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# Optimization of curcumin nanoemulsion for intranasal delivery using design of experiment and its toxicity assessment

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ABSTRACT

The objective of the study was to optimize curcumin nanoemulsion for intranasal delivery using design of experiment. Box–Behnken design was constructed using oil, surfactant and co-surfactant concentration as independent variables and their affect on response $y1$ (globule size) and $y2$ (zeta potential) were studied. The ANOVA test identified the significant factors that affected the responses. For globule size, percentage of oil, surfactant and co-surfactant were identified as significant model terms whereas for zeta potential, oil and co-surfactant were found to be significant. Critical factors affecting the responses were identified using perturbation and contour plots. The derived polynomial equation and contour graph aid in predicting the values of selected independent variables for preparation of optimum nanoemulsion with desired properties. Further, $2^4$ factorial design was used to study influence of chitosan on particle size and zeta potential. The formulations were subjected to *in vitro* cytotoxicity using SK-N-SH cell line and nasal ciliotoxicity studies. The developed formulations did not show any toxicity and were safe for intranasal delivery for brain targeting. *In vitro* diffusion studies revealed that nanoemulsions had a significantly higher release compared to drug solution. *Ex vivo* diffusion studies were carried out using sheep nasal mucosa fixed onto Franz diffusion cells. Mucoadhesive nanoemulsion showed higher flux and permeation across sheep nasal mucosa.



## 1. Introduction

Curcumin (diferuloyl methane) is a phenolic phytochemical obtained from rhizome of herb *Curcuma longa* L. commonly known as turmeric [1]. It has been found to exert beneficial effects on experimental models of Alzheimer's disease (AD) [2]. *In vitro* studies have shown that curcumin inhibit amyloid-β (Aβ) aggregation and Aβ induced inflammation [3–5]. Oral administration of curcumin in AD animal models has been found to inhibit Aβ deposition, Aβ oligomerization and tau phosphorylation in brain [3,6,7]. Curcumin has been found to decrease Aβ related inflammation and Aβ burden in amyloid precursor protein (APP) transgenic mice [6]. It also enhances activity of glutathione-S-transferase and inhibits nuclear factor κ beta (NFκβ). Activation of NFκβ increases the transcription of various inflammatory mediators [8,9]. Further, curcumin has been found to improve memory and cognitive deficits in rats [10]. Inspite of being a 'wonder molecule' the therapeutic efficacy of curcumin is limited by poor aqueous solubility, chemical instability in alkaline medium, rapid metabolism and poor absorption from gastrointestinal tract [11].

Delivery of drugs to brain for treatment of central nervous system (CNS) disorders is hindered by restrictive barriers like blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier [12]. One of the promising strategy to deliver neurotherapeutics to brain is intranasal delivery. It is a non-invasive technique for bypassing BBB and ensures direct delivery to CNS [13]. Many studies have indicated direct transport of drugs to cerebrospinal fluid and various parts of brain [14,15]. However, intranasal delivery is limited by rapid mucociliary clearance and poor nasal permeability of nasally applied drugs [13]. Alternative approaches that have been utilized to overcome these problems are use of chemical penetration enhancers and colloidal drug delivery systems (nanoemulsions, liposomes and nanoparticles) [16]. Most efforts in intranasal delivery have been focused on increasing the drug absorption, enhancing the nasal retention time and stability of the drug with the final goal of improving the therapeutic outcome. For treatment of CNS disorders like AD, these attempts include the design of mucoadhesive carrier system with improved drug delivery properties to the nasal cavity. Among these mucoadhesive nanoemulsions have been studied extensively. Nanoemulsions are heterogeneous systems consisting of fine oil-in-water dispersions stabilized by surfactant molecules. Moreover, they are kinetically stable without any apparent flocculation or

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coalescence during long-term storage [17]. Compared to micro emulsions, nanoemulsions are more robust in terms of destabilization due to dilution or temperature changes. The dilution of micro emulsions leads to decrease in globule size whereas it has no effect on nanoemulsion droplet size. Further, variation in temperature strongly affects structure and droplet size of micro emulsion [18,19]. Nanoemulsions have distinct advantages such as ease of preparation, biocompatible excipients, smaller globule size (<200 nm), enhanced solubility and dissolution rate and have found to increase mucosal permeability. This guarantees efficient absorption of oil droplets. Many lipophilic drugs have been studied using nanoemulsion delivery system, which justifies rationale of the study [20–22]. Nanoparticulate systems protect drug from chemical/biological degradation ensuring high concentration in CNS [23].

Nanocurcumin has been found to enhance the therapeutic efficacy of native curcumin as immunomodulatory, neuroprotective, anticancer, anti-malarial and anti-inflammatory agent [24–29]. Recently, micro emulsion based ion-sensitive *in situ* gel has been investigated for intranasal delivery of curcumin [30].

The traditional pharmaceutical development of any dosage form involving trial and error methodology is quite time consuming, expensive, unpredictable and laborious. Statistical optimization methods like design of experiment (DoE) are a systematic and scientific approach to study interaction between independent and dependent variables. Statistical design provides maximum information and interaction between variables in minimum number of runs [31]. Response surface methodology (RSM) is commonly used to estimate main effect, their interaction, quadratic effects and shape of response surface. Box–Behnken design (BBD) is one of commonly used RSM design and has an advantage of fewer number of runs when number of factors are three [32]. BBD is an independent, rotatable quadratic design with no embedded factorial or fractional factorial points where the variable combinations are at the midpoints of the edges of variable space and at the center [33].

The objective of the present study was to develop curcumin loaded nanoemulsion for intranasal delivery to CNS. The objectives also include identification of formulation variables affecting nanoemulsion formulation and its characteristics like globule size and zeta potential using DoE and optimization of an ideal batch. Further objective was to carry out toxicity studies of the developed formulations and study *in vitro* and *ex vivo* release behavior.

## 2. Materials and methods

### 2.1. Materials

Curcumin was obtained as a gift sample from Sanjivani Pharmaceutical Ltd. (Pune, India). Capmul MCM, Captex 300 (CTX 300), Captex 500 (CTX 500) and Captex 8000 (CTX 8000) were obtained as gift samples from Abitec, USA through Indchem International, Mumbai, India. Medium chain triglyceride (MCT) was a kind gift from Lipoid, Germany. Labrasol, Labrafac, Plurol oleique and Transcutol were obtained as gift samples from, Gattefosse, Mumbai, India. Cremophor RH 40 and Solutol HS 15 was a kind gift from BASF, Mumbai, India. Polyethylene glycol 200 (PEG 200), Polyethylene glycol 400 (PEG 400), Ethanol, Tween 20 and Tween 80 were purchased from S.D. Fine Chemicals, Mumbai, India. All other reagents used were of analytical grade.

### 2.2. Methods

#### 2.2.1. Solubility studies

Solubility of drugs in excipients is an important criterion for selection of components of nanoemulsion [17]. An excess amount of drug was added to 1 ml of oil, surfactant and co-surfactants in 5 ml stoppered vials and mixed using vortex mixer (Yorco Instruments, Delhi, India). The vials were placed in an isothermal shaker at 25 ± 0.5 °C (IKA®KS400i, Germany) for 72 h to reach equilibrium. The equilibrated samples were removed from shaker and centrifuged at 10,000 rpm for 15 min using centrifuge (Remi, India). The supernatant was taken and filtered through 0.2 μm membrane filter (HiMedia, India). The concentration of curcumin was found using HPLC as given in Section 2.2.2.

#### 2.2.2. HPLC and analytical method

The HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPDM20A; Shimadzu, Japan) and a 20 μL loop (Rheodyne). A C18 reverse-phase column (Phenomenex Gemini C18, 250 mm × 4.6 mm i.d., 5 μ) was utilized for drug separation, using acetonitrile–25 mM Potassium dihydrogen orthophosphate pH 4.5 (75:25, v/v) as mobile phase. The flow rate and UV wavelength were 1.0 ml $min^{-1}$ and 425 nm, respectively.

#### 2.2.3. Solution state stability

Standard solutions of curcumin (5–25 μg/ml) were prepared in simulated nasal fluid (SNF) pH 6.4 containing 1% SLS and stored at 37 ± 0.5 °C for 24 h. The samples were assayed for drug content by HPLC method at 1, 6, 12 and 24 h.

#### 2.2.4. Preparation of nanoemulsion

The nanoemulsions were prepared using spontaneous nanoemulsification method [17]. The oil phase was heated gently at 45–50 °C for 5 min. Surfactant and co-surfactant (smix) were mixed together and heated at same temperature. Oil and smix were mixed to form homogenous isotropic mixtures and were slowly titrated with aqueous phase to obtain clear and transparent nanoemulsions. The order of addition of excipients is very important for nanoemulsion formulation. Nanoemulsions are formed only if surfactant is mixed with oil phase followed by addition of water. Addition of surfactants to aqueous phase followed by addition of oil phase leads to formation of macroscopic emulsions. This is a key differentiation test from micro emulsions, which are not influenced by order of addition of excipients [18]. Drug loaded nanoemulsions were prepared in similar manner by adding the drug (12 mg) to oil phase. Curcumin solution was prepared in 20% (v/v) Tween 80 solution in distilled water.

#### 2.2.5. Design of experiment

Based on initial screening concentration of oil, surfactant and co-surfactant were found to be important parameters determining the globule size and zeta potential of nanoemulsions (data not shown). Box–Behnken design (BBD) was used to statistically optimize the independent variables: concentration of oil (10–18%), surfactant (25–35%) and co-surfactant (10–20%) and evaluate the main effects, interaction effects and quadratic effects of these formulation ingredients on globule size and zeta potential. Design-Expert® software (Version 8.0.7.1, M/s Stat-Ease, Minneapolis, USA) was used to conduct the study. A total of 17 experiments were designed by the software with 5 center points (in order to allow the estimation of pure error) and experiments were run in random order. Table 1 shows the coded and uncoded independent variables. Further to study the effect of chitosan on globule size and zeta potential $2^4$ factorial design was studied using concentration of oil, surfactant, co-surfactant and chitosan as independent factors. A total of 16 experiments were conducted as shown in Table 2.

#### 2.2.6. Preparation of mucoadhesive nanoemulsion

Mucoadhesive nanoemulsions were prepared by initially preparing nanoemulsion of drug using minimum volume of