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TO FORMULATE AND EVALUATE MULTIVITAMINS CONTAIN MICRO-EMULSION

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ABSRACT

Multivitamin microemulsion is prepared by using various API's like CO-Q_{10}, Vit E, Vit. C, Oleic acid, Castor oil, Tween 80, Tween 20, Propylene glycol, Polyethylene Glycol 400 etc. with the help of all these API's. Development of O/W microemulsion has been carried out by screening of components to find out excellent solubilisation and preservation for longer time. The preparation of O/W emulsion was carried out by phase- titration method. Characterization of micro-emulsion was done by simultaneous estimation of second order derivative spectra. The other evaluation parameters includes assay of Vit E, CO-Q10, Vit C, visual isotropy studies, viscosity, pH etc. The conclusion indicates on testing various parameters, the multivitamin microemulsion of CO-Q10, Vit E, Vit C offer an approved oral bioavaliabilty and enhance drug solubility, preservation, good and thermodynamically stability.

KEYWORDS: Multivitamin microemulsion, O/W emulsion, Phase- titration method, approved oral bioavaliabilty.

INTRODUCTION TO MICRO-EMULSION

Micro-emulsions are isotropic, thermodynamically stable, transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with a droplet size usually in the range of 10-100 nm. These homogeneous systems, which can be prepared over a wide range of surfactant concentration and oil to water ratio are all fluids of low viscosity.

Micro-emulsion have been widely studied to enhance the bioavailability of the poorly soluble drugs. They offer a cost effective approach in such cases. Micro-emulsions have very low surface tension and small droplet size which results in high absorption and permeation.

Solubility plays a vital role in achieving the therapeutic efficacy of a drug from a dosage form. Advances in the molecular screening techniques for identification of potential drug molecules investigated an increased number of new pharmaceutically active lipophilic compounds that are poorly water soluble. It is a great task for pharmaceutical scientist to formulate oral dosage forms of these drug candidates with sufficient bioavailability. Among the various approaches to improve oral bioavailability of these drug candidates, micro-emulsion based lipid formulations is one of the approaches used to improve the bioavailability of lipophilic drugs.[5]

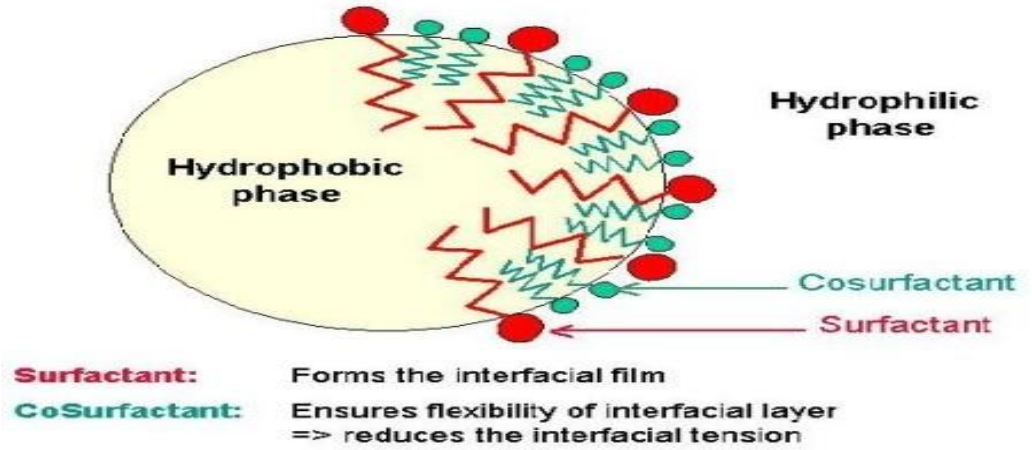

Fig. 1: Structure of Micro-emulsion.

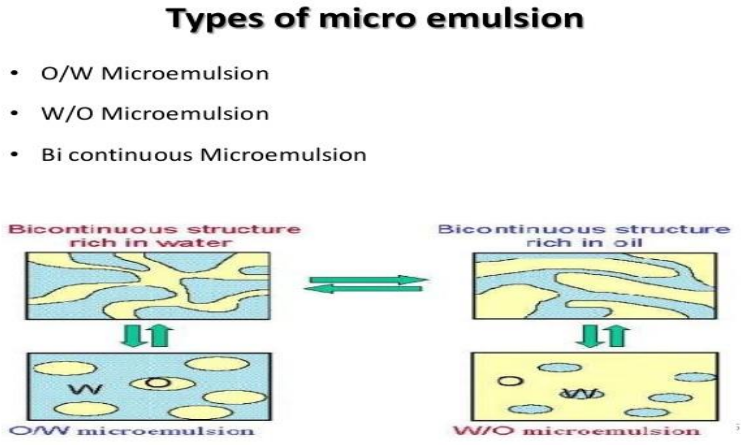

Fig. 2: Schematic representation of the three most commonly encountered micro emulsion microstructure.[6, 7, 8]

Micro-emulsions are thermodynamically stable, but are only found under carefully defined conditions. Characterizing the system by whether the domains are in droplets or continuous, results in three types of micro-emulsions:

Fig 3: Difference between Emulsion and Microemulsion.[9]

Table 2: Difference between Emulsion and Micro-emulsion.

Properties	Emulsion	Micro emulsion
Appearance	Cloudy	Transparent (or translucent)
Phases	Biphasic	Monophasic
Optical isotropy	Anisotropic	Isotropic
Proportion of dispersed phase	30-60%	23-40% without corresponding increase in viscosity
Interfacial tension	High	Ultra low
Microstructure	Static	Dynamic(interface is continuously and spontaneously fluctuating)
Droplet size	≥500 nm	20-200 nm
Energy requirement	Requires large energy input at the time of preparation	Forms spontaneously. So no energy requirement
Stability	Thermodynamically unstable (kinetically stable). Will eventually phase separate	Thermodynamically stable. Long shelf life
Nature	They are lyophobic	They are on the borderline between lyophobic and lyophilic colloids.
Preparation	Requires a large input of energy. Higher cost	Facile preparation, relatively lower cost for commercial production
Viscosity	Higher viscosity	Low viscosity with Newtonian behaviour

EXPERIMENTAL WORK

DEVELOPMENT OF O/W MICRO EMULSION

Screening of components for micro emulsions:

Screening of various oils such as Castor oil, Oleic acid; surfactants such as Tween 80, Tween 20, and co-surfactants such as Propylene glycol, Polyethylene glycol 400, was carried out to find out the suitable components which can be used as the oil phase, surfactant and co-

surfactant showing good solubilising capacity and can be used for micro-emulsion to provide excellent stabilization and preservation for longer duration of time.

Solubility studies

Two ml of each of the selected vehicles was added to each cap vial containing and excess of vitamins (100mg). After tightening the cap, the mixture was heated at 40°C in a water bath to facilitate the solubilisation. Mixing of the systems was performed using a vortex mixer. Formed suspensions were then shaken with a shaker at 25°C for 48 hours. After reaching equilibrium, each vial was centrifuged at 3000 rpm for 20 minutes, and excess insoluble DC in supernatant was discarded by filtration using a membrane filter (0.43 µm, 13 mm, what man, India). Aliquots of sample was taken and diluted with methanol to specific volume to give specific point concentration in calibration curve. Analysis of the vitamins was carried out on double beam U. V. Spectrophotometer at 272.6 nm, 261 nm and 284.6 nm wavelength.

Identification of Co-Q10, Vitamin E, and Vitamin C by UV spectral analysis: Preparation of solution for UV scan of Co-Q10

The sufficient quantity of Co-Q10 Powder(10mg) was dissolve in n-hexane and after suitable dilution final concentration of 10 µg/ml was prepare for UV scan and it was taken at room temperature and their corresponding obtained value of λmax 275 nm.

Preparation for the standard plots or calibration curve of Co-Q10

The accurately weigh quantity of Co-Q10 powder (10 mg) was dissolved in n-hexane so that it could be solubilized and then final volume was made to 100 ml in a volumetric flask. Now, this is called stock solution. The concentration of drug in that solution was 10 mg/100 ml i.e., 100 µg/ml. From stock solution different were taken out such as 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml in 10 ml volumetric flask and each of these were diluted to 10 ml n-hexane. The prepared solution were plotted calibration curve at specified wavelength 275 nm.

Preparation of solution for UV scan of Vitamin E

The sufficient quantity of Vitamin E Powder(10mg) was dissolve in n-hexane and after suitable dilution final concentration of 10 µg/ml was prepare for UV scan and it was taken at room temperature and their corresponding obtained value of λmax 280 nm.

Preparation for the standard plots or calibration curves of Vitamin E

The accurately weigh quantity of Vitamin E powder (10 mg) was dissolved in n-hexane so that it could be solubilized and before making the final volume solution was sonicated for 10 min and then final volume was made to 100 ml in a volumetric flask and this solution is filter. Now, this is called stock solution. The concentration of drug in that solution was 10 mg/100 ml i.e., 100 µg/ml. From stock solution different were taken out such as 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml in 10 ml volumetric flask and each of these were diluted to 10 ml n-hexane. The prepared solution were plotted calibration curve at specified wavelength 280 nm.

Preparation of solution for UV scan of Vitamin C

The sufficient quantity of Vitamin C Powder(10mg) was dissolve in n-hexane and after suitable dilution final concentration of 10 µg/ml was prepare for UV scan and it was taken at room temperature and their corresponding obtained value of λmax 261.6 nm.

Preparation for the standard plots or calibration curves of Vitamin C

The accurately weigh quantity of Vitamin C powder (10 mg) was dissolved in n-hexane so that it could be solubilized and before making the final volume solution was sonicated for 10 min and then final volume was made to 100 ml in a volumetric flask. Now, this is called stock solution. The concentration of drug in that solution was 10 mg/100 ml i.e., 100 µg/ml. From stock solution different were taken out such as 0.1 ml, 0.2 ml, 0.3ml, 0.4 ml, 0.5 ml in 10 ml volumetric flask and each of these were diluted to 10 ml n-hexane. The prepared solution were plotted calibration curve at specified wavelength 261.6 nm.

6.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Infrared spectroscopy was conducted using a Shimadzu FTIR 8300 spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm-1. Sample (drug alone) was mixed with potassium bromide (200-400 mg) and powder mixture was placed in a sample holder in the light path and the spectrum was obtained.

6.5 CONSTRUCTION OF PSEUDO TERNARY PHASE DIAGRAM[111]

The aim of the construction of pseudo ternary phase diagram was to find out the existence range of micro-emulsions and concentration ranges of its components. Pseudo-ternary phase diagrams were constructed separately for each combination of oils with each mixture of

surfactant and co-surfactant using ratio of 1:1, 2:1, 3:1 and 3:2 in order to identify o/w micro-emulsion regions and select the micro-emulsion formulations for each type of oil.

The pseudo ternary phase diagrams of investigated four quaternary systems Oleic acid/ Tween 80/ PG/ Water; Castor oil/ Tween 20/ PEG 400/ Water are represented in following figure. Phase behaviour investigation of these systems demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and co-surfactant concentration with which the transparent, one phase low viscous micro-emulsion system was formed.

The phase diagrams indicating the shaded micro-emulsion region (isotropic). The rest of the region on the phase diagram represents the turbid and conventional emulsion based on visual observation. Outside the micro-emulsion region, particularly for the compositions to the oil-water binary axis, there is insufficient surfactant to facilitate the formation of single micro-emulsion phase. In this case multiple phases may exist, the complexity of which increase with the number of components in the mixture. The single phase of micro-emulsion region is divided into w/o or o/w micro-emulsion phase by simply considering the composition, that is, whether it was oil-rich or water-rich. From the above phase diagrams it was found that, selected combination of oil phase, surfactant a co-surfactant, pseudo ternary study was done, the selected combination of phase it's upon the solubilisation capacity. In castor oil: Tween 20: PEG 400 with different ratio was not affected as much and micro-emulsion region was found as less compared to other systems. Oleic acid: Tween 80: PG different ratio (2:1, 3:1, 3:2) the solubilisation capacity of oleic acid with surfactant and co-surfactant having ratio (3:2) were found to be highest and the area occupied by the micro-emulsion region was also found to be more. It was found that the nature of the emulsion formed in aqueous medium depended on the concentration of hydrophilic surfactant Tween 80. The increase in concentration of Tween 80 resulted in an improvement in clarity of the emulsion and corresponding decrease in particle size. However higher concentration of Tween 80 caused formation of lipotropic liquid crystals gel phase with increase viscosity.

6.6 PREPARATION OF O/W MICRO-EMULSION BY PHASE TITRATION METHOD

Micro emulsion were prepared by dispersing required quantity of multivitamins in appropriate quantity of oil. The mixture was homogenized and to it, accurately weighed quantity of surfactant: co-surfactant ratio mixture was added slowly in oil portion with

stirring. The blend was mixed thoroughly using magnetic stirrer and water is drop wise added to the blend. The system was clear, transparent and micro emulsion are formed.

1. The quantity of oil phase, surfactant and co-surfactant in appropriate portion was selected based on the result of solubility study and observing phase data of pseudo ternary diagram.
2. The multivitamins micro emulsion were prepared.
3. The prepared formulation was stored in tightly closed glass vials which were stored at room temperature (48hrs). The formulations were observed for any sign of phase separation during this period.
4. From the pseudo ternary phase diagrams, it was observed that surfactant: co-surfactant. When used in (3:2) ratio was showing more micro-emulsion region. In each formulation 900 mg Co-enzyme Q, 900 mg Vitamin C, 150 mg Vitamin E was added, kept for equilibration and saturated solubility of all the formulation was determined. Then these formulations were subjected to various evaluation tests.

Table 4: Micro-emulsion composition (%w/w) of formulations from 1 to 8.

Composition	OME1	OME2	OME3	OME4	CME5	CME6	CME7	CME8
Co-Q10	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg
Vitamin E	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg	150 mg
Vitamin c	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg	900 mg
Oleic acid	4	4	4	4	-	-	-	-
Castor oil	-	-	-	-	4	4	4	4
Tween 80	45	50	48	49	-	-	-	-
Tween 20	-	-	-	-	45	50	48	49
Span 20	3	3	3	1	3	3	3	1
Propylene glycol	40	36	38	40	-	-	-	-
Polyethylene glycol 400	-	-	-	-	40	36	38	40
Water	8	7	7	6	8	7	7	6

7. RESULTS AND DISCUSSIONS

Table 5: Solubility data of Co-Q10, Vitamin E and Vitamin C in different components.

Components	Solubility in CoQ10 (mg/ml) (mean± S. D)	Solubility in Vitamin E (mg/ml) (mean± S. D)	Solubility in Vitamin C (mg/ml) (mean± S. D)
Oleic acid	23.816 ± 0.068	15.023 ± 0.025	24.741 ± 0.078
Castor oil	12.717 ± 0.061	12.068 ± 0.028	13.818 ± 0.077
Tween 80	19.410 ± 0.050	12.687 ± 0.051	20.118 ± 0.021
Tween 20	11.305 ± 0.138	10.318 ± 0.048	12.118 ± 0.112
Span 20	11.205 ± 0.128	10.235 ± 0.118	9.1011 ± 0.090
Propylene glycol	12.128 ± 0.012	9.234 ± 0.092	18.260 ± 0.040
Polyethylene glycol 400	8.684 ± 0.146	8.5840 ± 0.136	10.650 ± 0.168

7.1.2: Identification of Co-Q10, Vitamin E, Vitamin C by UV spectral analysis

1. Co-Q10

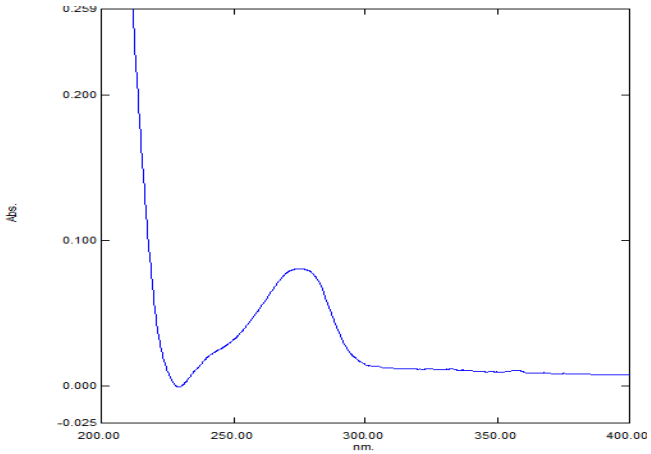

Fig. 7: UV Spectra of Co-Q10.

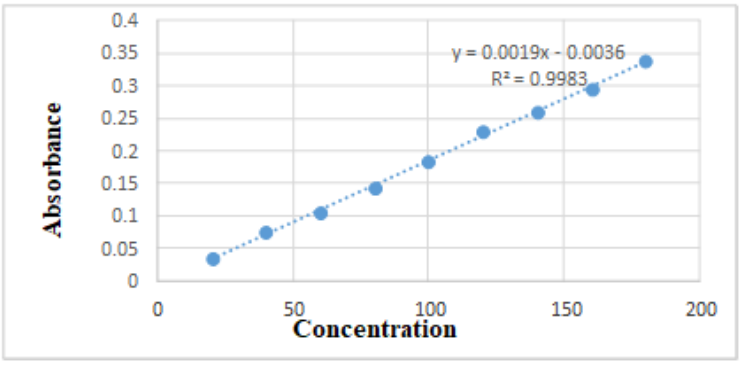

Fig. 8: Calibration curve of Co-Q10.

2. Vitamin E

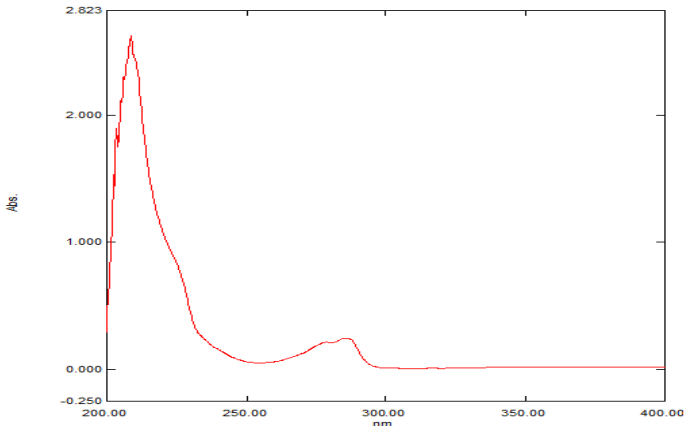

Fig. 9: UV Spectra of Vitamin E.

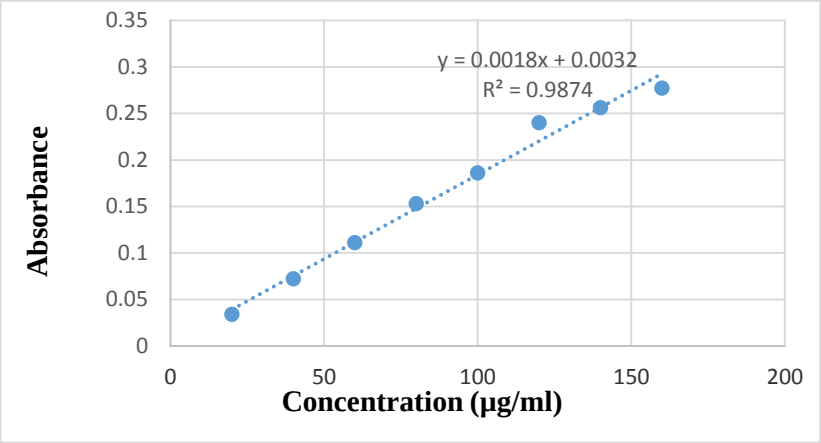

Fig. 10: Calibration curve of Vitamin E.

3. Vitamin C

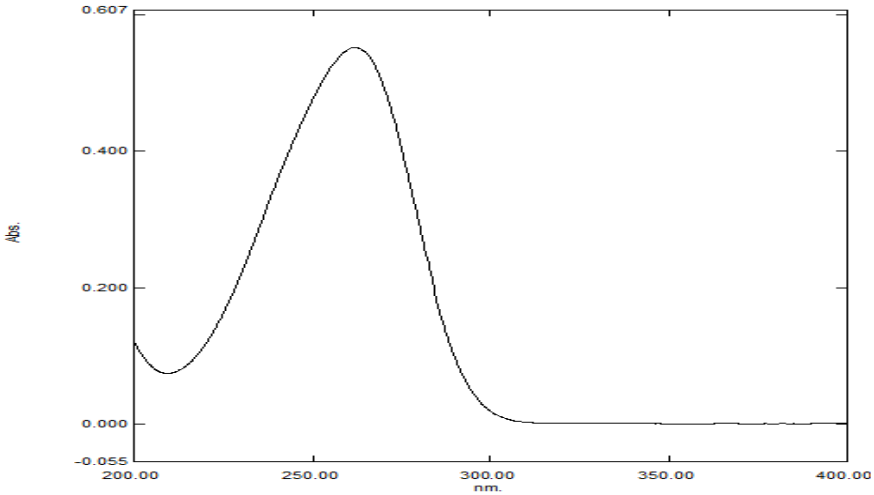

Fig 11: UV Spectra of Vitamin C.

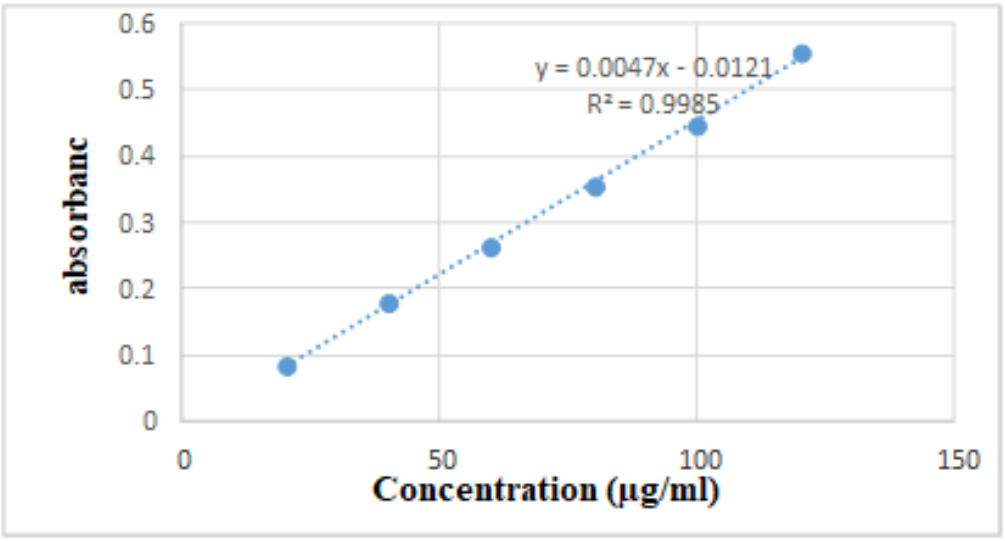

Fig 12: Calibration curve of Vitamin C.

7.1.3: Fourier Transform Infrared Spectroscopy (FTIR)

Further vitamin and multivitamins compatibility study investigated by FTIR spectroscopy. Pure Co-Q10, Vitamin E, and Vitamin C shows major peak around as given in Table 6, 7, 8, 9. And mixture of multivitamins spectra revealed no considerable change when compared with that of pure vitamins, proves that there is no interaction between vitamin and multivitamins.

1)

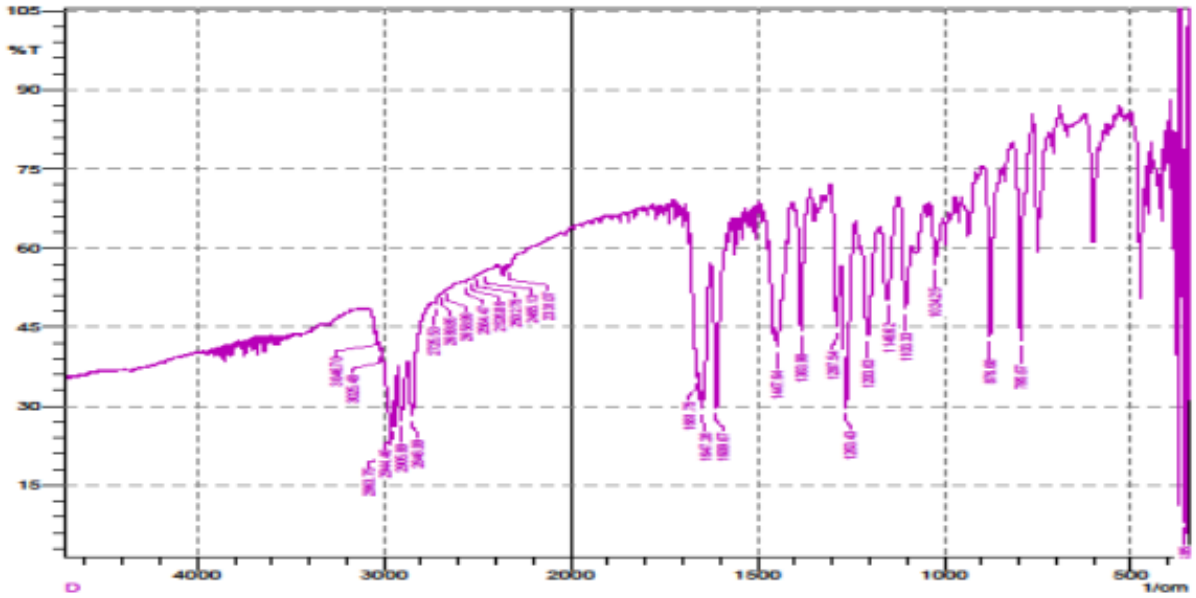

Fig. 13: FTIR spectra of pure Co-Q10.

Table 6: Showing the absorption peaks of pure C0-Q10.

Sr.no.	Peaks	Functional group
1.	795.67	CH_2 Rocking
2.	1103.33	C-O Stretch
3.	1447.64	C-H Bending
4.	1609.67	C=C Stretch
5.	1647.28	C=N Stretch
6.	1661.75	C=O Stretch for amides
7.	2502.75	S-H Stretch
8.	2963.75	C-H Stretch for alkanes
9.	3046.7	C-H Stretch for alkenes and aromatic

2)

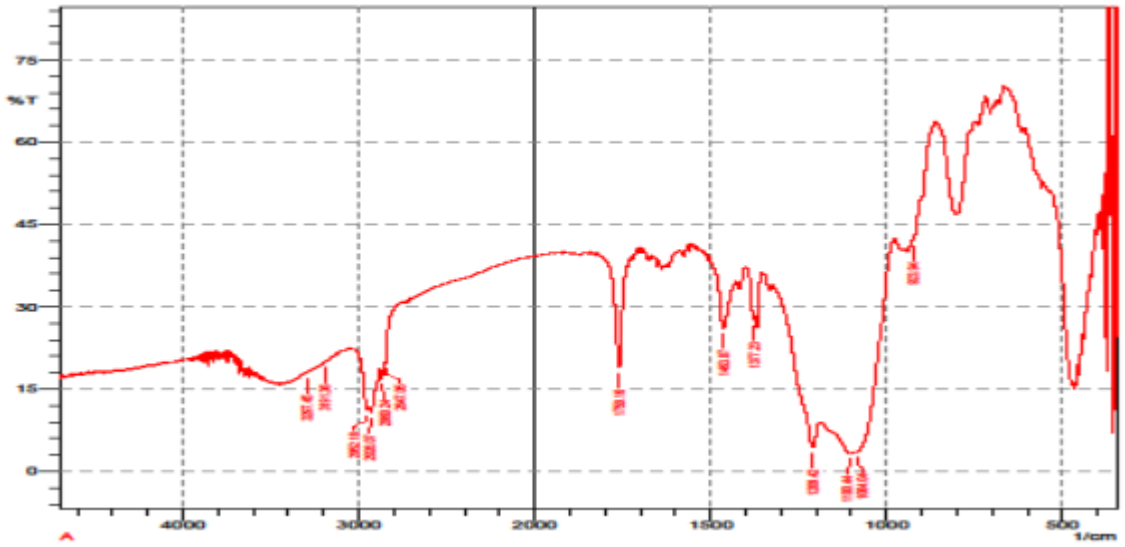

Fig. 14: FTIR spectra of pure Vitamin E.

Table 7: Showing the absorption peaks of pure Vitamin E.

Sr. No.	Peaks	Functional group
1.	1100.44	C-O Stretch
2.	1209.42	C-N Stretch
3.	1463.07	C-H Bending
4.	1759.16	C=O Stretch for vinyl esters
5.	2847.05	2 bands of C-H Stretch for aldehyde
6.	2928.07	C-H Stretch for alkanes
7.	3191.36	O-H Stretch
8.	3297.45	O-H Stretch or N-H Stretch or C-H Stretch for alkynes.

3)

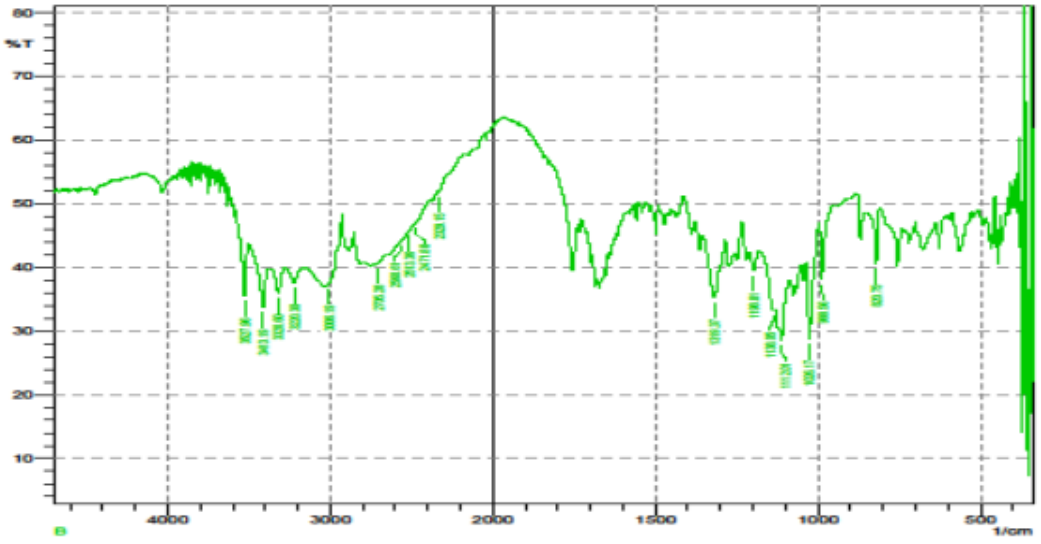

Fig. 15: FTIR spectra of pure Vitamin C.

Table 8: Showing the absorption peaks of Vitamin C.

Sr. No.	Peaks	Functional group
1.	1112.01	C-O Stretch
2.	2513.88	S-H Stretch
3.	2705.28	2 bands of C-H Stretch for aldehydes
4.	3006.3	C-H Stretch for alkenes and aromatics
5.	3220.3	N-H Stretch, C-H Stretch
6.	3320.6	O-H Stretch, C-H Stretch
7.	3527.96	O-H Stretch, N-H Stretch or C-H Stretch for alkynes.

4)

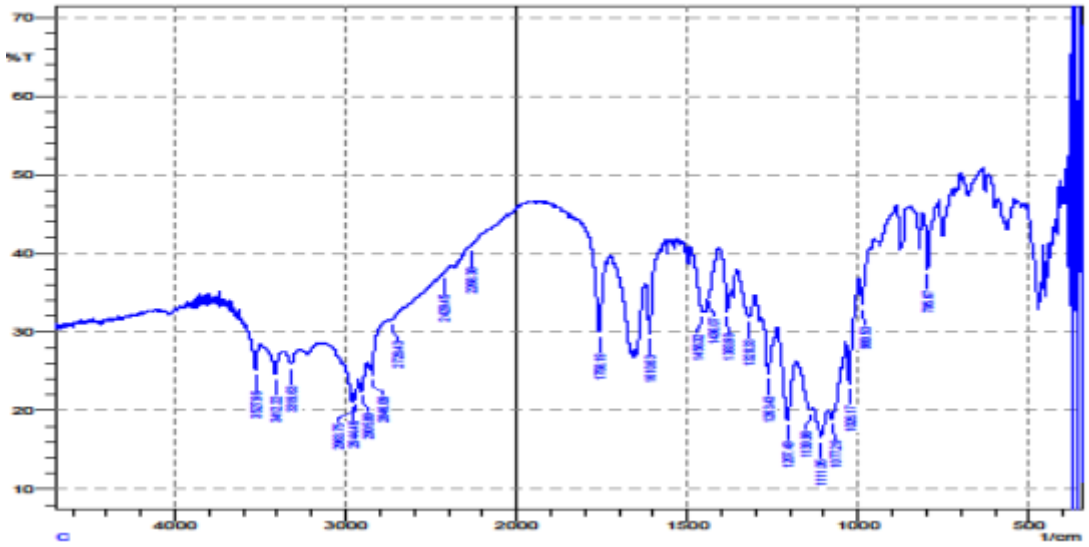

Fig. 16: FTIR spectra of Multivitamins.

Table: 9 showing the absorption peaks of Multivitamins.

Sr. No.	Peaks	Functional groups
1.	1111.05	C-O Stretch
2.	1436.07	C-H bending
3.	1610.63	C-C Multiple bond stretch for aromatic
4.	1758.19	C=O Stretch for vinyl esters
5.	2268.38	C=O Stretch
6.	2728.43	2 bands of C-H Stretch for aldehydes
7.	2944.46	C-H Stretch for alkanes
8.	3319.63	C-H OR N-H
9.	3527.96	O-H Stretch or N-H or C-H Stretch for alkynes

Pseudo ternary phase diagram

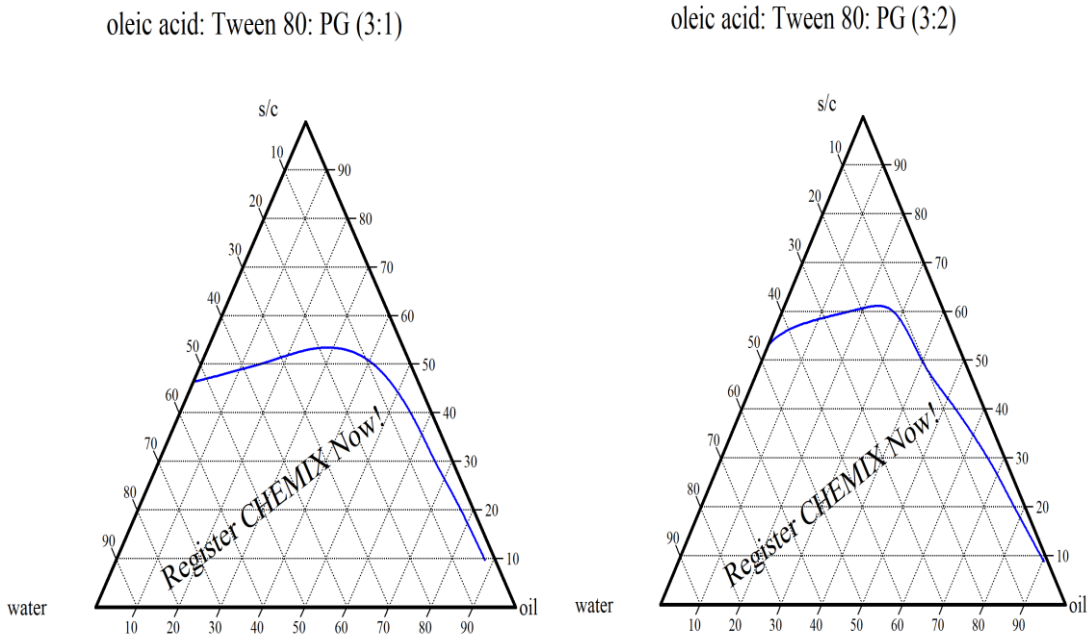

Fig. 17 Pseudo ternary phase diagram. Fig. 18 Pseudo ternary phase diagram.

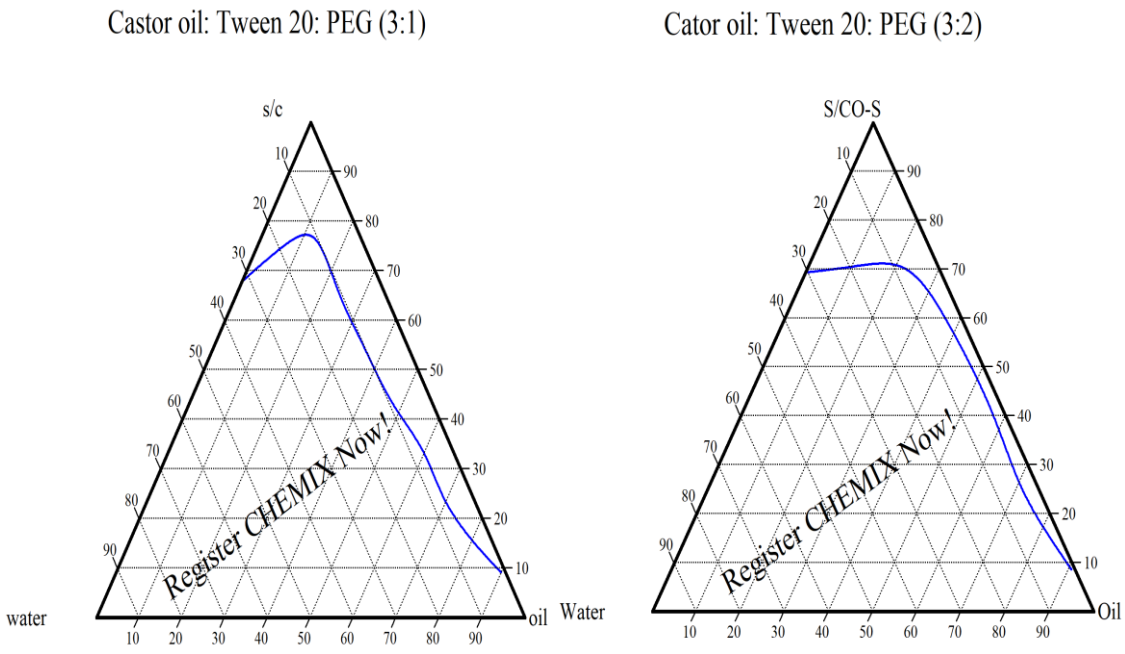

Fig. 19: Pseudo ternary phase diagram. Fig. 20 Pseudo ternary phase diagram.

Visual isotropy studies

Some of all the formulation batches of micro-emulsion were found to be clear, isotropic solution with no sign of precipitation.

Table 10: Results showing physical stability data of all the formulations.

Formulation Code	Visual inspection and microscopy	
	clarity	Phase separation
O-ME 1	Y	N
O-ME 2	Y	N
O-ME 3	Y	N
O-ME 4	Y	N
C-ME 5	N	Y
C-ME 6	Y	N
C-ME 7	Y	N
C-ME 8	N	Y

Viscosity and pH

The viscosity values of all the formulation batches are given in Table. The micro-emulsion samples observed Newtonian behaviour. Viscosity values of the micro-emulsion formulations increased as a result of increase in the concentration of surfactant in the micro-emulsion. It is well developmental that increasing the volume fraction of the dispersed phase in micro-emulsion brings in increases in dynamic viscosity.

The pH of all the formulation batches 1 to 8 was found to be in the range of 6.37 to 6.54 as shown in Table 10.

Table 11: Results showing Viscosity and pH of micro-emulsion.

Formulation Code	Viscosity(1 RPM) (Spindle no. 62)	pH
O-ME 1	2519 ± 0.14	6.37 ± 0.05
O-ME 2	2819 ± 0.22	6.54 ± 0.02
O-ME 3	3599 ± 0.09	6.22 ± 0.02
O-ME 4	3649 ± 0.4	6.40 ± 0.03
C-ME 5	4379 ± 0.21	5.81 ± 0.02
C-ME 6	3119 ± 0.6	6.30 ± 0.05
C-ME 7	4979 ± 0.99	6.00 ± 0.02
C-ME 8	1200 ± 0.12	6.54 ± 0.02

7.1.7: Drug content

Drug content was obtained by suitable dilutions.

1) Second derivative spectra of Vitamin E, CoQ_{10} and sample

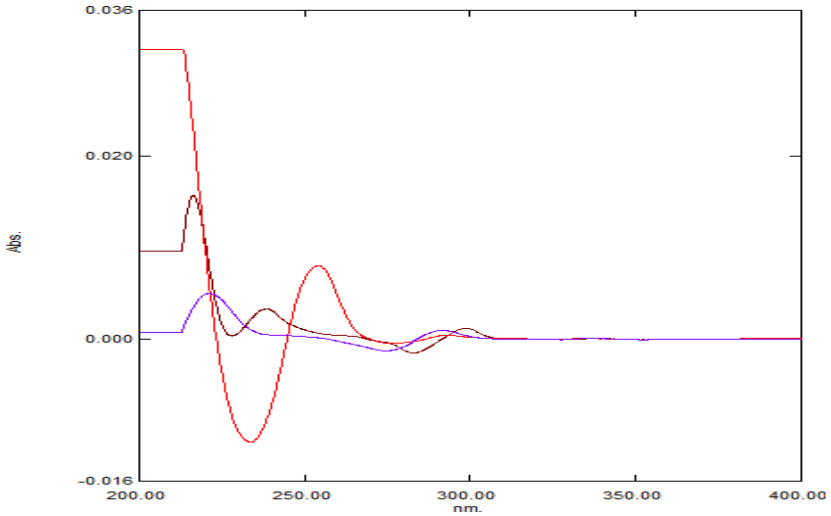

Fig 21: Second derivative spectra of Co-Q10, Vitamin E and sample.

2) Calibration graph of Vitamin E

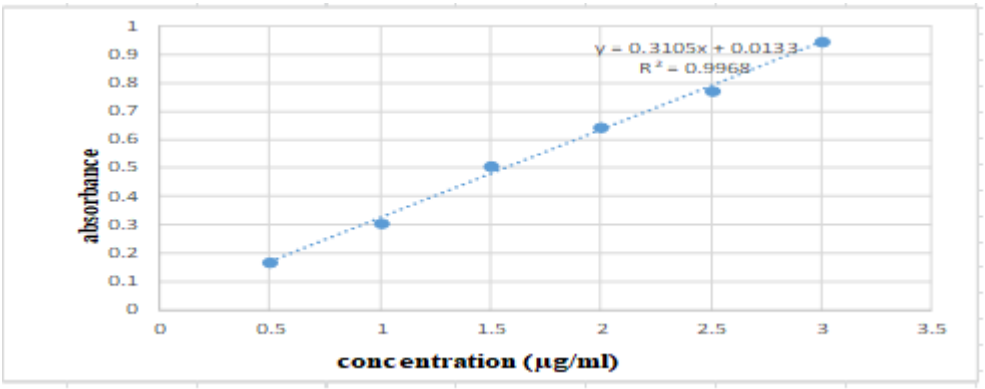

Fig. 22: Second derivative Calibration graph of Vitamin E.

1) Calibration graph of Co-Q_{10}

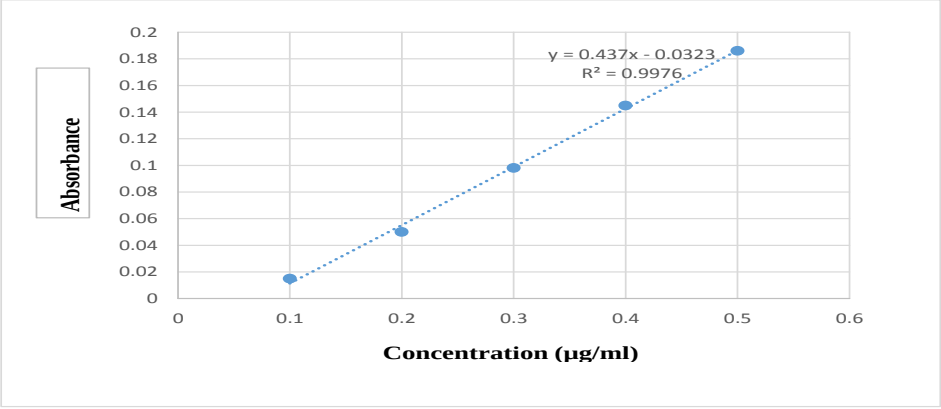

Fig 23: Second derivative Calibration graph of Co-Q10.

2) Calibration graph of Vitamin C

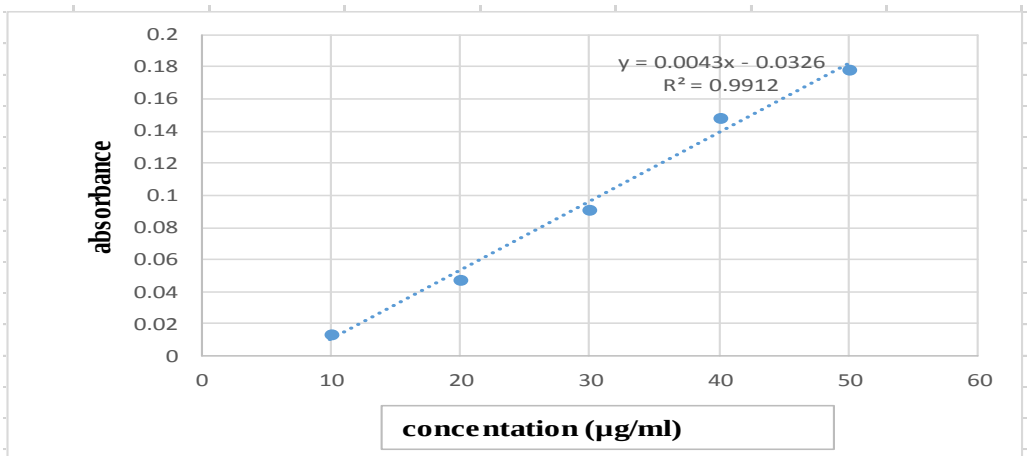

Fig 24: Second derivative Calibration graph of Vitamin C.

Table 12: Results showing the % Drug content.

Formulation Code	% Drug content of Co-Q10	% Drug content of Vitamin E	% Drug content of Vitamin C
O-ME 1	91.56 ± 0.42	83.68 ± 0.36	96.38 ± 0.48
O-ME 2	93.33 ± 0.45	84.52 ± 0.38	95.45 ± 0.46
O-ME 3	97.76 ± 0.44	88.76 ± 0.40	96.38 ± 0.48
O-ME 4	96.87 ± 0.48	87.07 ± 0.39	98.24 ± 0.49
C-ME 6	95.99 ± 0.46	86.22 ± 0.37	98.24 ± 0.49
C-ME 7	95.10 ± 0.46	87.91 ± 0.39	99.17 ± 0.50

Turbid metric evaluation

Formulation O-ME 1, O-ME 2, O-ME 3, O-ME 4, C-ME 6, C-ME 7 was subjected to dilution test with 0.1 N HCL and distilled water. It was observed that all formulations was stable and did not show any sign of phase separation.

1) -ve Sign indicate no phase separation 2) +ve Sign indicate phase separation

Table 13: Results of dilution test in different media.

Formulation Code	Phase separation	
	0.1 N HCL	Distilled water
O-ME 1	-	-
O-ME 2	-	-
O-ME 3	-	-
O-ME 4	-	-
C-ME 6	-	-
C-ME 7	-	-

In vitro release study

Table 14: In vitro drug release profile of formulations O-ME 3 and O-ME 4.

Time	O-ME 3			O-ME 4		
	Coenzyme Q 10	Vitamin E	Vitamin C	Coenzyme Q 10	Vitamin E	Vitamin C
30	19.60±0.13	18.60±0.13	17.90±0.11	26.23±0.40	27.83±0.70	29.13±0.55
1	26.32±0.26	27.30±0.27	29.80±0.29	38.54±0.13	37.55±0.14	39.56±0.16
2	37.97±0.13	36.86±0.13	39.40±0.14	48.10±0.26	49.14±0.27	50.12±0.29
3	49.33±0.40	47.89±0.40	50.55±0.50	60.32±0.13	62.33±0.14	62.33±0.14
4	66.28±0.53	68.30±0.56	69.31±0.54	70.07±0.53	73.08±0.56	75.18±0.57
5	78.88±0.13	77.55±0.14	79.90±0.19	80.68±0.26	82.18±0.26	84.11±0.24
6	89.77±0.26	87.80±0.27	89.90±0.29	88.73±0.13	89.13±0.19	89.99±0.25
7	97.25±0.13	96.40±0.14	97.99±0.19	93.56±0.26	95.80±0.27	97.55±0.25
8	98.67±0.53	98.96±0.55	98.86±0.52	94.88±0.80	97.13±0.90	96.18±0.96

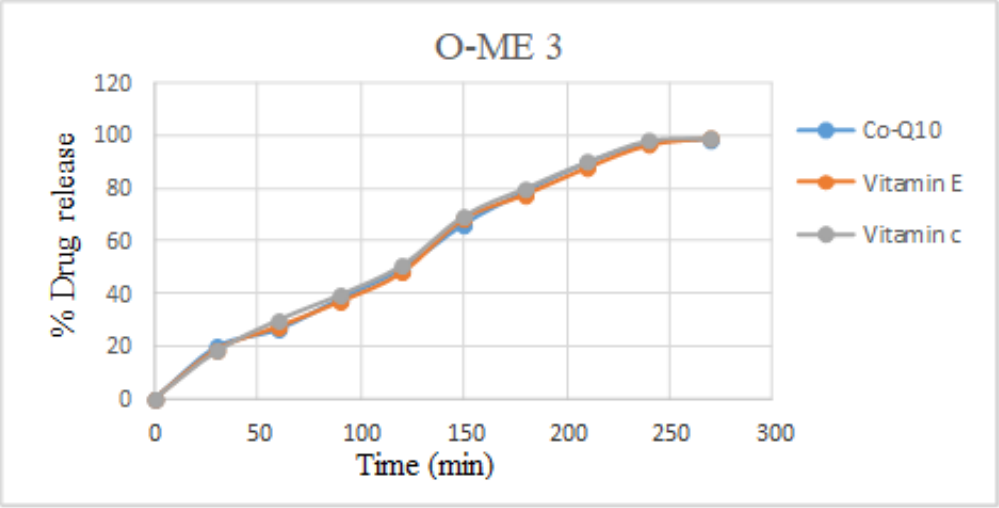

Fig. 25: Drug release graph of O-ME3.

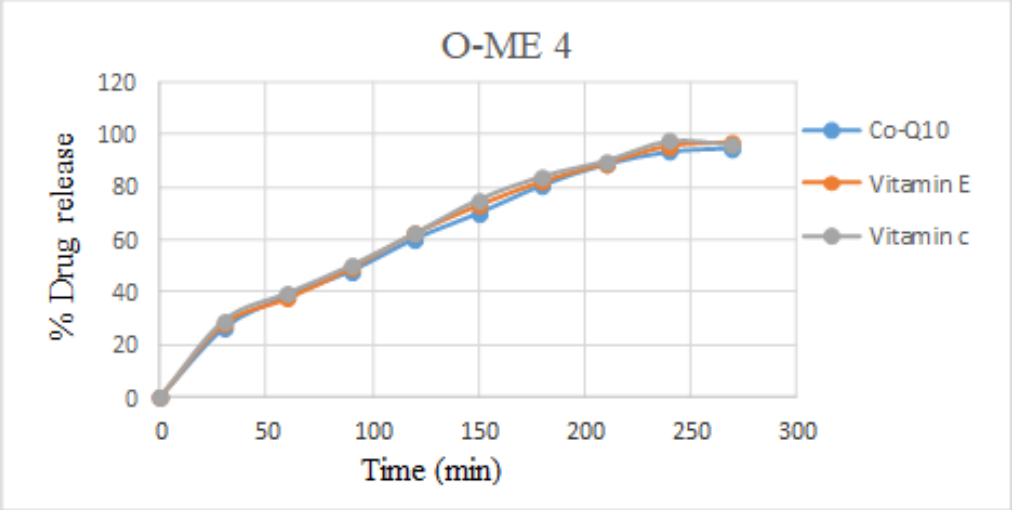

Fig. 26: Drug release graph of O-ME4.

Table 15: In vitro drug release profile of formulations C-ME 6 and C-ME 7.

Time	C-ME 6			C-ME 7		
	Coenzyme Q 10	Vitamin E	Vitamin C	Coenzyme Q 10	Vitamin E	Vitamin C
30	32.29±0.13	34.19±0.13	36.21±0.18	29.16±0.26	30.17±0.27	31.88±0.80
1	45.54±0.66	46.50±0.67	47.60±0.69	40.62±0.13	41.70±0.14	43.70±0.17
2	57.67±0.40	58.55±0.40	56.45±0.45	50.09±0.40	52.18±0.50	52.88±0.58
3	65.53±0.53	67.50±0.55	63.50±0.51	56.81±0.53	58.72±0.58	59.70±0.55
4	72.63±0.13	73.53±0.13	76.45±0.15	64.20±0.26	66.20±0.29	67.99±0.20
5	79.16±0.26	81.11±0.11	79.96±0.16	71.68±0.13	73.64±0.15	73.99±0.17
6	83.42±0.13	84.32±0.12	86.14±0.10	80.30±0.26	81.20±0.28	83.20±0.30
7	88.73±0.66	86.42±0.55	85.12±0.53	90.81±0.13	92.80±0.14	93.99±0.79
8	89.10±0.13	89.95±0.13	89.99±0.19	91.85±0.53	94.13±0.55	95.99±0.56

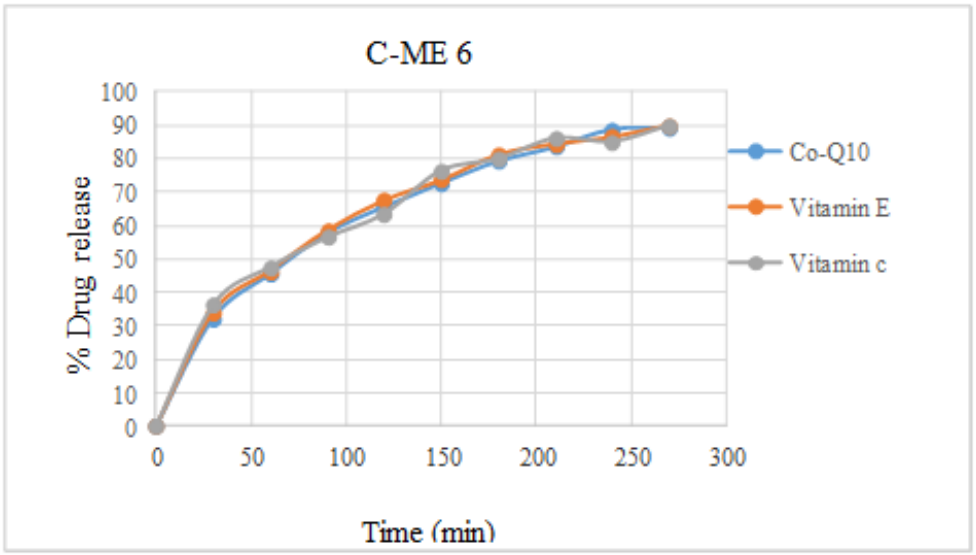

Fig. 27: Drug release graph of C-ME6.

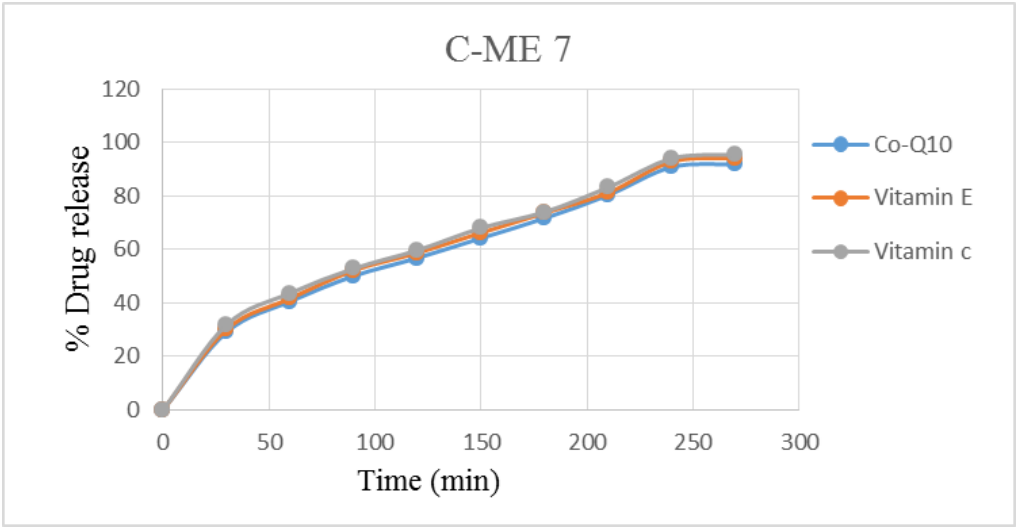

Fig. 28: Drug release graph of C-ME7.

Motic microscopy

Rough estimation of the optimized formulations O-ME 3, and C-ME 7 were taken and observed under the motic microscope. The images seen after measurement indicates that size of the particles was found to be micron range.

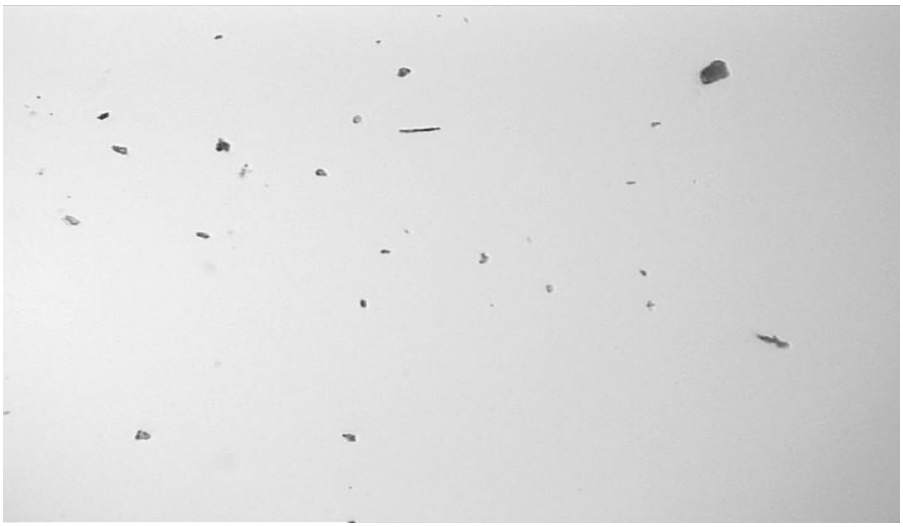

Fig. 29: Motic image of optimized formulation O- ME 3.

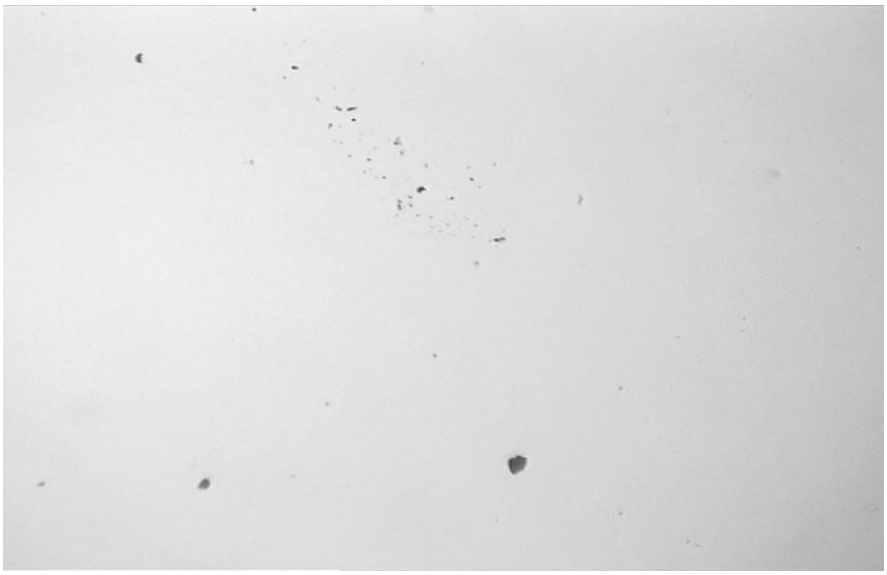

Fig. 30: Motic image of optimized formulation C- ME 7.

Droplet size/ Distribution and Zeta potential

Table 16: Particle size, Polydispersity index and Zeta potential of O-ME 3 and C-ME 7. Formulation.

Formulation code	Particle size	Polydispersity index	Zeta potential
O-ME 3	1934.7 nm	0.894	-0.07mv
C-ME 7	3277.5 nm	0.896	-0.076mv

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SZ-100

Measurement Results

Measurement Results

Date	: Tuesday, May 08, 2018 1:27:59 PM
Measurement Type	: Zeta Potential
Sample Name	: Sample A
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.894 mPa·s
Conductivity	: 0.178 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-0.7 mV	-0.000005 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean)	: -0.7 mV
Electrophoretic Mobility Mean	: -0.000005 cm²/Vs

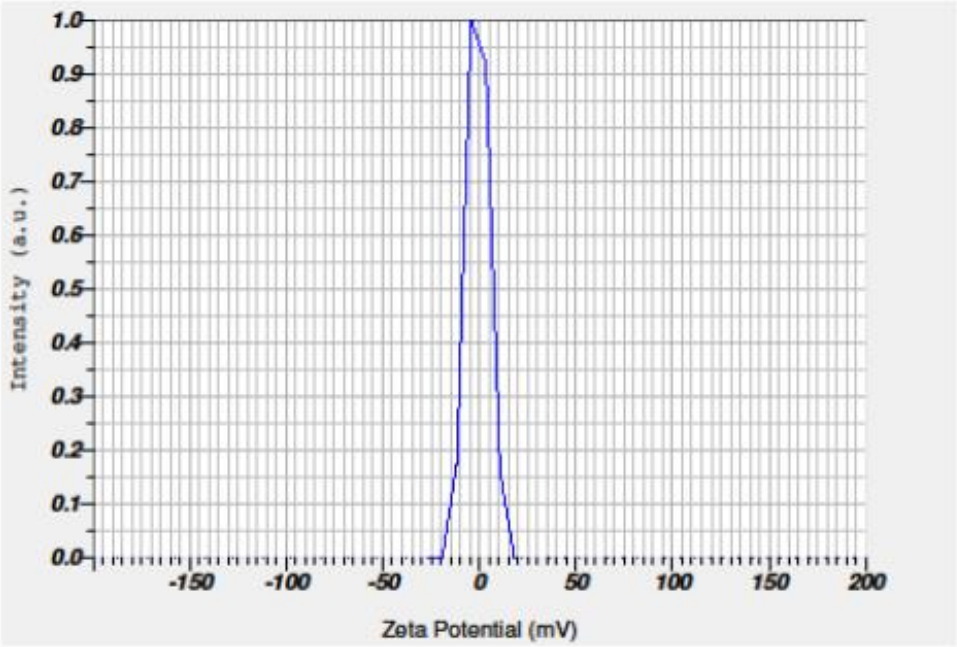

Fig. 31: Zeta potential of formulation O-ME 3.

HORIBA Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.20

SZ-100

Measurement Results

Date	: Tuesday, May 08, 2018 10:49:16
Measurement Type	: Particle Size
Sample Name	: Sample B
Scattering Angle	: 90
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.894 mPa·s
Transmission Intensity before Meas.	: 18334
Distribution Form	: Standard
Distribution Form(Dispersity)	: Monodisperse
Representation of Result	: Scattering Light Intensity
Count Rate	: 59 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.64	1.4 nm	0.2 nm	1.4 nm
2	0.36	6883.6 nm	352.3 nm	6944.4 nm
3	--	-- nm	-- nm	-- nm
Total	1.00	2480.6 nm	3310.7 nm	6944.4 nm

Cumulant Operations

Z-Average	: 1934.7 nm
PI	: 5.323

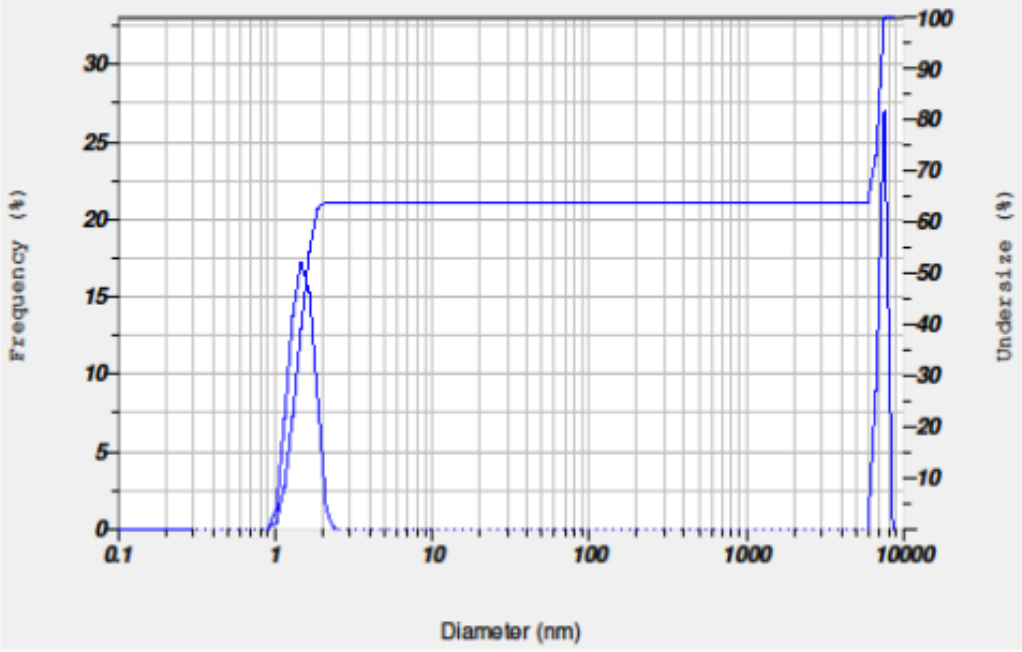

Fig. 32: Particle size of formulation O-ME 3.

HORIBA Scientific

HORIBA SZ-100 for Windows [Z Type] Ver2.2

SZ-100

Measurement Results

Measurement Results

Date	: Tuesday, May 08, 2018 1:16:02 PM
Measurement Type	: Zeta Potential
Sample Name	: Sample C
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.896 mPa·s
Conductivity	: 0.204 mS/cm
Electrode Voltage	: 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-0.7 mV	-0.000005 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean) : -0.7 mV

Electrophoretic Mobility Mean : -0.000005 cm²/Vs

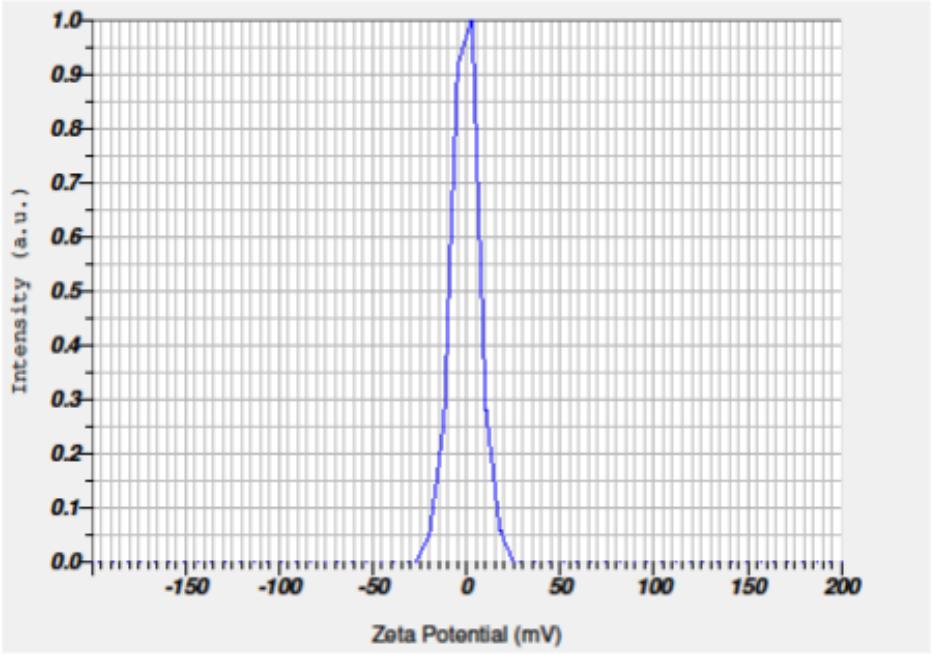

Fig. 33: Zeta potential of formulation C-ME 7.

HORIBA

HORIBA SZ-100 for Windows [Z Type] Ver2.20

SZ-100

Measurement Results

Date	: Tuesday, May 08, 2018 10:57:05
Measurement Type	: Particle Size
Sample Name	: Sample D
Scattering Angle	: 173
Temperature of the Holder	: 25.0 °C
Dispersion Medium Viscosity	: 0.894 mPa·s
Transmission Intensity before Meas.	: 5116
Distribution Form	: Standard
Distribution Form(Dispersity)	: Monodisperse
Representation of Result	: Scattering Light Intensity
Count Rate	: 1700 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	3034.5 nm	809.1 nm	2680.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	3034.5 nm	809.1 nm	2680.1 nm

Cumulant Operations

Z-Average	: 3277.5 nm
PI	: 1.072

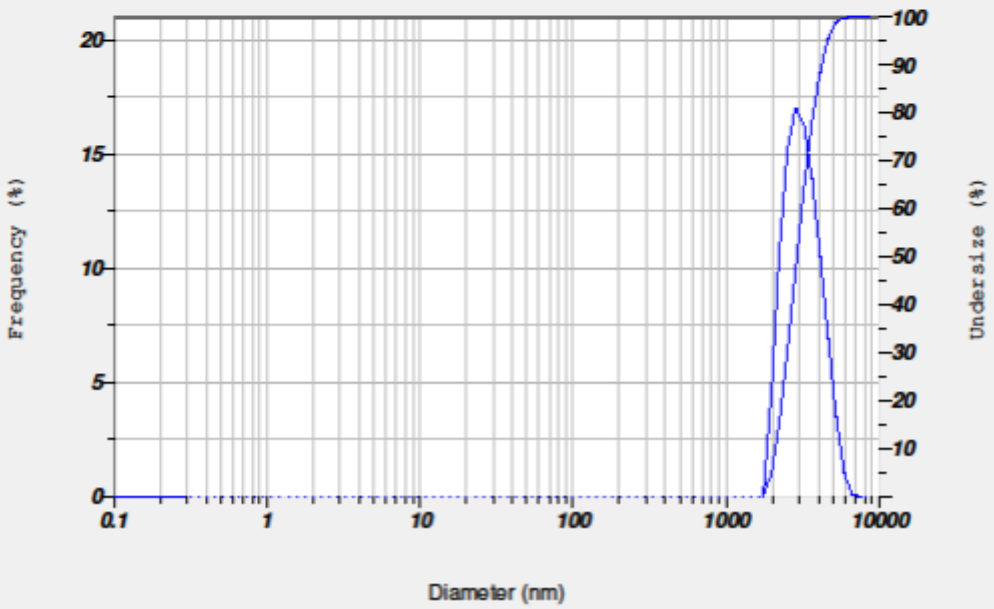

Fig. 34: Particle size of formulation C-ME 7.

7.1.12 Stability studies

Stability study was carried out as per ICH guideline. Formulation O-ME 1, O-ME 2, O-ME 3, O-ME 4, C-ME 6, C-ME 7 did not show any physical changes during the study period. This indicates that the prepared Micro-emulsion are stable at accelerated storage conditions. No drastic changes in colour and appearance were found.

Table 17: Results showing stability of selected formulations.

Formulation Code	Time in month	Appearance	Heating Cooling cycle	Centrifugation	Freeze thaw cycle
O-ME 1	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y
O-ME 2	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y
O-ME 3	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y
O-ME 4	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y
C-ME 6	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y
C-ME 7	0	Clear	Y	Y	Y
	1	Clear	Y	Y	Y
	2	Clear	Y	Y	Y
	3	Clear	Y	Y	Y

DISCUSSION

When we see the composition of the clear micro-emulsion formed, it is quite observable that the portion of oil is very less and they can be formed as o/w emulsions. Where polar head groups of surfactants are facing into droplets of water with fatty acid tails facing into oil phase.

In this research work aim was to formulate multivitamin micro-emulsion for the treatment of cardiovascular disease. We claim that as low HLB or high HLB surfactants were used it can be considered as o/w micro-emulsion as water phase continuous medium will be taken from GIT. But basically we have formulated o/w micro-emulsion and Tween 80/ Tween 20/ was added which favours continuous phase as oil.

Span 20 used in the formulation favours o/w micro-emulsion as oil required is very low. Co-surfactant take care of reduction of interfacial tension and helps in size reduction too. Tween having high HLB is also included so that this original micro-emulsion o/w is dispersed uniformly in oil phase from intestine.

The stability of such micro-emulsion will be logically more as the emulsion will not destabilized by aqueous biological system. As the biological system will not increase the phase volume of internal phase eventually phase separation or phase inversion will not occur.

SUMMARY AND CONCLUSION

Micro-emulsion can be formed by numerous oil, surfactant, co-surfactant and aqueous constituents. The main advantage of alcohol free micro-emulsion is that they are thermodynamically stable, have ease in formulation, and have relatively low cost of formulation preparation. Solubility is a key parameter for the oral bioavailability of fat soluble vitamins and water soluble vitamin. Diffusion of drug is the rate determining steps for oral absorption of these drugs, which can sufficiently affect their in-vitro absorption. Co-enzyme Q10 and Vitamin E are the drug with poor water solubility and high permeability and Vitamin C is water soluble with variable bioavailability. Therefore, many strategies have been worked out to improve its aqueous solubility as well as release rate from various dosage forms.

In the present study attempts were made for the improvement of oral bioavailability of co-Q10, vitamin E and vitamin C by enhancing its solubility and dissolution rate. Bioavailability enhancement was attempted to achieve by preparing micro-emulsion using oils, surfactants and co-surfactants in which vitamins has better solubility and are from the generally regarded as safe category.

Micro-emulsion systems were successfully prepared using oil phases such as oleic acid, castor oil. Tween 80, Tween 20 and span 20 were selected as a surfactant for all micro-emulsion systems. Propylene glycol, polyethylene glycol 400 were selected co-surfactant. The micro-emulsion formulation (O-ME2): OLEIC ACID: TWEEN 80: PG were found to have high thermodynamically stability, good phase clarity, small globule size (1934.7 nm, 3277.5 nm), zeta potential (-0.07 mv, -0.76 mv), shows that formulation is optically transparent.

Hence O-ME 3, C-ME 7 formulation is considered to be most satisfactory micro-emulsion. All the components in the system were safe. Vitamin and Multivitamins compatibility study was carried out using FTIR.

CONCLUSIONS

It may therefore be concluded that the micro-emulsion of co-Q10, Vitamin E, Vitamin C Offer an approved oral bioavailability and enhance drug solubility, preservation ,good thermodynamically stability and ease of manufacturing of lipophilic components without use of alcohol.

Thus micro-emulsion system can be used for preparing products with superior features such as having improved product bioavailability, stability or efficacy.

Therefore the present technique of improving solubility and bioavailability of fat soluble vitamins and water soluble vitamin has a huge commercial potential.

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