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Devlopment and Evaution of a Proprietary Herbal Formulation (C-95)

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ABSTRACT
Curcumin (CUR) is a potent antifibrinogenic drug which has been widely used in the treatment of various diseases including cancers and other inflammation and oxidative stress related pathologies. The aim of present study was to formulate, evaluate, and enhance the solubility profile of Curcumin (95%) and make a capsule dosage form. The preparation of physical mixtures (PM) and solid dispersion (SD) was successfully achieved from different solvent mixtures of acetone and ethanol. Various emulsifiers, surfactants, carrier such as Tween 80, PVP K30, PEG, Maltodextrin, HPMC were studied for formulation of C-95 capsule. The results showed that the dissolution of CUR from its solid dispersions at different ratios with the investigated hydrophilic polymers, as well as cyclodextrins, was clearly improved. The capsule showing acceptable solubility, disintegration time and in-vitro drug release was significantly improved. It concluded that using the TPGS and tween80 as potent emulsifier, the oral capsule of the C95, they would quite effective by giving fast onset action which having more solubilities and permeability.

KEYWORDS
Curcumin, C95, Turmeric, Curcuminoids, Solid dispersion, Solvent evaporation, antifibrinogenic drug

I. INTRODUCTION

Natural polyphenolic diferuloylmethane curcumin is obtained from the rhizomes of the plant Curcuma longa Linn. (Family: Zingiberaceae) has potential in the prevention and therapeutic interventions of several pathological conditions including respiratory diseases, inflammation, liver disorders and diabetic wounds. By reducing the mutagenesis effects of carcinogens, it protects rodents from a range of malignancies that are caused by carcinogens. There are other reports of curcumin's antirheumatic, hepato- and nephroprotective, thrombosis-suppressing, myocardial infarction-protective, and hypoglycemic properties.

[1]Curcumin has shown to be safe at very high dosages (12 g/d) in numerous investigations on both humans and animals, making it a promising drug for the treatment and prevention of a wide range of human diseases. Despite being safe and effective, curcumin has not yet received therapeutic agent approval because of its limited bioavailability, which poses a significant pharmacological barrier to clinical application. It is a hydrophobic substance with a weak solubility in water and little absorption. Fast systemic clearance and rapid metabolism significantly reduce its bioavailability. The water solubility of curcumin especially at acidic and physiological pH is extremely low (11 ng/ml). It undergoes rapidly hydrolysis under alkaline conditions and readily decomposed when exposed to bright light, high temperature or oxidative conditions. Curcumin is a BCS class IV molecule due to its low permeability and solubility in water.

[2]Effective pharmacokinetics is a surrogate for pharmacodynamics and is a determinant of therapeutic outcome. Although curcumin showed potential pharmacodynamics, it has poor pharmacokinetic characteristics. To boost curcumin's bioavailability, numerous efforts have been made to increase its solubility, absorption (permeability), and stability. Although many clinical trials for curcumin are currently ongoing, its clinical advancement of is hampered by its poor water solubility and short biological half-life and low bioavailability in both plasma and tissues. The oral bioavailability of curcumin and synthetic structural analogues of curcumin is very low (only 1% in rats) The motivation for building curcumin analogues is based upon its rapid metabolism and conjugation in the liver and its excretion through feces which limit the systemic bioavailability. In order to overcome these limitations, several approaches have been attempted including the combination of curcumin with adjuvants such as piper.

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II. TURMERIC (CURCUMA LONGA)

Turmeric, also known as Curcuma longa, belongs to the Zingaberaceae family of plants, which also includes ginger. The Persian word "kirkum," which means "saffron," is the source of the Latin name, which alludes to the rhizome's vivid yellow-orange hue. Although it comes originally from Southeast Asia, India has long utilized and grown it.

[3]Originating in South East Asia, turmeric has been used as a spice and a dye since ancient times. Bengal, China, Taiwan, Sri Lanka, and Java are the main places where it is grown. Peru. West Indian Islands and Australia.

Being organic, unprocessed, and inexpensive, it is still utilized in Hindu rituals and as a dye for sacred clothing. One of the least expensive spices is actually turmeric. Although the two spices are used similarly as dyes, saffron should never be substituted with either spice in food preparations. The Vedic culture of India, where it was employed as a culinary spice and had some religious significance, is where its use dates back over 4000 years.

Since pulverized turmeric has a colour that mimics a mineral pigment, the term is derived from the Latin terra merita, which means "meritorious soil." For more than 4,000 years, people have used turmeric (Curcuma longa) to treat a range of illnesses. Numerous studies have suggested that turmeric may be effective in treating a variety of diseases.

[4]There is evidence that the turmeric compound curcumin encourages the gallbladder to produce more bile. Curcumin also functions as a potent antioxidant, which is a substance that removes harmful substances from the body called free radicals. Free radicals can alter DNA, harm cell membranes, and even kill cells. Free radicals can be neutralized by antioxidants, which may also help lessen or even stop some of the harm they cause. Additionally, curcumin lessens inflammation by reducing the body's levels of the inflammatory enzymes COX-2 and LOX and prevents platelets from adhering to one another to create blood clots. It is an effective natural treatment for bronchial asthma. It works well to consume a teaspoon of turmeric powder with a glass of milk two or three times a day. It works best when you're not hungry. An effective intestinal antiseptic is turmeric.

Due to its high iron content, turmeric is helpful for anemia. Due to its antibacterial qualities, turmeric is a helpful treatment for persistent cough and throat irritations.80 percent of the turmeric crop grown worldwide is consumed in India. Indian turmeric is regarded as the greatest in the world due to its unique properties. In South East Asia, fresh spices are significantly more favoured than dried ones. The raw rhizome is grated and used to curries; in Thailand, it is often made into a paste for yellow curry. Turmeric has entered Ethiopian cuisine as a result of Indian influence. Turmeric is most frequently used to treat skin diseases and cleanse the blood, in addition to flavorings meals. Turmeric is a well-known Middle-Eastern spice, although few people are aware of its medical properties.To enhance digestion and lessen gas and bloating, it may be added to foods like rice and bean dishes. It is a cholagogue, facilitating bile excretion through the gallbladder and promoting bile synthesis in the liver. The body's capacity to metabolise fats is enhanced by this.

[5]Although turmeric is not used directly in Western cuisine, it is a component of many sauces and spice blends and is also used to give mustard paste a vivid yellow hue. Curcumin may be useful in halting the progression of Multiple Sclerosis, according to preliminary experiments conducted on mice. When fed curcumin, mice bred to manifest experimental autoimmune encephalomyelitis (EAE), a condition similar to MS, showed little or no signs of the condition, according to Vanderbilt University researchers. Curcumin-deficient mice later developed severe paralysis.

III. CHEMICAL CONSTITUENTS OF TURMERIC

Curcumin, desmethoxycurcumin and bisdemethoxycurcumin collectively known as curcuminoids (3-6%) are major polyphenolic compounds in turmeric rhizomes. The main colouring principle of turmeric rhizome was isolated in 19th century and named as ‘Curcumin’. Its chemical structure was determined by Roughley and Whiting.

[6]Other phenolic compounds present in turmeric rhizome are 1-hydroxy-1, 7-bis (4-hydroxy-3- methoxyphenyl)-(6E)-6-heptene-3, 5-dione; 1-(4- hydroxy-3, 5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1E, 6E)-1, 6-heptadiene-3, 4-dione; 1, 5-bis (4-hydroxy3-methoxyphenyl)-penta-(1E, 4E)-1, 4-dien3-one; 1-(4-hydroxy-3-methoxyphenyl-5-(4-hydroxyphenyl)-penta-(1E, 4E)-1, 4-dien3-one; 1-(4-hydroxy-3-methoxyphenyl)-7- (3, 4-

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dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione and 1, 7-bis (4-hydroxyphenyl)-1, 4, 6-heptatrien-3-one.
The pale yellow to orange-yellow volatile oil (4-6%) obtained from turmeric consists of a number of mono- and sesquiterpenes. The sesquiterpenes were named as curcumenone; dehydrocurdione; (4 S, 5 S)-germacrone 4, 5-epoxide; bisabola 3, 10-diene 2-one; arturmerone; bisacumol; bisacurone; curcumenol; isoprocurcumenol; zedoaronediol; procurcumenol; epiprocurcumenol; germacrone-13-al; 4- hydroxybisabola-2, 10-diene-9-one; 4, 5-dihydrobisabola-2, 10-diene; 4-methoxy5-hydroxybisabola-2, 10-diene-9-one; 2, 5-dihydroxybisabola-3, 10-diene and procurcumadiol. Some other compounds named as curlone; α-turmerone; βturmerone; terpinolene; α-phellandrene; curcumadiol; labda-8 (17)-diene-15, 16- dial and three acidic polysaccharides isolated on a column of DEAE Sephadex A25 were named as Ukon A, B and C. They were composed of L-arabinose, Dxylose, D-galactose, D-glucose, L-rhamnose, D-galacturonic acid in the molar ratio 12:4:12:1:4:10 (Ukon A), 12:4:12:1:2:4 (Ukon B) and 8:3:614:2:3 (Ukon C).

[7]The polysaccharide, named as Ukon D is composed of L-arabinose, Dgalactose, D-glucose and D-mannose in the molar ratio of 1:1:12:2. The water-soluble peptide is named as turmerin with an amino acid composition as aspartic acid/aspargine, glutamic acid/glutamine, serine, glysine, argenine, proline, alanine, tyrosine, valine, methionine, leucine, iso- leucine and phenylalanine in the ratio: 1:2:3:8:1:1:1:3:2:6:3:4:5:3. Niranjan et al reported that the leaves are excellent natural sources of carotenoids with maximum amount present in the middle followed by lower and upper leaves.

IV. BIOLOGICAL ACTIVITY OF TURMERIC

[8]Turmeric has a wide range of pharmacological effects that have been documented. One of its key ingredients, curcumin, is in charge of many of its biological effects. It exhibits anti-parasitic, antispasmodic, anti-inflammatory, anticarcinogenic and gastrointestinal effects in vitro whereas it has shown antiparasitic and anti-inflammatory activity through oral application in animal models. The various pharmacological activities of turmeric/ compounds/ extracts are shown in Table 1.

BIOLOGICAL ACTIVITY OF TURMERIC COMPOUNDS AND EXTRACTS

Table-1

Turmeric/compounds/extracts	Activity
Curcumin	Anti-inflammatory
Curcumin	Antioxidant
Curcumin	Anti-tumorandanti-cancer
Curcumin	Anti-HIV
Curcumin	Antimutagenic
Curcumin	Anti-fungal
Curcumin	Antidiabetic
Curcumin	Antifibrinogenic
Curcumin	Woundhealing
Curcumin	Lipidlowering
Curcumin	Radioprotective
Curcumin	Immunomodulating
Demethoxycurcumin	Antioxidant
Bisdemethoxycurcumin	Antioxidant

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Diacetylcurcumin	Anti-inflammatory
Triethylcurcumin	Anti-inflammatory
Tetrahydrocurcumin	Anti-inflammatory
	Antidiabetic
Turmericoil	Anti-bacterial,anti-fungal
Turmericpowder	Antidiabetic
	Anti-inflammatory
	Anti-tumorandanti-cancer
Ethanolextract	Anti-protozoal
	Anti-fungal
Acetoneextract	Anti-fungal
	Antioxidant
Methanolextract	Antioxidant
Waterextract	Antioxidant

V. CURCUMIN EXTRACTION:

a) Conventional extraction using Soxhlet:

[9]Turmeric rhizomes were dried in an oven at 105 °C for three hours. To create a homogenous powder with a particle size of 0.18 mm, dried rhizomes were triturated in a mortar and then filtered through a sieve with mesh size 80. In order to avoid moisture absorption, the turmeric powder was kept in the refrigerator. The Soxhlet extraction, as the reference method, was performed as follows: 15 g ground turmeric powder was weighed and embedded in a thimble and put in the Soxhlet apparatus which was gradually filled with acetone as the extraction solvent. The extraction experiment was carried out at 60 °C within 8 h. Upon completion of the extraction, the acetone was separated from the extract using rotary evaporator (Stuart RE300) under vacuum at 35 °C. The residue (oleoresin) was dried and weighed; then dissolved in 10 ml methanol for calculation of curcumin content using HPLC. In all extraction experiments acetone was used as the extraction solvent due to its high solubilization capacity

b) Microwave-assisted extraction of curcumin:

For microwave-assisted extraction of curcumin, 0.5 g of turmeric powder was weighed and dissolved in 10 ml acetone and put in microwave chamber (domestic Samsung microwave). Acetone which was used as extraction solvent has good dissipation factor (tan δ =0.5555) which can be heated up to high extent and dissipate the microwave energy. Different microwave operating powers between 100 and 450 W and various irradiation periods between 0.5 and 3 minutes were used for extraction. The samples were subjected to microwave irradiation in an intermittent way of irradiation-cooling irradiation for extraction time of up to 3 min, because longer irradiation time and higher power caused boiling of solvent. After that, the solvent was separated through 0.45 µm filter and evaporated under vacuum, the residue was weighed and dissolved in 10 ml methanol for HPLC analysis.

c) Ultrasound-assisted extraction of curcumin:

An ultrasonic bath (Elmasonic S 10 H, Elma Schmibauer GmbH, Germany) with tank capacity of 0.8 liter was used for extraction of curcumin. The bath power was 90 W with 37 kHz frequency. For extraction experiment, 10 ml of acetone were used to dissolve 0.5 g of turmeric powder before it was placed in an ultrasonic bath. The extraction took place at a temperature of 25 to 40 °C for between 10 and 40 minutes. Parafilm was placed on top of the Erlenmeyer containing the sample to prevent solvent loss during the experiment. The solvent was evaporated under vacuum while the extract was filtered through a 0.45µm filter. The residue was weighed as oleoresin and dissolved in 10 ml methanol for HPLC analysis to determine its curcumin content.

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d) Enzyme-assisted extraction of curcumin:

[10]In this set of experiments, 1 g of turmeric powder was weighed in 250 ml Erlenmeyer flask and mixed with 100 ml water followed by addition of 50 ml McIlvaine's buffer pH 5. α-Amylase and amyloglocosidase enzymes were used for turmeric hydrolysis because more than 80% of turmeric is composed of carbohydrate which can be hydrolyzed by these type of enzymes. Different concentrations of α-amylase and amyloglocosidase varying between 1-5% w/w of turmeric for each enzyme was added to the described mixture. Water is poor extraction solvent but necessary for enzyme activity. The flask was sealed with cotton and aluminum foil and shaken in shaker incubator (IKA IC4000) at 130 rpm and incubation temperature of 65 °C which was the suitable temperature for high activity of α-amylase and amyloglocosidase. The turmeric powder was hydrolyzed at different incubation times of 2-8 h. After pretreatment with enzymes, the suspension was lowered and the water was separated from the turmeric powder; the precipitated turmeric was then dried at 60 °C for 6 hours before curcumin was extracted using 10 ml acetone across a range of periods from 1 to 5 hours. After that, the solvent was removed from the turmeric and evaporated using a rotary evaporator.The residue was weighed as oleoresin and dissolved in 10 ml methanol for analysis of its curcumin content.

VI. EXPERIMENTATION

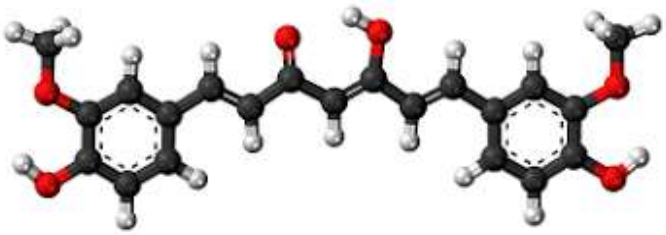
AIM & OBJECTIVES:

- The aim of present study to formulate, evaluate and enhancement the solubility profile of Curcumin (95%) and make a capsule dosage form.
- C-95 is a BCS class -IV drug, having low solubility and low permeability.
- **objective:** Enhancement of drug solubility with a proper dosage form for oral drug delivery.

DRUG PROFILE OF C-95

- NAME-Curcumin 95% (C-95)
- IUPAC NAME- (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione
- **Formula-** $C_{21}H_{20}O_6$

Structure of Curcumin



Sl. No	Properties	Range
1	Molarmass	368.38g/mol
2	CASID	458-37-7
3	Eliminationhalf-life	6-7hours
4	Bioavailability:Oral	0.47%
5	BCS	IV
6	pH	8
7	Melting Point	183°C

VII. CALIBRATION A STANDARD CURVE(C-95)

Calibration curves can be used to predict the concentration of an unknown sample.
PREPARATION OF STANDARD CURVE

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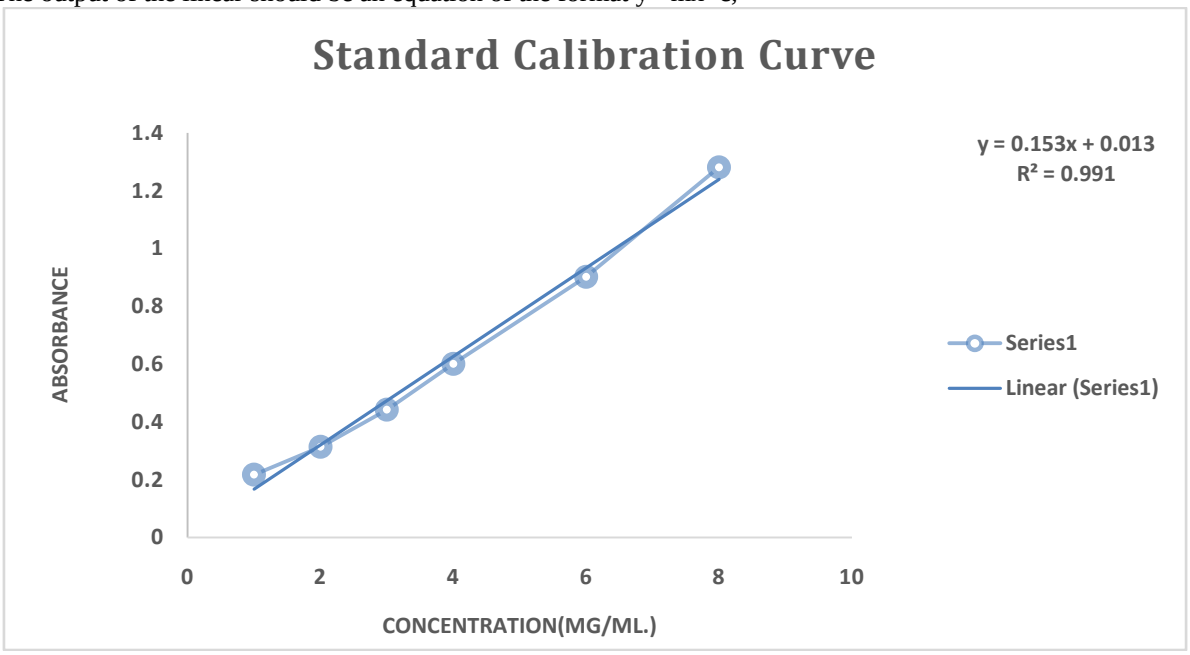
Data table

Concentration (1µg/10ml.)	Absorbance
1	0.217
2	0.313
3	0.442
4	0.601
6	0.902
8	1.281

Set the wavelength at 424 nm
The output of the linear should be an equation of the format y= mx+c,



VIII. PREFORMULATION STUDIES OF API C-95

Physical evaluation

Sl. No	Parameter	Results
1	Bulk density	0.4 mg/ml
2	Tap density	0.59 mg/ml
3	Carr's index	32.2%
4	Housner's ratio	1.475
5	Angle of repose	35

Differential Scanning Calorimetry (DSC)

The different scanning calorimetry measurements were performed by thermal analyzer. These studies were used to determine melting point drug and excipients All the accurately weighed samples were placed in sealed aluminium pans at a scanning rate of 10°c/min from room temperature up to 20-220°c. An empty aluminium pan was selected as reference.

a) Atmosphere: Nitrogen inert.
b) Heating rate: 10ºC/min
c) Gas flow rate: 20ml/min
d) Temperature range: 30-200ºC
e) Sample size: 0.5 mg

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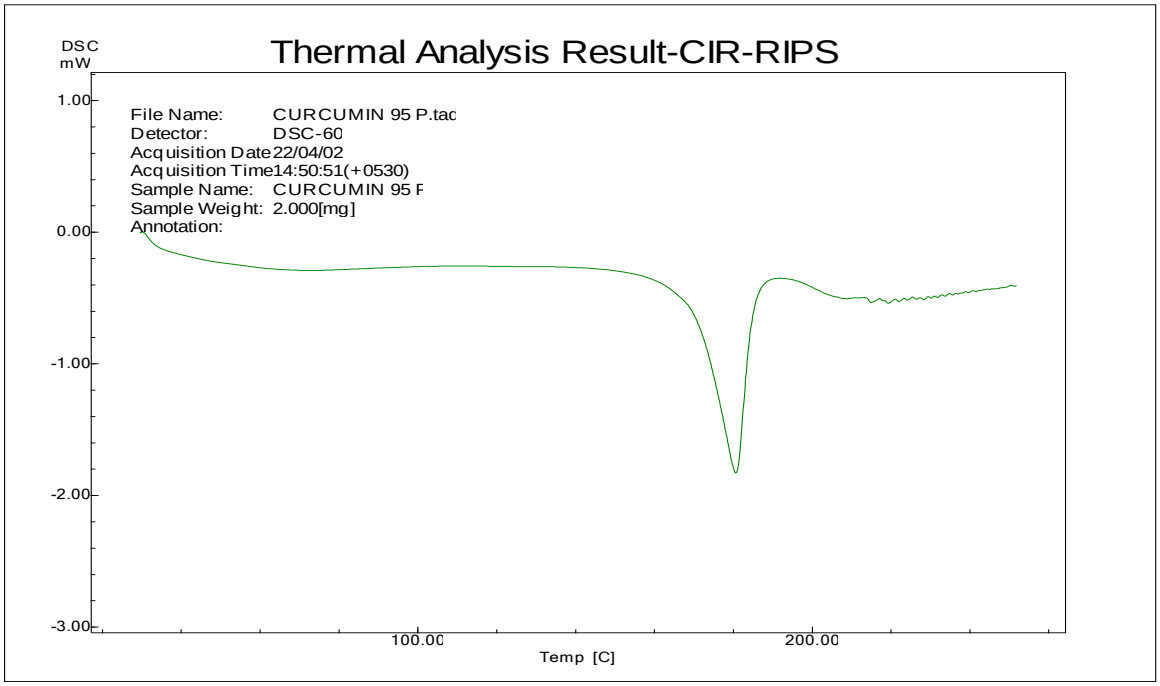
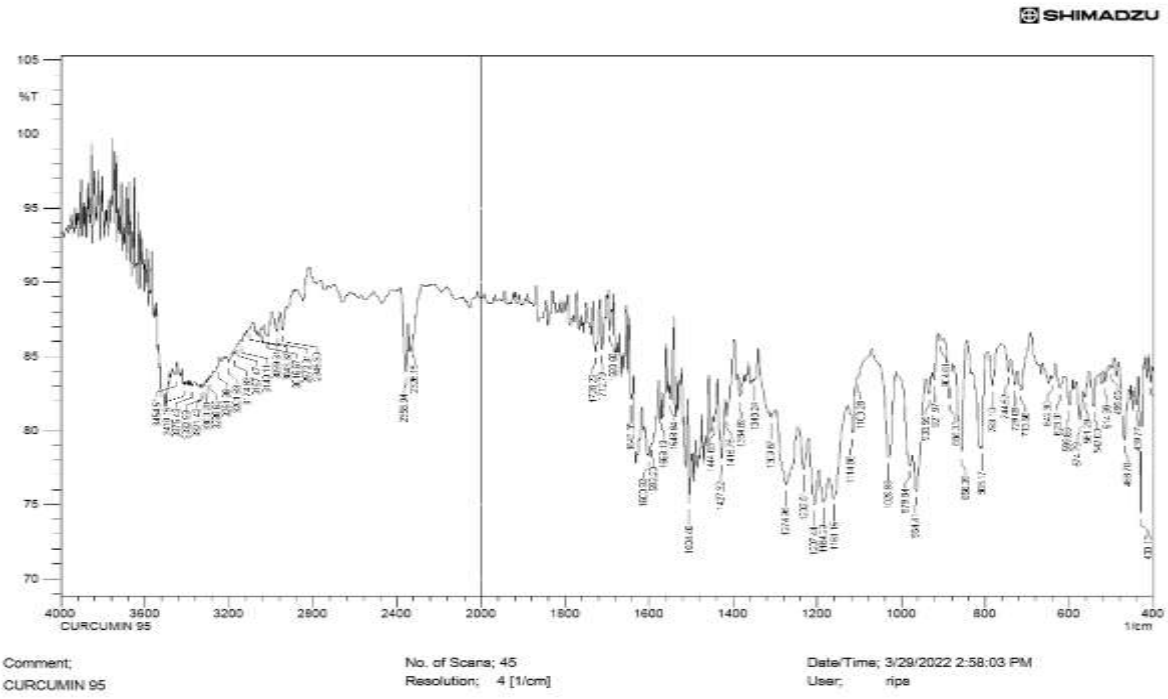


Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to determinate the chemical interaction, also used to scan test sample and observe chemical properties. In this analysis drugs-excipients compatibility studies.



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Wavenumber (Cm-1)	Characteristic Functional Group Feasible
3508	O-H stretching
16	C=O, C=C
1601	Aromatic C=O
1429	Phenol, C-O
1272	Enol

IX. MATERIALS AND METHODS

CUR was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Tween 80 was purchased from Merck Life Sc. PVT.LTD. PEG 4000 was purchased from LOBA CHEMIE PVT.LTD. TPGS was purchased from Antares Health Products, Inc.

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Quantification of CUR

The CUR content was analyzed using ultraviolet (UV)–visible spectroscopy at 432 nm (Shimadzu 1800 UV–visible spectrophotometer, Shimadzu Co. Ltd., Kyoto, Japan). The analytical method was validated according to International Conference of Harmonization guidelines in terms of specificity, linearity, accuracy, and precision.

X. METHOD OF PREPARATION

SOLID DISPERSION

Take distil water in a glass biker & kept in magnetic stirrer. Add TPGS in distil water stirring to form a clear solution. Then add Tween 80 to this solution stirring & giving heat to form a clear solution. Then add PEG 4000 to this solution to form a clear solution. Then add C-95 in this solution & stirring for 30min.

SOLVENT EVAPORATION

Take the excipients & drug mixture in a stenless steel tray & kept in vacuum dryer at 60°C temperature in 12hr.

Grinding & Shifting Method

Griding the dry flacks, then shifting through 170 mesh.

XI. PROCEDURE:

PEG 4000, TPGS & Tween 80 mix in distil water in 30°C (15min)

↓

Then add C-95 in the mixture mix it 30min (60°C)

↓

Dry this slurry using V.D. (60°C) (12hr)

↓

Mill the dry flacks & pass it in 170 mess get the FFP

XII. FORMULATION OF C-95

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Formulation of c-95 contain below excipients:

Ingredients(g)	T1	T2	T3	T4	T5
C-95 (API)	8.80	8.80	8.80	8.80	8.80
Tween 80	0.20	0.20	0.20	0.20	0.20
PVP K30	0.50	0.50	0.50	0.50	-
PEG 4000	-	-	-	0.50	0.50
HPMC K15	-	0.50	0.50	-	-
Maltodextrin	0.50	-	-	-	-
TPGS	-	-	-	-	0.50

XIII. SOLUBILITY STUDIY OF C-95 PH 6.8 BUFFER

- Add the formulation of C-95(500 mg) in the beaker and compare to the standard.
- Take 400 ml of 6.8 buffer as dissolution media.
- Set the temperature of the media to 37° c
- Add the C-95 formulations in to the dissolution media.
- According to the time interval (1 hr,2hr,3 hr,4hr,5hr……) 5 ml of sample of each container was taken simultaneously.
- After taking the sample, the sink condition was maintained by 6.8 buffer.
- The absorbance was measure by the u.v spectrophotometer of the taken sample.

DRUG PRODUCT	API AND F-9 CAPSULE
DISSOLUTION MEDIA	6.8 BUFFER 400 ml
TIME INTERVAL	1 HR
DISSOLUTION APPARETUS	Dissolution Tester (USP II) Electro lab (TDT06L)
TEMP	37°C
DOSE OF DRUG	500MG
TIME OF STUDY	5HR

In a particular time period, how much absorbance is measure is listed in below table.

Solubility StudiyOf Formulation In Phosphate Buffer Ph-6.8

Time (hr)	Standard conc.	T-1 conc.	T-2 conc.	T-3 conc.	T-4 conc.	T-5 conc.
0	0	0	0	0	0	0
1	0.1576	0.2405	1.0104	0.5081	0.3486	2.0305
2	0.1679	0.3589	1.141	0.6226	0.3152	2.0697
3	0.1717	0.3499	0.6226	0.5422	0.3248	2.1128
4	0.1164	0.3627	0.6264	0.638	0.6908	1.9076
5	0.2213	0.2978	0.6142	0.5795	0.3936	2.0003

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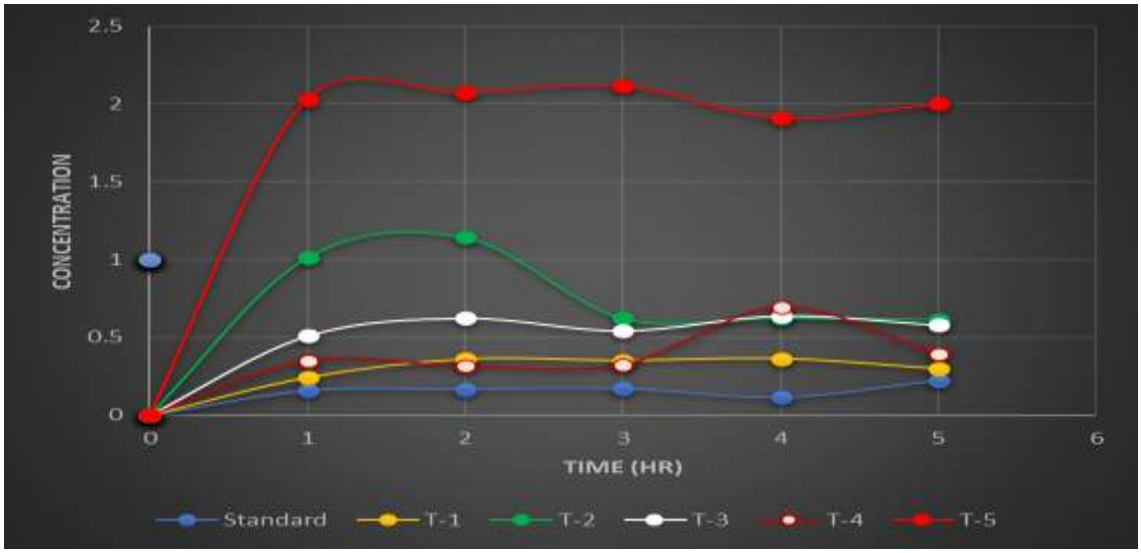

XIV. OBESERVATIONS FROM THE TESTS

After observing the above graph ,we finalised the T-5 formulation having a grater solibility than other formulation.so we take the T-5 formulation for further study.

XV. BRIEF DESCRIPTION ABOUT T-5 FORMULATION

FormulationT-5			
S. No.	**Ingredients**	**Quantity for 100gm**	**Quantity for 10gm**
1	C-95 (API)	88.00	8.80
2	TPGS	5.00	0.50
3	Tween 80	2.00	0.20
4	PEG 4000	5.00	0.50
Total		**100.00**	10.00

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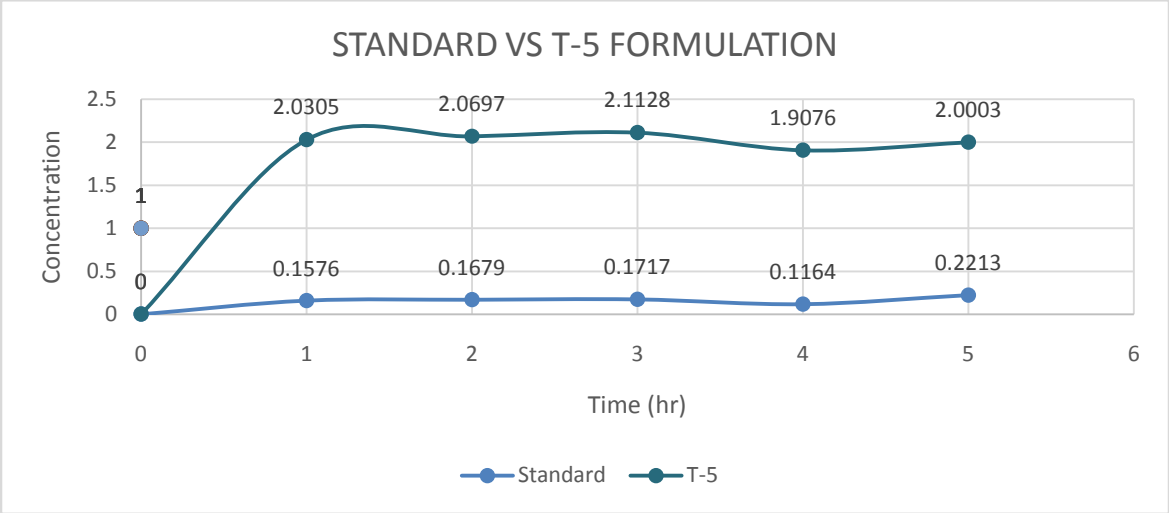
FormulationT-5			
S. No.	**Ingredients**	**Quantity for 100gm**	**Quantity for 10gm**
1	C-95 (API)	88.00	8.80
2	TPGS	5.00	0.50
3	Tween 80	2.00	0.20
4	PEG 4000	5.00	0.50
Total		**100.00**	10.00

DISSOLUTION PROFILE OF API Cap. &T-5 IN phosphate buffer pH-6.8

TIME (HR)	API (concentration(µg/ml))	**Formulation -5** (concentration(µg/ml))
0	0	0
1	0.1576	2.0305
2	0.1679	2.0697
3	0.1717	2.1128
4	0.1164	1.9076
5	0.2213	2.0003



DRUG RELEASE FORMULA

Amount of drug released mg/ml = Concentration × Dissolution bath volume × Dilution factor/1000

% Drug Relese = Drug Relese / Drug Taken ×100

For API

Amount of drug released mg/ml = 0.2213× 400/1000 = 0.08852mg/ml

% Drug Release = 0.08852/500 × 100 = 0.017704%

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For T-5

Amount of drug released mg/ml = 2.0003× 400/1000

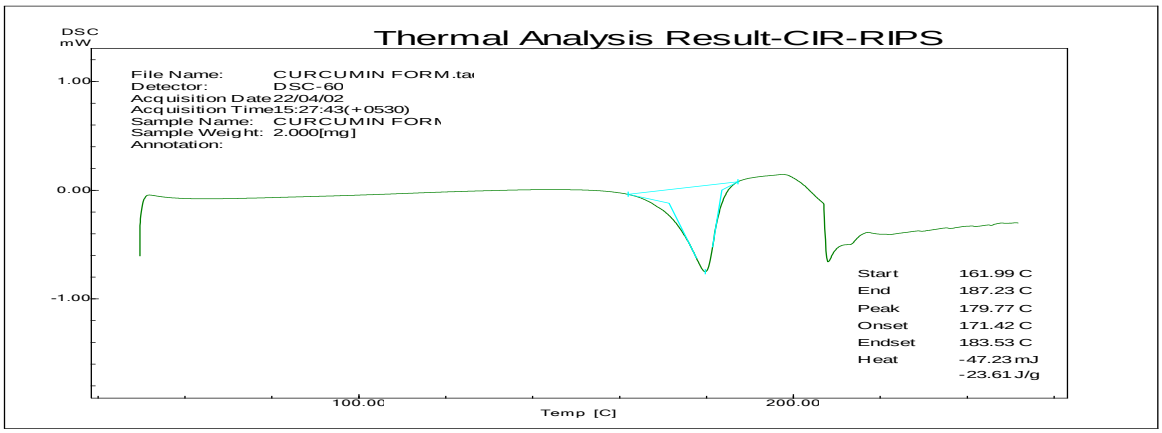
= 0.80012mg/ml

% Drug Release = 0.80012/500 × 100

= 0.160024 %

ANALYTICAL STUDY OF T-5 FORMULATION

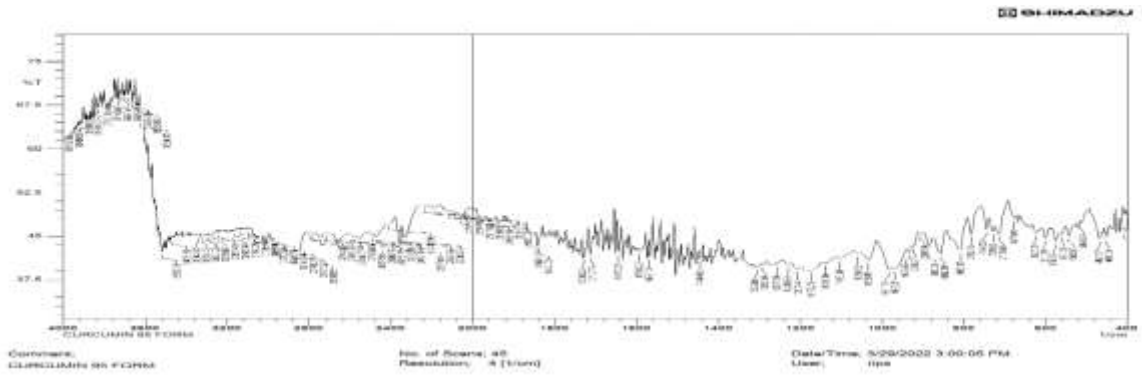
Differential Scanning Calorimetry (DSC)



The melting point of the extract was found to be 175.1°C which is in accordance with the standard monograph of curcumin which confirms the presence of curcumin in the extract of Turmeric.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR of the sample was performed using FTIR instrument (IR affinity-1 SHIMAZDU) equipped with OPUS spectrum software at Roland institute of pharmaceutical sciences, Berhampur. Drug sample was vacuum dried for 12 hours before performing IR studies. The dried sample was placed and further processed for IR spectra (FTIR, Germany).



XVI. CONCLUSION

Looking back on this project, the overall outcome of results to be observed. This can be evaluated by looking at how well our objectives were met.Our first objective is to Enhance the solubility of C-95.Normal curcumin is not water soluble, it is a BS-IV drug, so in this research we prepare a highly soluble C-95 formulation that is T-5.

The formulation is highly stable and ready for preparing any suitable dosage form. Its 90% more soluble than standard.The stability study of this c-95 formulation is not completed yet, it is in the ongoing process. This recearch is very important for the future of the pharma industry as well as our education.

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