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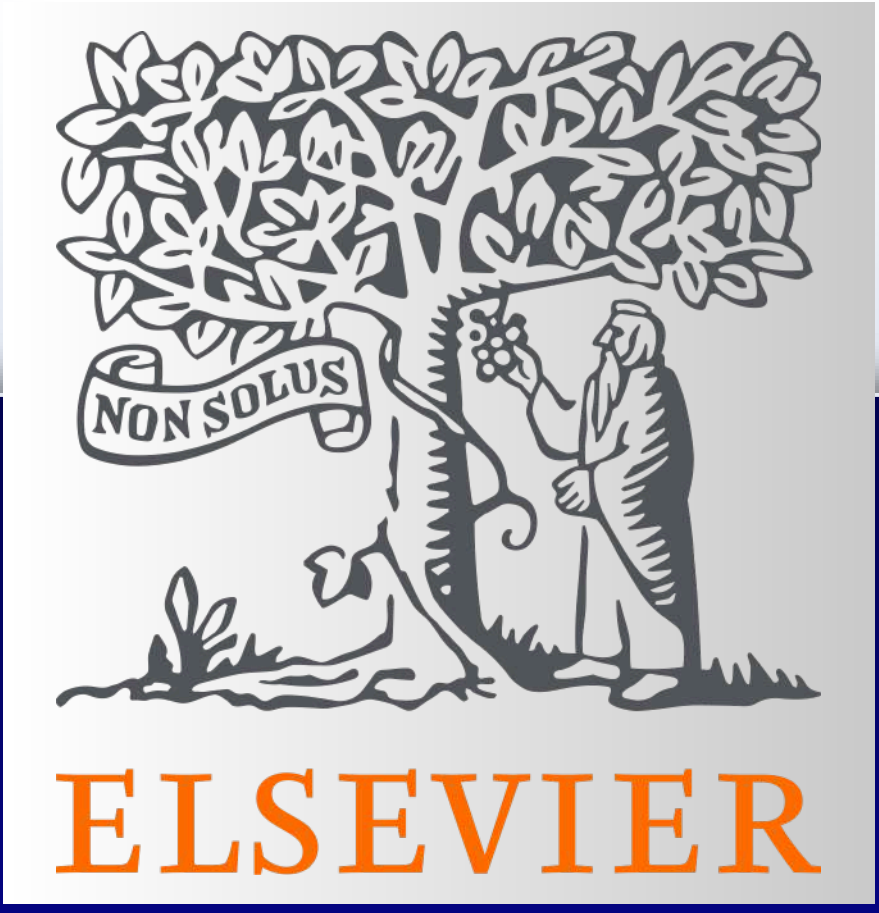

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FORMULATION AND EVALUATION OF CURCUMIN LOADED POLYMERIC NANOPARTICLES AND DETERMINATION OF ITS ANTI-CANCER ACTIVITY BY MTT ASSAY

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ABSTRACT

Aim: The aim of the present investigation is to prepare curcumin nanoparticles using the nano precipitation technique. The main objective is to regulate the drug release, maximise therapeutic effectiveness.

Methodology: The drug is incorporated into the polymer (such as Eudragit S 100 and ethyl cellulose) by adjusting the drug-polymer concentrations and organic-aqueous ratios. Total 10 formulations were prepared and % yield, % drug content, and in vitro drug release were evaluated.

Results: Out of all the formulations ,F3 was considered to be the best formulation with minimum particle size of 219.4nm, drug content of 99%, %entrapment efficiency of 98.23%, zeta potential value (-47.1mv), In vitro drug release of 46.11% which was sustained up to 12 hours. The drug release kinetic study of the best formulation was studied.

Conclusion: F3 was considered for the determination of anti-cancer activity in vitro in MCF-7 Breast cancer cell line by MTT assay. The results indicated that the prepared formulation demonstrated anti- cancer activity with an IC50 value of 38.5µg.

Keywords: Curcumin, Eudragit S 100, Ethyl cellulose, nano precipitation technique, Entrapment efficiency.

INTRODUCTION

Various advantages of nano sizing include decreased fed/fasted variability, decreased patient-to-patient variability, enhanced solubility, increased oral bioavailability, increased rate of

dissolution, increased surface area, less amount of dose required, and more rapid onset of therapeutic action. [1]

Polymeric nanoparticles are colloidal particles composed of a biocompatible or biodegradable lipid matrix. These bio actives are entrapped in the polymer matrix as particulates enmeshed or solid solutions or may be bound to the particle surface by adsorption or chemically. The nanoparticles loaded with bio actives could not only deliver drugs to specific organs but delivery rate in addition could be controlled as being bystanders, burst, controlled, pulsatile or modulated.

Curcumin has many pharmacological effects that mainly includes anti-inflammatory activity, antibacterial, antioxidant, anticancer activity and also has plasma half-life of 7 hrs and poor bioavailability (3%) due to extensive first pass metabolism. So nanoparticles were designed. Curcumin is a phyto polyphenol pigment isolated from the plant curcuma longa, commonly known as turmeric, which has many potential pharmacological effects including anti-inflammatory, antioxidant, anti-bacterial, anti-cancer activities. Curcumin blocks the formation of reactive oxygen species, possesses anti-inflammatory properties as a result of inhibition of cyclooxygenase (COX) and other enzymes involved in inflammation, and disrupts cell signal transduction by various mechanisms.[3]

There is a scope to further improve bioavailability, solubility and so attempts have been made to prepare curcumin nanoparticles using Eudragit S100 and EC as polymers and the anticancer activity can be better established for curcumin nanoparticles.

METHODOLOGY

In order to obtain particles in nano range with good stability the nano precipitation procedure was optimized for three process variables i.e.; concentration of surfactant, stirring speed,stirring time.[3,4]

Optimization Of Process Variables By Nanoprecipitation Technique

In order to obtain particles in nano range with good stability the above procedure was optimized for various process variables and formulation[5,6].

1. Concentration of Surfactant

To optimize the concentration of the surfactant, trials were made by preparing the formulations (C1, C2, C3) by varying the concentration of surfactant used. The concentrations of surfactant that were used in the trials included 0.1%, 0.3%, 0.5% v/v respectively as shown in table 1

Table:-1 Optimization of concentration of surfactant

Trials	Concentration of Surfactant
C1	0.1% V/V Span 60
C2	0.3% V/V Span 60
C3	0.5% V/V Span 60

2. Speed of agitation

To optimize the speed of agitation, trials were made by preparing the formulations (D1, D2, D3) by varying the speed of agitation [7,8]. The agitation speeds that were used in the trials included 200 rpm, 500 rpm, 700 rpm respectively as displayed in table 2.

Table:-2 Optimization of speed of agitation

Trials	Speed of Agitation
D1	200 rpm
D2	500 rpm
D3	700 rpm

3.Time of agitation

To optimize the duration of agitation, trials were made by preparing the formulations (E1, E2, E3) by varying the time periods of agitation. The varied time periods of agitation used in the trails were 3hrs, 5hrs, and 7 hrs respectively as shown in table 3.

Table:-3 Optimization of time of agitation

Trials	Agitation Time
E1	3 hours
E2	5 hours
E3	7 hours

- The yield of polymeric nanoparticles were obtained with the use of 0.1% v/v Span60 surfactant solution was more when compared to the higher concentrations of surfactant (0.3% v/v and 0.5% v/v).
- On comparing the three different stirring speeds (200, 500, 700 rpm) for the preparation of drug loaded polymeric nanoparticles, at 200rpm – No nanoparticle formation was observed. At 500 rpm particles were observed, at 700rpm particles were broken. So stirring speed of 500rpm was considered to be best.
- On comparing the three different periods of agitation time (3, 5, 7 hours) for the preparation of drug loaded polymeric nanoparticles, at 3hrs- No Nano particle formation was observed ,at 5hrs of stirring time nanoparticles were observed.

Methodology

Ten formulations were created by adjusting the drug to polymer ratio in order to observe how the concentrations of the drug and polymer affected the nanoparticles. In dichloromethane, weighed amounts of curcumin and Eudragit S100/Ethyl cellulose were dissolved. Then, 50ml of an aqueous phase containing surfactant was added to the organic phase while being continuously stirred at 500 rpm.The end point was seen as the emergence of precipitate. Five hours were spent stirring the solution once the endpoint was reached. A vacuum was used to remove the precipitate from the solution.Five hours were spent stirring the solution once the endpoint was reached [9,10]. A vacuum was used to remove the precipitate from the solution. The formulations were shown in table 4.

Table 4:-Formulation table of CUR NPs of Nano precipitation technique

S.No	Formulation code	Amount of drug(mg)	Amount of polymer(mg)	Drug: polymer ratio	Organic to aqueous phase ratio
1.	F1	100	100 (ES)	1:1	1: 10

2.	F2	100	200 (ES)	1:2	1: 10
3.	F3	100	300 (ES)	1:3	1: 10
4.	F4	100	100 (EC)	1:1	1: 10
5.	F5	100	200 (EC)	1:2	1: 10
6.	F6	100	300 (EC)	1:3	1: 10
7.	F7	100	100 (ES)	1:1	1:5
8.	F8	100	100 (EC)	1:1	1:5
9.	F9	100	100 (ES)	1:1	1:15
10.	F10	100	100 (EC)	1:1	1:15

RESULTS AND DISCUSSION

All the formulations were evaluated for percentage yield,% drug content, % Entrapment efficiency, loading capacity, Invitro dissolution studies, zeta potential, mean particle size(nm). The formulations results are as follows.

1. Percentage yield

The yields of the prepared nanoparticles were calculated. The dried nanoparticles were weighed and the yield of nanoparticles.[11,12]

%yield= amount of nanoparticles obtained/theoretical amt× 100

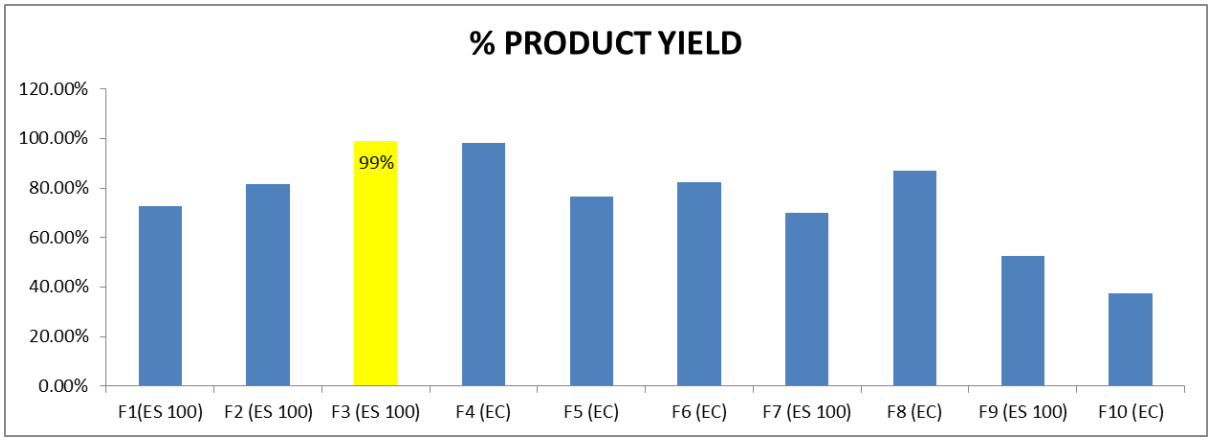

Fig. 1: Percentage product yields of CUR loaded polymeric nanoparticles

The percentage yield of F3 formulation was found to be 99% as shown in figure 1

2. **Drug content:** To determine the amount of drug in the synthesised nanoparticles, the drug content of the polymeric NPs is assessed. To determine the precise amount of drug present in the product, an assay process called "drug content" is used. The required amount of drug was weighed and crushed then transferred into the beaker containing the methanol which makes 1000µg/ml concentration and kept for stirring by using a magnetic stirrer for 2 hours. Then the sample was analysed at the respective λmax in UV visible spectrophotometer.[13,14]

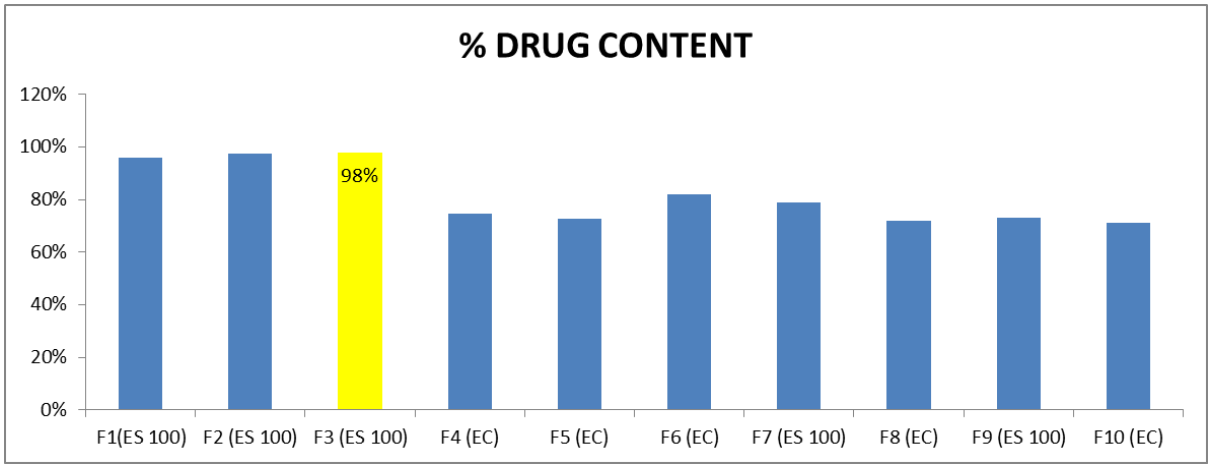

Fig.2: Percentage drug content of CUR loaded polymeric nanoparticles

The drug content of the F3 formulation was found to be 98% as shown in figure 2

3. **Loading capacity**

%L.C = Total drug added-un entrapped drug/ Weight of nanoparticles taken for test ×100

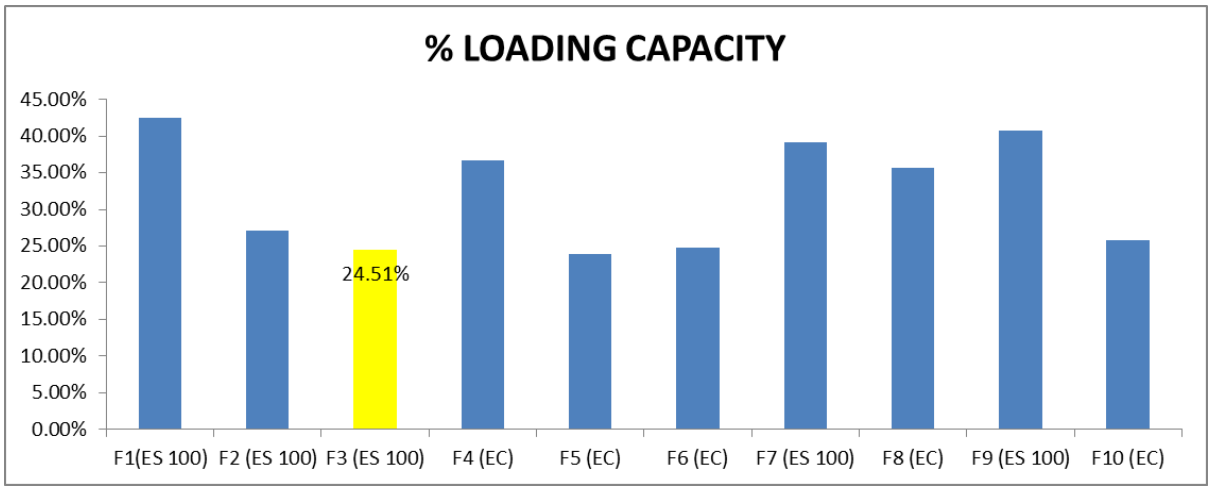

Fig.3: Percentage loading capacity of CUR loaded polymeric nanoparticles

The loading capacity of the formulation F3 was found to be 24.51% respectively as shown in figure 3.

4. Entrapment efficiency

It refers to the percentage amount of drug entrapped in nanoparticles.

% E.E = Total drug added - entrapped drug/ Total drug added ×100

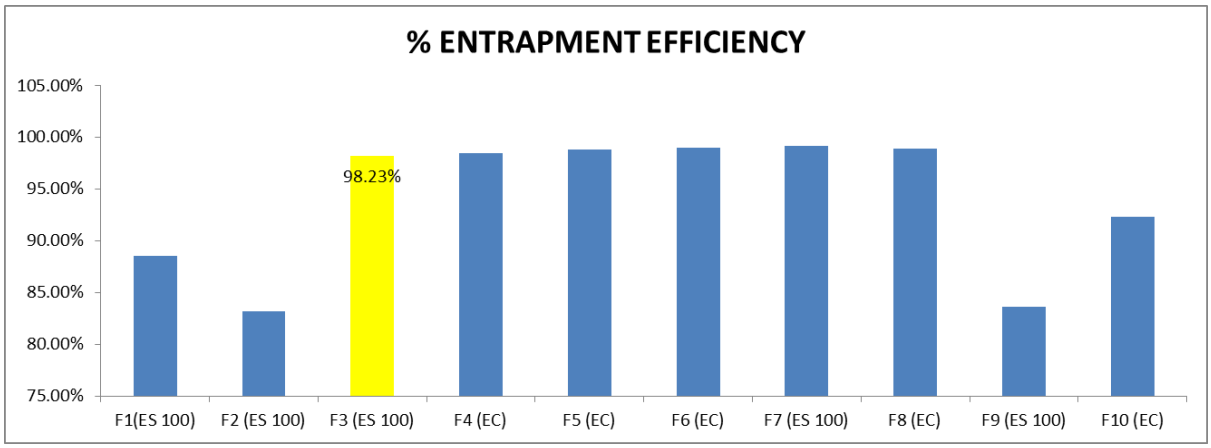

Fig.4: %Entrapment efficiency of CUR loaded polymeric nanoparticles

From the figure 4 the entrapment efficiency of F3 formulation was found to be 98.23%.

5. Comparison of mean particle diameter of CUR loaded polymeric nanoparticles :

Particle size analysis was determined by Malvern instrument and characterized for particle size using particle size analyzer[15,16]

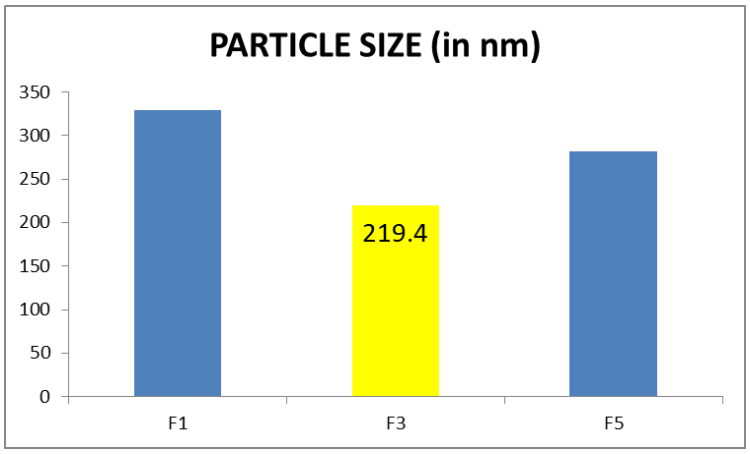

Fig:5 Comparison of mean particle diameter of CUR NPs prepared.

From the above fig 5, the mean particle sizes of formulations F1, F3, and F5 were observed to be 329.8nm, 219.4nm, and 281.7nm respectively. All the obtained formulations were found to be in nano range.

6. Determination of Size Distribution and Zeta Potential

Zeta potential is an important parameter to evaluate for stability of colloidal dispersion systems. It was determined by the Malvern instrument.[17,18]

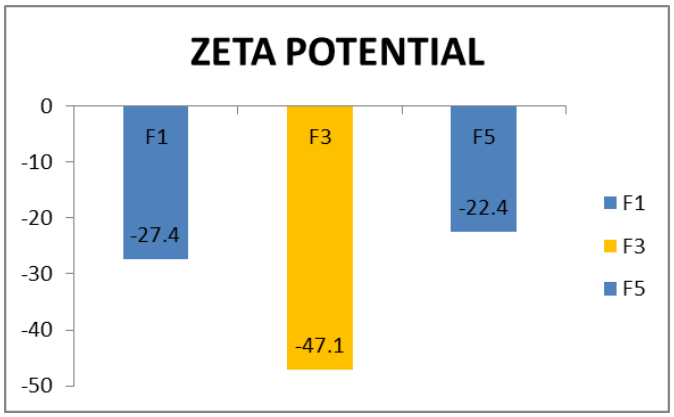

Fig:6 Comparison of Zeta potential of CUR Nanoparticles

From the above fig 6, the zeta potential of formulations F1, F3, and F5 were observed to be -27.4, -47.1, -22.4 respectively.

7. Invitro dissolution studies

An orbital shaker was used to perform in vitro drug release kinetics. Each formulation's 50mg dosage was precisely measured out and placed into a 250ml conical flask with 50 ml of pH 7.4 phosphate buffer solution. They were maintained in an orbital shaker at 100 rpm and 37°C. At predetermined intervals, aliquots of 5 ml buffer were removed, and the medium was replenished with the same volume of buffer. The withdrawn samples were centrifuged at 3000 rpm for 15 min. The supernatant sample was collected. This study was carried out for a time period of 12 hrs with all the prepared formulations. Using an ELICO UV Spectrophotometer to measure the absorbance at each wavelength, the concentration of drug release was determined.[19,20]

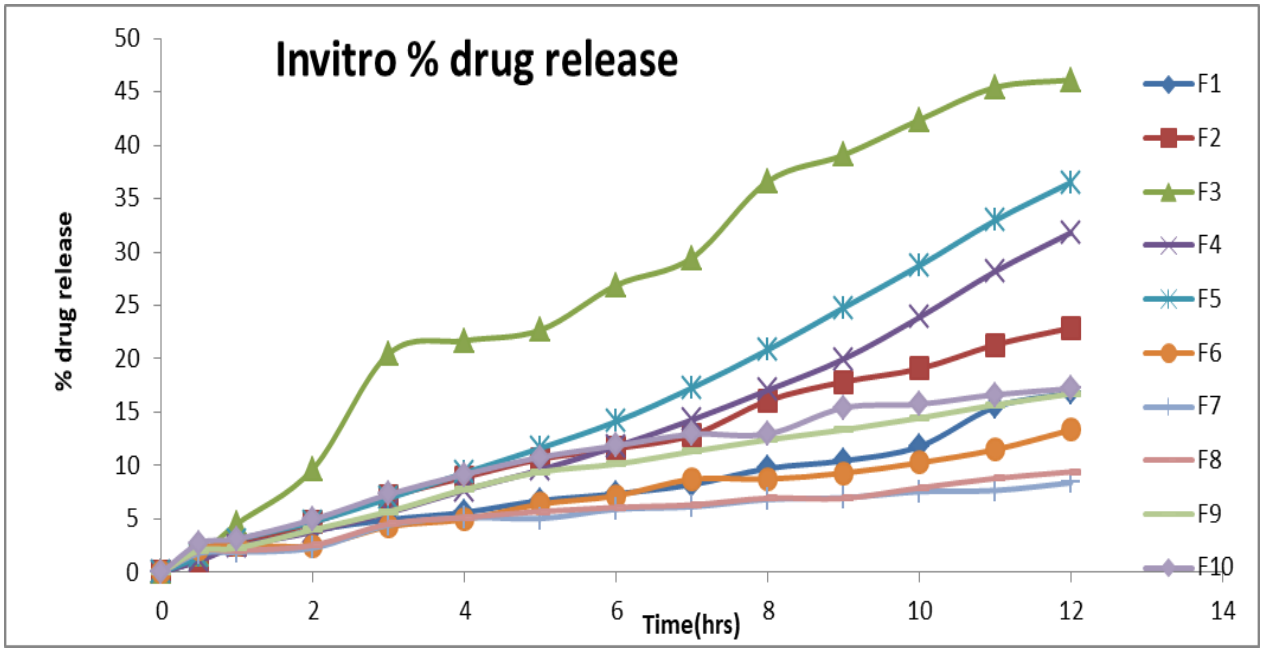

Fig.7: Invitro % drug release of CUR loaded polymeric nanoparticles

In vitro drug release studies were performed for a period of 12 hrs withdrawing aliquots of each formulation for every 1 hr including the first half an hour. At the end of 12hrs, the maximum release of drug was respectively 46.11%. The % drug release of F2 and F3, formulations were found to be 22.89%, 46.11% respectively. The result was displayed in figure 7.

IN VITRO CYTOTOXICITY ASSAY

Out of all the 10 formulations, the best formulation was considered for the determination of the anti-cancer activity in the breast cancer cell line.The anti-cancer activity was determined in vitro by MTT assay. The percentage of viability was displayed in table 5.[21,22]

Table 5:- In-vitro-cell viability studies

Concentration (µg)	% Viability
5	85.7
10	69.46

25	49.85
50	30.1
100	16.57

The IC50 value of the given curcumin nanoparticle formulation F3 was found to be 38.5µg as depicted in the table 6 and figure 8

Table 6:- Cytotoxicity effect of F3 on growth of MCF-7 Breast cancer cell line

Concentration (µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
5	0.565	14.13	85.7	
10	0.457	30.54	69.46	
25	0.328	50.15	49.85	
50	0.198	69.9	30.1	
100	0.109	83.43	16.57	
Untreated	0.658	0	100	
Blank	0	0	0	38.5

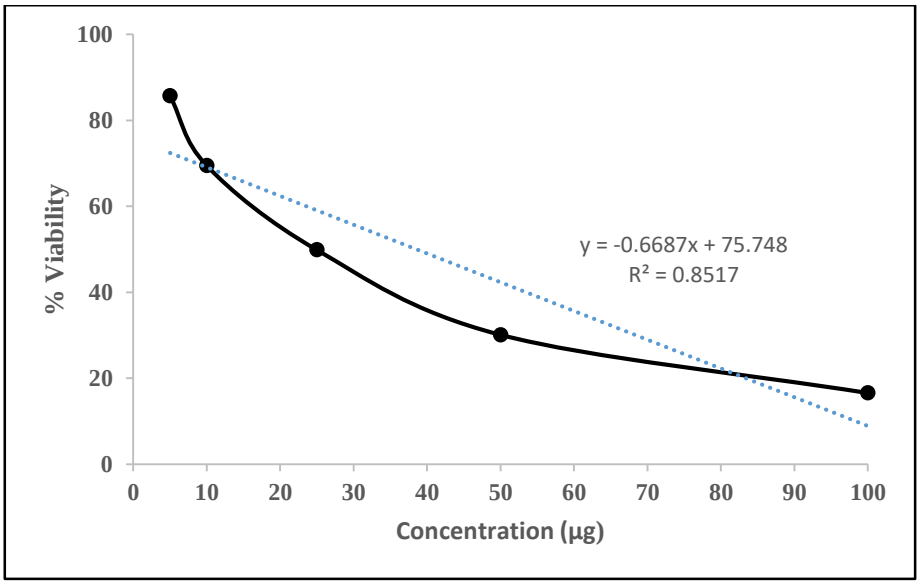

Fig.8: Cytotoxicity effect of F3 on growth of MCF-7 Breast cancer cell line.

DISCUSSION OF CURCUMIN LOADED NANOPARTICLES BY NANO PRECIPITATION

By employing nano precipitation technique, 10 formulations were prepared by varying organic to aqueous phase ratios with two polymers (Ethyl cellulose and Eudragit S 100) at various drug polymer ratios of 1:1, 1:2 and1:3 and organic to aqueous phase ratio of 1:5, 1:10, and 1:15. The prepared formulations are evaluated for % drug content, % product yield,% Entrapment efficiency and In vitro drug release studies and characterized for mean particle diameter and zeta potential.

Increase in polymer concentration has shown better results when compared with the lower concentrations. The formulation having higher drug to polymer ratio (1:3) was observed to have high %drug content, % entrapment efficiency, and also particle size is decreasing and maximum drug release was observed which is considered to be good.

Effect of Organic to aqueous phase ratio

Trials were made to know the impact of org:aq phase ratio upon the evaluation parameters. That is why one formulation was prepared less than the 1:10 and one formulation was prepared above the 1:10 ratio, two trails were made. But, 1:10 ratio is considered as the perfect org: aq phase ratio for the formulation of NPs.

Formulations F1 to F6 the org: aq ratio was 1:10, and F7, F8 the org: aq phase ratio was 1:5, F9 and F10 was 1:15. In this, 1:5 organic: aqueous phase ratio is not considered as the best ratio for the preparation of NPs, the drug release is observed to be very very poor. Upon increasing the ratio from 1:5 to 1:10, it is giving good drug release with the drug release of 46.11%, with good drug content of 99%, %entrapment efficiency of 98.23%, good particle size (219.4nm), zeta potential value (-47.1mv), also 1:15 org: aq phase ratio is considered to be very high, which is showing negative impact upon the % drug release.

Comparison of polymers (Eudragit S 100 and Ethyl cellulose)

On comparison of polymers Ethyl cellulose and Eudragit S 100 in each ratio, Eudragit S100 was considered as the best polymer in case of Nano precipitation technique as the formulation prepared using Eudragit S 100 showed minimum particle size, maximum drug content, Entrapment efficiency and in vitro drug release with good stability.

The higher drug: polymer ratio 1:3 and at the 1:10 org: aq ratio the formulations have shown

the highest drug content, entrapment efficiencies in both the formulations F3 and F6 of ES100 and EC respectively.

But, the formulation prepared by the Ethyl cellulose was having the less drug content, low entrapment efficiency, poor drug release when compared with the formulation prepared by the Eudragit S 100.

CONCLUSION

Increase in polymer concentration has shown better results when compared with the lower concentrations. 1:10 ratio is considered as the perfect org: aq phase ratio for the formulation of NPs. The formulation prepared using Eudragit S 100 showed minimum particle size, maximum drug content, Entrapment efficiency and in vitro drug release with good stability.The formulation F3 containing the drug to polymer ratio 1:3 and org: aq ratio constant (1:10) showed better results.

Upon comparison, based on all parameters, F3 was considered to be best formulation F3 was considered to be the best formulation with good drug content of 99%, % entrapment efficiency of 98.23%, good particle size (219.4nm), zeta potential value (-47.1mv), In vitro drug release of 46.11% which was sustained up to 12 hours. The drug release kinetic studies of the best formulations indicated that the release of the drug followed Zero order kinetics and showed non-fickian diffusion mechanism.The anti-cancer activity was determined in vitro by MTT assay. The IC50 value of the given curcumin nanoparticle formulation F3 was found to be 38.5µg.The nanoparticles obtained were observed to have the better anti-cancer activity and the objectives were fulfilled.

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