, as well as the bronchodilator effect [2]. When theophylline was first introduced into asthma therapy, it was used as a bronchodilator, and early dose-response studies showed an increasing acute bronchodilator response. Bronchial asthma is a chronic inflammatory disease of the airways. Bronchial asthma is a state of obstructive airway obstruction that is not related to any other disease, and hyperreactivity (hyperresponsiveness) of the airways against various stimuli [2,3]. Asthma is a serious social health problem for people of all age groups in the world. Asthma is a disease that significantly restricts the quality of life when it is uncontrolled, requires constant medical care and treatment, and can even lead to death [4]. Although it is claimed that there are approximately 300 million asthma patients worldwide today, the absence of a precise and generally accepted definition of asthma makes it difficult to make a reliable comparison of reported frequency values from various parts of the world [5,6]. Until recent years, asthma was considered a disease characterized by airway smooth muscle spasm, and bronchodilator drugs were

used primarily in the treatment. Today, the main goal of asthma treatment is to suppress chronic inflammation in the airways, thereby controlling and maintaining the disease [2].

Drugs used in the treatment of asthma are divided into two as controlling and relaxing drugs. Controlling medicines are mainly used daily and long-term to keep asthma under control through its anti-inflammatory effects. Relaxing drugs, on the other hand, are medicines that act rapidly, correct bronchoconstriction, relieve symptoms, and are used when necessary [6]. Theophylline preparations especially provide controlled release, and they are included in the list of controlled medicines in the treatment of asthma.

The Global Initiative for Asthma Guidelines, 2002 guidelines for asthma, recommend that theophylline can be used as an add-on therapy to patients not controlled by low doses of inhaled corticosteroids but recommend long-acting β<sub>2</sub>-agonists as more effective and with fewer adverse effects [7]. Several clinical studies have demonstrated that adding theophylline to inhaled corticosteroids in patients with mild to moderate asthma who are not controlled gives equivalent or better asthma control than doubling the dose of inhaled corticosteroids [8,9].

Theophylline was first used in the clinic in 1907 by Minkowski and a group of doctors as a diuretic in cardiac and renal edema following extraction from tea leaves by the

German biologist Albrecht Kossel in 1888 [10]. It was added for the first time to The United States Pharmacopoeia's (USP) contents in 1918. With the development of pharmacokinetic science in the 1970s and 1980s, the pharmacokinetic properties of theophylline, the plasma level, and the relationship between bronchodilator effect and toxicity were intensively investigated. Considering this information, theophylline has become a commonly used drug in the treatment of asthma [11].

Microemulsions (MEs) are thermodynamically stable and translucent systems widely used for the systemic delivery of the drugs. In pharmaceutical fields, the interest in MEs is increasing; thus, they are applied to various administration routes. These systems form spontaneously at a certain concentration of oil, water, and surfactant. MEs often require the addition of cosurfactants to obtain an appropriate fluidity and flexibility of the interface. Due to their unique characteristics such as transparency, small droplet diameter (10-140 nm), high stability, and low surface tension, MEs are also one of the popular drug delivery systems for the controlled release of the active ingredient. Having very low inter-surface tension is essential in their formation and stability [12-14].

There are three types of MEs, including water-in-oil (w/o), oil-in-water (o/w), and bi-continuous systems. In w/o or o/w type of MEs, the droplets are surrounded by a film made of surfactant and cosurfactant molecules. The main advantages of this type of system are the simple preparation method, low energy requirement, and also high encapsulation efficacy. The critical parameter for ME preparation is the correct selection of the oil phase and the surfactant/cosurfactant component in appropriate ratios.

Recently, there has been considerable interest in the ME formulations for the delivery of hydrophilic as well as lipophilic drugs as drug carriers because of its improved drug solubilization capacity, long shelf life, ease of preparation and improvement of bioavailability. The present study deals with the progress of polysorbate-based theophylline MEs in drug delivery.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Theophylline powder,  $\geq 99\%$ , anhydrous; isopropyl palmitate (IPP); Tween<sup>®</sup>20 and propylene glycol were provided from Sigma-Aldrich. All other chemicals were of analytical grade and used without further purification.

### 2.2. Methods

#### 2.2.1. Analytical method validation

Method validation studies of theophylline were carried out according to the International Conference on Harmonization (ICH) guideline Q2(R1) [15]. The described method was validated with respect to linearity, precision, accuracy, the limit of detection (LOD) and quantitation (LOQ). The analysis method was developed by using a UV-Spectrophotometer, and absorbance values were obtained at 295 nm.

The stock solution of theophylline (1 mg/mL) was prepared by dissolving 50 mg of drug in 50 mL of Simulated

Intestinal Fluid (SIF) (pH 6.8) prepared according to USP 29 [16]. The standard solutions were stored at  $4 \pm 1^\circ\text{C}$  in a clear glass volumetric flask and light protected with aluminum foil. Theophylline concentrations in the working solution chosen for the calibration curves were 25, 50, 75, 100, 125, and 150  $\mu\text{g/mL}$ .

#### 2.2.2. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were obtained with a water titration method at room temperature ( $25^\circ\text{C}$ ). For each phase diagram, the oil phase and the surfactant mixture ( $S_{\text{mix}}$ ) were mixed and different ratios of oil:surfactant mixtures were prepared from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Water was added drop by drop to each mixture carefully until the mixture became turbid at a certain point. The mixture that remained transparent and homogeneous after stirring was assigned to the monophasic area of the phase diagram. This process was repeated in the same way for each phase diagram [17,18].

#### 2.2.3. Preparation of microemulsion formulation

The pseudo-ternary phase diagram of the largest area was selected to determine the concentration range of the components in the ME. ME formulations for further studies were selected from the weight center of these pseudo-ternary diagrams [19].

#### 2.2.4. Globule size, zeta potential and polydispersity index

The mean droplet size and size distribution (polydispersity index; PDI) of MEs were characterized by photon correlation spectroscopy (Nano ZS, Malvern, UK). Three individual runs were performed on each sample. The size of droplets is expressed as Z-average diameter and reported as the mean diameter. The surface charge of MEs was determined by measuring the zeta potential of MEs using the electrophoretic light-scattering technique (Nano ZS, Malvern, UK). For each sample, three individual runs were performed for which zeta potential was calculated as the mean value.

#### 2.2.5. Centrifugation

ME formulation was centrifuged at 3000 rpm for 30 minutes and then was examined for whether the system was monophasic or biphasic.

#### 2.2.6. *In vitro* drug release studies

*In vitro* release tests were performed using dialysis membrane methods. The medium was 50 mL of Simulated Intestinal Fluid (SIF) pH 6.8 [16], and the temperature was kept at  $37 \pm 0.5^\circ\text{C}$ . MEs containing the same amount of theophylline prepared based on the solubility study were directly introduced into the dissolution medium. While stirring the release medium using the magnetic stirrer at 100 rpm, 1 mL of dissolution medium was withdrawn at the predetermined period (5 min, 15 min, 30 min, 60 min, 90 min, 2 h, 3 h, 4 h, 5 h). After each sampling, 1 mL of fresh medium was added to the incubation medium. The amount of released active agent in the medium collected was determined by UV-spectroscopy, and cumulative release percentage of theophylline was calculated [20,21].

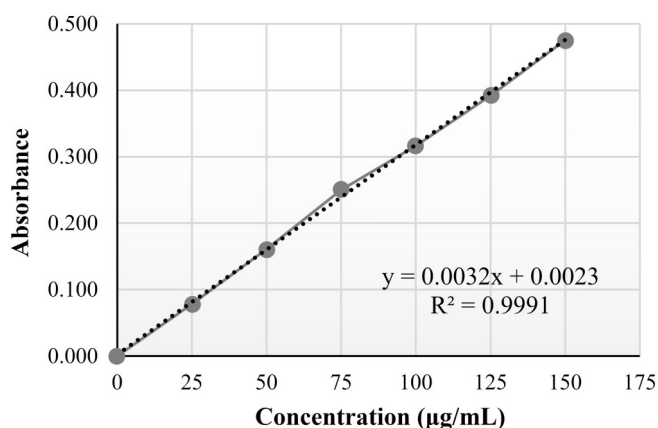
### 3. RESULTS AND DISCUSSION

#### 3.1. Analytical Method Validation

The linearity between absorbance and concentration was analyzed using a calibration curve obtained from standard solutions of theophylline (25 to 150 µg/mL). Linearity was evaluated by linear regression analysis, which was calculated by the least-square regression analysis. The determination coefficient ( $R^2$ ) of 0.9991 was obtained. Linearity test results are shown in **Table 1**, and the regression curve is presented in **Figure 1**. In this study, the range was observed linearly to the highest concentration, and the  $R^2$  of 0.9991 was highly significant [18].

**Table 1.** Series and absorbance values of the linearity study

Conc. (µg/mL)	Absorbance			Mean	SD
	SET 1	SET 2	SET 3		
25	0.077	0.081	0.076	0.078	0.003
50	0.172	0.154	0.158	0.161	0.009
75	0.271	0.245	0.233	0.250	0.019
100	0.333	0.320	0.295	0.316	0.019
125	0.421	0.388	0.366	0.392	0.028
150	0.513	0.485	0.429	0.475	0.043



**Figure 1.** Regression profile of theophylline

25, 75, 125 µg/mL theophylline solutions were analyzed three times in order to evaluate method precision, standard deviation (SD), and relative standard deviation (RSD). Results of precision and repeatability tests on different concentrations are given in **Table 2**.

Since the RSD% values for both intermediate precision and repeatability were <2%, the method developed for theophylline was found to be precise according to the suggestions in ICH Q2(R1) guidelines [22].

The accuracy of an analytical method is the closeness of test results obtained by the method to the true value and is defined recovery. The prepared three standard solutions (50, 100, 150 µg/mL) were analyzed three times to evaluate the accuracy of the method developed. As given in **Table 3**, the recoveries of theophylline at various concentrations were obtained between 97-106%. Since the percentage of recovery has been found between the acceptance criteria, which is 90-110%, the analysis system for the determination of assay is verified. Low values of standard deviation denoted good repeatability of the measurement [23].

**Table 2.** Precision results of theophylline on three different days

	Absorbance			Concentration (µg/mL)		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
25 µg/mL	0.076	0.107	0.076	23.031	32.719	23.031
	0.078	0.106	0.078	23.656	32.406	23.656
	0.078	0.108	0.077	23.656	33.031	23.344
	<b>Mean</b>			23.448	32.719	23.344
			<b>SD</b>	0.361	0.313	0.313
			<b>RSD</b>	1.539	0.955	1.339
75 µg/mL	0.274	0.25	0.241	84.906	77.406	74.594
	0.271	0.245	0.230	83.969	75.844	71.156
	0.269	0.240	0.229	83.344	74.281	70.844
	<b>Mean</b>			84.073	75.844	72.198
			<b>SD</b>	0.786	1.563	2.081
			<b>RSD</b>	0.935	2.060	2.882
125 µg/mL	0.419	0.389	0.361	130.219	120.844	112.094
	0.420	0.386	0.366	130.531	119.906	113.656
	0.425	0.390	0.370	132.094	121.156	114.906
	<b>Mean</b>			130.948	120.635	113.552
			<b>SD</b>	1.005	0.651	1.409
			<b>RSD</b>	0.767	0.539	1.241

Limit of detection (LOD) of an analytical procedure is the minimum amount of analyte which can be detected in a sample, while the limit of quantitation (LOQ) is the lowest concentration of an analyte in a test and can be determined with acceptable precision and accuracy under the specified test conditions. LOD and LOQ were determined using the calibration curve method, according to ICH Q2(R1) recommendations. The LOD and LOQ of the proposed method were calculated using the following equations:

$$\text{LOD} = 3.3 \times \sigma/s$$

$$\text{LOQ} = 10 \times \sigma/s$$

where,

$\sigma$  = the standard deviation of the response

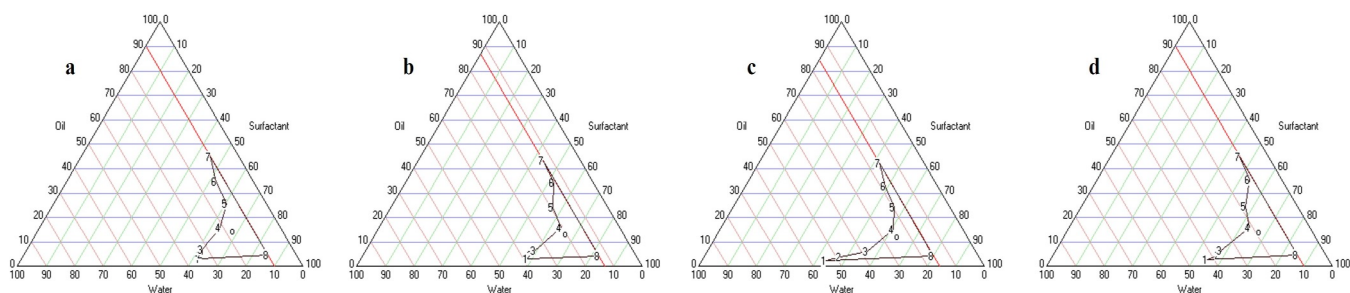
$s$  = slope of the calibration curve [24].

LOD and LOQ for theophylline were found to be 0.260 µg/mL and 0.786 µg/mL, respectively.

**Table 3.** Accuracy results of theophylline

	Absorbance	Conc. (µg/mL)	Recovery %
50 µg/mL	0.157	48.344	96.688
	0.154	47.406	94.813
	0.163	50.219	100.438
	<b>Mean</b>		97.313
		<b>SE</b>	2.864
100 µg/mL	0.333	103.344	103.344
	0.337	104.594	104.594
	0.334	103.656	103.656
	<b>Mean</b>		103.865
		<b>SE</b>	0.651
150 µg/mL	0.512	159.281	106.188
	0.514	159.906	106.604
	0.512	159.281	106.188
	<b>Mean</b>		106.326
		<b>SE</b>	0.241

SE: Standard error



**Figure 2.** Pseudo-ternary phase diagrams of microemulsions surfactants:cosurfactant ratio **a:** 2:1, **b:** 3:1, **c:** 4:1, **d:** 5:1

### 3.2. Construction of Pseudo-Ternary Phase Diagrams

MEs are transparent, thermodynamically stable and isotropic oil, water, surfactant, and cosurfactant mixtures. Several studies have shown that ME formulations provide improved oral and dermal drug delivery properties [25,26]. Many ME formulations are based on short- and medium-chain alcohols, while others are based on poloxamer or propylene glycol as cosurfactants in combination with polysorbates [25].

It is known that large proportions of surfactant may cause irritation and toxicity. It is important to determine the surfactant concentration and to use the minimum concentration required for the development of the ME formulation [27].

In this study, all the ingredients were pharmaceutically acceptable and generally regarded as in the safe category. Nonionic surfactants are generally considered as having low toxicity and irritancy and, therefore, regarded to be suitable for oral administration.

Pseudo-ternary diagrams are equilateral triangles that define the compositional phase behavior of the system. The vertices represent pure ingredient or two ingredients, the edges indicate two-ingredient systems, and the interior represents all three ingredients in the system. Pseudo-ternary phase diagrams of the ME area of existence with isopropyl palmitate, Tween<sup>®</sup>20, propylene glycol, and water mixtures and the final compositions are presented in **Figure 2**. The ME formulations 5:1 ratio was selected, which showed larger ME regions. The complete composition of oil, surfactant, cosurfactant, and the aqueous phase were shown in **Table 4**.

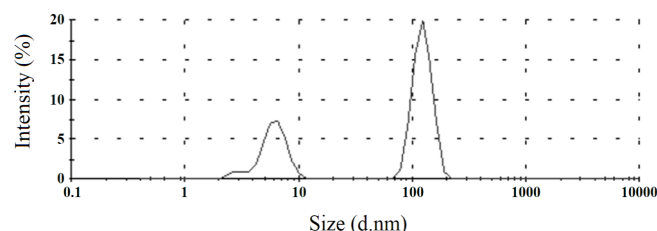
**Table 4.** Composition of optimum microemulsion formulation

Components	%
Isopropyl palmitate	14.5
Tween <sup>®</sup> 20	57.4
Propylene glycol	9.6
Water	18.6

### 3.3. Characterization of Microemulsion Formulation

The final ME formulation maintained the predetermined characteristics after the addition of theophylline. Blank formulation and formulation including the active agent were clear transparent liquids with a homogenous appearance, no precipitation, good fluidity, and a yellow tinge at room temperature. When physical stability was observed, MEs showed no phase separation after centrifugation. Besides, there was no sign of turbidity or creaming.

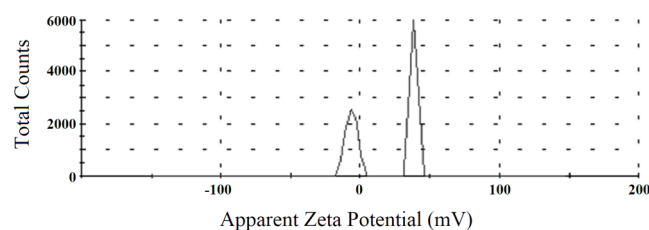
The zeta-size analyzer was employed to determine the globule size of the ME formulation (**Figure 3**). The globule size was found to be  $203.6 \pm 0.2$  nm. This shows that the optimized formulation was nano-sized and could be termed as nano formulations. Smaller emulsion globule size results in lower emulsification time and a larger surface area provided for the drug absorption. The small globule size and good dispersibility of the ME indicated that the bioavailability and absorption of theophylline could be improved. There was no significant change in globule size and PDI after active agent incorporation [28-30].



**Figure 3.** Globule size analysis of 5:1 microemulsion formulation

The globule size and polydispersity index have a significant impact on the physical stability of colloidal drug delivery systems when used to orally. The polydispersity index varies from 0.0 to 1.0 and is a measure of particle homogeneity. The globules are more homogeneous if the polydispersity index value is closer to zero [28,31]. The small polydispersity index of ME showed uniformity in the size distribution of globules, polydispersity index below 0.3 could be used as an indication of uniformity of globules. This result used as a determination of the stability of MEs [31].

The zeta potential is a method to measure the surface charge of particles or globules. Zeta potential is used for predicting dispersion stability, and its value depends on the physicochemical property of the drug, polymer, vehicle, presence of electrolytes, and their adsorption [29]. **Figure 4** indicates the value of the zeta potential of the optimized ME. Zeta potential is an essential property of the formed dispersion, and large zeta potential value of nanostructures was an important factor for physical stability.

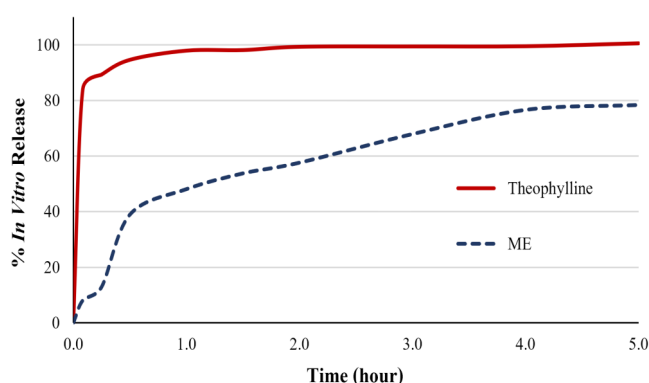


**Figure 4.** Zeta potential of 5:1 microemulsion formulation

The values of zeta potential indicate that the system is relatively stable. The zeta potential values of ME may approach neutral charge due to the presence of the nonionic surfactant. The ME shows positive zeta potential, which is important for achieving the interaction of the gastrointestinal system for effective and prolonged theophylline delivery [30].

### 3.4. In Vitro Drug Release Studies

The dialysis bag is the most commonly used technique in the literature for drug release measurements [32]. The *in vitro* dissolution study of theophylline loaded formulation was compared with the dissolution profile of the pure theophylline. The cumulative release percentages at the regular time were calculated and showed in **Figure 5**. In the figure, the cumulative drug release percentage after 1 hour was 40% and 95% for ME formulation and pure active agent solution in SIF pH 6.8, respectively.



**Figure 5.** *In vitro* cumulative drug release profile

Experimental results showed that ME formulations release more than 75% of the drug during 5 hours of study period. These drug release behaviors confirm the sustained release of theophylline in the ME formulation, which enhances the drug retention time. The sustained release of this formulation is consistent with the literature [28-30].

## 4. CONCLUSION

In this study, we successfully prepared theophylline loaded ME formulation sized around 200 nm. This study shows that the o/w ME with good clarity, ideal stability, and proper characterization, can be prepared successfully using Tween®20 as the surfactant and propylene glycol as the cosurfactant. Tween®20:propylene glycol in the weight ratio of 5:1 was the surfactant providing greater area. The *in vitro* studies demonstrated the potential of developed ME for oral delivery of theophylline.

## AUTHOR CONTRIBUTIONS

Concept: TÇ, DO, UMG, ED; Design: TÇ, DO, UMG, ED; Supervision: TÇ, DO, UMG, ED; Materials: TÇ, DO, UMG, ED; Data Collection and/or Processing: TÇ, DO, UMG, ED; Analysis and/or Interpretation: TÇ, DO, UMG, ED; Literature Search: TÇ, DO, UMG, ED; Writing: TÇ, DO, UMG, ED; Critical Reviews: TÇ, DO, UMG, ED.

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## CONFLICT OF INTEREST DECLARATION

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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