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Formulation and Evaluation of Nanoemulsion for Non-Steriodal Anti-inflammatory Drug

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Abstract: ***The present work aimed to develop nanoemulsion of mefenamic acid for improving its efficacy, stability, and permeability for the treatment of rheumatoid arthritis. A nanoemulsion was prepared using aqueous titration method composed of capmul oil as the oil phase, tween 60 as surfactant and Polyethylene glycol 400 as cosurfactant and evaluated. The aqueous phase titration method was employed for constructing the existing zone of nanoemulsion in pseudoternary phase diagrams representing three axes of the aqueous phase, oil, and mixture of surfactant and cosurfactant. The prepared nanoemulsions were placed in tightly closed glass vials and stored at ambient temperature. These drug-loaded formulations were subjected to physical stability tests [centrifugation stress (5000 rpm, 30 min), heating cooling stress (0 and 45ºC, eight cycles) and freeze-thawing stress (-21 and 25 ºC, ≥48 h)], appearance, percent transmittance, refractive index, Electrical conductivity. In conclusion, nanoemulsion of mefenamic acid was developed to a satisfactory level in terms of physical stability tests, appearance, percent transmittance, refractive index, Electrical conductivity , lower surfactant concentration. The present study endorsed nanoemulsion of mefenamic acid to be a promising choice over conventional formulations for the treatment of rheumatoid arthritis.***

Keywords: Mefenamic acid, rheumatoid arthritis, nanoemulsion, capmul oil

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease causing inflammation in the synovial membrane of joints with the migration of activated phagocytes and other leukocytes into synovial and periarticular tissue. About 1% of the whole world population is attacked by rheumatoid arthritis1. A recent study indicated that increased oxidative stress and/or defective antioxidant status also contributes to the pathology of rheumatoid arthritis. It is also reported that there is an increased state of oxidative stress in RA, in which the use of antioxidant supplementation in such patients is proposed. Mefenamic acid, the newest member of the oxicam class, is a nonsteroidal anti-inflammatory drug (NSAID). It interferes in the synthesis of prostaglandins from arachidonic acid by the inhibition of the cyclooxygenase isozymes. It is a COX-2 inhibitor which also antagonizes certain actions of prostaglandins which play an important role in pain and inflammation. It is classified as BCS class-II drug having high lipophilicity and poor solubility.

The main objective of the investigation is to formulate nanoemulsion of mefenamic acid in order to overcome the drawbacks associated with the other dosage forms and in order to improve the overall efficacy of the drug. The prepared nanoemulsion will thereby improve the overall efficacy of mefenamic acid in the improvement of rheumatoid arthritis.

2. Materials and Methods

2.1 Materials

Mefenamic acid (MA) was provided by Gennova Biopharmaceutical Ltd. (Pune). Tween series surfactants (S.D.Fine Chemicals, Mumbai, (India), Polyethylene glycol series co-surfactants (Gattefosse, France) Capmul MCM(Gattefosse, France) . All chemicals were of analytical grade.

2.2 Methods

2.2.1 Formulation development and optimization

Authentication of excipients

The authentication of procured oils (Capmul oil 90, oleic acid, soyabean oil, sunflower oil, fish oil, TPGS), surfactants (Tween 20, Tween 40, Tween 60, Labrasol, Span 80) and cosurfactants () used for formation of nanoemulsions was checked by determine their Organoleptic Properties, Melting point determination, Solubility study of mefenamic acid in different solvents. The solubility of drugs to be determined by taking 10 ml of various medium 0.1 N HCI, acetate buffer and phosphate buffer and then cumulative accumulation of drug was done to obtained the saturated drug solution, maintained at 47 and continually shaken in to mechanical shaker up to 24 hours. Sample were withdrawn, filtered by using a whatman filter paper, suitably diluted and assayed by UV spectrometer.

Screening of components

The solubility of mefenamic acid in different oils (capmul oil, oleic acid, clove oil, castor oil,), surfactants (Tween 80, Labrasol) and cosurfactants (Polyethylene glycol 200, Polyethylene glycol 400, Polyethylene glycol 600, Trascutol), an excess amount of drug was added to 1 ml of the selected oils, surfactants and cosurfactants separately in 5 ml stoppered vials to produce supersaturated solutions which were then mixed using vortex mixer and then kept for 72 hours at 25±1°C in isothermal shaker to reach

equilibrium. After 72 hours, the equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatants were taken and filtered through the 0.45µm membrane and analyzed by UV spectrophotometer at 286nm after suitable dilution, if necessary. The experiment was done in triplicate13.

Spontaneous emulsification method

The aqueous phase titration method was employed for constructing the existing zone of nanoemulsion in pseudoternary phase diagrams representing three axes of the aqueous phase, oil, and mixture of surfactant and cosurfactant. To construct phase diagram, the screened surfactant was blended with cosurfactant in different Smix ratio (1:1, 2:1, 3:1, 1:2, 1:3,) with increasing concentration of surfactant to cosurfactant. The selected Smix ratio was further treated with oil at different ratios (1:9, 2:8, 3:7, 4:6, 1:2, 1:3, 1:3:5, 1:5, 1:6, 1:7 and 1:8) so that maximum ratio could be covered in order to obtain a precise phase diagram. In order to obtain a clear, isotropic and physical stable nanoemulsion with low viscosity, slow titration with water was done for each ratio of oil and Smix. The volume of distilled water was recorded.

The prepared nanoemulsions were placed in tightly closed glass vials and stored at ambient temperature. These drug-loaded formulations were subjected to physical stability tests [centrifugation stress (5000 rpm, 30 min), heating cooling stress (0 and 45ºC, eight cycles) and freeze-thawing stress (-21 and 25 ºC, ≥48 h)].

Table1: Solubility of mefenamic acid in various oils, surfactants and co-surfactants

S. No.	Ingredients (Oils)	Solubility (mg/mL)	Ingredients (Surfactants)	Solubility (mg/mL)	Ingredients (Co- surfactants)	Solubility (mg/mL)
1	Capmul MCM EP	70.15±2.25	Tween 20	20.21±1.15	Polyethylene glycol 200	27.5±2.35
2	Clove oil	60.23±4.20	Tween 80	30. 50±2.35	Polyethylene glycol 400	34.21±2.35
3	Soyabean oil	47.5±1.15	Tween 60	34.23±4.20	Polyethylene glycol 600	17.98±1.96
4	Sunflower oil	40.5±2.35	Labrasol	32.21±2.35	Trascutol	37.5±1.15
5	Fish oil	37.98±1.96				
6	Oleic acid	10.21±2.42				

Characterization of mefenamic acid nanoemulsion

a) Centrifugation study

The selected formulations were centrifuged at the 3000, 4000, and 5000 rpm for 30 mins and observed for phase separation, creaming or cracking. The formulations which showed maximum stability (No creaming, cracking, and phase separation) were selected and studied for heating-cooling cycle and freeze-thaw cycles[92].

b) Heating cooling cycles

It is used to observe the stressed effect of heating and cooling on the nanoemulsions stability. In this study the formulations were kept at 45^0c and at 0^0C temperature for not less than 48 h for each temperature cycle[93].

c) Freeze-thaw cycles (Accelerated ageing)

This test was performed for accelerated stability testing of nanoemulsion formulations. were exposed at two different temperatures i.e. -21^0C and 21^0C for each temperature cycles not more than 24 h. For the better estimation of accelerated stability studies six such cycles should be run for each batch of formulation. The formulations which showed the maximum stability were selected for further study[94].

d) Appearance

The appearance of the nanoemulsion formulations was determined by visual examination of the formulation under light alternatively against white and black backgrounds and turbidity were checked. The nanoemulsion was clear transparent, easily flowable liquid[91, 90].

e) Percent transmittance (Clarity)

Percentage transmittance was checked against distilled water using UV-visible spectrophotometer at 650 nm (Double beam UV spectrophotometer,V-630 Jasco, Japan) by dilution of 1 mL of the formulation with distilled water up to 100 mL (100 times) and as such, and percent transmittance of all formulations was determined in triplicate.

f) Refractive index

Refractive index (RI) being an optical property is used to characterize the isotropic nature of the nanoemulsion, RI was determined using an Abbe type refractrometer and it was observed that the nanoemulsion formulations were chemically stable and remained isotropic in nature, thus having no drug excipient interactions. The observation of formulation shows that as the concentration of the oils increases in the formulation, the RI of formulation increases. Refractive index of all formulations was determined in triplicate.

g) Electrical conductivity

Type of nanoemulsion (O/W or W/O) and the stability of the nanoemulsion (Phase inversion on storage) can be determined by electrical conductivity (σ). Electrical conductivity of nanoemulsion was measured by using Digital conductometery by placing a conductivity electrode in the nanoemulsion formulation which calibrated by using Nacl solution. If nanoemulsion formulation show conductivty that indicate given nanoemulsion formulation was o/w type nanoemulsion because in these type of emulsion, oil was small oil droplet phase and water was continuous phase that reason electrical conduce pass through the continuous phase but nanoemulsion formulation does not show conductivity that indicate the given nanoemulsion is w/o type nanoemulsion because in these type of emulsion water was small oil droplet phase and oil was continuous phase that reason electrical conduce does not pass through the continuous phase. Electrical conductivity is directly proportional to the percentage of water. Higher the electrical conductivity more will be the percentage of water, which allows more freedom for

mobility of ions. Electrical conductivity of all formulations was determined in triplicate.

3. Results and Discussion

Table 2: Appearance study-Drug+ oil (Capmul MCM)

Sr.no.	Time (Day)	Appearance study of mefenamic acid and Capmul MCM	
		Blank capmul MCM	Mefenamic acid+capmul MCM
1	0	Clear, transparent colourless liquid	Clear, transparent colourless liquid
2	7		
3	15		
4	30		
5	60		
6	90		

Table 3: Appearance study-Drug+ surfactant (Tween 60)

Sr.no.	Time (Day)	Appearance study of mefenamic acid and tween-60 mixture	
		Blank tween-80	Mefenamic acid + tween-60
1	0	Slightly yellowish liquid	Slightly yellowish red liquid
2	7		
3	15		
4	30		
5	60		
6	90		

Table 4: Appearance study- Drug+ Co-surfactant (PEG 400)

Sr. no.	Time (Day)	Appearance study of mefenamic acid and PEG-400 mixture	
		Blank PEG-400	Mefenamic acid + PEG-400
1	0	Clear, transparent colorless liquid	Clear, transparent colorless liquid
2	7		
3	15		
4	30		
5	60		
6	90		

Nanoemulsion was checked for transparency to turbidity. Nanoemulsion remained clear and transparent, slightly yellowish easily flowable liquid formulation. Slightly yellow color of formulation appeared due to presence of synthetic oils and polysorbate derivatives as surfactants. Above parameter indicate that formulated nanoemulsion was clear transparent, slightly yellowish easily flowable liquid formulation as such as main characteristics of nanoemulsion.

Table 5: Percentage transmittance (%)

S. no	Ratio (O:S)*	Percentage transmittance (%)**				
		Smix ratio (S:CoS)				
		01:01	02:01	03:01	01:02	01:03
1	01:09	97.12±0.01	96.80±0.05	97.07±0.07	94.27±0.06	93.85±0.03
2	02:08	92.60±0.18	95.72±0.21	97.29±0.13	86.41±0.34	89.49±0.34
3	03:07	91.54±0.35	77.68±0.19	92.52±0.87	77.45±0.07	83.50±0.18
4	04:06	90.58±0.39	74.39±0.29	90.69±0.17	74.42±0.30	78.32±0.25
5	05:05	47.36±0.31	67.35±0.19	87.21±0.19	65.41±0.04	60.20±0.15

The clarity of nanoemulsion was checked by transparency, measured in terms of transmittance (%T). In case of systems having %T values less than 95% suggest less clarity of nanoemulsion. This may be due to greater droplet size of the formulation, due to higher droplet size, oil globules might have reduced the transparency of nanoemulsion and thereby values of %. The observed transparency of the system is due to the fact that the maximum size of the droplets of dispersed phase is not larger than $1/4^{th}$ of the wavelength of visible light. Thus, nanoemulsions scattered little light and therefore transparent or translucent.

Table 6: Conductivity study

Sr.no.	Ratio (O:S)*	Conductivity study (mS/cm)				
		Smix ratio (S:CoS)				
		01:01	02:01	03:01	01:02	01:03
1	01:09	0.160±0.011	0.168±0.010	0.140±0.002	0.167±0.002	0.170±0.015
2	02:08	0.165±0.002	0.170±0.013	0.145±0.015	0.175±0.008	0.174±0.009
3	03:07	0.176±0.019	0.175±0.012	0.151±0.013	0.182±0.012	0.182±0.012
4	04:06	0.180±0.012	0.177±0.016	0.156±0.017	0.184±0.007	0.187±0.014
5	05:05	0.185±0.017	0.180±0.017	0.158±0.013	0.186±0.006	0.190±0.007

Type of nanoemulsion (o/w or w/o) and the stability of the nanoemulsion (Phase inversion on storage) can be determined by electrical conductivity (σ). The conductivity of the formulations is given in the above Table no 9.31. nanoemulsion formulation shows conductivty that indicate given nanoemulsion formulation was o/w type nanoemulsion because in these type of emulsion, oil was small oil droplet phase and water was continuous phase that reason electrical conduce pass through the continuous phase but nanoemulsion formulation does not show conductivity that indicate the given nanoemulsion is w/o type nanoemulsion because in these type of emulsion water was small oil droplet phase and oil was continuous phase that reason electrical conductance does not pass through the continuous phase. The lowest electrical conductivity was found for 1:9 ratios of all Smix ratio and the highest electrical conductivity was found for 5:5, 4:6,3:7 ratio of 1:1, 2:2, 3:1, 1:2,1:3 of Smix ratio respectively. This indicated that the formulation was o/w type. Electrical conductivity is directly proportional to the percentage of

water. Higher the electrical conductivity more will be the percentage of water, which allows more freedom for mobility of ions.

Table 7: Refractive index

Sr.no.	Ratio (O:S)*	Refractive index**				
		Smix ratio (S:CoS)				
		01:01	02:01	03:01	01:02	01:03
1	01:09	1.365±0.001	1.361±0.0050	1.374±0.0050	1.360±0.001	1.351±0.0015
2	02:08	1.366±0.0025	1.365±0.0020	1.382±0.0001	1.367±0.0015	1.357±0.0030
3	03:07	1.372±0.0015	1.370±0.0025	1.387±0.0010	1.375±0.002	1.363±0.0010
4	04:06	1.376±0.0011	1.382±0.0015	1.390±0.005	1.382±0.0025	1.365±0.0025
5	05:05	1.385±0.0020	1.392±0.0015	1.393±0.0015	1.391±0.001	1.372±0.0015

Refractive index (RI) being an optical property is used to characterize the isotropic nature of the nanoemulsion. The RI of the selected formulations was determined using an Abbes refractometer. Refractive index supposes of might have be in the range of 1.351-1.393. The results given indicate RI values increased with increase in concentration of oil and corresponding decrease in aqueous content. It was observed from the above table value of selected nanoemulsion formulations were chemically stable and remained isotropic in nature, thus having no drug excipient interactions.

4. Discussion

Formulation and evaluation of nanoemulsions

In an attempt to enhance solubility and penetration that will be effective in inflammatory disorder in patients suffering from rheumatoid arthritis, nanoemulsion of mefenamic acid was formulated. Nanoemulsion have emerged as one of the most interesting novel delivery systems. Drug delivered through nanoemulsion has better adhesion on the surface on the surface of the skin and high solubilizing capacity which leads to larger concentration gradient towards the skin hence influences better skin penetration. The aqueous phase titration method was used for the development of nanoemulsion formulations by using probe sonicator. The solubility of the drug in the oil phase is an important criterion for the selection of oils for the ability of nanoemulsion to maintain the drug in the solubilized form which is greatly influenced by the solubility of the drug in the oil phase. Usually, the oil which has the maximum solubilizing potential for a selected drug candidate is selected as an oily phase for the formulation of nanoemulsions. This helps to achieve maximum drug loading in the nanoemulsions. Surfactants are used for stabilizing the systems. Three types of surfactant are anionic, cationic, and nonionic. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower critical micelle concentration. Therefore, proper selection of surfactants becomes a crucial factor. Hydrophilic surfactant and cosurfactant are considered to lower the interfacial tension and to lower the necessary energy to form the nanoemulsions, therefore improving the stability. Cosurfactants are added to obtain nanoemulsion systems at low surfactant concentration. Based on the results of solubility and miscibility studies, Capryol 90: TPGS (1:1) was selected as an oil phase along with Tween 60 and Transcutol-P as surfactant and cosurfactant respectively for the development of nanoemulsion. Pseudoternary phase diagrams were constructed using Capmul oil: TPGS(1:1), Tween 60 and Transcutol-P as oil, surfactant, and cosurfactant respectively. The maximum nanoemulsion area was found in the Smix 3:1 ratio (surfactant: cosurfactant) as compared to rest Smix ratios. The drug-loaded stable formulations were subjected to characterization parameters (Appearance study, percent transmittance, refractive index, conductivity study etc).

5. Conclusion

In conclusion, nanoemulsion of mefenamic acid was developed to a satisfactory level in terms of physical stability tests [centrifugation stress (5000 rpm, 30 min), heating cooling stress (0 and 45ºC, eight cycles) and freeze-thawing stress (-21 and 25 ºC, ≥48 h)], appearance, percent transmittance, refractive index, Electrical conductivity.. The present study endorsed nanoemulsion of mefenamic acid to be a promising choice over conventional formulations for the treatment of rheumatoid arthritis.

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