ORIGINAL ARTICLE

Development of Multiple Emulsion of Andrographolide for Taste Masking

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Abstract

Aim: The objective of this study was to develop and evaluate water-in-oil-in-water multiple emulsions for taste masking of andrographolide isolated from *Andrographis paniculata* leaves. **Materials and Methods:** Andrographolide (diterpenoid lactone) is the major active bitter glycoside and has been found to possess remarkable hepatoprotective activity. Andrographolide isolated was effectively entrapped into a multiple emulsion to facilitate the delivery of andrographolide in liver cells. **Results and Discussion:** This also improved its therapeutic efficacy during hepatic damage. Multiple emulsions prepared were stabilized using a combination of hydrophilic as well as hydrophobic surfactants in ratio of 4:1. This is significant in achieving stable multiple emulsions. Multiple emulsions prepared were of optimum globular size and drug content was found to be around 80%. **Conclusion:** This may be attributed to their enhanced bioavailability, taste masking, and sustained release as well as physicochemical stability which prolong the activity of andrographolide in the multiple emulsions, which could be safer and more effective.

Key words: *Andrographis paniculata,* hepatoprotective, multiple emulsions

INTRODUCTION

Taste masking is the main aspect in the development of the dosage form, especially in the formulations for pediatric and geriatric, bedridden, and non-cooperative patients. The main challenge to the compounding pharmacist is to mask the taste of obnoxious and bitter drugs so that the patient can receive optimal therapeutic value of their medication.[1] Multiple emulsions are successfully formulations for masking the bitter taste efficiently. Multiple emulsions are good approach for taste masking of bitter drugs.[2] Water-in-oil-in-water (W/O/W) multiple emulsions are made with small (nanometer-sized) aqueous droplets entrapped within larger oil droplets, which are again stabilized and dispersed in a continuous aqueous phase. As the reservoir phase is present inside the droplets of another immiscible phase that can be used to prolong the release of active ingredients, multiple emulsions find many applications in pharmaceutical and cosmetic industries.[3] Multiple emulsions also offer the advantage of encapsulating several active agents in a single formulation and sequestering the different agents in selective compartments for enhanced stability.[4] *Andrographis paniculata* is a highly bitter plant and its constituents are used for variety of multifunction, especially as hepatoprotective agent.[5] Moreover, it possesses low aqueous solubility and poor oral bioavailability.[6,7] In nanotechnology, the multiple emulsions have been used in targeted drug delivery system as well as taste masking of bitter drugs.[8-10] The main purpose of the present work is to mask the bitter taste of andrographolide through encapsulation in multiple emulsions.

MATERIALS AND METHODS

Materials

Pure standard andrographolide was purchased from Sigma-Aldrich. Fresh leaf material of *A. paniculata* was procured from Vindhya Herbal, Bhopal, in November and authenticated from Vindhya Herbal, Bhopal. Tween 80 and Span 80 (CDH, New Delhi) were used for emulsification. The olive oils

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(Modi Naturals Ltd., New Delhi) were used for oil phase of multiple emulsions. Fresh leaf material of *A. paniculata* was obtained from Vindhya Herbal, Bhopal, in November and authenticated. The leaf material was air-dried under shade for a day. It was powdered and passed to 40 mesh and stored in an air-tight container until further use.

Methods

Extraction and isolation of andrographolide

Andrographis paniculata leaves were dried at room temperature and the dried leaves were crushed and sieved. The leaf powder (100 g) was macerated in methanol and kept at room temperature (25 ± 2°C) for 7 days with shaking. After 7 days, they are filtered. After filtration, the solution was evaporated through water bath with continuous stirring until half solvents are left and kept for 1 day. The dark green crystalline mass was obtained which was washed with toluene until most of the coloring matter was removed from the crystals. Then, the toluene was completely removed from the crystals.[11,12]

Preformulation studies

Identification and qualitative estimation of isolated andrographolide

The isolated crystals of *A. paniculata* were subjected to qualitative analysis using ultraviolet (UV) spectrophotometer (Shimadzu, UV-1700), infrared (IR) spectrophotometer (Bruker's Fourier transform IR [FT-IR]), and high-performance thin-layer chromatography (CAMAG Linomat 5).

- UV spectroscopy: Spectrophotometric study was performed on UV-visible spectrophotometer (Model: UV-1700, Shimadzu). 10 mg of standard and extracted andrographolide was accurately weighed separately and dissolved in 10 ml of methanol in different volumetric flasks. Further, aliquot of 40 µg/ml and 20 µg/ml was prepared by diluting the stock solutions of standard and extracted andrographolide simultaneously. The resultant solutions were scanned on UV spectrophotometer in the range of 200–400 nm. The maximum absorbance of standard drug and crystal of andrographolide was found out at 227 nm.
- IR spectroscopy: IR spectra of standard drug and extracted drug were recorded to determine the presence of andrographolide in the extracted crystals. The study was performed on Bruker's FT-IR, the spectra of extracted crystals show no major deviation, in comparison to the spectra of standard drug.
- High-performance thin-layer chromatography (HPTLC): HPTLC study of the standard and test material was performed at Minor Forest Produce Processing and Research Centre, Bhopal. The chromatographic estimation was performed using CAMAG Linomat5, CAMAG TLC Scanner, and CAMAG Visualizer with solvent chloroform:methanol (8:2).

Calibration curve of standard andrographolide

Andrographolide (5 mg) was accurately weighed and dissolved in few ml of ethanol and volume was made up to 10 ml with methanol in volumetric flask (10 ml) (Stock solution A). From stock solution A, dilution between 10 µg/ml and 50 µg/ml was prepared, respectively. Absorbance was measured using UV-visible spectrophotometer at λ_{max} 227 nm.

Solubility of crystal of andrographolide

The isolated crystals of andrographolide were studied solubility by methanol, ethanol, acetone, chloroform, water, and toluene.[13-16]

Drug interaction with excipients

Drug-excipient interaction was studied using UV spectrophotometer. Standard solution of drug was taken separately in different vials and different excipients (Tween 80, Span 80, and Triton X) were added to it and change in the maximum wavelength and absorbance was measured.

Partition coefficient of the crystal of andrographolide

Partition coefficient studies are carried out to find out extent of drug transfer in the aqueous and the other non-aqueous layer in which was taken 5 ml water and 5 ml octanol and mix 10 mg of crystal of andrographolide was dissolved in a separating funnel. After 24 h, separate the water phase and octanol, then observe by UV spectrophotometer.

Determination of bitterness threshold value of andrographolide

Threshold value for bitterness is the minimum concentration of a substance that evokes perception of bitter taste. Andrographolide is an intensely bitter drug. Its threshold value was determined in five healthy human volunteers. First of all, the volunteers were said to rinse their mouth with water, then 10 ml of the aqueous solution of andrographolide of the different concentrations from 10 to 40 µg/ml were given at different time to keep it in their mouth for 10–15 s and then spit out. In between two administrations, an interval of 1 h was elapsed so that proper taste result could be obtained. The perception of the degree of bitterness of the drug solution by the volunteer after each administration was noted.[17]

Formulations

Preparation of multiple emulsions - after determinations of required HLB and optimum surfactant blend W/O/W, multiple emulsions were prepared through a two-step emulsification process. For the preparation of primary emulsion, oil phase consisting of olive oil and Span 80 was mixed. Aqueous phase consisting of andrographolide crystals was also mixed. Aqueous phase was added to the oil phase drop by drop with triturate until the clicking sound was heard. Thus, primary emulsion was obtained. For obtaining

the multiple emulsions, primary emulsion was added to the aqueous phase containing Tween 80 drop by drop with constant stirring.

CHARACTERIZATION OF MULTIPLE EMULSIONS

Microscopic Analysis of Multiple Emulsions

Multiple emulsions were analyzed under the microscope to confirm the multiple characters. A drop of multiple emulsions was placed on the glass slide, diluted with water, and covered by a glass cover. A drop of immersion oil was placed on the cover slide and observed under the microscope.

Table 1: Multiple emulsion formulations prepared through two-step emulsification process

W/O/W phase ratio	Primary-secondary surfactant ratio	Formulation
20:30:45	1:1	F1
30:40:30	1:1	F2
30:20:50	2:1	F3
25:33:33	2:1	F4
20:30:45	2:1	F5
30:20:50	3:1	F6
20:30:45	3:1	F7
25:33:33	3:1	F8
25:33:33	4:1	F9

Table 2a: Optimization ratio of stable multiple emulsions

W/O/W phase ratio	Primary-secondary surfactant ratio	Formulation
25:33:33	2:1	F4
25:33:33	3:1	F8
25:33:33	4:1	F9

Active ingredient (andrographolide), sweetening agent, and flavoring agent were added in every formulation. Considering the entire ingredient, a general formula for the multiple emulsions is being mentioned below

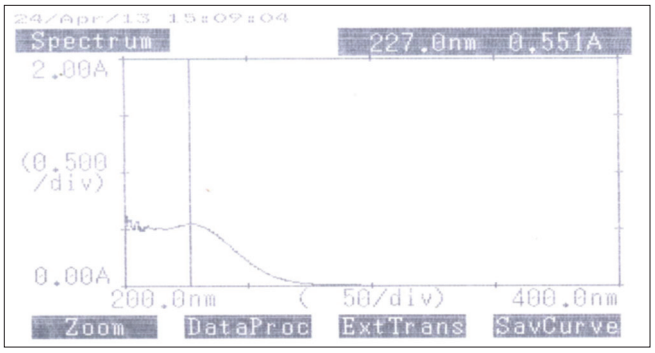

Figure 1: Ultraviolet spectra of standard andrographolide (40 µg/ml)

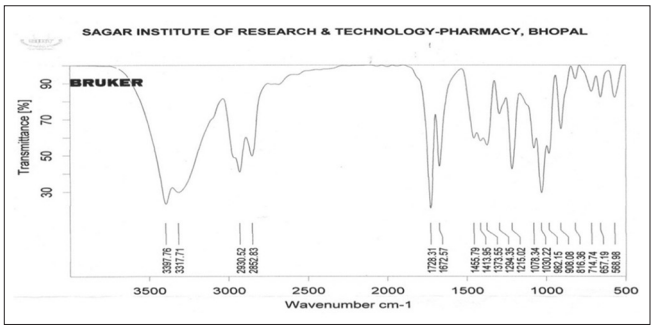

Figure 2: Graph showing of infrared spectrum of crystals of andrographolide

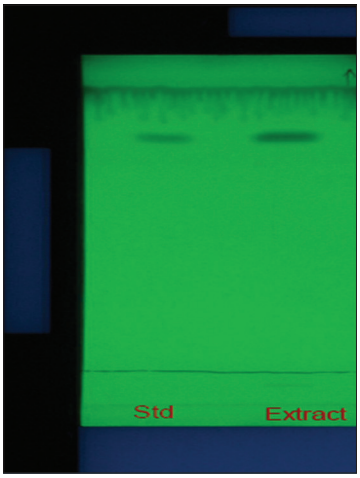

Figure 3: High-performance thin-layer chromatography of the standard andrographolide and crystal of andrographolide

Table 2b: General formula for preparing multiple emulsions

Ingredients	Prescribed amount								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Drug	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg
Oil	30 ml	40 ml	20 ml	33 ml	30 ml	20 ml	30 ml	33 ml	33 ml
Water	65 ml	60 ml	70 ml	58 ml	65 ml	70 ml	65 ml	58 ml	58 ml
Span 80	10.5 ml	10.5 ml	14 ml	14 ml	14 ml	14 ml	14 ml	14 ml	14 ml
Tween 80	10.5 ml	10.5 ml	7 ml	7 ml	7 ml	4.67 ml	4.67 ml	4.67 ml	3.5 ml
Aspartame	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
Vanilla	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Macroscopic Analysis

Macroscopic analyses of stabilized formulations were studied their organoleptic characteristics (color, consistency, and appearance) and homogeneity (creaming and phase separation). Multiple emulsions kept at different storage conditions, i.e., color, liquefaction, and phase separation were noted at various intervals, i.e., 1 day, 2 days, 5 days, 10 days, 20 days, 30 days, and 60 days.

Number of Globules

Number of globules per cubic millimeter can be measured using a hemocytometer cell after appropriate dilution of the multiple emulsions. The globules in five groups of 16 small squares (total 80 small squares) were counted. The total number of globules in per cubic mm is calculated using the formula:

Numberofglobules/mm^3=NumberofGlobules*Dilution*4000/ Number of small squares counted

Entrapment Efficiency (EE)

Percentage EE (% EE) was determined by taking freshly prepared W/O/W multiple emulsions and immediately centrifuged (by centrifuge REMI) at 4000 rpm for 10 min. 1 ml of the aqueous phase (the lower layer) was withdrawn using 2 ml hypodermic syringe and diluted with distilled water. The solution was filtered and drug content was analyzed by UV spectrophotometer at 227 nm. The

Table 3: Absorbance of different aliquots of the standard andrographolide in 227 nm

Concentration µg/ml	Absorbance	Statistical parameters
10	0.318	Correlation coefficient r=0.999
20	0.618	
30	0.933	Slope m=0.030
40	1.256	Intercept c=−0.007
50	1.522	Equation of line, Y=0.030 X−0.007

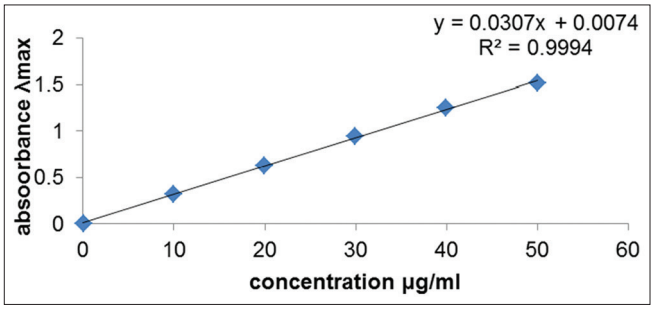

Figure 4: Standard curve of standard andrographolide

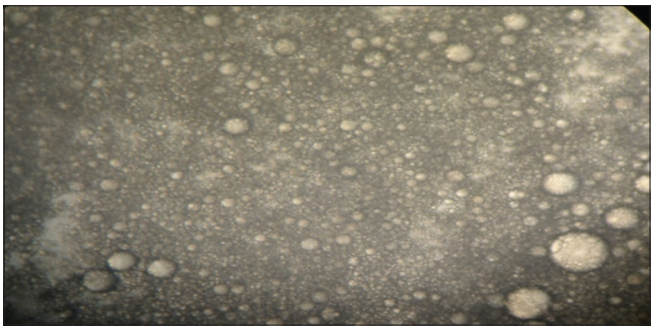

Figure 5: Multiple emulsions F9 (a) (F9 [a] - formulation after 20 days)

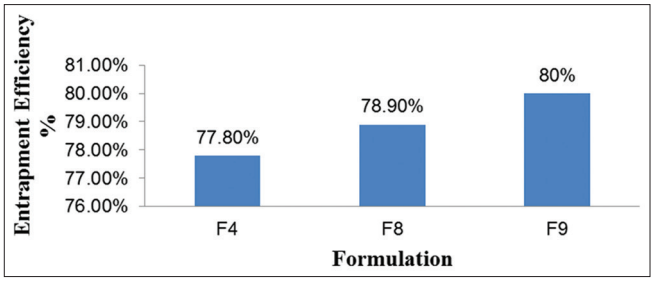

Figure 6: Graph of entrapment efficiency percentage of the crystal of andrographolide

Table 4: Estimation of bitterness threshold value of andrographolide

Concentration of andrographolide (µg/ml)	Perception of bitter taste				
	Volunteer I	Volunteer II	Volunteer III	Volunteer IV	Volunteer V
10	−	−	−	−	−
15	−	−	−	−	−
20	+	+	−	−	+
22	+	+	−	+	+
25	+	+	+	+	+
30	++	++	++	++	++
35	+++	+++	+++	+++	+++
40	+++	+++	+++	+++	+++

−No taste, +: Slight bitter, ++: Bitter, +++: Intensely bitter

encapsulation efficiency was determined by the following equation:

% EE=[(Total drug incorporated–Free drug)/Total drug]×100

Stability Studies

Phase separation is a phenomenon by which one phase of emulsion gets separated due to coalescence. Percentage phase separation is the volume of phase in percentage separated from the total volume of emulsion after storage. 10 ml of freshly prepared w/o/w emulsion is kept in 10 ml of graduated cylinder and allowed to stand for defined period at 40°C.

In Vitro Drug Release Study

The *in vitro* drug release study was carried out on a simple dissolution cell using cellophane membrane (thickness - 200 mm and breaking strength - 2.7 kg/cm). Before release studies, the cellophane membrane was soaked in 0.1 N HCL for 24 h, washed frequently 4 times by changing distilled water. 1 ml freshly prepared multiple emulsion was added to donor chamber, made up of a hollow glass tube, and membrane was tied on bottom end of the tube with a nylon string. This tube was dipped into 100 ml of phosphate buffer saline pH 7.4 and was stirred at 75 rpm/min on a magnetic stirrer and maintained at 37°C which acted as receiving chamber. Aliquots of 1 ml were collected from receiving chamber at predetermined time intervals and the drug content was determined on UV spectrophotometer at 227 nm after suitable dilution.

Table 5: Results of organoleptic characteristics (color, consistency, and appearance) and homogeneity (creaming and phase separation)

Time (days)	Liquefaction			Color			Phase separation		
	F4	F8	F9	F4	F8	F9	F4	F8	F9
1	–	–	–	W	W	W	–	–	–
2	–	–	–	W	W	W	–	–	–
5	–	–	–	W	W	W	–	–	–
10	++	++	–	W	W	W	++	++	–
20	++	++	–	Yw	Yw	W	++	++	–
30	++	++	–	Yw	Yw	W	++	++	–
60	++	++	–	Yw	Yw	W	++	++	–

–: No change; W: White; YW: Yellowish-white; ++: More change

Table 6: Number of globules of the formulations

Formulation	Number of globules
F4	230,300/mm^3
F8	236,000/mm^3
F9	245,000/mm^3

Value expressed in mean

Zeta potential

Zeta potential gives an indication of the potential stability of the colloidal system. The zeta potential, size distribution, and globules sizes are determined using the zeta potentiometer (Malvern Instruments Ltd.). For molecules and particles that are small enough, a high zeta potential will confer stability.[18-21]

Evaluation of Taste Masking

- Panel testing (human subjects) - The taste evaluation was done by panel testing. The selected panel of five healthy human volunteers was requested to taste the taste masked by keeping in the mouth, for 30 s then evaluated by the panel and the results were compared between standard solution and multiple emulsion.
- Spectrophotometric method - 10 ml of the taste-masked formulation is mixed with 10 ml of distilled water in 10 ml syringe by revolving the syringe, end to end, 5 times in 30 s. The test medium is then filtered through a membrane filter, followed by spectrophotometric determination of the concentration of the drug in the filtrate.[22,23]

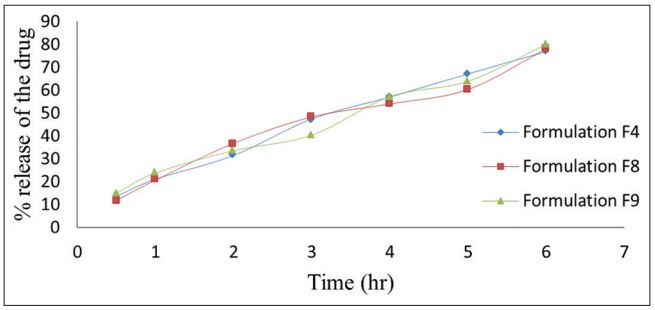

Figure 7: Release of Andrographolide in different formulations

Table 7: Stability studies of formulations

Formulation	1st day	5th day	10th day	20th day	30th day	45th day
F4	++	++	+	-	-	-
F8	++	++	+	-	-	-
F9	++	++	++	++	++	++

++: More stable, +: Phase separation, –: Completely phase separation

4. RESULTS AND DISCUSSION

Multiple emulsions (F1-F9) were prepared through a two-step emulsification process as shown in Table 1. Out of all the formulations (F1-F9), the three formulations F4, F8, and F9 were found to be stable as shown in Table 2a and b. Results of preformulation studies such as identification of extracted andrographolide crystals by UV spectroscopy, FT-IR, and HPTLC are shown in Figures 1-3. Calibration curve of andrographolide was prepared in ethanol at λ_{max} 227 nm as shown in Table 3 and Figure 4. The isolated andrographolide shows bitterness >20 µg/ml while it shows no taste <15 µg/ml in majority of the volunteers. Hence, further, dilution between 15 µg/ml and 20 µg/ml was prepared and asked to evaluate it again by the volunteers. The results found are mentioned in Table 4.

Prepared multiple emulsions were characterized for various parameters. Results for microscopic and organoleptic analysis are shown in Figure 5 and Table 5, respectively. Results indicate no change in organoleptic properties such as color, consistency, and appearance within 60 days. A number of globules for optimized formulations were found to be within 230,300/mm^3–245,000/mm^3 as shown in Table 6.

Drug loading efficiency of multiple emulsions for optimized formulations was found to be 77.8%, 78.9%, and 80%, respectively, as shown in Figure 6. EE shows minute differences in between the optimized formulations. The ratio of surfactants is continuously increased for stability and final F9 formulation is optimized for 60 days, due to different concentrations show different in EE.

Optimized emulsions showed no signs of instability for 45 days as shown in Table 7. *In vitro* drug release of the optimized formulations was calculated and it was found that formulation F9 exhibited maximum release of around 80%

Table 8: *In vitro* release study

Time (h)	Cumulative % of drug release		
	F4	F8	F9
30 min	13.65	11.9	15.09
1	21.15	20.74	23.85
2	31.50	36.81	33.58
3	47.44	48.34	40.36
4	57.05	54.04	57.22
5	67.19	60.34	63.96
6	76.9	78.06	80.01

Table 9: Evaluation of the taste of multiple emulsions

Formulation	Perception of bitter taste				
	Volunteer I	Volunteer II	Volunteer III	Volunteer IV	Volunteer V
F4	–	+	++	–	–
F8	–	–	+	++	–
F9	–	–	+	–	–

–: No taste, +: Slight bitter, : ++: Bitter

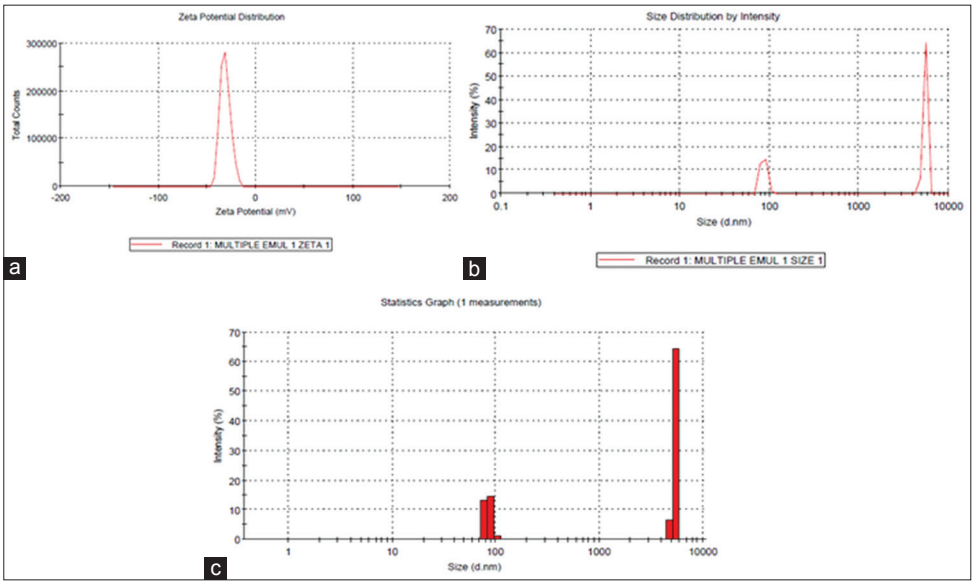

Figure 8: (a) Zeta potential report of multiple emulsions. (b) Size distributions report by intensity of multiple emulsions. (c) Size statistics report by intensity of multiple emulsions

at the end of 6 h as shown in Table 8 and Figure 7. The drug is delivered in the site (especially in liver) from globules of multiple emulsions. Hence, the absorption of globules is fast and 80% drug released in the site and therapeutic efficacy is high as compare from conventional preparation. Andrographolide has poor solubility and low bioavailability. In this project work enhances the bioavailability of andrographolide and increases the therapeutic efficacy.

The zeta potential of the formulation was found to be 31.1 which show better electrokinetic property and stability of the formulation. At the similar time, about 29% of the globules were having the average size of 86.29 nm while 71% of the globules were having the size of approximately 5.4 µ [Figure 8]. At the same time, it has been observed that bitter taste of Andrographolide was not sensed by majority of the volunteers as shown in Table 9.

Drug-loaded multiple emulsions for each formulation were found out that F4, F8, and F9 having 7.8%, 78.9%, and 80%, respectively.

CONCLUSION

Multiple emulsions have been anticipated to have numerous uses including their use for enhancement of bioavailability or as a sustained drug delivery system. However, their inherent instability needs to be overcome before finding their potential application in pharmaceuticals. Multiple emulsions are often stabilized using a combination of hydrophilic and hydrophobic surfactants whose ratio is important in achieving stable multiple emulsions. Andrographolide has low bioavailability and is a highly bitter drug. Few studies have been reported for enhancement of the bioavailability of poorly water-soluble drugs and masking their bitter taste by formulating as multiple emulsions. The objective of present work was the development and evaluation of multiple emulsion of andrographolide for potential oral drug delivery. The preformulation studies were carried out using FTIR to find out the various functional groups present and also to detect any interaction between drug and surfactant. Organoleptic characteristics of multiple emulsions formulated were satisfactory. The investigations presented lead us to conclude that the multiple emulsions can be successfully prepared using andrographolide and non-ionic surfactants such as Tween 80 and Span 80 by two-step emulsification methods. The drug release from the formulations was sustained up to 6 h which thereby reduce dose frequency and improved patient compliance.

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