COMPARATIVE STUDY ON FORMULATION AND EVALUATION OF MICROEMULSION BASED GEL OF CURCUMIN WITH DIFFERENT COMBINATIONS OF POLYMERS

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ABSTRACT

The present study was developed microemulsion based gel of curcumin polymer which will be useful in further drug delivery. The prepared microemulsion based gel was subjected to various tests like size distribution study, zeta potential, and soon. The microemulsion was clear, stable, an isotropic mixture of oil, water, and surfactant frequently in combination with a cosurfactant. It helps to solubilize the lipophilic drug moiety and showed rapid and efficient penetration of the skin. The microemulsion had stability problems due to having low viscosity, but this can be overcome by incorporation into topical DDS which causes improved viscosity and hydrating stratum corneum which increase dermal drug permeation and the skin flux. The Curcumin loaded micro-emulsion developed and optimized on the basis of findings of physico-chemical characterization, was further loaded into the Carbopol 934 and HPMC gel base. The gel based micro-emulsion was expected to improve the overall pharmaceutical and pharmacokinetic features of the drug candidate in terms of ease of dermal application and improved transdermal drug penetration. The developed formulations were characterized by physico-chemical parameters such as globule size, zeta potential, viscosity, pH, FTIR studies, etc. The finding of FTIR studies released the absence of drug potential drug – excipient interaction and ruled out any possible physico-chemical incompatibility b/w curcumin and excipients. The proposed Curcumin loaded micro-emulsion was formulated using Castor oil as the oil phase, Tween 80 and methanol as Surfactant and co-surfactant respectively. Due to all these factors within the major group of semisolid preparations, the use of transparent gels has expanded in pharmaceutical preparations. In spite of the many advantages of gels, a
major limitation is in the delivery of hydrophobic drugs. So to overcome this limitation microemulsion based approach being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels. Hydrophobic drugs can be incorporated into the microemulsion based gel using drug/oil/water emulsions.

INTRODUCTION
The oral route is the most accessible, most convenient route for invasive administration and the primary route of the drug delivery for the chronic treatment of many diseases. In current years, new chemical entities exhibit poor aqueous solubility which in turn leads to low oral bioavailability. Formulation of poorly aqueous soluble drugs is a challenging job to the pharmaceutical scientists as result of modern drug discovery technique, and oral delivery of such drugs is frequently associated with low bioavailability, high inter-subject variability and lack of dose proportionality. Improving oral bioavailability of drugs such as solid dosage forms remains a challenge for the formulation scientist due to some problems. A poor bioavailability can be due to poor solubility, degradation in GI lumen, poor membrane penetration and pre-systemic elimination. The formulation technique plays a vital role in overcoming this shortcoming of poorly water-soluble drugs, to encounter these problems, various formulation strategies are reported including use of surfactants, pulverization, crystal polymorphism selection, salt formation, solid dispersion, mixed pulverization, complex formation agent like cyclodextrin, emulsion, microemulsion, liposome, particle size, nanoparticles, micro and nanospheres, lipid carriers, use of prodrug, drug derivatization, solution phase studies and permeation enhancers to improve the dissolution rate of the drug. Different approaches have been used for avoiding these problems. One of the most popular methods is lipid-based formulation such as oils, surfactant dispersions, self-emulsifying formulations, emulsions, and liposomes (Singh et al., 2014).

1. Curcumin
In the country like India, every kitchen has one of the spices named ‘haldi’ without it food remains incomplete. This ‘haldi’ is popularly known as turmeric. The botanical name of this popular spice is Turmeric or Curcuma longa, which belongs to the Zingiberaceae or ginger family, not only in food but turmeric is also used for many medicinal purposes in India in the form of Ayurvedic, Unani and Siddha medicines. Turmeric is the boiled, dried, cleaned and polished rhizomes of Curcuma longa. After harvesting the whole rhizomes are collected. These rhizomes are transported as entire rhizomes. They are usually like fingers 2 to 8 cm
long and 1 to 2 cm wide having bulbs and splits. The dried rhizomes are further processed and reprocessed to obtain the turmeric powder.

The main constituent of turmeric is curcumin. Curcumin is a polyphenol that gives turmeric its color. Curcumin is a polyphenol and is lipophilic in nature, hence insoluble in water and also in ether but soluble in ethanol, dimethylsulfoxide, and other organic solvents. Curcumin is stable at the acidic pH of the stomach. Curcumin, a yellow compound isolated from its rhizome, may be responsible for much of the bioactive effects. Recent research shows that curcumin may inhibit carcinogenesis and angiogenesis (Arbiser et al., 1998; Thaloor et al., 1998) and may have a potential to improve chronic inflammatory conditions in obesity. Other investigations have also shown that curcumin inhibited the proliferation of cisplatin-resistant ovarian cancer cells through the induction of superoxide generation, G(2)/M arrest and apoptosis (Weir et al., 2007).

The solvents maintained in table 1.1 are considered suitable for preparation of microemulsion properties.

Table 1.1 Various Solvents Used in Preparation of Microemulsion Properties.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isopropanol</td>
<td>In the curcumin manufacturing process, isopropyl alcohol is used as a processing aid for purifying curcumin.</td>
</tr>
<tr>
<td>Ethyl acetate/dichloroethane</td>
<td>With a restriction placed on the use of chlorinated solvents, such as it is found that ethyl acetate, owing to its polarity, is a reasonable replacement providing an acceptable quality of product and commercially viable yields.</td>
</tr>
<tr>
<td>Acetate</td>
<td>This is used as a solvent in the curcumin manufacturing process.</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>This is not currently used in commercial productions. However, it is listed in EC Directive 95/45/EC and has potential as a substitute for chlorinated solvents.</td>
</tr>
<tr>
<td>Methanol</td>
<td>This solvent is occasionally used as a processing aid for purification.</td>
</tr>
<tr>
<td>Ethanol</td>
<td>This solvent is used sparingly because curcumin is entirely is entirely soluble in ethanol.</td>
</tr>
</tbody>
</table>

2. Microemulsion

Micro-emulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a co-surfactant. Microemulsion acts as potential drug carrier systems for oral, topical, and parenteral administration. They offer the advantage of spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, and improved drug solubilization and bioavailability.
2.1. Methods of Preparation of Microemulsion (Patel et al., 2013)

2.1.1. Phase Titration Method

The microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when the different components are mixed. Microemulsions are formed along with various association structures (including emulsions, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. The understanding of their phase equilibrium and demarcation of the phase boundaries are essential aspects of the study. As quaternary phase diagram (four component system) is time-consuming and difficult to interpret, the pseudo-ternary phase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component (Fig. 3.1). The region can be separated into w/o or o/w microemulsion by merely considering the composition, which is, whether it is oil-rich or water-rich. Observations should be made carefully so that metastable systems are not included.

Fig. 1.1: Pseudoternary Phase Diagram of Oil, Water, and Surfactant Showing Microemulsion Region.
2.1.2. Phase Inversion Method

Phase inversion of microemulsion occurs upon addition of an excess of the dispersed phase or in response to temperature. During phase inversion, drastic physical changes occur, including changes in particle size that can affect drug release both \textit{in vivo} and \textit{in vitro}. These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, this can be achieved by changing the temperature of the system, forcing a transition from an o/w microemulsion at low temperature to a w/o microemulsion at high temperature (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. This method is referred to as phase inversion temperature (PIT) method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone. Additionally, a transition in the spontaneous radius of curvature can be obtained by changing the water volume fraction. By successively adding water into oil, initially, water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a w/o microemulsion to an o/w microemulsion at the inversion locus. Short-chain surfactant forms flexible monolayers at the o/w interface resulting in a bicontinuous microemulsion at the inversion point.

Three types of microemulsions are most likely to be formed depending on the composition

1) Oil in water microemulsions wherein oil droplets are dispersed in the continuous aqueous phase;
2) Water in oil micro-emulsions wherein water droplets are dispersed in the continuous oil phase;
3) Bi-continuous micro-emulsions wherein micro-domains of oil and water are inter-dispersed within the system.

3. Microemulsion Based GEL

The present work aims to prepare microemulsion based gel (MBG) may be formulated to enhance the drug release, onset of action to provide the more topical effect of curcumin. Rapid drug release on specific site of skin compared to conventional dosage form can be achieved by this approach (Shah, \textit{et al.}, 2014) microemulsion is a colloidal dispersion composed of an aqueous phase, oil phase, surfactant and co-surfactant at appropriate ratios,
which is single optically isotropic and thermodynamic stable liquid solution with droplet diameter, usually within the range of 10-100 nm. (Joshi B, \emph{et. al.}, 2011).

The concept of microemulsion was introduced in the early 1940s by Hoar and Schulman, who generated a clear single-phase solution by titrating a milky emulsion with hexanol. They prepared the first microemulsion by dispersing oil in an aqueous surfactants solution and adding alcohol as a co-surfactant, leading to a transparent, stable formulation (Lapasin, \emph{et al.}, 2001). The structure of microemulsion droplet has been described in Fig. 1.1.

Recently, the concept of microemulsion based gel formulation becomes popular as novel drug delivery system. Most of the conventional topical agents, like ointment, creams and lotion have many disadvantages. They are very sticky and exhibit stability problems. Moreover, they also have lesser spreading coefficients and need to apply with rubbing. The present stability problems also (Patel, \emph{et al.}, 2014). Due to the factors mentioned above within the significant group of semisolid preparations, the use of microemulsion based gels have expanded both in cosmetics and in pharmaceutical preparations. Though the conventional semisolid dosage forms have many advantages of gel, they have significant limitations with hydrophobic drugs. So to overcome these limitations, microemulsion based gel approach is being used, so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through microemulsion based gels (Ansel, \emph{et al.}, 1999).

In the microemulsion based gel, the drug particle size is maintained to the micron range. It is soluble in oil, surfactant and co-surfactant and has a diameter in the range of 100-1000A (10-100 nm). Also, the small size of the drug droplets in microemulsion yields large interfacial, from which the drug can be quickly released into an external phase when it is area through the skin at the particular site of action in specific diseases, maintaining the concentration in the external phase close to initial levels for topical drug delivery system. (Singh V, \emph{et al.}, 2011).
The microemulsion based gel formulation has been well accepted for drugs with poor aqueous solubility and high permeability, classified as Class II drugs by BCS system (Fig. 1.2). The rate and extent of absorption of class II compounds are highly dependent on the performance of the formulated product. These drugs can be successfully formulated for oral administration, but care needs to be taken with formulation design to ensure consistent bioavailability (Mehta et al., 2011).

3.1. Method of Preparation of Microemulsion Based Gel.

**STEP 1:** Formulation of microemulsion either o/w or w/o.

**STEP 2:** Formulation of gel base.

**STEP 3:** Incorporation of microemulsion into gel base with continuous stirring.
Fig. 1.4: Flow Chart for Preparation of Microemulsion Based Gel.

3.2. Advantages OF MBG (Trommer et al., 2006)

(i) **Better stability:** Other Transdermal preparations are comparatively less stable than microemulsion based gel. Like powders are hygroscopic, creams show phase inversion or breaking and ointment shows rancidity due to an oily base and normal topical emulsion shows creaming effect. The microemulsion based gel does not show any above problems and gives better stability.

(ii) **Better loading capacity:** Other novel approaches like niosomes and liposomes are of nano size and due to vesicular structures may result in leakage and result in lesser entrapment efficiency. But gels due to the vast network have a comparatively better loading capacity of the drug.

(iii) **Production feasibility and low preparation cost:** Preparation of microemulsion based gel is comprised of more straightforward and short steps which increase the feasibility of the production. There are no specialized instruments needed for the production of microemulsion based gel. Moreover, the materials used are easily available and cheaper. Hence, decreases the production cost of microemulsion based gel.

(iv) **Incorporation of hydrophobic drugs:** Most of the hydrophobic drugs cannot be incorporated directly into the gel base because the solubility act as a barrier and a problem occurs during the release of the drug, mainly class VI drug. Micro-emulsion based gel helps in the incorporation of hydrophobic drugs into the oil phase, and then oily globules are dispersed in an aqueous phase resulting in o/w emulsion. And this emulsion
can be mixed into gel base. This may be proving better stability and release of a drug that is merely incorporating drugs into gel base. E.g., ketoconazole, fluconazole, etc.

(v) **No intensive sonication:** Production of vesicular molecules needs intense sonication which may result in drug degradation and leakage. But this problem is not seen during the production of microemulsion based gel as no sonication is required.

(vi) **Controlled release:** Microemulsion based gel can be used to prolong the effect of drugs having shorter t1/2.

### 3.3. Disadvantages of MBG (Trommer, et.al, 2006)

A. The larger particle size drugs not easy to absorb through the skin.
B. The poor permeability of some drugs through the skin.
C. Can be used only for drugs which require minimal plasma concentration for action.
D. The possibility of allergenic reactions.
E. An enzyme in the epidermis may denature the drugs.

### 3.4. Application of MBG

1) Enhance the transdermal permeation of drugs significantly compared to conventional formulations such as solutions, gels or creams.
2) They can incorporate both hydrophilic (5-fluorouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate, etc.) and lipophilic drugs (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide, etc.) and enhance their permeation.
3) A significant amount of drug can be incorporated in the formulation due to the high solubilizing capacity.
4) Increase thermodynamic activity towards the skin, the permeation rate of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favor partitioning into the stratum corneum.
5) Using different internal phase, changing its portion in the microemulsion, the surfactant and co-surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers.
6) The percutaneous absorption of a drug will also increase due to the hydration effect of the stratum corneum if the water content in the microemulsion is high enough.
4. **Components of MBG**

i. **Oils:** The oils used for the preparation of microemulsion having the capacity to solubilize the drug. For externally applied microemulsions, mineral oils, either alone or combined with soft or hard paraffin, are widely used both as the vehicle for the drug and their occlusive and sensory characteristics. Widely used oils in oral preparations are non-biodegradable mineral and castor oils that provide a local laxative effect, and fish liver oils or various fixed oils of vegetable origin (E.g., arachis, cottonseed, and maize oils) as nutritional supplements. Some are as light liquid paraffin, isopropyl myristate, isopropyl stearate, isopropyl palmitate, propylene glycol, etc.

**Examples:** Olive oil, Peanut oil, Sesame oil, Soybean oil, Sunflower oil, Triacetin, Paraffin oil, oleic acid, castor oil, corn oil, ethyl oleate, polyoxy castor oil, etc.

ii. **Aqueous Material:** This forms the aqueous phase of the microemulsion. Most commonly, water is used as an aqueous phase. The pH of the aqueous phase always needs to be adjusted due to its considerable impact on the phase behavior of microemulsion. The commonly used agents are water, alcohols, etc.

iii. **Surfactant (Emulsifier):** Surfactant molecules consist of two-part, polar head group region and non-polar headgroup region. They are classified into four categories according to the nature of hydrophilic group within the molecule: Anionic surfactant, Cationic surfactant, Non-ionic surfactant, an Ampholytic surfactant. Surfactant reduces the interfacial tension between two immiscible liquids and makes them miscible. When surfactants are incorporated in oil and water mixture, then their polar heads are self-associated towards water phase, and non-polar tails towards oil phase or they can quickly locate at the interface, which is thermodynamically very stable.

**Examples:** Tween 80, Tween 20, Cremophor RH40, Labrafil M1944CS, Cremophor EL, Span 80.

iv. **Co-surfactant (Co – Emulsifier):** Relatively high concentration (usually, more than 30% w/w) are needed to produce a useful microemulsion for the topical drug delivery system. Organic solvents: which is Suitable for topical administration (ethanol, propylene glycol (PG), polyethylene glycol (PEG), etc.) may help to dissolve large amounts of either the hydrophilic surfactant or the drug in the lipid base. These solvents sometimes
play an essential role in the co-surfactant in the microemulsion systems. Polymeric liquid and semi-solid excipients can be used alone or in mixture with other lipid excipients to improve the solubilizing power of formulation. Among the polymeric glycol based excipients, PEGs are the versatile, well characterized and widely applied class of solubilizers which are available as both liquid and thermo softening semisolid.

Examples: Propylene glycol, PEG 200, PEG 400, Ethanol, Transcutol HP.

v. Polymers: Polymer present in 5 to 40% w/w, which is not ionizable at physiological pH and able to form a matrix. Examples are hydroxypropyl ethyl cellulose, ethyl cellulose, etc. (Giri et al., 2013).

vi. Gelling agents: Addition of gelling agent to these formulations gives a gelled structure. The gelling agent is of two types: natural and synthetic. Incorporation of gelling to a system makes it thixotropic. According to the Swedish national encyclopedia: thixotropy is "property of viscous (viscid) or gel-like product turning more liquid as the long time and the more vigorous, which is deformed (i.e., stirring)." It is generally accepted thixotropy the phenomenon of the fluid which shows a reversible structural. Carbopol polymers are polymers of acrylic acid cross-linked with polyalkenyl ethers or divinyl glycol. They are produced from primary polymer particles of less a diameter. Each particle can be viewed as a network structure of polymers chains interconnected via crosslinking. Carbomers readily absorb water and get hydrated and swell. Besides it's hydrophilic in nature, its cross-linked structure and it's insolubility in water make carbopol a potential candidate for use in the controlled release drug delivery system. Effect of gelling agent has been studied on the release rate of the drug from microemulsion based gel. It has been found. That is an inverse correlation b/w the concentration of gelling agent and the extent of drug released. Others type including synthetic, semi-synthetic, natural gelling agent can also be employed.

vii. Penetration Enhancers: Penetration enhancers are the agents which increase the penetration power of the drug through the skin. To promote absorption of drugs through skin barrier, vehicles often include penetration enhancing ingredients which temporarily disrupts the highly ordered structure of stratum corneum skin barrier, fluidize the lipid channels between corneocytes, alter the partitioning of the drug into skin structures, or otherwise enhance delivery into the skin.
5. MATERIALS AND METHODS

Formulation Development of MBG

Various composition of MBG (Table 1.2) were prepared

Drug (curcumin, 2gm) was dissolved in the oleic acid (oil phase). Then add tween 80 and methanol was added as surfactant and co-surfactant respectively. All the ingredients were mixed well. Then water was added drop by drop and mixed well on the magnetic stirrer. The transparent microemulsion was formed. Now for preparation of Gel base (aq phase), Carbopol 934 and HPMC was suspended in water and hydrated it for overnight. pH was adjusted around 6 to 6.5 using triethanolamine. For the preparation of microemulsion based gel, microemulsion and 1% Carbopol 934 gel and 1% HPMC were mixed in the ratio of 1:1.

Table 1.2: Composition of Microemulsion Based Gel of Curcumin.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
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</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>HPMC</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Carbopol 934</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Tween 80</td>
<td>3.1</td>
<td>3.1</td>
<td>3.1</td>
<td>3.1</td>
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<tr>
<td>Methanol</td>
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<td>PEG</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Castor oil</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
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<td>0.9</td>
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<tr>
<td>Oleic acid</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td>Triethanolamine for pH</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
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</tr>
<tr>
<td>Distt. Water</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
</tbody>
</table>

5.1. Methods

5.1.1. Preformulation Studies

Preformulation studies were carried out to standardize a spectrophotometric method of estimation for curcumin and to investigate any possible drug-polymer interactions. The drug-polymer interaction was studied by carrying out Fourier transform infrared (FTIR) spectral studies. It is the first step in the rational development of any dosage forms.

Identification of the pure drug and polymers was performed using infrared spectroscopy. IR spectroscopy by potassium bromide method was carried out on drug, polymer, ingredients and physical mixture of drug-ingredients.

About 2 mg of each sample was ground thoroughly with previously dried KBr at 120°C for 30 min. Uniformly mixed with drug and kept in the sample holder and compressed under ten tones pressure in a hydraulic press from a transparent pellet. The pellet was scanned from
4000 to 400 cm$^{-1}$ in a spectrophotometer and peaks obtained were recorded. Pure, wholly dried KBr was used as blank and before running the sample (Bassler and Morril, 1981; Devendra and Dhananjay, 2007).

5.1.1.1. Identification and Characterization of curcumin (Nazzal, et al., 2002)

- **Organoleptic properties:** Curcumin was tested for organoleptic properties such as appearance, color, taste, etc.

- **The melting point determination:** The melting point of a compound is the temperature at which it changes from a solid to liquid. The melting point determination of the obtained sample of curcumin was done by an open capillary method.

- **Clarity and color of solution:** Clarity and color of curcumin solution were determined by preparing the solution of 0.5 g of curcumin dissolved in 10 mL methanol. Results were compared with specifications given in Herbal Pharmacopoeia and certificate of analysis provided by drug sample provider.

- **Solubility profile:** About 10 mg of curcumin was taken, and solubility was studied in different solvents (Indian Pharmacopoeia, 1996; Martin, 2001).

5.1.1.2. UV Spectroscopy

The stock solution of curcumin was prepared by dissolving accurately 10 mg of curcumin in methanol in a 100 ml volumetric flask to obtain a concentration of 100μg/ml. The UV spectrum was recorded in the range of 220-430 nm on Shimadzu UV-visible spectrophotometer (Shimadzu-1800, Japan) at 1 cm, slit width (Patel, P.A., 2008).

5.1.1.3. IR spectrum interpretation

The infrared absorption spectrum of pure curcumin was recorded on FT-IR spectrophotometer and the spectrum analysis was done for functional groups.1 mg of the drug was mixed with 100 mg of potassium bromide in a mortar by trituration and the mixture was compressed into a pellet at 10 ton/cm$^2$ in a pellet maker. The sample was scanned at 4000 cm$^{-1}$- 400 cm$^{-1}$. The IR spectra obtained was found concordant with the IR spectrum of curcumin.

5.1.1.4. Determination of the solubility of drugs in oils, surfactants, and co-surfactants

The solubility of curcumin was studied in an aqueous and non-aqueous solvent. Different oils, surfactants, co-surfactants (table 4.3) were taken separately in the small vial and an excess amount of a drug was added to each vial. The vials were tightly closed and were
stirred continuously for 72 h using mechanical shaker at 25°C. Then oils, surfactant and cosurfactants were centrifuged at 1000 rpm for 10 min to separate un-dissolved drug. A supernatant was taken and diluted with methanol and solubility was quantified with UV spectroscopy (Shimadzu 1800) at 425 nm.

5.1.2. Quantitative Estimation of Drug

5.1.2.1. Preparation of calibration curve of curcumin in methanol

The standard curve in methanol was prepared to analyze the drug content in different oils, surfactants, and co-surfactants during solubility study of curcumin. From this solution, 10 ml was withdrawn and diluted to 100 ml with methanol. From this stock solution, serial dilutions were made to obtain the solutions in the concentration ranging from 2-18 μg/ml. The absorbance of the solution was measured at 425 nm, and the calibration curve was plotted. (Palet et al., 2011)

5.1.2.2. Preparation of calibration curve of curcumin in phosphate buffer pH 8

10 mg of curcumin was dissolved in 10 ml solution of phosphate buffer pH 8 in the volumetric flask, and the volume was made up to 100 ml using the phosphate buffer. From this solution, 10 ml was withdrawn and diluted to 100 ml with phosphate buffer pH 7.5. From this stock solution, serial dilutions were made to obtain the solutions in the concentration ranging from 2-18 μg/ml. The absorbance of the solution was measured at 425 nm, and the calibration curve was plotted.

5.1.2.3. Drug Excipients Interaction Study

Drug-excipient compatibility studies were designed to ensure the stability of the final formulation. The physical mixture of drug and excipients in the ratio of 1:1 were placed in glass vials, sealed and stored at ±40°C and ±75% relative humidity. The samples were drawn at the predetermined time interval of 7, 15, 21, 30 and 45 days and examined for physical and chemical integrity. Parameters such as color changes, odor or gas formation, liquification and caking were analyzed. The excipients the showed minimal or no degradation when kept with drug after 45 days were chosen for further studies since they were compatible with a drug.

5.1.3. Evaluation of Microemulsion Based GEL

- **pH:** The pH of microemulsion the prepared was determined using pH meter. The pH meter was calibrated using pH buffer solution (pH 4 and pH 7) and the pH of each microemulsion prepared was determined in triplicate.
• **Dilution test:** A small amount of microemulsion was placed on a clean glass slide. A drop of water added to the microemulsion and was mixed with the help of glass rod and their transparency was assessed visually.

• **Viscosity measurements:** The viscosity of each of the prepared microemulsion containing different percentages of water content was determined by using digital Brookfield viscometer equipped with spindle No. S64 at a speed of 10 rpm (Brookfield Viscometer, model LVT, U.S.A.).

• **Determination of percentage drug content:** The drug content of the microemulsion formulation was determined by dissolving 1 ml of the formulation in 10 ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer keeping Methanol as control at wavelength 425 nm.

• **Droplet size and particle size measurement:** Zeta potential must be negative or neutral, which indicate that droplets of a microemulsion having no charge that is system is stable. Zeta potential was determined by using zetasizer. Zeta potential is mainly useful for assessing flocculation since electrical charges on particles influence the rate of flocculation. The polydispersity index (PDI) was also determined using zetasizer.

• **Measurement of droplet size the Micro-emulsions:** Droplet size of novel formulations was measured by use of dynamic light scattering based zetasizer nano- ZS instrument (Malvern, master sizer SM 2000k, United Kingdom) at room temperature using the neon laser at 425 nm.

• **Refractive index and percent transmission:** Refractive index and percent transmittance prove the clearness of formulation. The refractive index of micro-emulsion gel was measured by refractometer and compared with that of water. The percent transmittances of the system were measured at a particular wavelength using UV-vis spectrophotometer keeping the distilled water as a blank. If the refractive index of a system was similar to that of water, formulation showing transmittance >99% was assumed to be transparent in nature.

• **Percent Transmittance:** Transparency of micro-emulsion formulation was determined by measuring the percentage transmittance at 425 nm with purified water taken as blank through UV spectrophotometer.

• **Transmission Electron Microscopy:** Transmission electron microscopy characterized the microstructure of the prepared curcumin microemulsion (TEM Hitachi (H-7500)). The microemulsion was prepared at ambient conditions. Morphology and structure of oil
globules were determined with the aid of TEM. The microemulsion systems were dried on a microscopic carbon coated grid and viewed under the microscope after staining at the suitable magnification. Photo-micrographs of the globules were then taken.

6. RESULT AND DISCUSSION

6.1. Identification and Characterization of Curcumin

Table 1.3: Characterization of Curcumin.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Specifications</th>
<th>Result</th>
</tr>
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<tr>
<td>Appearance</td>
<td>Yellowish orange</td>
<td>Yellowish orange</td>
</tr>
<tr>
<td>Taste</td>
<td>Tangy, a little sour</td>
<td>Tangy, a little sour</td>
</tr>
<tr>
<td>Melting range</td>
<td>178-181º C</td>
<td>180º C</td>
</tr>
<tr>
<td>Solubility</td>
<td>Practically insoluble in water, highly soluble in methanol, ethanol</td>
<td>Practically insoluble in water, highly soluble in methanol, ethanol</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>NMT 2.0%</td>
<td>1.0%</td>
</tr>
</tbody>
</table>

Discussion: The results of the characterization of curcumin complied with the specifications given in certificate of analysis provided by the supplier.

6.2. Solubility of Drug in Oils, Surfactants, and Co-Surfactants

UV spectroscopy determined the concentration of curcumin in various oils, surfactants, and co-surfactants at room temperature, and results are shown in Tables 5.2, 5.3 and Figs. 5.1, 5.2 respectively.

Table 1.4: Solubility of Curcumin in Various Oils.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Oil</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soya bean oil</td>
<td>0.142±0.016</td>
</tr>
<tr>
<td>2</td>
<td>Paraffin oil</td>
<td>0.124±0.015</td>
</tr>
<tr>
<td>3</td>
<td>Peanut oil</td>
<td>0.257±0.023</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl Oleate</td>
<td>0.366±0.033</td>
</tr>
<tr>
<td>5</td>
<td>castor oil</td>
<td>0.50±0.05</td>
</tr>
<tr>
<td>6</td>
<td>oleic acid</td>
<td>1.02±0.03</td>
</tr>
</tbody>
</table>
Fig. 1.5: Solubility of Curcumin in Various Oils.

Table 1.5: Solubility of Curcumin in Various Surfactants and Co-Surfactants.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Surfactant and Co-Surfactant</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tween-60</td>
<td>1.143±0.084</td>
</tr>
<tr>
<td>2</td>
<td>Tween-80</td>
<td>1.351±0.115</td>
</tr>
<tr>
<td>3</td>
<td>Cremophor RH40</td>
<td>1.922±0.238</td>
</tr>
<tr>
<td>4</td>
<td>PEG 400</td>
<td>1.441±0.168</td>
</tr>
<tr>
<td>5</td>
<td>PEG 200</td>
<td>1.03±0.03</td>
</tr>
<tr>
<td>6</td>
<td>Propylene glycol</td>
<td>0.49±0.05</td>
</tr>
</tbody>
</table>

Fig. 1.6: Solubility of Curcumin in Various Surfactants and Co-Surfactants.
Discussion: From the results of solubility study of curcumin in different oils, surfactants and cosurfactant, it was found that curcumin was more soluble in Oleic acid, ethyl oleate, cremophor RH40, Tween 80 and PEG 400 than other vehicles. Hence Oleic acid was selected as oil, and from Tween 80, Propylene glycol, cremophor RH40 and PEG 400 might be chosen as surfactant and co-surfactant after screening their emulsification ability with Oleic acid so that optimal SMEDDS will be formed with improved drug loading capabilities.

6.3. Compatibility Studies: FTIR

IR Spectrum of Microemulsion Based Gel Formulation of Curcumin.

![IR Spectrum of MBG Formulation of Curcumin.](image)

Fig. 1.7: IR Spectrum of MBG Formulation of Curcumin.

6.4. Analytical Method Development

Calibration Curve of Curcumin by UV Spectrophotometric Method

Table 5.5 and Fig. 5.12 represent the absorbance data and standard plot of curcumin in methanol respectively. Beer-Lambert’s law was obeyed over the range of 1-8 μg/ml, and the data was found to fit the equation.

\[
y = 0.034x + 0.003
\]

\[R^2 = 0.996\]
Table 1.6: Calibration Curve Data of Curcumin in Methanol.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.155</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.289</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.440</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.610</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.756</td>
</tr>
</tbody>
</table>

![Calibration curve of curcumin in methanol](image)

**Fig. 1.8**: Standard Curve of Curcumin in Methanol.

6.5. Globule Size And Zeta Potential Determination

![Size Distribution by intensity](image)

**Fig. 1.9**: Particle Size Distribution Curve of Curcumin Microemulsion.
Table 1.7: Results of Optimized Microemulsion Gel Batch.

<table>
<thead>
<tr>
<th>Atch</th>
<th>pH</th>
<th>Viscosity (cps)</th>
<th>Globule Size (nm)</th>
<th>Zeta Potential (Mv)</th>
<th>Poly Dispersity Index (PDI)</th>
<th>% Drug Content</th>
<th>% Transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6.36</td>
<td>70.46</td>
<td>85.90</td>
<td>-7.36</td>
<td>0.126</td>
<td>91.25</td>
<td>97.36±0.13</td>
</tr>
<tr>
<td>F2</td>
<td>6.19</td>
<td>74.24</td>
<td>89.5</td>
<td>-7.3</td>
<td>0.113</td>
<td>93.12</td>
<td>96.05±0.48</td>
</tr>
<tr>
<td>F3</td>
<td>6.45</td>
<td>67.92</td>
<td>82.90</td>
<td>-10.7</td>
<td>0.245</td>
<td>94.52</td>
<td>98.42±0.72</td>
</tr>
<tr>
<td>F4</td>
<td>6.58</td>
<td>80.60</td>
<td>80.0</td>
<td>+2.05</td>
<td>1.036</td>
<td>89.67</td>
<td>97.84±0.06</td>
</tr>
<tr>
<td>F5</td>
<td>6.25</td>
<td>71.95</td>
<td>98.2</td>
<td>-11.86</td>
<td>0.168</td>
<td>85.34</td>
<td>96.29±0.28</td>
</tr>
<tr>
<td>F6</td>
<td>6.72</td>
<td>68.83</td>
<td>104</td>
<td>+2.74</td>
<td>2.051</td>
<td>90.54</td>
<td>95.67±0.34</td>
</tr>
</tbody>
</table>

7. SUMMARY AND CONCLUSION

7.1. Summary

A preliminary comparative study not reported in this paper was used for selection of appropriate oil and surfactant, which would aid comparative study of curcumin in the dosage form and allow for retaining the drug in solution even after dilution with water.

Since the transdermal transport of a drug involves interactions among solubility, partition, and diffusion processes, in the present study relationships between the vehicle composition and curcumin solubility were investigated. The solubility of curcumin in the various polymer, oils, surfactants, and co-surfactants was studied to determine the optimal components as a vehicle for transdermal delivery. Different oils including castor oil, oleic acid.

A microemulsion gel formulation comprising of carbopol 934, HPMC, and water was deemed promising as a successful topical delivery system of curcumin for the treatment of
fungal skin infections. Although, the gel preparation was more effective in vitro drug release as an antifungal agent than the normal skin cream.

Curcumin was identified and characterized as per requirements of an official monograph (Herbal Pharmacopoeia, 2002). The $\lambda_{\text{max}}$ was obtained at 425 nm. IR spectroscopy also identified the drug. The IR spectrum of drug sample was found to agree with the standard IR spectra of pure drug given in the official monographs. A calibration curve of curcumin was prepared in methanol in the concentration range of 2-10 μg/ml by measuring absorbance at 425 nm. The correlation coefficient of the calibration curve was found to be 0.996 indicating good linearity.

A series of microemulsion gel were prepared in the formula with varying concentrations of castor oil, HPMCC, carbopol 934 and PEG. Droplet size distribution of suitably diluted MBG formulation with water was determined using Photon correlation spectrometer. The zeta potentials of the optimized formulation were less than -11 mV, indicating excellent stability. Polydispersity index below 0.11 indicates good uniformity in the droplet size distribution after dilution with water.

1 ml of MBG was dissolved in excess methanol, and curcumin content in the extract was analyzed spectrophotometrically (UV spectrophotometer) at 425nm. Drug content of various formulations was found to be in the range of 89.67 - 94.52%.

**7.2. CONCLUSION**

A microemulsion prepared was clear, stable, isotropic mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. Formulating Curcumin as a microemulsion was found to be useful for transdermal application with sufficient drug loading and drug release profiles. Also, it was found to serve the purpose improves solubility and gel-based microemulsion would cater to need of patient compliance.

**8. REFERENCE**


