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Formulation and *In-vitro* evaluation of controlled polyherbal microemulsion for the treatment of diabetes mellitus

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Abstract

Aim of present study was to formulate and evaluate microemulsion for oral drug delivery of *Tinospora cordifolia*, *Momordica charantia* and *Trigonella foenum graecum* to enhance bioavailability of extracts. Oil–in-water microemulsions were prepared by high mixer homogenizer method. Microemulsion was formulated using Liquid paraffin oil as oil phase; Tween 80 and Span 20 were used as surfactant and cosurfactant respectively, on the basis of solubility studies. Aqueous phase titration method was used to construct phase diagrams. Formulations were selected from phase diagrams. Prepared microemulsions were subjected to various parameters like: thermodynamic stability tests, drug content, viscosity, percent transmittance, electroconductivity, droplet size and *in-vitro* release studies. Results showed that all the formulations had good stability. Viscosity of F1 optimized formulation was found to be 4.86cPs, percent transmittance was found to be 98.32% and drug content was found to be 89.62%, 97.78% and 99.75% of *Momordica charantia*, *Trigonella foenum graecum* and *Tinospora cordifolia* respectively. Particle Size through SEM was found to be 861nm-1.18µm. Optimized formulations were successful in control release for 18 hours. Formulation F1 showed 89.28%, 92.31%, 99.03% drugs release of *Momordica charantia*, *Trigonella foenum graecum* and *Tinospora cordifolia* respectively.

Key-words: Diabetes, Polyherbal, Microemulsion, *Momordica charantia, Trigonella foenum graecum, Tinospora cordifolia* etc.

Introduction

The oral route is mostly preferred route for 90% of all drugs in human beings. Oral administration of therapeutic agents remains the favored rout due to the excellent accessibility, and patient compliance as well as the preferred alternative route of drug administration for non-invasive drug delivery among the other various routes [1]. 50% of the drug compounds are facing problems for oral delivery because of the high lipophilicity (Low hydrophilicity) and low permeability of the drug itself [2]. Controlled drug delivery is one which delivers the drug at a predetermined rate, for locally or systemically, for a specified period of time. It provides the continuous oral delivery of drugs at predictable and reproducible kinetics for predetermined period throughout the course of GIT [3]. **Diabetes Mellitus (DM)** is an endocrine system chronic disorder of carbohydrate, fat and protein metabolism, which is characterized by increased fasting and post-prandial blood sugar levels [4]. It is also defined as a disease, where human body does not properly produces or utilizes insulin [5].

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Due to rising incidences of obesity and reduced activity levels diabetes mellitus raises in developed countries of world at rate of about 10% per year (especially type 2DM). DM is expected to continue as major health problem owing its serious complications like end stage renal diseases, gangrene of lower extremities and blindness in adults [6]. Signs and symptoms of diabetes mellitus are increased or extreme thirst, increased appetite, increased fatigue, increased or frequent urination, unusual weight loss and blurred vision [7, 8, 9]. **Microemulsions** are kinetically stable, transparent (translucent) dispersion of oil and water that are stabilized by an interfacial film of surfactant and cosurfactant molecules [10]. Microemulsions consist of fine oil-in-water dispersions, having droplets of size range of 0.1 - 100µm [11]. The particles can exist in oil-in-water and water- in-oil forms, where the core of the particle is either oil or water, respectively. Microemulsions are made from surfactants, which are approved for human consumption and common food substances that are "Generally Recognized as Safe" (GRAS) by the FDA. Microemulsions are also known to as miniemulsions, ultrafine emulsions and submicron emulsions [12]. Microemulsion is a heterogeneous mixture of lipid and aqueous phase and stability are achieved by using emulsifying agents [13, 14, 15].

Momordica charantia belongs to the family *Cucurbitaceae*. Fruits are known as *bitter melon, bitter gourd, balsam pear, karela or African cucumber*. It consists of alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, lauric acid, linoleic acid, linolenic acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicin, momordicinin, momordicosides, momordin, momordolo, pipecolic acid as chemical constituents. It was found that the leaves of karela are nutritious sources of calcium, magnesium, potassium, phosphorus, iron and B vitamins [16]. Charantin Alcoholic extract was more potent than Tolbutamide. Subcutaneous injection of charantin decreases blood glucose level and inhibited gluconeogenesis [17]. Bitter gourd can also recover partially destroyed cells and stimulates pancreatic insulin secretion [18].Charantia stimulates glycogen storage by liver and improves peripheral glucose uptake [19].

Tinospora cordifolia belongs to the family *Menispermaceae*. It is also known as *giloya, gulvel, amrit*. Its chemical constituents are tinosporone, tinosporic acid, cordifolisides A to E, syringen, berberine, giloin, gilenin, crude giloininand, columbin, chasmanthin, palmarin, syringine apiosylglycoside, isocolumbin, palmatine etc [20, 21]. Insulin secretion is promoted by giloe due to inhibition of gluconeogenesis and glycogenolysis. Stem extract of ethyl acetate, dichloromethane, chloroform and hexane inhibits salivary amylase and glucosidase enzymes, which increases post-prandial glucose level and showed antidiabetic activity [22]. Significant reduction in blood glucose, hepatic glucose-6-phosphatase, serum acid phosphatase, increase in body weight and total haemoglobulin is caused by oral administration of aq. root extract [23]. The isoquinoline alkaloid rich fraction of stem has been showed insulin-mimicking and insulin-releasing effect both *in-vitro* and *in-vivo* [24].

Trigonella foenum graecum belongs to *Fabaceae* family. It is also known as *methi*. It contains Trimethylamine, Neurin, Trigonelline, Choline, Gentianine, Carpaine and Betain, Isoleucine, 4-Hydroxyisoleucine, Histidine, fenugrin B, fenugreekine, trigofoenosides A-G, Yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, Coumarin, lipids, vitamins, minerals, 28% mucilage, 5 % of a stronger-swelling, bitter fixed oil as chemical constituents [25]. Administration of deffated seeds (1.5-2g/kg) caused reduction in fasting and postprandial blood levels of glucose, glucagon, somatostatin, insulin, triglycerides and increased HDL-cholesterol levels in diabetic dogs [26, 27]. Anti-hyperglycemic effect of the extracts, powder and gum of seeds and leaves have been delayed gastric emptying caused by the high fiber content, inhibition of carbohydrate digestive enzymes and stimulation of insulin secretion [28]. Hepatic and renal glucose-6-phosphatase and fructose-1, 6-biphosphatase activity is reduced by methi [29].

Material and Methods

Materials: Authentified herbal extracts of *Momordica charantia, Tinospora cordifolia* and *Trigonella foenum graecum* were purchased from Sri Herbasia Biotech., Amritsar (Punjab). Synthetic chemicals Liquid Paraffin, Span 20, Tween 80, Propyl paraben and Methyl paraben were purchased from Central Drug House Pvt. Lt., New Delhi.

Preformulation studies:

Maximum Wavelength of *Momordica charantia*, *Tinospora cordifolia* and *Trigonella foenum graecum*: Stock Solution (1000 µg/ml) was prepared in 6.8 pH Phosphate buffer. This solution was diluted with same solvent to obtain concentration of 100µg/ml. The resultant solution was scanned in the range of 200-520 nm on double beam UV-spectrophotometer. The resultant was plotted in chart in the range of 200-520nm.

Drug - Excipient compatibility studies: The IR spectrum of drug substances were authenticated by using IR spectroscopy. The presence of characteristic peaks associated with specific structural characteristics of the drug molecule was noted. Fourier transform infrared analysis (SHIMADZU) was conducted to study the drug-drug, drug-excipient interactions. Scanned the samples in the range of 400-4000cm^{-1} [30].

Solubility studies: The solubility of herbal drug extracts in various solvents was measured. Solubility was determined by taking 10 mg of drug sample in 10 ml of solvent as water, Methanol, Ethanol, Castor oil, Isopropyl myristate, Liquid paraffin, Linseed oil, Tween 20, Tween 80, Span 20, Span 80, 0.1 N HCl , pH buffer 6.8 in small test tubes and well solubilized by shaking. The samples were centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through Watman filter paper. The filtrate was solubilized in suitable solvent, diluted with the suitable solvent (solvent used for analysis) and the concentration of drug extracts were determined using UV-Visible spectrophotometer at suitable wavelengths [30].

Formulation of Microemulsion:

Pseudo-Ternary Phase Diagram Study: The relationship between the phase behavior of a mixture and its composition can be analyzed with the help of phase diagram. Liquid paraffin light (oil), Tween 80 (surfactants), and Span 20 (co-surfactant) were selected to study the phase diagrams in detail. Pseudoternary phase diagrams were constructed separately for each Smix ratio to identify the o/w microemulsion regions [31].

The pseudoternary phase diagrams were developed using the aqueous titration method surfactant (Tween 80) and co-surfactant (Span 20) were mixed (Smix) in different volume ratios (1:1,1:2,1:3,1:4,2:1,3:1,4:1). These Smix ratios were chosen to show the increasing concentration of the co-surfactant with respect to surfactant and increasing concentration of surfactant with respect to co-surfactant for the detailed study of the phase diagrams in the microemulsion formulation. Liquid paraffin light optimized as an oil phase based on the basis of solubility study. For each phase diagram, oil (Liquid paraffin) and specific Smix ratios were mixed in different volume ratios from 1:9 to 9:1. 13 different combinations of oil and Smix (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1) were made to study the boundaries of the phases precisely formed in the phase diagrams. 5ml of aqueous phase was added at each interval upto 70ml under magnetic stirring and visually observed for phase clarity and flowability [32, 33].

Selection of placebo microemulsion formulations: Different formulations were selected from the microemulsion region from each constructed phase diagram so that herbal extracts of *Momordica charantia*, *Tinospora cordifolia* and *Trigonella foenum graecum* could be incorporated into the oil phase. Following criteria was used for the selection of different formulations from the phase diagrams:

- 15gm of herbal extracts of *Momordica charantia*, *Tinospora cordifolia* and *Trigonella foenum graecum* were selected as dose for incorporation into the oil phase.
- Depending on the solubility of the drug in the oil, the concentration of oil can be calculated so that it solubilizes the drug (single dose) completely.
- From each phase diagram, different concentrations of oil were selected at a difference of 5% (10%, 15%, 20%, etc) from the microemulsion region.
- Herbal drug extracts effect on the phase behavior and microemulsion area of the phase diagram was checked [34].

Formulation of drug loaded microemulsions formulation: Microemulsion was formulated using high mixer homogenization method.15gm of herbal extracts were dissolved in the liquid paraffin oil for the preparation of drug loaded microemulsions. The required amount of Smix was added into the drug loaded oil phase. The aqueous phase was injected in the organic phase under homogenizer; this led to instantaneous formation of an o/w emulsion in the external aqueous phase and subsequent formation of microdroplets. The homogenizer was maintained at 8000 rpm during 30min [35].

The composition of microemulsion formulations was shown in the table no. 1 and formulation was represented in figure 1.

Post formulation studies:

Thermodynamic stability tests

Selected formulations were subjected to different thermodynamic stability tests.

Heating cooling cycle: Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 hours were conducted, and the formulations were examined for stability at these temperatures [30].

Centrifugation: Formulations were centrifuged at 3500 rpm for 30 min by using centrifugator. The formulations that did not show any phase separated were further taken for tests [36].

Freeze thaw cycle: Between –4˚C and +25˚C temperature, three freeze thaw cycles were done for formulations with storage at each temperature for not less than 48 hours and observed for any phase separation. Formulations which passed these thermodynamic stress tests were further taken for the dispersibility tests [30].

Dispersibility tests: Dispersibility tests were done using a dissolution apparatus 2. 1ml of each formulation was added to 500 ml of water at 37±0.5˚C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. *In-vitro* performance of the formulation was visually assessed using the following grading system:

Grade A: Clear or bluish appearance rapidly forms within 1 min.

Grade B: Slightly less clear emulsion having bluish white form rapidly.

Grade C: Fine milky emulsion formed within 2 min.

Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).

Grade E: Poor or minimal emulsification with large oil globules present on the surface [37].

Electroconductivity study: Prepared the tested microemulsions were with a 0.01 N aqueous solution of NaCl instead of distilled water, for the conductivity measurements. The formulations were measured by an electroconductometer (Conductivity meter 305, Systronic) [37].

Percent transmittance: The percent transmittance of emulsion was measured at specific wavelength using UV spectrophotometer (Systronic) keeping distilled water as blank [38].

Viscosity: Viscosity of the samples was measured as such without dilution using Redwood viscometer. Clean the viscometer with the help of suitable solvent e. g; Acetone, petroleum spirit etc. and properly dry to remove any trace of solvent. Level the instrument with the help of the leveling screws on the tripod. Fill the bath with water for determining viscosity at 37˚C and room temperature. Keep the brass ball in position so as to seal the orifice. Pour the emulsion under test carefully into the oil cup upto the trip of the indicator. Keep the 50 ml flask in position below the jet. When temperature of emulsion has become steady in oil cup, lift the ball valve and simultaneously start stop watch, allow the emulsion to fill in the flask upto 50 ml mark. Stop the stop watch and note down the time, in seconds. Replace the ball valve in position to seal the cup to prevent over flow of the emulsion [39].

Drug content: The drug content was calculated by using UV-visible spectrophotometer. The formulation was diluted to required concentration using 6.8pH buffer as solvent and the absorbance was measured at specific wavelength against a solvent blank [38]. The drug content was calculated as:

$$\text{Drug content} = \frac{\text{Analyzed content}}{\text{Theoretical content}} \times 100$$

Scanning electron microscopy (SEM): By using magnifications from 10X to 100,000X; the size analysis, topographical and elemental information were measured. A concentrated aqueous dispersion of nanoparticles was finely spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer (20 nm thick). The surface morphology of the nanoparticles was observed by SEM using S-3400N scanning electron microscope [34].

***In-vitro* drug release studies:** The *in-vitro* drug release of *Momordica charantia, Tinospora cordifolia and Trigonella foenum graecum* from the microemulsion formulation was determined by using dialysis bag method. 0.1N HCl and pH 6.8 buffer were used as medium for *in-vitro* release studies. 5ml of formulation was placed in the dialysis bag(single dose containing 250mg of each herbal extract of *Momordica charantia, Tinospora cordifolia and Trigonella foenum graecum)*, which was immersed in 90ml of 0.1 N HCl for 2hrs and than replaced with pH 6.8 buffer maintained at 37°C and stirred with a magnetic stirrer. 5ml of samples were withdrawn at regular time intervals (15, 30, 45, 60, 90, 120 etc. minutes). In order to maintain sink conditions, an equal volume of medium was replaced. The samples were analyzed by the UV-Visible spectrophotometer at appropriate wavelength to determine the concentration [30].

Results and Discussion

Preformulation Results:

Determination of λmax:

λmax of *Momordica charantia* (karela), *Trigonella foenum graecum* (methi) and *Tinospora cordifolia*(giloe) were found to be222.5, 223.3, and 260nm respectively. It is represented in figure 2.

Drug - Excipient compatibility studies:

Drug-excipients compatibility studies were confirmed by carrying out FTIR studies. There was none of the extra peak found in the graph. The graphs are presented in figure 3 & 4. Thus, FTIR studies showed that there was no drug-excipient interaction.

Solubility studies:

Solubility of *Tinospora cordifolia, Momordica charantia* and *Trigonella foenum graecum* drug extracts were found to be 811±0.4µg/ml, 383±0.6µg/ml & 121.22±0.3g/ml respectively in liquid paraffin oil. Solubility of all herbal extracts was found to be high in Tween 80 and Span 20 as compared to other surfactants. Thus, Liquid paraffin oil was selected as the oil phase for the development of the formulation. Solubility study of herbal extracts in different solutions was given in table no. 2.

Post Formulation Studies:

Thermodynamic stability tests and Dispersibility tests:

Formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. The results showed that F1, F2, F3 formulations had a very good physical stability. Formulations that passed dispersibility test in Grade A and B were taken for further study, as Grade A and B formulations will remain as microemulsions when dispersed in GIT. All 5 formulations passed the dispersibility test under different grades which is represented in table no. 3..

Electroconductivity test:

Conductivity of the optimized formulations was found in range of 0.07 - 011 MHOS/cm at 20 m (Table No. 4). From the viscosity and the electroconductivity study it can be concluded that the system is of o/w type.

Evaluation of Drug content, viscosity and percent transmittance:

Drug content of the optimized formulations was found to be 89.62%, 97.78% and 99.75% of *Momordica charantia*, *Trigonella foenum graecum* and *Tinospora cordifolia* respectively i.e. highest in F1 formulation (Table No. 5).

The drug content varied for upto 7.13% between formulations F1 to F5. It was observed that viscosity of all the formulations is less than 9 cPs. Formulation F1 has the minimum viscosity (4.86 cPs), perhaps because of its higher aqueous content. Lower viscosity is an ideal characteristic of the o/w microemulsion. The percentage transmittance of the optimized formulation F1 was found to be 98.32% and formulation F5 was found to be 98.18%.The results of percentage transmittance revealed that all the formulations were nearly transparent.

Scanning Electron Microscopy:

The SEM studies were carried out to get more insight about the morphology of the microemulsion systems. SEM images showed the diameter of formulation F1 was found to be 861nm-1.18µm (Figure 5). This showed that the formulated formulation was micro in size as diameter ranges between 0.1µm to 100µm.

***In-vitro* release studies:**

Percent cumulative drug release was calculated for Karela, Methi, Giloe in F1, F2 & F3 formulations. *In-vitro* Drug release is presented in table no. 6. The highest release was found to be 97.60, 89.28 and 92.31 % of *Tinospora cordifolia*, *Momordica charantia* and *Trigonella foenum graecum* respectively obtained in case of formulation F1 can be detected by performing dissolution studies for 18 hours. F1 showed better control release rate in comparison to other 3 different microemulsion formulations. An *in-vitro* release graph for each drug in different formulations is depicted in figure 6, 7 & 8. Results of different plots of formulation F1 for each drug are presented in table no. 7. Regression coefficient of *Trigonella foenum graecum*, *Momordica charantia*, and *Tinospora cordifolia* for zero order release plot was found to be 0.978, 0.992, and 0.986 respectively. Regression coefficient of *Trigonella foenum graecum*, *Momordica charantia* and *Tinospora cordifolia* for Higuchi Plot was found to be 0.972, 0.946, and 0.985 respectively. Regression coefficient of *Trigonella foenum graecum*, *Momordica charantia* and *Tinospora cordifolia* for Korsmeyer- Pappas Model was found to be 0.929, 0.944 and 0.921 respectively. Thus the formulation showed zero order release kinetics. Thus formulated microemulsion follows zero order release rate. The graphs are presented in figure 9-17.

Conclusion

Polyherbal microemulsion was formulated containing *Momordica charantia*, *Trigonella foenum graecum* and *Tinospora cordifolia* for the treatment of diabetes mellitus. Viscosity of F1 microemulsion formulation was found to be 4.86 cPs and percent transmittance was 98.32%.

Particle size of formulation F1 was found to be 861nm-1.18µm. *In-vitro* release profile indicated that there was zero order release of optimized formulation. Thermodynamic stability studies (heating cooling cycle, centrifugation and freeze thaw cycle stress tests) indicated that F1, F2, F3 formulations were stable.

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Fig. 1: Microemulsion Formulation

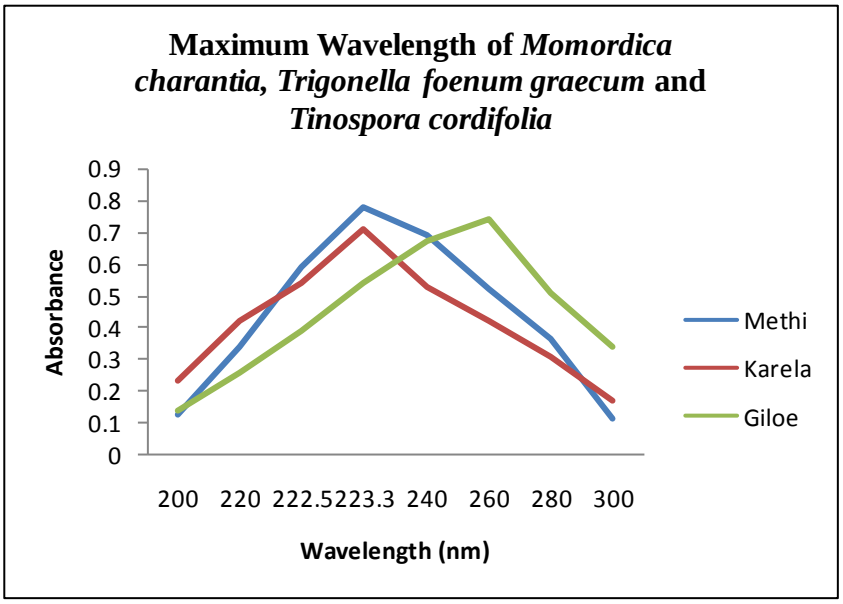

Fig. 2: Maximum Wavelength of *Momordica charantia* (Karela), *Tinospora cordifolia* (Giloe) and *Trigonella foenum graecum* (Methi)

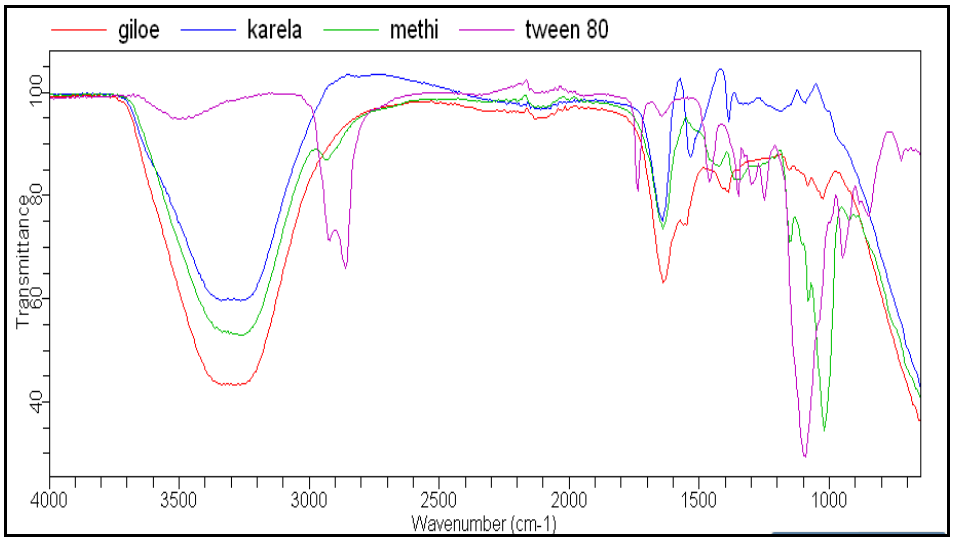

Fig. 3: Drug-Polymer Interaction Studies through FTIR

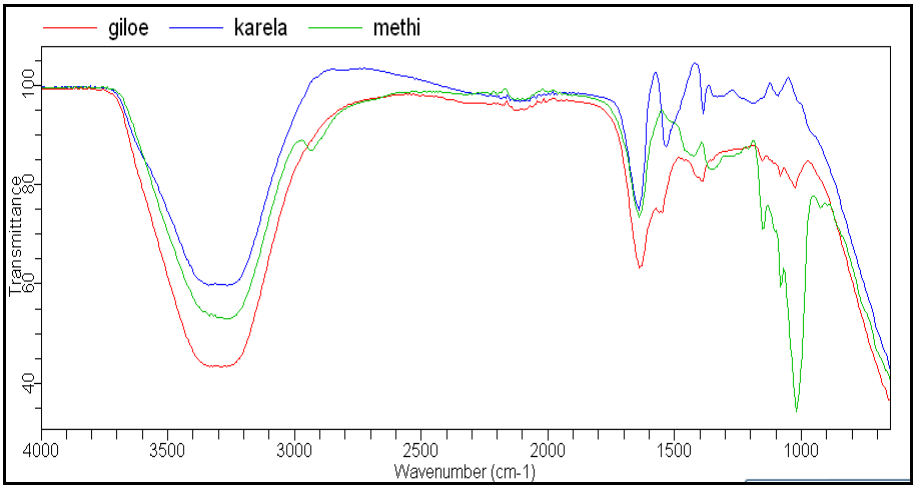

Fig. 4: Drug-Drug Interaction Studies through FTIR

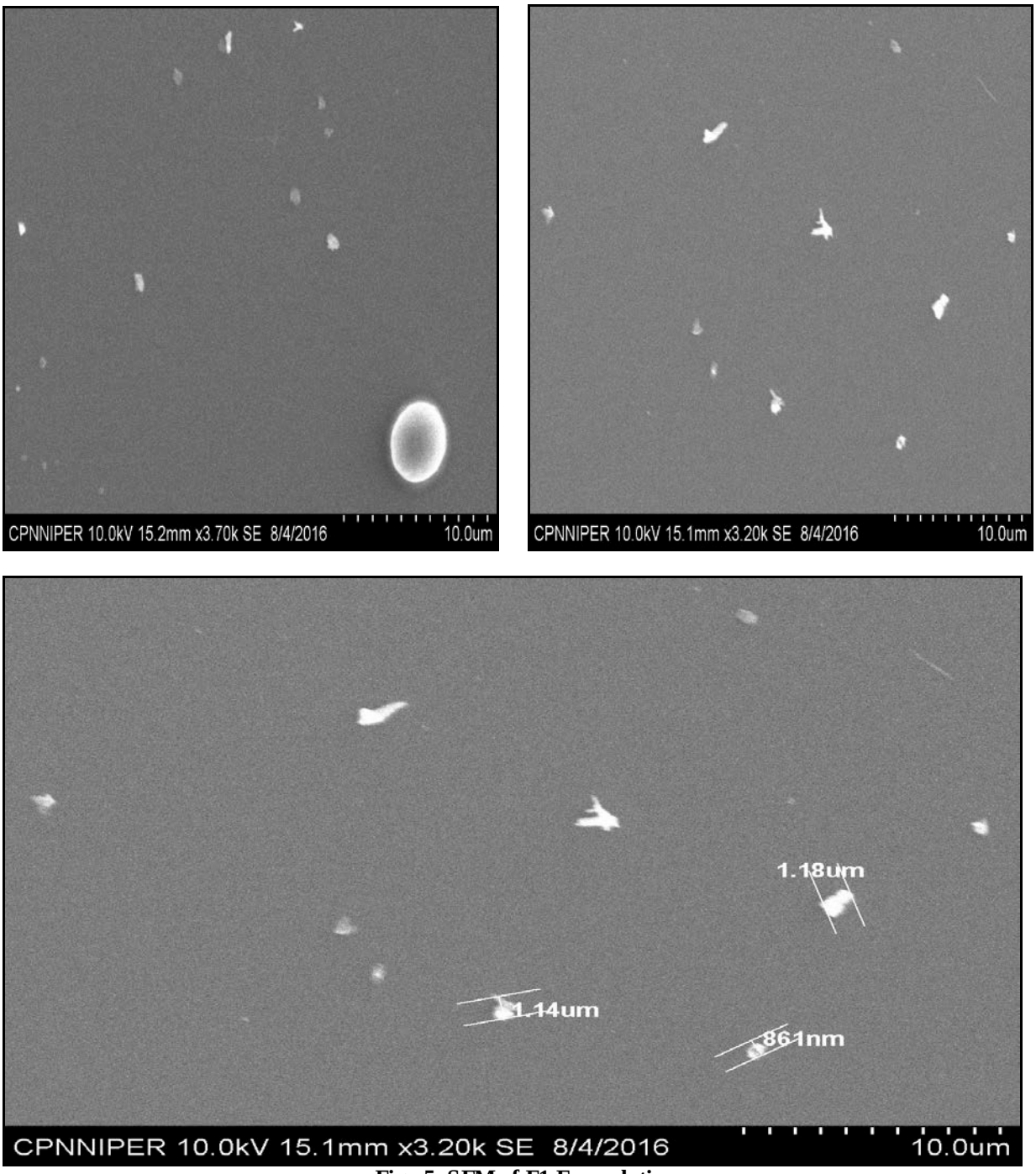

Fig. 5: SEM of F1 Formulation

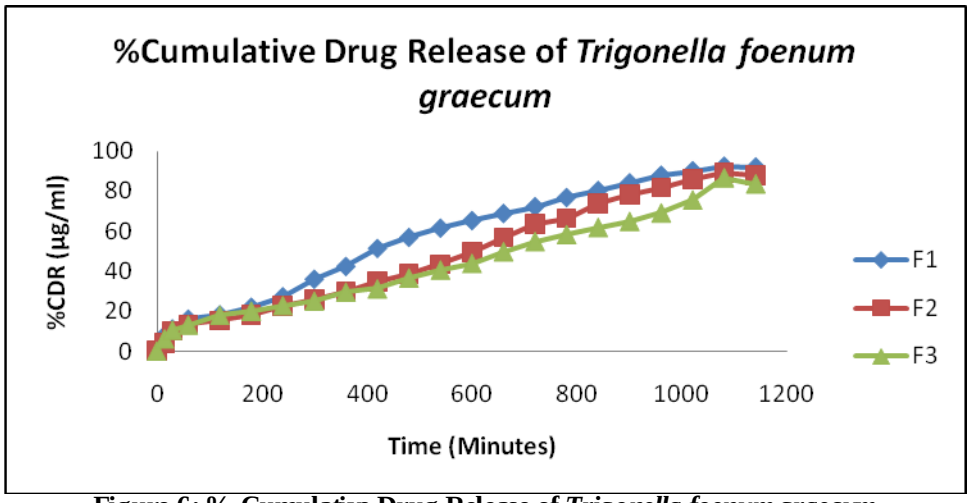

Figure 6: % Cumulative Drug Release of *Trigonella foenum graecum*

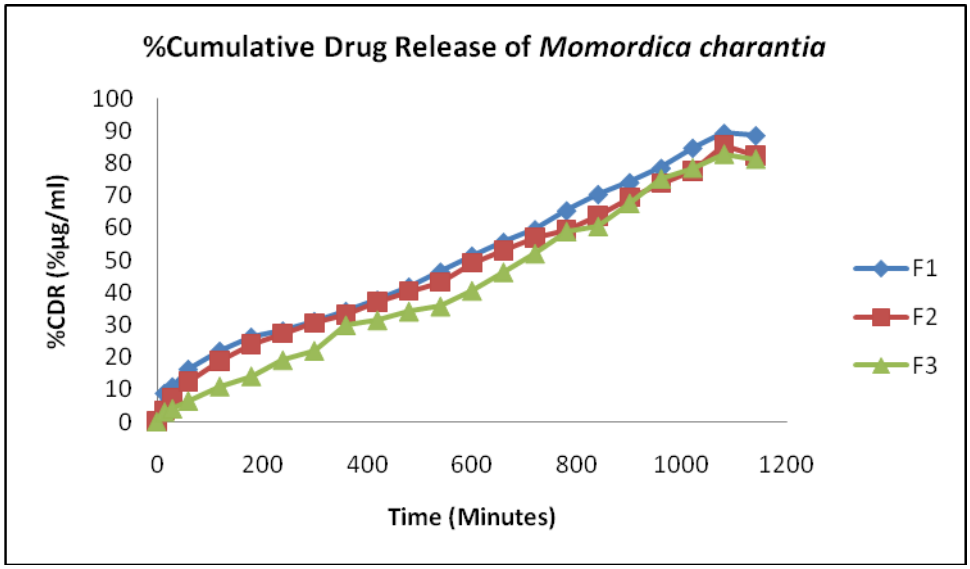

Figure 7: % Cumulative Drug Release of *Momordica charantia*

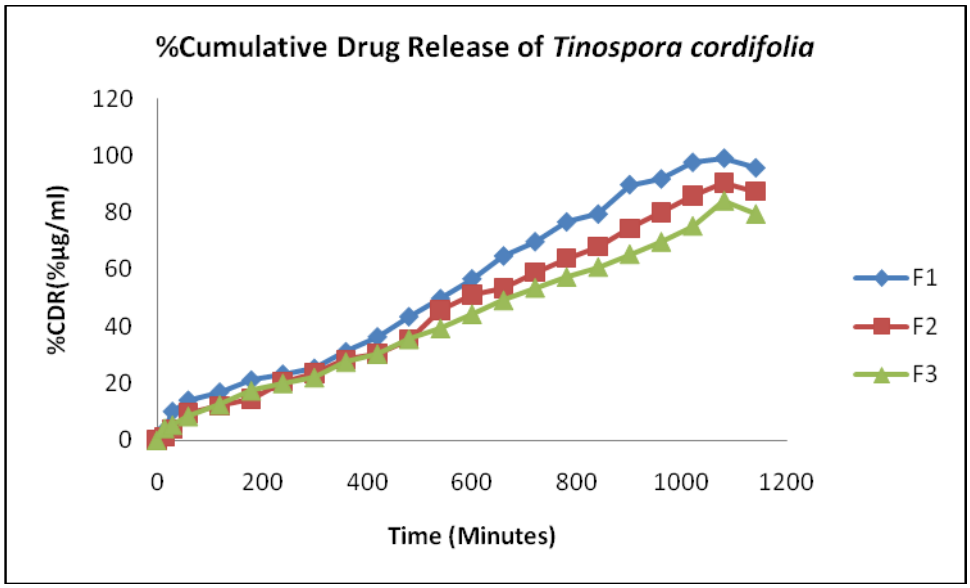

Figure 8: % Cumulative Drug Release of *Tinospora cordifolia*

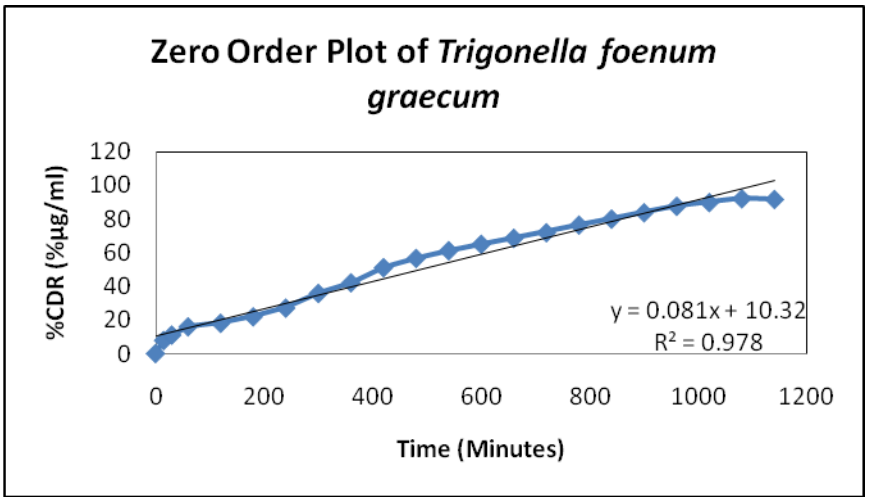

Figure 9: Zero Order Release Plot of *Trigonella foenum graecum* of F1 Formulation

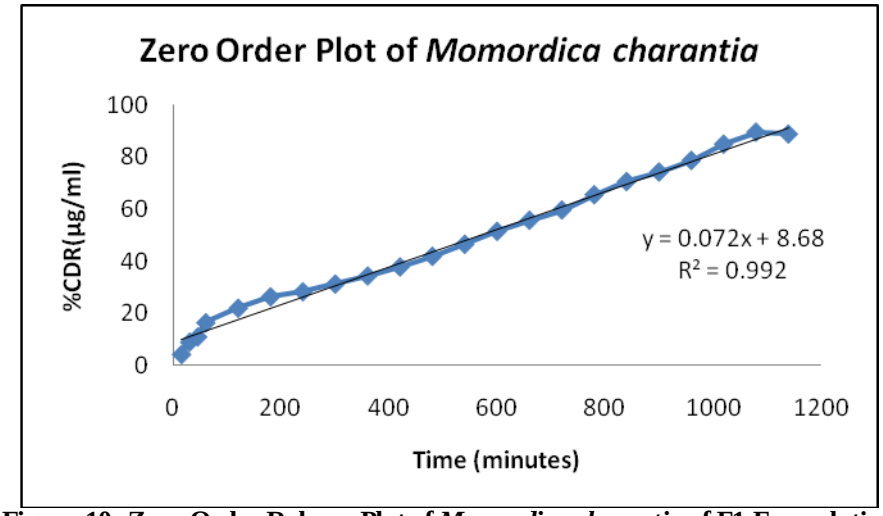

Figure 10: Zero Order Release Plot of *Momordica charantia* of F1 Formulation

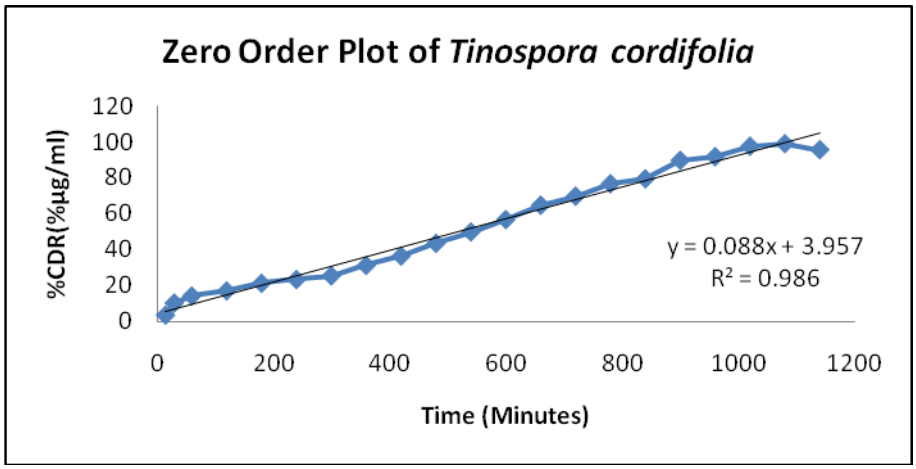

Figure 11: Zero Order Release Plot of *Tinospora cordifolia* of F1 Formulation

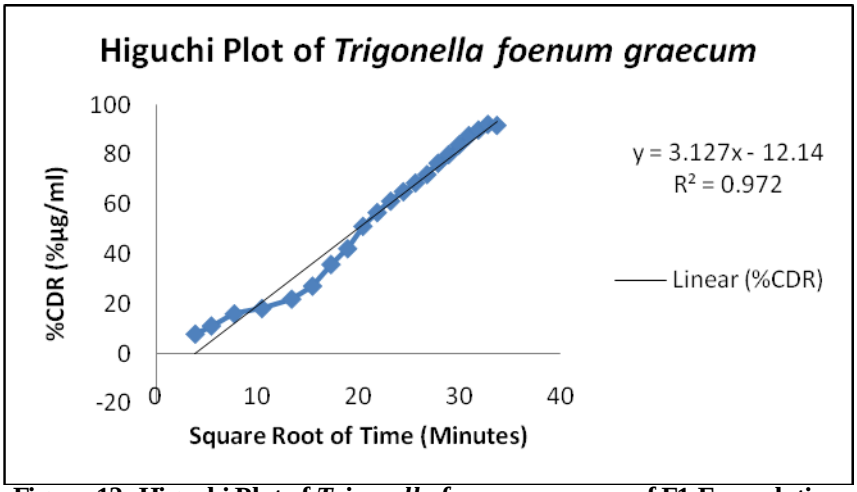

Figure 12: Higuchi Plot of *Trigonella foenum graecum* of F1 Formulation

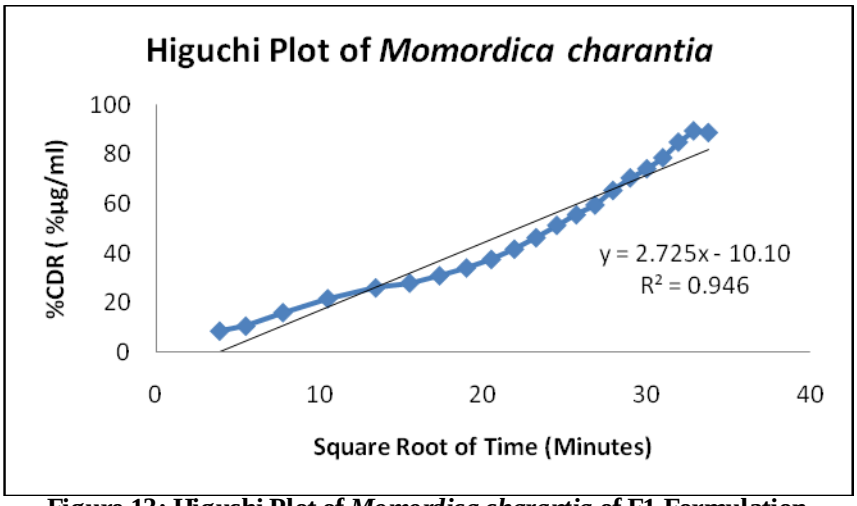

Figure 13: Higuchi Plot of *Momordica charantia* of F1 Formulation

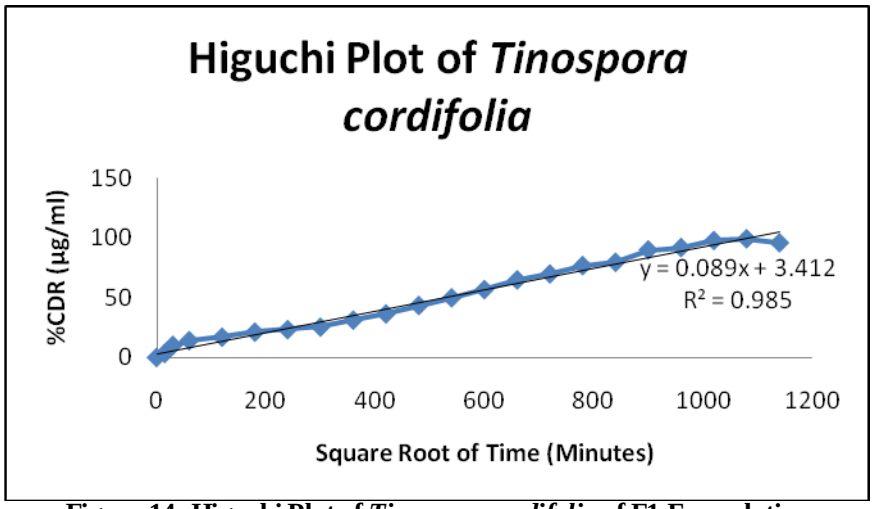

Figure 14: Higuchi Plot of *Tinospora cordifolia* of F1 Formulation

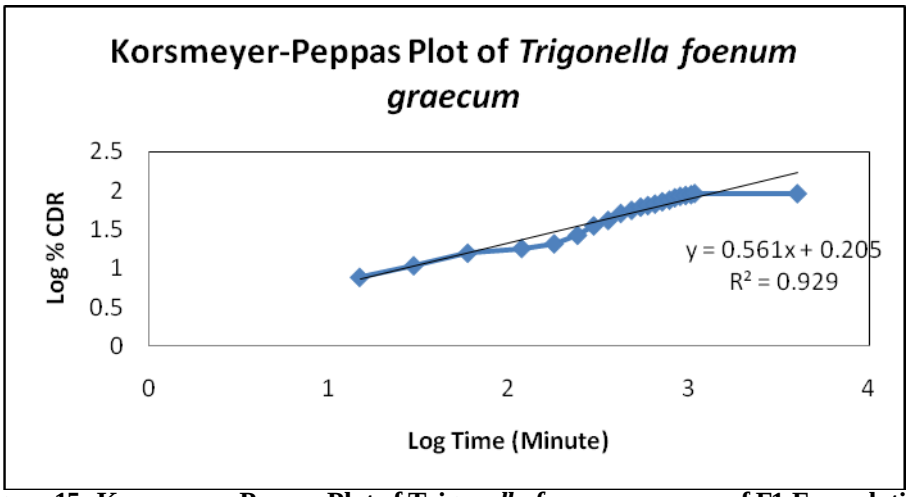

Figure 15: Korsmeyer -Peppas Plot of Trigo*nella foenum graecum* of F1 Formulation

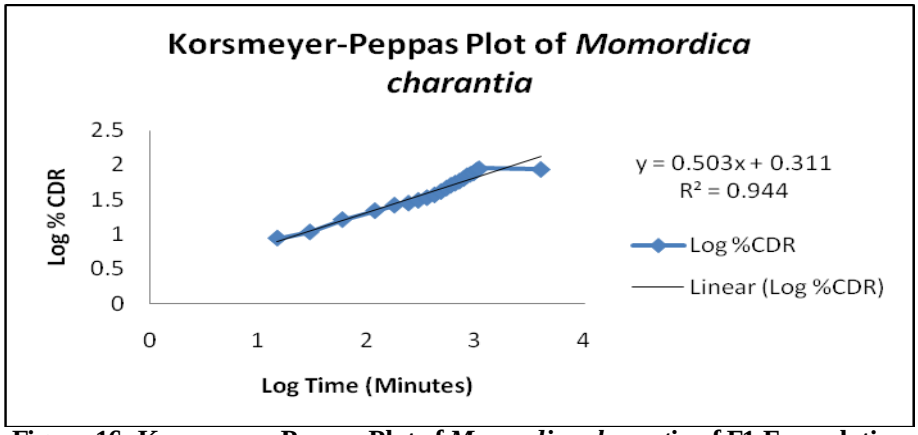

Figure 16: Korsmeyer -Peppas Plot of *Momordica charantia* of F1 Formulation

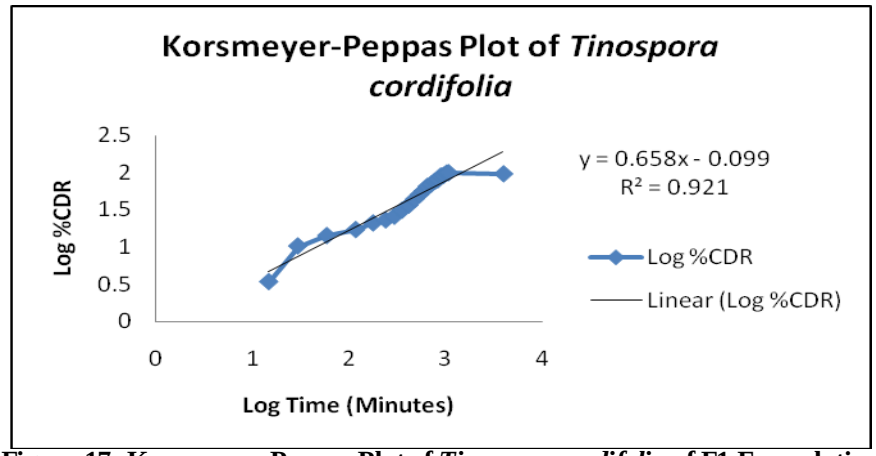

Figure 17: Korsmeyer -Peppas Plot of *Tinospora cordifolia* of F1 Formulation

Table No. 1: Composition of selected microemulsion formulations:

Formulation Code	Liquid paraffin (% v/v)	Smix (% v/v)	Water (% v/v)	Smix Ratio	Herbal Drug Extracts (% wt/v)	Methyl paraben (% w/w)	Propyl Paraben (% w/w)
F1	10	20	70	1:1	15	0.2	0.3
F2	15	20	65	1:1	15	0.2	0.3
F3	10	30	60	1:2	15	0.2	0.3
F4	15	30	60	1:2	15	0.2	0.3
F5	10	20	70	2:1	15	0.2	0.3

Table No. 2: Solubility of *Tinospora cordifolia* in different solutions:

Sr. No.	Solution	Solubility(µg/ml)		
		Tinospora cordifolia	*T. foenum graecum*	*Momordica charantia*
1.	Distilled water	636.4±0.5	330.67±0.5	976±0.3
2.	0.1 N HCl	618.8±0.4	326.56±0.6	930±0.3

3.	Ethanol	565.4±0.2	166.22±0.09	788.68±0.4
4.	6.8 pH Buffer	833.5±0.6	211.56±0.2	771.67±0.2
5.	Methanol	913±0.2	158±0.7	966.67±0.1
6.	Liquid paraffin	811±3.8	121.22±0.7	945±0.5
7.	Isopropyl myristate	475.5±0.3	98.4±0.2	326.56±0.2
8.	Linseed oil	301±0.9	56.56±0.4	198.4±0.1
9.	Castor oil	158±0.5	65.79±0.3	48.67±0.5
10.	Tween 20	595±2.08	29.94±0.85	158.70±1.23
11.	Tween 80	706±5.3	85.94±2.25	250.45±0.8
12.	Span 20	573±3.05	63.99±0.79	233.57±0.57
13.	Span 80	338±2.2	49.71±0.95	122.34±1.07

Table No. 3: Thermodynamic stability test results of F1 to F5 formulations:

Formulation Code	Heating Cooling Cycle	Centrifugation	Freeze Thaw Cycle	Dispersibility Grade
F1	++	++	++	A
F2	++	++	++	B
F3	++	++	++	A
F4	+	+	+	B
F5	+	+	+	C

Here, ++ = Very Good, + = Good

Table No. 4: Electroconductivity test results of formulations (F1 to F5):

Formulation Code	Conductivity at 20m (MHOS/cm)	Conductivity at 2m (MHOS/cm)	Conductivity at 200µ (MHOS/cm)
F1	0.11	0.133	134.7
F2	0.10	0.129	128.9
F3	0.09	0.124	125.1
F4	0.07	0.115	117.3
F5	0.08	0.120	121.8

Table No. 5: Drug content, viscosity and percent transmittance tests results of F1 to F5 formulations:

Formulation Code	Drug Content (%)			Viscosity (cPs)	Percent Transmittance (%)
	M. charantia	*T. foenum graecum*	*T. cordifolia*		
F1	89.62	97.78	99.75	4.86	98.32
F2	88.07	95.98	98.45	5.26	97.58
F3	84.31	91.04	95.85	5.69	93.2
F4	88.59	93.49	96.56	8.14	93.4
F5	85.48	92.43	89.22	7.43	98.18

Table No. 6: *In-vitro* release study i.e. % Cumulative amount of drug release from microemulsion formulation

TIME (MINUTE)	F1			F2			F3		
	K	M	G	K	M	G	K	M	G
0	0	0	0	0	0	0	0	0	0
15	4.03	7.79	3.41	3.41	3.95	1.20	2.99	6.13	4.21
30	8.77	11.06	10.15	7.44	9.98	3.97	3.97	10.56	5.36
60	16.19	15.94	14.06	12.32	13.15	9.63	6.38	13.09	8.45
120	21.78	18.09	16.94	18.83	15.07	11.98	10.86	17.98	12.47
180	26.14	21.09	21.15	23.88	17.85	14.55	13.98	20.04	17.45
240	28.07	27.11	23.22	27.19	22.35	20.53	18.94	22.78	20.05
300	31.03	35.86	25.31	30.42	25.75	23.45	21.86	25.04	22.07
360	34.15	42.2	31.1	32.92	29.78	28.34	29.75	29.56	27.56
420	37.6	51.23	36.32	36.94	34.73	30.40	31.29	31.24	30.35
480	41.69	56.75	43.41	40.31	38.60	35.45	33.98	36.62	35.57
540	46.31	61.17	49.77	43.07	43.33	45.65	35.54	40.69	39.36
600	51.26	65.17	56.68	48.97	49.34	50.98	40.36	43.98	44.36
660	55.52	68.71	64.73	52.72	56.74	53.18	46.08	49.87	49.24
720	59.45	72.1	69.73	56.86	63.47	58.97	51.87	54.98	53.67
780	65.29	76.65	76.68	59.09	66.2	63.86	58.69	58.43	57.45
840	70.35	80.17	79.43	63.63	73.55	67.97	60.26	61.99	60.91
900	74.01	83.99	89.75	69.20	78.29	74.35	67.42	65.03	65.46
960	78.45	87.81	91.76	73.63	81.75	79.99	74.95	69.28	69.81
1020	84.7	90.06	97.6	77.28	85.81	85.88	78.31	75.77	75.39
1080	89.28	92.31	99.03	85.40	89.13	90.38	82.59	86.93	84.27
1140	88.57	91.92	95.67	82.15	87.75	87.43	81.09	83.90	79.60

Here, K = Karela, M = Methi, G = Giloe

Table No. 7: Results of Plots of Formulation F1:

Model/Plot	Parameter	*Trigonella foenum graecum*	*Momordica charantia*	*Tinospora cordifolia*
Zero Order	Slope	0.081	0.072	0.088
	Intercept	10.32	8.68	3.957
	R^2	0.978	0.992	0.986
Higuchi Plot	Slope	3.127	2.725	0.089
	Intercept	12.14	10.10	3.142
	R^2	0.972	0.946	0.985
Korsmeyer – Pappas Plot	Slope	0.561	0.503	0.658
	Intercept	0.205	0.311	0.099
	R^2	0.929	0.944	0.921

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