

Formulation and evaluation of medicated microemulsion for topical application

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Abstract Backgrounds

Microemulsion is a clear thermodynamically stable dispersion of two immiscible liquids with carefully adjusted emulsifier(s). It can be used as a vehicle for many different drugs and dosage forms.

Methods

To formulate a microemulsion for topical use from cheap components a pseudoternary phase diagrams were constructed with formulae consisting of paraffin oil, cosurfactant (sorbitol or glycerol), surfactant (Brij97) and water. The existence of microemulsion regions was demonstrated.

Indomethacin was added to the best formulae, which produce microemulsions in gel form and evaluating them for their physical characters, release rate, physical and chemical stability and Pharmacodynamic.

Results

Fifty five formulae produce stable microemulsions in gel form. On incorporation of indomethacin only four formulae remain stable, clear formulae. They were used for further studies. Their order of drug release rate was first order. The four formulae were stable when stored at room temperature, or under stress. Shelf life of them would be minimum of 492 days and maximum of 712 days.

Indomethacin significantly inhibits edema, induced in rat paw by different percentage. Effect of the prepared microemulsions were between the effect of commercial injection form, highest effect, and commercial topical form, lowest effect.

Conclusions

The microemulsion formulae prepared with paraffin oil, brij97, sorbitol or glycerol and water showed acceptable physical properties, drug release, stability and Pharmacodynamic effect.

Keywords: indomethacin; microemulsion; Paraffin oil; water; surfactant; cosurfactant.

Introduction

Indomethacin is a potent anti-inflammatory, analgesic and antipyretic drug. Indomethacin has been used effectively in the management of patients with the moderate to severe rheumatoid arthritis, osteoarthritis, acute painful shoulder and acute gouty arthritis.¹

Microemulsion is clear thermodynamically stable dispersion of two immiscible liquids with carefully adjusted emulsifier(s). It is an isotropic systems of infinite stability, usually consists of four components, which are surfactant, cosurfactant, oil phase and aqueous phase. Optimization of the microemulsion preparation is dependent on these four components. The formation of microemulsion is determined by changing the concentration of these four interacting variables. Only specific components combination can produce transparent system. Microemulsion has been used as a vehicle for several dosage forms to enhance the absorption of drug.²⁻⁵ It has also been used as a mobile phase in HPLC.⁶⁻⁸

A number of steps are required to formulate a microemulsion for therapeutic use. After selecting the components (usually oil, surfactant and cosurfactant and hydrophilic medium) and after some preliminary tests, a series of phase diagrams are made to establish the different zones (microemulsion, coarse emulsion, etc.) usually by physical methods^{9, 10} in the absence of the drug. Phase diagrams are typically constructed in three dimensions, either maintaining the fourth component constant or maintaining the ratio between two of the components fixed. After defining the microemulsion domain, the second step is to define the domain in the presence of the drug, since it is known that addition of a drug to a microemulsion alters the

percentages of the components of the mixture vary and may affect the stability of the system. ¹¹

The aim of present investigation was to formulate stable microemulsion from cheap components suitable for topical application containing suitable non-volatile carrier for Indomethacin with good physical and chemical stability and of improved Pharmacodynamic.

Materials and methods

Materials

Brij 97, glycerin, sorbitol 70% and liquid paraffin (Sigma Chemical Company, USA). Indomethacin base (Merck Sharp and Dohme, USA).

Screening of Oils and Surfactant for Microemulsion

Suitable oil and surfactant that possess good solubilizing capacity on indomethacin were identified by using solubility studies in various oil and surfactant solutions. Solubility of indomethacin in oils and surfactant solutions was determined by adding excess amount of drug and continuously stirring for at least 72 hours at 30°C. The mixtures were centrifuged (2500 rpm for 30 min), and supernatant was filtered through 0.45 mm membrane filter. Drug concentration in the filtrate was determined by using an HPLC after appropriate dilution with methanol.

Formulation

A. Preparation of plain microemulsions

Different blends of the surfactant (Brij97) and cosurfactant at different volume ratios was prepared using the above mentioned materials. Each of these blends was allowed to stand in a water bath at 90°C for 5 minutes and then stirred using a magnetic stirrer at high speed. Aliquots of each of these blends were mixed with oil to give (Brij97+ cosurfactant): oil weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5 and 4:6. The mixtures of Brij97, cosurfactant and oil were diluted with water at 90°C. The amount of water added was varied to give water content in the range of 0-90%. The systems were allowed to cool to room temperature and stored for 24h before further observations were made.

B. Testing the gel formation of each mixture

The test was performed by tilting a jar containing the microemulsion to an angle of 90°, the one that does not show a change in the upper surface after tilting to this angle within few seconds were considered gel.

C. Representing the results of the gel test in a Phase Diagram:

Pseudo-three component phase diagram was constructed with one axis representing water, one representing oils and the third representing a mixture of surfactant and cosurfactant at fixed weight ratios. The mixture that show clear solution after preparation indicating microemulsion formation were recorded in the Phase Diagram as fluid like microemulsions and gel-like microemulsions.

Formulation and Evaluation of indomethacin gel-like microemulsions

The aim of this part was to formulate an indomethacin gel-like microemulsions (IGMs) by selecting the best concentrations of surfactant, cosurfactant, oil and water, which produce the best gel-like microemulsions (IGMs) in the previous part and evaluating them after the addition of indomethacin.

Preparation of the indomethacin gel-like microemulsion:

This step was done as in the previous part for the plain microemulsion with the immersion of 100-mg indomethacin base in the amount of oil determined for preparation of 10gm of the microemulsion to prepare the medicated microemulsion.

The prepared IGMs were tested for:

A. Physical character.

The prepared IGMs were examined when fresh for:

1. Phase separation.
2. Clarity.
3. Gel formation.

B. Determination of the release rate of indomethacin from different gel-like microemulsion formulae.

The test was carried out using USP dissolution tester containing 900 ml of 5.5-pH medium at 37± 0.5°C and paddle rotating at 50 r.p.m. 20 gm of the chosen gel-like microemulsion was placed in a cup of a fixed surface area covered with a cellulose membrane. The cup was placed in the dissolution medium with 10 gm weight to settle it down at the bottom of the dissolution medium. Aliquots were withdrawn every 10 minutes for 2 hours.

The aliquots were diluted with equal volume of methanol to be analyzed spectrophotometrically at 318.5 nm for their indomethacin contents. Kinetic Study of release data of IGMs was done for the determination of release order of indomethacin from its formulae.

The obtained data were compiled in tables and statistically treated by the least squares method to identify the order of drug release whether being zero, first or diffusion release model.

C. Stability of the selected IGMs.

The prepared IGMs were subjected to shelf life and accelerated stability:

1. Shelf Life Stability: The prepared IGMs were stored at room temperature in a glass bottle and examined periodically for their for their chemical stability (Drug content) and physical stability namely:

I- Appearance and Clarity test.

II- Phase Separation and Centrifugation test at 20000 r/min for 30 min at 25°C.

III- pH testing.

IV- Viscosity measurement.

Pharmacodynamic of indomethacin from different IGMs formulae

The Pharmacodynamic of Indomethacin of different microemulsions was tested by observing the effect of the formulae on reducing local edema induced in the rat paw by injection of the carrageenan (Carrageenan Sodium, BDH, England) as irritant.

Carrageenan induced rat paw edema was selected for the present study according to the method described by Winter et al.¹² Adult male albino rats weighing 160-220g were used. They were fasted over night and then uniformly hydrated by giving 3 ml water/rat through gastric intubations to reduce variation to edema response.¹³ Animals were divided into 7 groups each of 6 animals. The control group was given saline solution containing few drops of Tween 80. Indomethacin (5 mg/kg) was given parenterally 1 hour before induction of inflammation to the group selected for testing injection form as commercial product of indomethacin. Inflammation was induced by a subcutaneous injection of 0.5 ml of 1% carrageenan solution into the planter tissue of right hind paw before the application of the topical drug to the animals. The topical drugs were applied to rats every 1-hour to ensure contact of the formulae to the inflamed hind paw of the rats. The mean increase in weight of carrageenan injected paws of rats treated with the drug (W_t) as well as that of the control group (W_c) were calculated by subtracting the weight of the left hind paw from the weight of the right hind paw. The percentage inhibition of inflammation for each group was calculated from the mean effect in control and treated animals according to the following equation: -

$$\% \text{ Inhibition in Edema weight} = ((W_c - W_t) / W_c) \times 100$$

The test was carried out on:-

1. IGMs
2. Commercial Injection form of Indomethacin.
3. Commercial topical form of Indomethacin.

Results and Discussion

Microemulsions prepared by using only pharmaceutically acceptable ingredients are limited. Therefore, with a view to developing a suitable microemulsion system for topical delivery of indomethacin, the solubility of indomethacin in various oils and nonionic surfactant solutions were determined. Highest solubility of the drug was observed with paraffin oil followed by isopropyl myristate, castor oil, soya bean oil, and olive oil. On the other hand, solubility of the drug in Brij 97 was greater than that in Tween 20, 40, 60 and 80; they are known to be unaffected by pH and ionic strength variations. The use of paraffin oil as oil in the following experiments was not only due to its solubilizing effect on indomethacin but also due to its low price.

On preparing the microemulsions at different ratios of their constituents, it was found that the microemulsions formed at surfactant: cosurfactant ratio of 9:1 was easily formulated and that at ratio of 3:1 were difficult to be formulated.

Microemulsion was formed at high surfactant concentration and its formation was decreased as oil content increases. In all the systems the increase in the paraffin oil content leads to a shift of the microemulsion region towards the surfactant apex which suggests that excess surfactant molecules were required to cover an expanded hydrophobic core.

The microemulsion occurred in the consistency of fluid; as the water concentration increased to a certain level it started to gel till a certain concentration, where it returned back to fluid again. That differed from surfactant : cosurfactant ratio to another and from surfactant : cosurfactant : oil ratio to another.

From the preliminary tests it was clear that the oil contents as well as the ratio of the surfactant to the cosurfactant control the formation of the microemulsion. The effect of the surfactant/cosurfactant weight ratios may be explained by the opposing effects of the surfactant and cosurfactant on the interfacial film. Addition of more surfactant causes the interfacial film to condense with subsequent decrease in the interfacial tension¹⁴ while the addition of the cosurfactant would cause decrease in the amount of oil incorporated in the system due to the increase in the polarity of the system.^{15, 16}

Fifty five formulae of microemulsions differ in the proportion of surfactant, oil, cosurfactant and water produce gel-like microemulsions (IGMs). These formulae were selected to proceed to the next testing procedure.

The addition of indomethacin and the storage had clearly affected the consistency of the 55 selected microemulsions. This is consistent with the previously published work.¹¹

Incorporation of indomethacin in IGMs led to many changes. All the gel-like microemulsions, except 11 formulae, show phase separation. Upon storing the 11 stable IGMs for one month at room temperature, only 4 IGMs remained stable, clear gel-like microemulsions. The compositions of these 4 formulae are listed in table 1.

Table 1. The composition of the best IGMs.

Cosurfactant in formulae 4 and 8 was sorbitol 70% and in Formulae 43 and 44 was glycerol

Formula No.	Brij 97 % v/v	Cosurfactant % v/v	Oil % v/v	Water % v/v
4	30.4	3.6	4	60
8	28.8	3.2	8	60
43	40.5	13.5	6	40
44	33.75	11.25	5	50

These formulae were used for further study. The release rates of indomethacin from gel-like microemulsions of the four selected formulae are shown in figure 1. The kinetic of all the four formulae show a drug release of first order release. The highest release rate was obtained from formula 8; the lowest release rate was obtained from formula 44. Formula 8 had the highest release rate followed by formula 4 then Formula 43 then formula 44. That could be due to the presence of the glycerol in formulae 43 and 44 which may retard the release more than sorbitol in formulae 8 and 4.

All the tested IGMs remain physically stable when stored at room temperature for one year and at 40, 50, 60°C for 3 months. A slight increase in pH value especially those samples maintained at 60°C and the increase can be considered to be within the usual limits. Regarding Viscosity Measurement, the relationships between shear stress and shear rate, when fresh and after shelf life stability appeared to be plastic with thixotropic properties for the four formulae. The formula of the highest viscosity range is formula 8 and the formula of the lowest viscosity range is formula 4. The formulae could be ranked from the highest to the lowest viscosity range as follow:

Formula8 > Formula 44 > Formula 43 > Formula 4

All the four formulae show a drug degradation of first order Kinetic. The degradation rate constants (K value) for the microemulsions studied were obtained at different temperatures assuming a first order kinetics and were used to get the K_{20} value of the accelerated chemical stability test.

There was no significant difference between the K_{20} value obtained from the accelerated chemical stability test and the K_{20} value of the shelf life stability test.

It should be noted that the shelf life of the microemulsions would be minimum of 492 days (1.349 years, formula 4) and maximum of 712 days (1.952 years, formula 8). Formulae 43 and 44 had a shelf life of 642 days (1.759 years) and 568 days (1.556 years), respectively.

Hence, on stability tests the four formulae showed good shelf life stability. Formulae 8 showed the best stability.

The difference in rheological properties of the IGMs between the fresh and after the shelf life stability was insignificant, as there were a slight increase in the viscosity and the shear stress. That can be considered to be within the usual limits. That different could be due to a slight evaporation of the aqueous phase of the formulae.

In the animal study, indomethacin significantly inhibits the carrageenan-induced paw edema by different percentage as shown in table 2.

Table 2. Anti-inflammatory activity of different formulation of Indomethacin using carrageenan-induced paw oedema in rats.

Formulation	Mean oedema weight in mg (M ± SD)	% Inhibition of control value
Control	336.133 ± 7.178	--
4	145.233 ± 7.081	56.79
8	96.833 ± 1.363	71.19
43	130.233 ± 6.763	61.26
44	92.967±0.936	72.34
Injection	62.633±1.298	81.37
Gel	202.2±1.021	39.85

The effect of formulae 4 and 43 were less than formulae 8 and 44 and that might be due to the viscosity effect.

The effect of IGMs is between the effect of the commercial injection form (higher effect) and the effect of the commercial topical gel form (lower effect). These results showed that formulation of indomethacin in microemulsion dosage form enhanced its absorption. This is consistent with most of the previous studies that recommended the microemulsion as a drug delivery vehicle.¹⁷

Conclusion

From the above results we can conclude that gel-like microemulsion formulations prepared with paraffin oil, brij97, sorbitol or glycerol and water showed acceptable physical properties, drug release, stability and Pharmacodynamic effect. The best indomethacin gel-like microemulsions was formula 8, which consist of (28.8% v/v) Brij97, (3.2% v/v) Sorbitol 70%, (8% v/v) paraffin oil and (60% v/v) water. This formula shows the highest stability, release rate and viscosity range and gives comparatively superior anti-inflammatory activity.

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