Preparation And In-Vitro Evaluation Of Stable Liquid Formulation Of Dehydro Ascorbic Acid For Oral Drug Delivery System

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Abstract
Deep eutectic solvents (DESs) and other eutectic solutions have found extensive use in a variety of fields, in the interest of academics, researchers, engineers, medical professionals, and pharmacists. Eutectic works as a synthesis pathway for drug carriers and enhances drug penetration and dissolution. Due to its special qualities, which include chemical and thermal stability, DESs have been thoroughly investigated as possible drug delivery systems to date. DESs, a novel class of eutectic mixtures, are presently receiving a lot of scientific and technological attention as less expensive substitutes for ionic liquids (ILs) and organic solvents. The current study's objectives were to determine photostability, enhance bioavailability, and investigate the feasibility of deep eutectic solvents as a viable option for creating a stable formulation of dehydroascorbic acid.

Keywords: In-Vitro, Liquid Formulation, Dehydro Ascorbic Acid, Oral Drug Delivery System

Introduction

Vitamin C, or dehydroascorbic acid, may be an innate vitamin. Strong reducing and inhibiting, antioxidants protect against microbial infections, aid in detoxification processes, and help create sclera protein in connective tissue, fibrous tissue, teeth, bones, skin, and capillaries. Vitamin C, which is present in citrus and other fruits as well as vegetables, cannot be produced or stored by humans and must be consumed through food. An antioxidant is a material that inhibits or delays the oxygen-induced degradation of another material. Artificial and natural antioxidants are wont to slow the deterioration of hydrocarbon and rubber, and such antioxidants as water-soluble vitamin (Dehydroascorbic Acid), butylated hydroxyl tolune (BHT), and butylated hydroxyl anisole (BHA) are additional to foods to stop them from turning into rancid or type discoloring. Within the body, nutrients comparable to beta-carotene, a vitamin A precursor, vitamin C. vitamin E, and atomic number 34 are found to act as antioxidants. Oral medication is that the most typical sort of drug administration as a result of blessings equivalent to convenience of drug administration via the oral route, patient preference, cost effectiveness, and simple large-scale producing of oral indefinite quantity forms. Furthermore, orally administered drugs is targeted to explicit regions inside the (GI) tract for localized treatment of pathological conditions equivalent to abdomen and large intestine cancers, infections, inflammations, gut diseases, gastro- duodenal ulcers, and esophageal reflux disorders.[1,2]

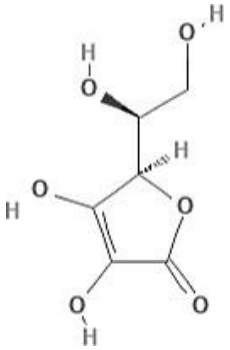

Figure 1: Chemical Structure of Dehydroascorbic Acid

Objective of the Study

To formulate immediate release of the drug that improves photo degradation of drug and inhibit the oxidative degradation of the drug

MATERIALS AND METHODS

Dehydro ascorbic Acid was arranged from Marksan Pharmaceutical Ltd., Goa, all excipients were of analytical grade list of instruments and chemicals listed in below table 1 & 2.

Materials and Equipment

Table 1: List of Instruments

S. No.	Instruments	Manufacturer
01.	UV spectrophotometric	Shimadzu, Japan
02.	Weighing balance,(CY220)	Shimadzu, Japan
03.	Rotary Evaporator	Popular India, Mumbai
04.	Ultra bath Sonicator	PCI, Mumbai
05.	Dissolution apparatus	Lab india Ltd. Mumbai, India
06.	Magnetic Stirrer	Remi Equipments, Mumbai
07.	Microscope	Biolux-CTX(2), Kyowa
08.	pH Meter	Ohaus,USA
09.	Melting Point Apparatus	Remi Equipment, Mumbai
10.	Infra red spectrophotometer (FTIR)	PerkinElmer, Germany
11.	Cooling Centrifuge	Remi Equipments, Mumbai

Table 2: List of Chemicals

S. No	Materials	Source
01.	Dehydroascorbic Acid	Marksan Pharmaceutical Ltd., Goa
02.	Choline Chloride	Spectrum Chemical Mfg Corp
03.	Citric Acid	Central Drug House (P)Ltd.
04.	Oxalic Acid	Central Drug House (P)Ltd.
05.	Urea	Fischer Scientific India Pvt. Ltd.
06.	Gallic Acid	Loba Chemie, Mumbai
07.	Chloroform	Avarice Laboratories Pvt. Ltd., U.P
08.	Potassium Dihydrogen orthophosphate	Fisher Scientific India Pvt. Ltd.
09.	Disodium hydrogen orthophosphate	Thomas Baker
10.	Sodium Chloride	Thomas Baker

Pre-formulation studies

Organoleptic Characteristics

The organoleptic studies like general appearance like nature, color, odour etc. were performed by visual observations.[3]

Melting point

Melting point apparatus is employed for the determination of melting point of the drug. [4]

Determination of Absorption Maxima of Dehydroascorbic Acid

Absorption maxima (λmax) of drug were determined by UV Spectrophotometer (Shimadzu Pharma.Spec1800). Stock solution of 1000 µg/ml was prepared by dissolving10mg Dehydroascorbic Acid in distilled Water in 10 ml volumetric flask and the volume was made up to mark with distilled water. The dilutions were scannedfrom400–200 nm with UV spectrophotometer.[5]

Preparation of UV Calibration Curve in Distilled Water

Then, the aliquots of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml were taken in 10 ml volumetric flask and the volume was made up to mark with distilled water to get the concentrations of 50, 100, 150, 200, 250 and 300µg/ml. The dilutions were determined for absorbance at λmax with UV spectrometer.[6]

Solubility Studies

For solubility study, excess amount of drug was taken in thoroughly cleaned test tubes containing 1 ml of various solvents (Methanol, Ethanol, Acetone, Chloroform, 0.1N HCl, water, PBS pH 6.8 and 7.4) and test tubes were tightly closed. These test tubes were shaked on water bath shaker for twenty-four hrs at 25°C temperature. After 24 hrs, each sample was centrifuged 15,000 rpm and supernatant was withdrawal then supernatant was filtered and filtrates were suitably diluted and determined spectrophotometrically.[7]

Partition Coefficient of Drug

Shake flask method

The partition coefficient determination study was performed by using shake flask method. Excess amounts of the drug (Dehydroascorbic Acid) dissolved in 10 ml of two solvents (n- octanol: Water) together (1:1) and placed for twenty-four h. After 24 hrs, the 2 layers were separated and centrifuge for 15 min at 15,000 rpm. The AOC was taken in HPLC at the respective λmax after appropriate dilution.[8]

FTIR of Dehydroascorbic Acid and Excipients

The KBr disc was prepared using 1 mg of Dehydroascorbic Acid/ excipients plus drug in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and Dehydroascorbic Acid was mixed and subjected to hydraulic pressure to make disc. This disc was placed in FT-IR chamber spectrum was recorded within the 4000 - 400 cm-1 region.[9]

Drug-excipients Compatibility Study by FTIR

The compatibility of drug with excipients was ascertained by FT-IR. FTIR was used as tool to detect any physical and chemical interaction between drug and excipients. Drug and various excipients were mixed thoroughly in ratio of 1:1. Samples were scanned by FTIR under the range of 400-4000 cm-1. The spectra of pure drug and drug with excipients were compared to see any incompatibility and physical changes.

Preparation of Deep Eutectic Solvent Mixture of Dehydroascorbic Acid

To prepare a Deep Eutectic Solvent Mixture (DESM), choline chloride and selected carboxylic acids were mixed at different molar ratios. The mixtures were sealed in vials, and heated in an oven at 75℃ until homogenous solutions were formed. Subsequently, these samples were stored at room temperature and only those samples that remained liquid were tested as room-temperature solvents for model poorly soluble drugs. [10,11]

Table 3: Screening of Carboxylic Acid for Deep Eutectic Solvent Mixture of Dehydroascorbic Acid

Formulation Code	Carboxylic Acid Used	Molar Ratio	Ascorbic Acid	Choline Chloride	Carboxylic Acid
F1	Citric Acid	1:1	40mg	69.81 mg	96.062 mg
F2		1:10	40mg	69.81 mg	960.62 mg
F3	OxalicAcid	1:1	40mg	69.81 mg	45.014 mg
F4		1:10	40mg	69.81 mg	450.14 mg
F5	GallicAcid	1:1	40mg	69.81 mg	85.06 mg
F6		1:10	40mg	69.81 mg	850.6 mg
F7	Urea	1:1	40mg	69.81 mg	30.03 mg
F8		1:10	40mg	69.81 mg	300.3 mg
F9	Menthol	1:1	40mg	69.81 mg	78.134 mg
F10		1:10	40mg	69.81 mg	781.34 mg

Table 4: Screening of Solvent Mixture for Deep Eutectic Solvent Mixture of Dehydro ascorbic Acid with Urea

Formulation Code	Solvent	Molar Ratio	Drug (mg)	Choline Chloride	Urea
F11	Glycerol:water	01:01	40	69mg	30mg
F12	Glycerol:water	01:10	40	69mg	300mg
F13	Glycerol:water	01:20	40	69mg	600mg
F14	Propyleneglycol:Water	01:01	40	69mg	30mg
F15	Propyleneglycol:Water	01:20	40	69mg	600mg
F16	Propyleneglycol:Water	01:10	40	69mg	300mg

Table 5: Composition of different Deep Eutectic Solvent Mixture of Dehydroascorbic Acid with Urea at different Temperature Conditions

Formulation Code	Solvent	Molar Ratio	Drug (mg)	Choline Chloride	Urea	Temperature Conditions
F17	Glycerol:water	1:20	40	69mg	600mg	4℃
F18	Glycerol:water	1:05	40	69mg	150mg	25℃

F19	Glycerol:water	1:10	40	69mg	300mg	37°C

Evaluation of Deep Eutectic Solvent Mixture of Dehydroascorbic Acid

Appearance of the Solution

The prepared solution was subjected to evaluation of the physical appearance of the solution and the change being witnessed with time. The change is color was observed for a period of a month. The appearance of the solutions was monitored gradually (day0, day 3, day 7, day 15 and day 30). The appearance was determined and noted down until the color fades or turn yellow.[12]

pH of the Solution

For pH measurements, the freshly prepared solutions were kept at 25±2°C for a period of 30 min. After pH was measured at this temperature, each solution was stored for a period of one month and the pH of the solutions were monitored gradually (day 0, day 3, day 7, day 15 and day 30). The pH was determined using digital pH meter.[13,14]

Drug Content

Once the pH is being measured, the solution containing Dehydroascorbic Acid was estimated for the drug content present in the solution. The drug concentration in the DESM supernatant determined spectrophotometrically on a UV-Vis spectrophotometer and was performed at predestined intervals (day 0, day 3, day 7, day 15 and day 30). The absorbance was measured spectrophotometrically by diluting the aliquots in water.[14,15,16]

In-Vitro Drug Release Study

This study was performed through Dissolution Apparatus USP type I. The in vitro release of Dehydroascorbic Acid from the DESM liquid formulation was examined using USP basket dissolution apparatus (USP Type I). Simulated gastric fluid (0.1N HCl, pH: 1.2, without enzymes)(900 ml) was used as the dissolution medium and maintained at 37 ± 0.5 ℃ at a rotation speed of 100 rpm. An aliquot of 5 ml of the solution was withdrawn at pre- determined time intervals time (1mins, 5 mins, 10 mins, 15 mins, 20 mins, 25 mins and30 mins) and replaced by 5 ml of fresh dissolution medium. Samples were assayed spectrophotometrically at 264 nm. All experiments were performed in triplicate.[17,18]

Drug release kinetic studies

In the present study, raw data obtained from in vitro release studies was analysed, wherein data was fitted to different equations and kinetics model to calculate the percent drug release and release kinetics of from DESM of Dehydroascorbic Acid. The kinetic models used were a Zero-order equation, First-order, Higuchi's model and Korsmeyer-Peppas equation.[19,20,21]

RESULTS AND DISCUSSION

Pre-formulation Studies

The aim of pre-formulation studies is to investigate the physical and chemical properties of a drug substance. The selected drug Dehydroascorbic Acid was subjected for investigation of physical characterization parameters such as:

Organoleptic properties
- UV-visible spectra
- FT-IR spectra
- Melting point
- Solubility
- Partition coefficient[22]

Organoleptic properties

Organoleptic properties of drug Dehydroascorbic Acid found to be as per I.P. monograph. The Organoleptic properties of Dehydroascorbic Acid were found to the given in Table. [6]

Table 6: Organoleptic Properties of Dehydroascorbic Acid

Sr.No.	Properties	Inferences
1.	Colour	White
2.	Odour	Aromatic, sometimes Odourless
3.	Form	Amorphous Powder
4.	Taste	Tasteless

Melting Point

The melting point of a substance is the temperature at which the solid phase gets converted to liquid phase under the one atmosphere of pressure. The melting point determination implies the purity of drug. Melting point of Dehydro ascorbic

Acid was determined by capillary tube method and was found to be quite similar to the reported melting point as shown in Table 7.

Table 7: Melting Point of Dehydroascorbic Acid

Drug	Reference M.P.	Observed M.P.
Dehydro ascorbic Acid	192-197°C	193.67±1.528°C

Determination of absorption maxima by UV spectroscopy

The result of UV spectrum of Dehydro ascorbic Acid is shown in figure.[23]

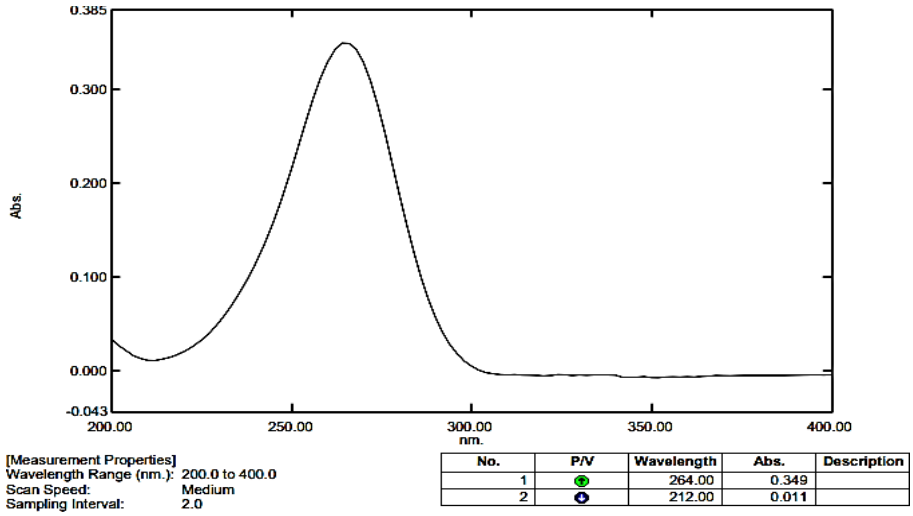

Figure 2: UV spectrum of Dehydroascorbic Acid

Table 8: Absorption maxima (λ_{max}) of Dehydro ascorbic Acid

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
Dehydro ascorbic Acid	264 nm	255 nm

Preparation of calibration curve of Dehydroascorbic Acid in Distilled Water

The standard stock solution of Dehydroascorbic Acid (100µg/ml) was prepared in distilled water. This solution was diluted with distilled water to obtain suitable dilutions (5-30µg/ml) and analysed spectrophotometrically at 264 nm. The results obtained are shown below in Table 9 and graphically shown in Figure. The standard curve of Dehydroascorbic Acid as shown in graph indicated the regression equation y=0. 0.031x-0.0694 and R^2 value is 0.9987, which shows good linearity as shown in Tables, respectively.[24]

Table 9: Calibration curve of Dehydroascorbic Acid in Distilled Water

Sr. No	Concentration(µg/ml)	Absorbance	Statistical data
1.	5	0.220±0.008	
2.	10	0.374±0.005	R^2=0.9987
3.	15	0.537±0.005	
4.	20	0.699±0.002	Regression equation y=0.031x-0.0694
5.	25	0.856±0.004	
6.	30	0.983±0.012	

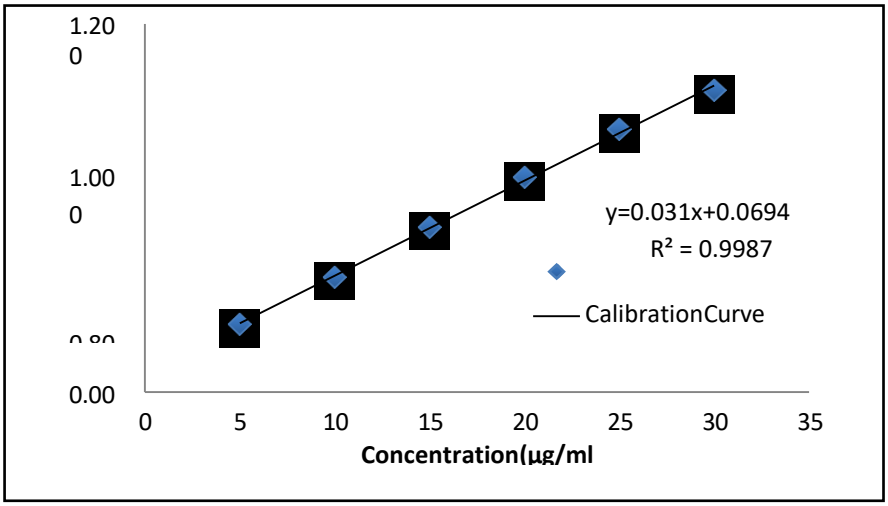

Figure 3: Calibration curve of Dehydroascorbic Acid in Distilled Water

The calibration curve for Dehydroascorbic Acid was obtained by using the 5 to 30 µg/ml concentration of Dehydroascorbic Acid in distilled water. The absorbance was measured at 264 nm. The results obtained are shown above in Table and graphically shown in Figure. The standard curve of Dehydroascorbic Acid as shown in graph indicated the regression equation y=0.031x-0.0694 and R^2 value is 0.9987, which shows good linearity as shown in table, respectively.

Solubility studies

Solubility of drug in various solvents, were carried out in order to screen for the components to be used for formulation development. Analysis of the drug was carried out on UV Spectrophotometer at 282 nm. [25]

Figure 4: Solubility in different solvents

Table 10: Solubility studies of Dehydroascorbic Acid for different solvents

Sr.No	Solvent	Solubility(mg/ml)
01.	Chloroform	0.705±0.003
02.	Methanol	5.194±0.174
03.	0.1NHCl	10.936±0.162
04.	PBSpH6.8	16.547±0.145
05.	Ethanol	19.703±0.145
06.	PBSpH7.4	20.303±0.126
07.	Water	26.789±0.964

Value is expressed as mean± SD; n=3

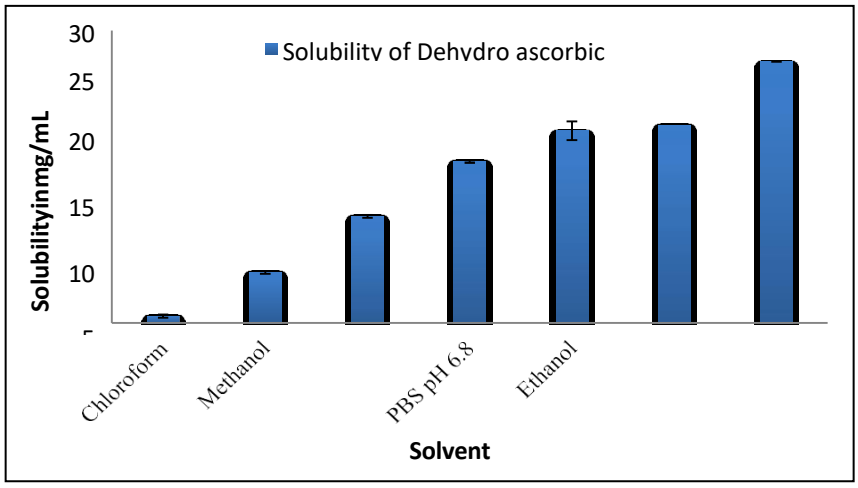

Figure 5: Solubility study of drug in different solvents

Partition coefficient determination

Partition coefficient of the Dehydroascorbic Acid was determined using n-octanol and water. Log P greater than one indicates that the drug is lipophilic in nature, whereas those with partition coefficients less than one are indicative of a hydrophilic drug.

Table 11: Partition coefficient determination of Dehydroascorbic Acid

Partition coefficient of drug	Solvent system	Log P Values	Reference
Dehydroascorbic Acid	n-octanol: water	-1.56±0.050	-1.56

Value is expressed as mean± SD; n=3

The partition coefficient of Dehydroascorbic Acid in n-octanol:water was found to be 1.419±0.007, this indicates that the drug is lipophilic in nature(Table) which is similar to the literature.

FTIR Studies

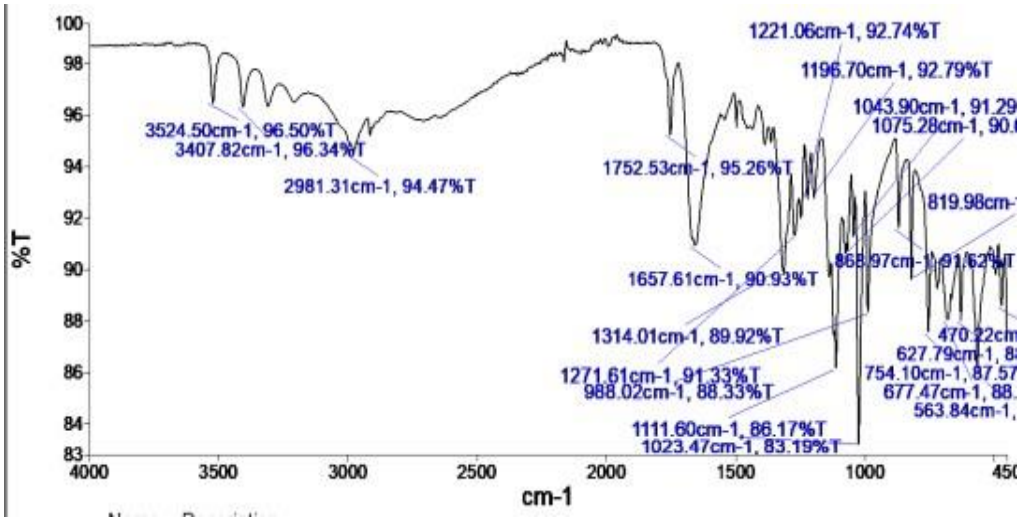

Figure 6: FTIR spectrum of Dehydroascorbic Acid

Table 12: FTIR interpretation of Dehydro ascorbic Acid

Reported(cm^{-1})	Observed(cm^{-1})	Characteristics Peaks
3410	3407.82	OH stretching
1750	1752.53	Stretching vibrations of the C=O of the five -membered lactone ring
1665	1657.61	stretchingvibration ofC=C
1322	1314.01	Characteristi cpeak of enol-hydroxyl
1277	1271.61	C-O-C stretching
1222	1221.06	C-C(=O)-O stretching

1113	1111.60	C-O-C stretching
871	868.97	C-Cring stretching
757	754.10	OHout-of-plane deformation

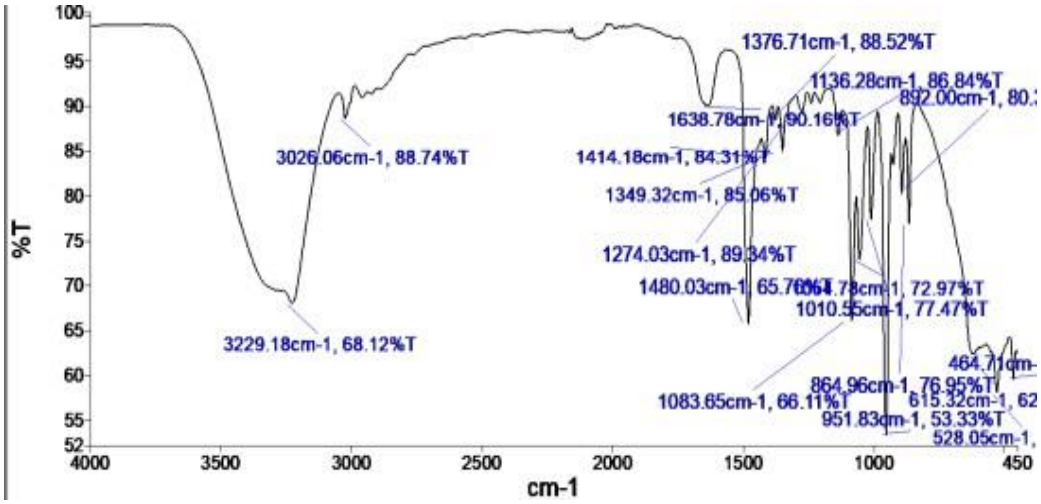

Figure 7: FTIR Spectrum of Choline Chloride

Table 13: Interpretation of FTIR spectrum of Choline Chloride

Reported(cm^{-1})	Observed(cm^{-1})	Characteristics Peaks
3025.82	3026.06	C–H vibration
1634.16	1637.02	Halogenated organic compounds
1441.65	1413.70	O–H vibration
1348.26	1349.05	C=O vibration

The FTIR spectra of Choline Chloride were shown in the Figure and Table. The principal IR absorption peaks of Choline Chloride at 3026.06 cm^{-1} (C–H vibration),1637.02cm^{-1}(halogenatedorganiccompounds),1413.70cm^{-1}(O–Hvibration) and 1349.05 cm^{-1} (C=O vibration) were all observed in the spectra of Choline Chloride were found to be similar to cited peaks. These observed principal peaks confirmed the purity and authenticity of the Choline Chloride.

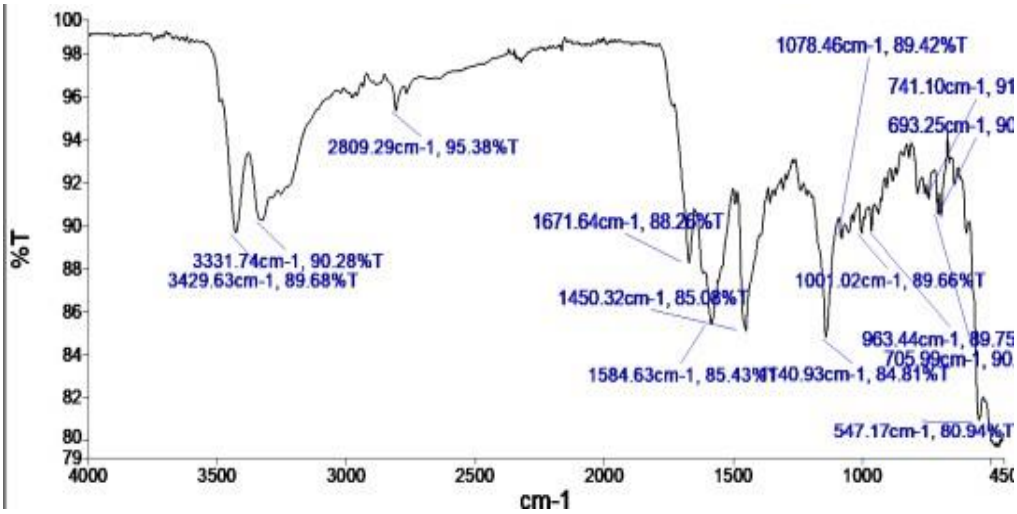

Figure 8: FTIR Spectrum of Urea

Table 14: Interpretation of FTIR spectrum of Urea

Reported(cm^{-1})	Observed(cm^{-1})	Characteristics Peaks
3448	3429.63	N-H stretching
1519	1584.63	NH_2bending
1450	1450.32	–CH_3 bending vibration
1182	1140.93	C-C stretching

The FTIR spectra of Urea were shown in the Figure; Table. The principal IR absorption peaks at 3429.63 cm^{-1} (N-H

stretching), 1584.63 cm^{-1} (NH_2 bending), 1450.32cm^{-1} (–CH_3 bending vibration) and1140.93cm^{-1} (C-C stretching) were all observed in the spectra of Urea were found to be similar to cited peaks. These observed principal peaks confirmed the purity and authenticity of the Urea.

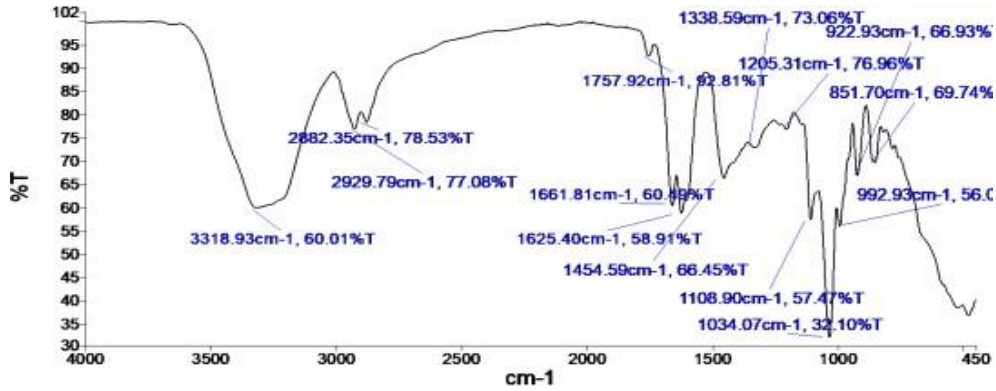

Figure 9: FTIR Spectrum of Physical mixture

Table 15: Interpretation of FTIR spectrum of Physical mixture

Reported(cm^{-1})	Observed(cm^{-1})	Characteristics Peaks
1750	1757.92	ibrations of the C=O of the five- membered lactone ring
1665	1661.81	Stretching vibration ofC=C
1634	1625.40	Halogenated organic compounds
1348	1338.59	C=O vibrations
1450	1454.59	-CH_2bendingvibrations
1182	1108.90	C-C stretching

FTIR of Pure drug and physical mixture studies were carried out to eliminate the possibility of interaction between drug and excipients used with analytical method of drug estimation. All the spectrum peaks revealed that corresponding peaks of drugs are present in the above spectra along with excipients peaks. Hence no interaction was observed in this mixture.[26,27]

Evaluation of Deep Eutectic Solvent Mixture of Dehydro ascorbic Acid[28,29]

a. Appearance of the Solution

The solutions prepared were subjected to pH estimated and was recorded as shown in table.

Table 16: Appearance of DESM of Dehydroascorbic Acid with different Carboxylic Acid

Formulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F1	Transparent	Transparent	Transparent	LightYellow	Yellow
F2	Transparent	Transparent	Transparent	LightYellow	Yellow
F3	Transparent	Transparent	LightYellow	LightYellow	Yellow
F4	Transparent	Transparent	LightYellow	LightYellow	Yellow
F5	Transparent	Transparent	Transparent	Transparent	LightYellow
F6	Transparent	Transparent	Transparent	Transparent	LightYellow
F7	Transparent	Transparent	Transparent	Transparent	LightYellow
F8	Transparent	Transparent	Transparent	Transparent	LightYellow
F9	Transparent	Transparent	Transparent	Transparent	Transparent
F10	Transparent	Transparent	Transparent	Transparent	Transparent

Table 17: Appearance of DESM of Dehydro ascorbic Acid with Urea

Formulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F11	Transparent	Transparent	Transparent	Transparent	Transparent
F12	Transparent	Transparent	Transparent	Transparent	Transparent
F13	Transparent	Transparent	Transparent	Transparent	Transparent
F14	Transparent	Transparent	Transparent	Transparent	LightYellow
F15	Transparent	Transparent	Transparent	Transparent	LightYellow
F16	Transparent	Transparent	Transparent	Transparent	LightYellow

Table 18: Appearance of DESM of Dehydro ascorbic Acid with Urea at different Temperature Conditions

Formulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F17	Transparent	Transparent	Transparent	Transparent	Transparent
F18	Transparent	Transparent	Transparent	Transparent	Transparent
F19	Transparent	Transparent	Transparent	Transparent	Transparent

(a) Refrigerator2ºC

(b) Room Temperature 25ºC

(c) Orbital shaker37ºC

Figure 10: Solution put at different temperature condition (2ºC, 25ºC, 37ºC)

b. pH of the Solution

The solutions prepared were subjected to pH estimated and was recorded as shown in table.

Table 19: pH data of DESM of Dehydroascorbic Acid with different Carboxylic Acid

nulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F1	3.4±0.058	3.4±0.058	3.2±0.100	3.2±0.100	3.2±0.100
F2	3.4±0.058	3.7±0.115	3.2±0.115	3.2±0.115	3.2±0.115
F3	3.4±0.100	3.4±0.100	3.3±0.100	3.3±0.100	3.3±0.100
F4	3.4±0.058	3.4±0.100	3.2±0.100	3.2±0.100	3.2±0.100
F5	3.4±0.100	3.4±0.115	3.1±0.115	3.1±0.115	3.1±0.115
F6	3.4±0.058	3.3±0.058	3.2±0.058	3.2±0.058	3.2±0.058
F7	3.4±0.058	3.4±0.058	3.3±0.058	3.3±0.058	3.3±0.058
F8	3.4±0.100	3.4±0.058	3.3±0.100	3.3±0.100	3.3±0.100
F9	3.4±0.058	3.4±0.058	3.3±0.058	3.3±0.058	3.3±0.058
F10	3.4±0.058	3.4±0.100	3.2±0.100	3.2±0.100	3.2±0.100

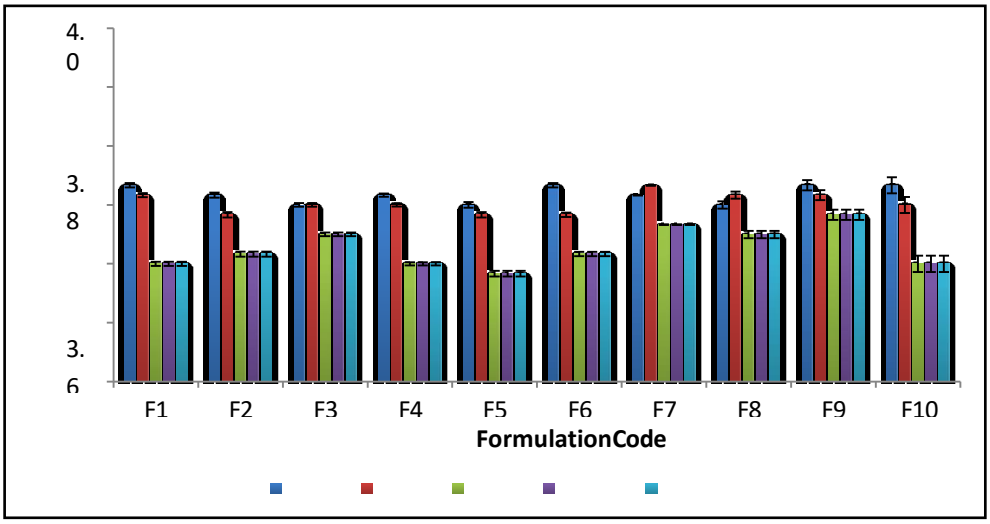

Figure 10: pH data of DESM of Dehydroascorbic Acid with different Carboxylic Acid.

Table 20: pH data of DESM of Dehydroascorbic Acid with Urea

nulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F11	3.4±0.058	3.4±0.058	3.2±0.100	3.2±0.100	3.2±0.100
F12	3.4±0.058	3.4±0.058	3.2±0.100	3.2±0.100	3.2±0.100
F13	3.4±0.058	3.4±0.100	3.3±0.058	3.3±0.058	3.3±0.058
F14	3.4±0.058	3.4±0.100	3.1±0.058	3.1±0.058	3.1±0.058
F15	3.4±0.058	3.4±0.100	3.3±0.058	3.2±0.058	3.2±0.100
F16	3.4±0.058	3.4±0.100	3.3±0.100	3.2±0.058	3.2±0.058

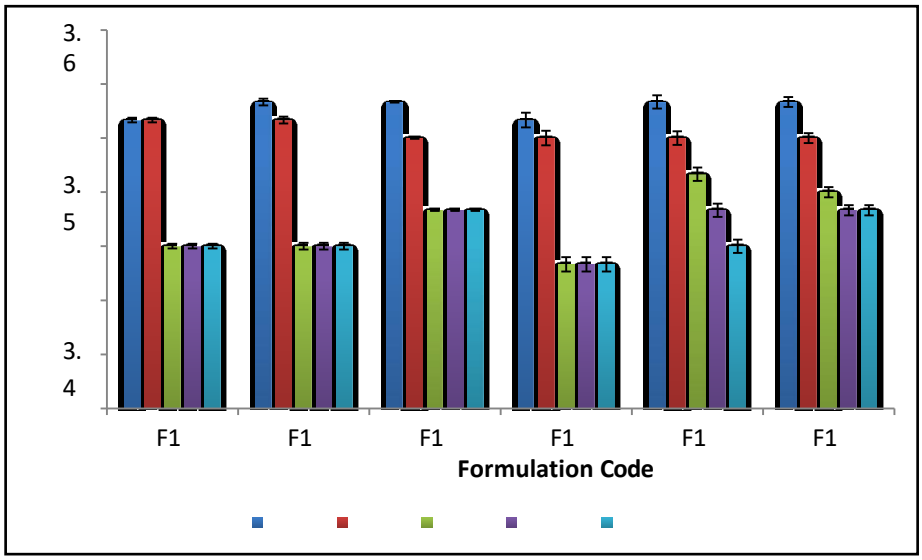

Figure 11: pH data of DESM of Dehydroascorbic Acid with Urea

Table 21: pH data of DESM of Dehydroascorbic Acid with Urea at different Temperature Conditions

nulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F17	3.4±0.058	3.3±0.058	3.3±0.058	4.2±0.100	4.3±0.200
F18	3.4±0.058	3.4±0.058	3.2±0.100	3.3±0.265	3.2±0.100
F19	3.4±0.058	3.4±0.100	3.3±0.100	3.2±0.058	3.2±0.058

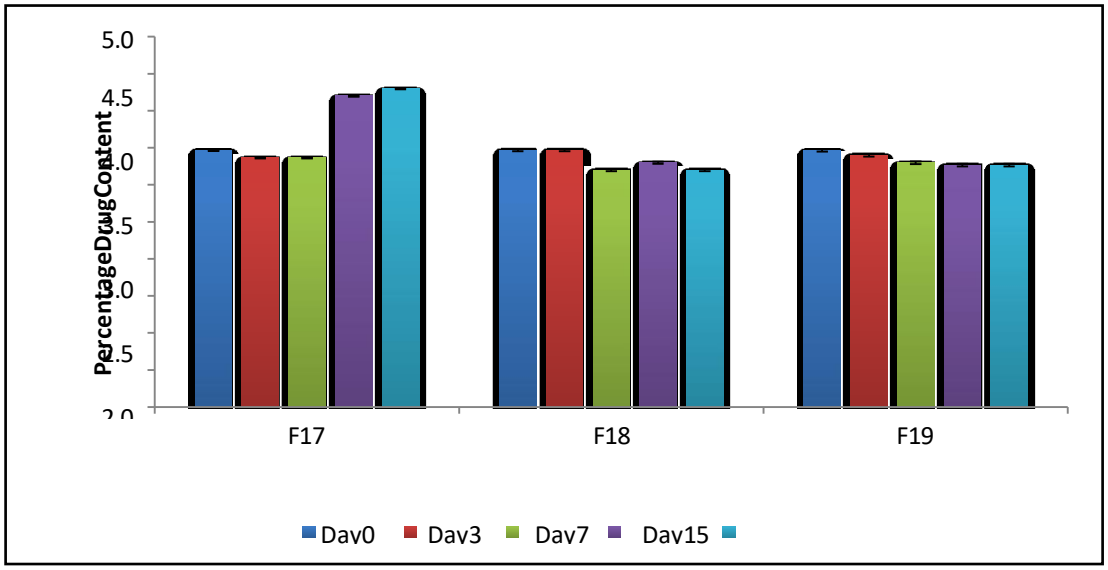

Figure 12: pH data of DESM of Dehydroascorbic Acid with Urea at different Temperature Conditions

Discussion: The pH values of formulations were found to be in the range of 3.1and 3.5respectively as shown in Table. All the solution was observed to be having a comparable pH and was found to have stable pH value.

Drug Content

The drug content of Dehydro ascorbic Acid in the DESM in all the formulation is shown in Table

Table 21: Percentage Drug Content of Dehydroascorbic Acid DESM with different Carboxylic Acid

nulation Code	Day 0	Day 3	Day 7	Day15	Day 30
F1	97.88±0.758	88.46±0.219	75.40±1.375	69.49±0.417	62.40±0.158
F2	96.87±0.437	92.10±0.244	76.62±0.974	70.10±0.175	60.53±0.919
F3	94.85±0.758	92.73±0.152	82.12±0.152	67.10±0.244	59.55±0.303
F4	91.31±1.157	89.65±0.087	85.91±0.152	58.08±0.306	56.94±0.720
F5	98.18±1.403	82.98±1.434	82.20±0.200	51.79±0.500	48.94±0.303
F6	97.32±0.191	80.61±0.152	79.24±0.303	59.97±0.116	49.57±0.374
F7	97.32±0.231	84.47±0.273	73.13±0.231	67.40±0.653	51.72±0.044
F8	99.62±1.261	84.27±0.687	76.24±0.342	70.51±0.116	55.83±0.330
F9	97.95±0.854	97.20±0.076	95.05±0.231	93.66±0.306	90.63±0.342
F10	98.87±0.206	96.59±0.200	94.27±0.158	92.75±0.158	93.13±0.214

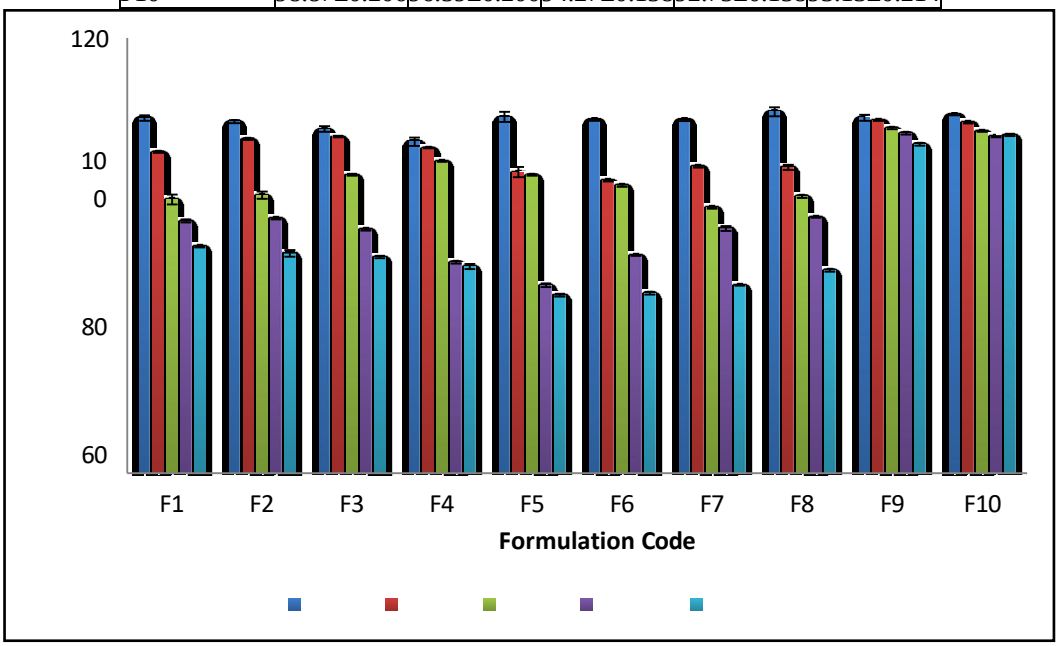

Figure 13: Percentage Drug Content of Dehydroascorbic Acid DESM with different Carboxylic Acid

Table 23: Percentage Drug Content of DESM of Dehydro ascorbic Acid with Urea at different Temperature Conditions

nulation Code	Day 0	Day 3	Day 7	Day 15	Day 30
F17	98.96±0.342	93.56±0.200	92.65±0.076	90.53±0.200	89.92±0.347
F18	99.65±0.087	96.74±0.379	95.73±0.158	94.17±0.076	93.56±0.131
F19	99.49±0.087	95.79±0.163	95.00±0.152	93.69±0.342	89.92±0.422

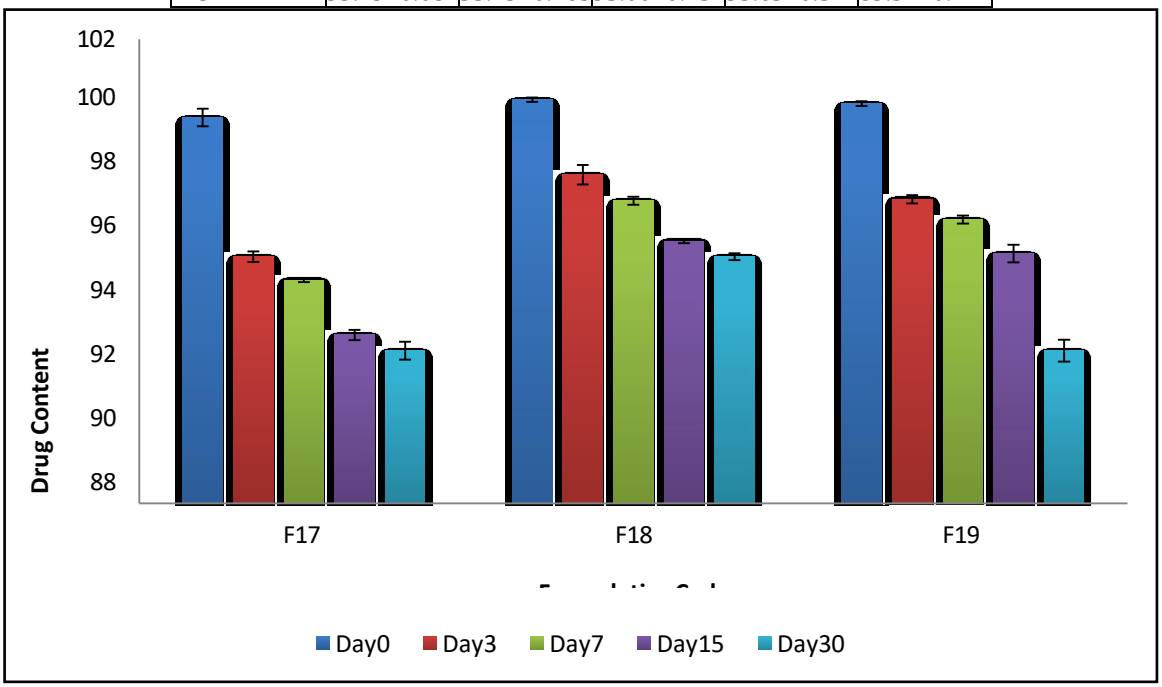

Figure 14: Percentage Drug Content of DESM of Dehydro ascorbic Acid with Urea at different Temperature Conditions

From the table, it was found that maximum percentage drug content of Dehydroascorbic Acid in DESM was found to bein case of urea. The degradation of the drug was found to be minimum when a molar solution of choline chloride and urea was being incorporated in preparation of the stable liquid formulation of Dehydroascorbic Acid.

***In-vitro* release Kinetics**

In-vitro drug release kinetic study data of formulation F18was given below.

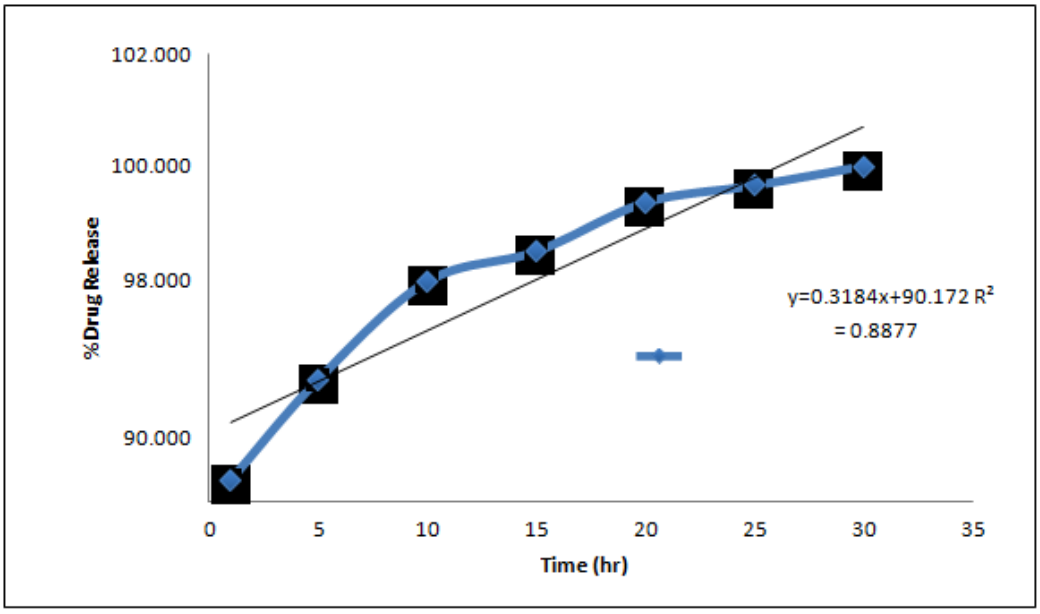

Figure 15: Zero order graph of formulation F18

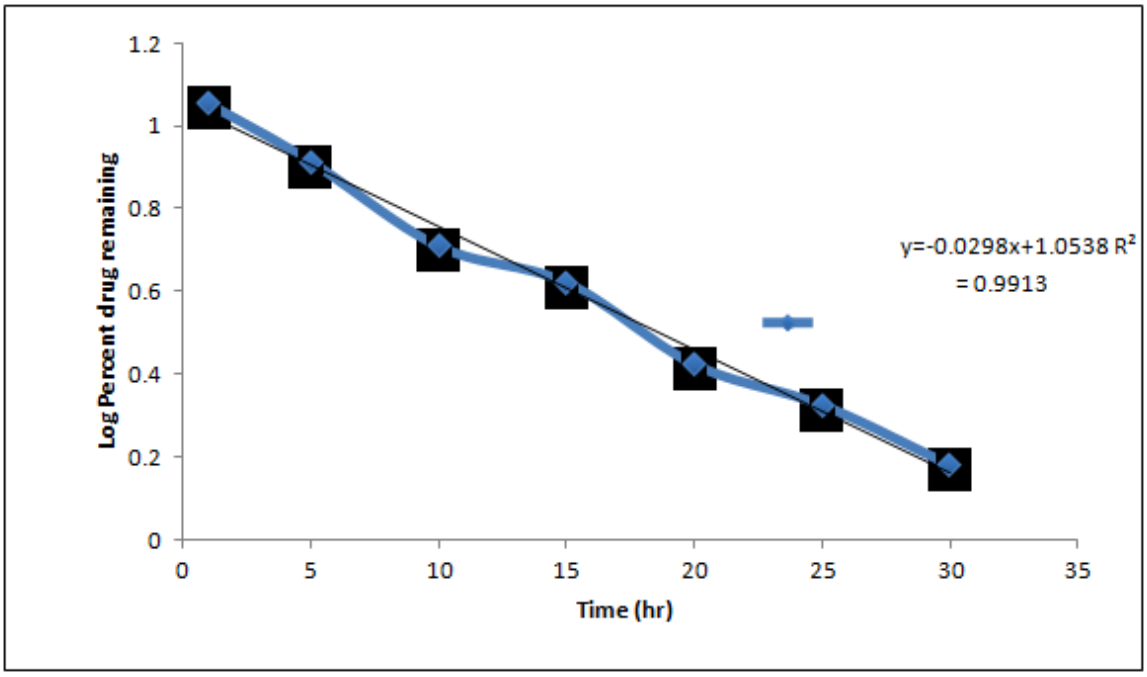

Figure 16: First order graph of formulation F18

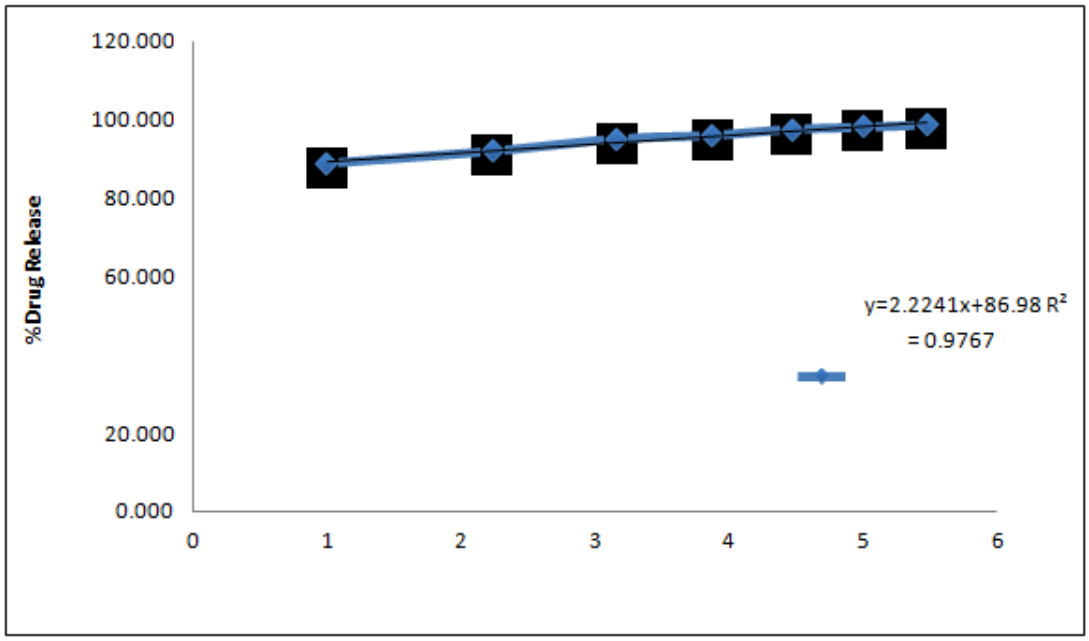

Figure 17: Higuchi order graph of formulation F18

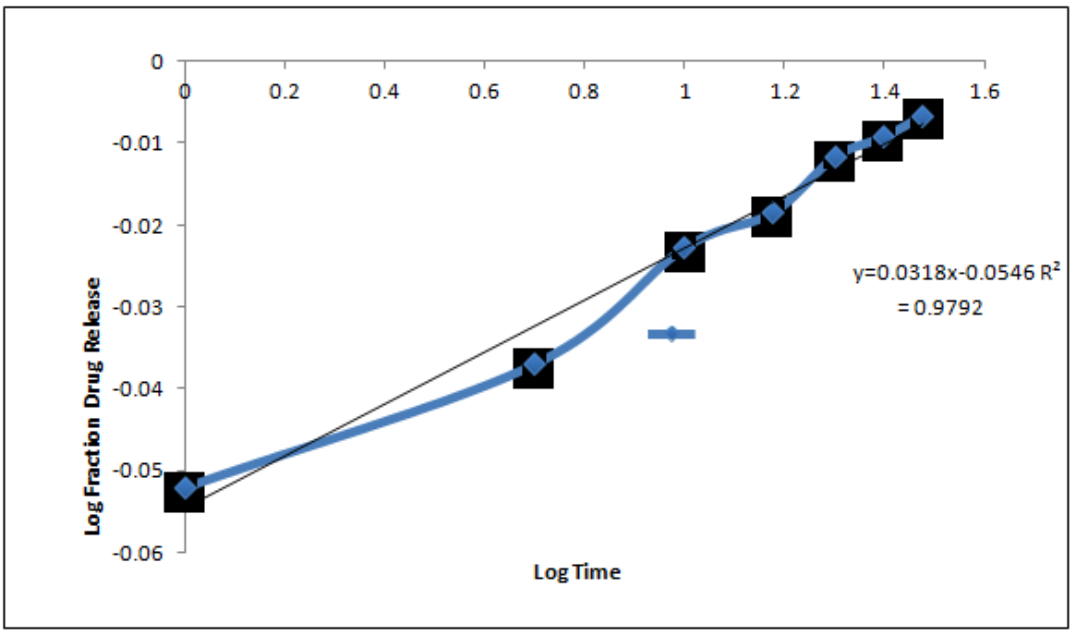

Figure 18: Korsmeyer of formulation F18

Table 24: Kinetics equation parameter of formulation F18

nulation Code	Zero order		First order		Higuchi		Peppas	
	K_0	R^2	K_0	R^2	K_0	R^2	K_0	R^2
F18	0.3184	0.8877	-0.0298	0.9913	2.2241	0.9767	0.0318	0.9792

The calculated regression coefficients for zero order, first order and Higuchi models and Korsmeyer Peppas were shown in Table above it was found that the *in vitro* drug release of F18 was best explained by first order model as the plot showed the highest linearity. The value of R^2 found to be 0.9913 signifying the highest for the first order model.

SUMMARY AND CONCLUSION

Aim of this study was to identify the most significant factors in the liquid formulation of Dehydroascorbic Acid with Deep Eutectic Solvent used as the solvent against oxidative degradation. On physicochemical evaluation, melting point of Dehydroascorbic Acid was found to be 193.67±1.528°C.On UV Spectrophotometric analysis absorption maxima were found to be 264 nm in distilled water. Drug was freely soluble in distilled water, Phosphate buffer pH 6.8, ethanol, Phosphate buffer pH 7.4, 0.1N HCl, methanol and less soluble in chloroform. The partition coefficient of Dehydroascorbic Acid in n-octanol: water was found to be -1.56±0.050, this indicated that the drug is hydrophillic in nature. On FTIR spectroscopy analysis there was no incompatibility between drug and lipid.

An attempt is made to prepare liquid formulation of Dehydroascorbic Acid by an approach of ep Eutectic Solvent (DES) using quaternary ammonium salt such as Choline Chloride and different hydrogen donor group such as Urea, Citric Acid, Oxalic Acid, Gallic Acid and Menthol. Among which Urea was seen to provide stable formulation with overcome stability of Dehydroascorbic Acid.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Abbott, A. P., Boothby, D., Capper, G., Davies, D. L., & Rasheed, R. K. (2004). Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. Journal of the American Chemical Society, 126(29), 9142-9147.
2. Abbott, A. P., Capper, G., & Davies, D. L. (2003). Rasheed; RK; Tambyrajah, V. Chem. Commun, 70-71.
3. Abbott, A. P., Capper, G., & Gray, S. (2006). Design of improved deep eutectic solvents using hole theory. Chemphyschem: a European journal of chemical physics and physical chemistry, 7(4), 803-806.
4. Abbott, A. P., Harris, R. C., Ryder, K. S., D'Agostino, C., Gladden, L. F., & Mantle, M. D. (2011). Glycerol eutectics as sustainable solvent systems. Green Chemistry, 13(1), 82-90.

5. Abdelhamid, H. N. (2016). Ionic liquids for mass spectrometry: Matrices, separation and microextraction. TrAC Trends in Analytical Chemistry, 77, 122- 138.
6. Ahmad, I., Ali Sheraz, M., Ahmed, S., Shad, Z., &Vaid, F. H. M. (2012). Photostabilization of Dehydroascorbic Acid with citric acid, tartaric acid and boric acid in cream formulations. International journal of cosmetic science, 34(3), 240-245.
7. Akar, G., &BarutçuMazı, I. (2019). Color change, Dehydroascorbic Acid degradation kinetics, and rehydration behavior of kiwifruit as affected by different drying methods. Journal of food process engineering, 42(3), e13011.
8. Alamgir, A. N. M. (2018). Vitamins, nutraceuticals, food additives, enzymes, anesthetic aids, and cosmetics. In Therapeutic Use of Medicinal Plants and their Extracts: Volume 2 (pp. 407-534). Springer, Cham.
9. Singh S, Dadabhau GD, Singh K, a systematic review of antiulcer drug its pharmacodynamic & pharmacokinetic properties: nizatidine, Eur. Chem. Bull. 2022, 11(8), 1159-1164.
10. Ali, M. R., Rahman, M. Z., & Saha, S. S. (2014). Electroless and electrolytic deposition of nickel from deep eutectic solvents based on choline chloride.
11. Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., & Khan, F. H. (2020). Understanding oxidants and antioxidants: classical team with new players. Journal of food biochemistry, 44(3), e13145.
12. Anju, V.S., &Kuriachan, M.A. (2017). Formulation and Evaluation of Dehydroascorbic Acid LoadedMicrospongeBasedGelforTopicalSustainedDelivery.International Journal of Pharmacy & Pharmaceutical Research, 10(2), 69-93.
13. Singh S, Dadabhau GD, Singh K, review on sustained release dosage form: a novel approach and its evaluation, journal of survey in fisheries sciences, 2022, 8(3), 570-577.
14. Azim zzadeh-Sadeghi, S., & Yavari, I. (2020). Choline chloride/pentaerythritol: a deep eutectic solvent for the synthesis of pyran and chromene derivatives. Journal of the Iranian Chemical Society, 1-7.
15. Baka, E., Comer, J. E.,&Takács-Novák, K. (2008). Studyof equilibrium solubility measurementbysaturationshake-flaskmethodusinghydrochlorothiazideasmodel compound. Journal of pharmaceutical and biomedical analysis, 46(2), 335-341.
16. Banu, R.J., Kumar, M., &Jaykar, B. (2019). Isolation and Characterization of Urea from the Ethanolic Extract of Leaves of Butea Monosperma (Lamb). International Research Journal of Pharmacy, 10(2), 114-17.
17. Bergua, F., Castro, M., Muñoz-Embid, J., Lafuente, C., & Artal, M. (2021). Hydrophobic eutectic solvents: Thermophysical study and application in removal of pharmaceutical products from water. Chemical Engineering Journal, 411, 128472.
18. Bergua,F.,Delso, I.,Muñoz-Embid,J.,Lafuente,C.,&Artal,M.(2021).Structure and properties of two glucose-based deep eutectic systems. Food Chemistry, 336, 127717.
19. Berthod, A., &Carda-Broch, S. (2004). Determination of liquid–liquid partition coefficients byseparation methods.Journal of chromatographyA,1037(1-2), 3-14. https://doi.org/10.1016/j.chroma.2004.01.001
20. Boyd, B. J., Bergström, C. A., Vinarov, Z., Kuentz, M., Brouwers, J., Augustijns, P.,&Jannin,V.(2019).Successfuloraldeliveryof poorlywater-solubledrugsboth depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems. European Journal of Pharmaceutical Sciences, 137, 104967.
21. Carriazo, D., Serrano, M. C., Gutiérrez, M. C., Ferrer, M. L., & del Monte, F. (2012). Deep-eutectic solvents playing multiple roles in the synthesis of polymers and related materials. Chemical Society Reviews, 41(14), 4996-5014.
22. Casay, G., Quattrocchi, O., Hauck, W., Hernandez-Cardoso, A., & Belsky, J. (1997). USP melting point reference standards: Evaluation of parameters thataffect the melting point. In USP Pharmacopeial Forum, PF39 (4) (Vol. 3).
23. Chatwal, G. R., & Anand, S. K. (1979). Instrumental Methods of Chemical Analysis: (for Hons. and Post-graduate Students of Indian and Foreign Universities). Himalaya publishing house.
24. Chaurasia, G. (2016). A review on pharmaceutical preformulation studies in formulation and development of new drug molecules. International Journal of Pharmaceutical Sciences and Research, 7(6), 2313.
25. Singh S, Dadabhau GD, Singh K, formulation development of sustained release antiulcer drug and study of pre-formulation parameters and its characterization, JPTCP, 30(8), 610-622.
26. Corvis, Y., Menet, M. C., Négrier, P., Lazerges, M., &Espeau, P. (2013). The role of stearic acid in Dehydroascorbic Acid protection from degradation: a heterogeneous system for homogeneous thermodynamic data. New Journal of Chemistry, 37(3), 761-768.
27. Cui, Y., Li, C., Yin, J., Li, S., Jia, Y., & Bao, M. (2017). Design, synthesis and properties of acidic deep eutectic solvents based on choline chloride. Journal of Molecular Liquids, 236, 338-343.
28. D'Agostino, C., Harris, R. C., Abbott, A. P., Gladden, L. F., & Mantle, M. D. (2011). Molecular motion and ion diffusion in choline chloride based deep eutectic solvents studied by 1 H pulsed field gradient NMR spectroscopy. Physical Chemistry Chemical Physics, 13(48), 21383-21391.
29. Singh S, Dadabhau GD, Singh K, Formulation, Development and investigation of matrix type sustained release tablet of antiulcer drug by using soluble polymer as a drug release retarding agent, International journal of membrane science and technology, 2023, 10(4), 2593-2603.