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Qualitative And Quantitative Evaluation And Determination Of Kinetic Model For Release Of CUR SEDDS

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***Abstract*:**

Chitosan microspheres and Eudragit-coated chitosan microspheres of curcumin were prepared by the emulsion cross-linking method. These prepared microspheres were also evaluated for their various quality control parameters. The formulated microspheres were examined for particle size, shape, surface morphology, percentage yield, drug loading, entrapment efficiency (EE) and degree of swelling.
SEM photomicrograph of chitosan microspheres indicated that the cross-linked chitosan microspheres exhibited rough surface and spherical shape while SEM photomicrograph of Eudragit coated chitosan microspheres revealed smooth and spherical. The size of microspheres was found to increase (36.84µm to 77.25µm) with increase in chitosan concentration. Further thecoating with Eudragit also showed significant increase in the size of microspheres. The mean particle size of the coated microspheres increased from 93.27µm to 129.74µm, which may be due to the corresponding increase in the chitosan concentration that resulted in larger emulsion droplets.

Key Words: Release Kinetics, Spectroscopy, Chromatography, Models.

INTRODUCTION

Colonic formulations should be designed in such a way that they provide a 'burst release' or sustained/ prolonged release when they reach the colon. Designing a formulation depends upon various factors like pathology, pattern of disease and affected parts of the lower GIT or physiology and physiological composition of healthy colon if formulation is not intended for localized treatment, physiochemical and biopharmaceutical properties of the drug like solubility, stability and permeability at intended site of delivery and the desired release profile of active ingredient.
Presently, the inflammatory diseases like chronic colitis, ulcerative colitis and Crohn's diseases are treated with glucocorticoids namely dexamethasone and methyl prednisolone and many anti- inflammatory drugs by oral and parenteral route produces systemic side-effects including adenosuppression, immunosuppressant, cushinoid symptoms and bone resorption. Therefore, if colon targeted drug delivery system is used, it decreases the therapeutic dose and reduces the systemic side-effects caused due to high doses. Conventional therapies for

colon diseases are not very effective, as the drug does not reach the site of action at therapeutic concentration. Therefore, this treatment requires relatively large doses to compensate drug loss during passing GIT which causes undue side-effects. Due to this, colon-specific drug delivery system is required.

MATERIALS AND METHODS

MATERIALS

Curcumin was received as a gift sample from Asoj Soft Caps Private Limited Halol - Vadodara Rd, Halol, Khandiwada, Gujarat, India. Chitosan (low mol. wt., viscosity 20-200 cP) and Eudragit S100 (Evonik Rohm Pharma, Germany, Viscosity 50–200 mPa s, mol. wt. 135,000) were received as a gift sample from Matrix laboratory, Hyderabad. The deacetylation degree of chitosan according to the specifications from the provider was higher than 80%. Span 80 (Mol. wt. 428.6, viscosity 1200-2000 mPa s) and liquid paraffin, anhydrous zinc chloride, carboxy methyl cellulose were purchased from Loba chemie Mumbai, India. All chemicals and reagents used were of analytical grade. Double distilled water was used throughout the study.

METHOD

Experimental

Determination of kinetic model for release of CUR SEDDS

Different kinetic models were applied on release data for categorizing the kinetics of drug release(Table 1). On the basis of correlation co-efficient the release data seem to better fit with Higuchi model, thus suggested diffusion as the mechanism for drug release. Among the Eudragit coated CUR- chitosan microsphere formulations the F6 showed the best release pattern with highest r^2 value of 0.9404. Further the release data was fitted for release exponent of Peppas model which confirmed that the formulations showed super case II diffusion kinetics. The kineticprofiles of F5-F8 formulations are shown in Fig. 1, 2, 3 and 4.

Table 1: Release kinetics for dissolution data of different formulations (F5- F8)

Formulation Code	Correlation co-efficient R^2 value				
	Zero order	**First order**	**Higuchi model**	**Peppas model**	**N**
F5	0.8904	0.7707	0.9254	0.8682	1.054
F6	0.9093	0.8254	0.9404	0.8693	1.026
F7	0.9044	0.8218	0.9349	0.8655	1.069
F8	0.8955	0.8311	0.9229	0.8719	1.030

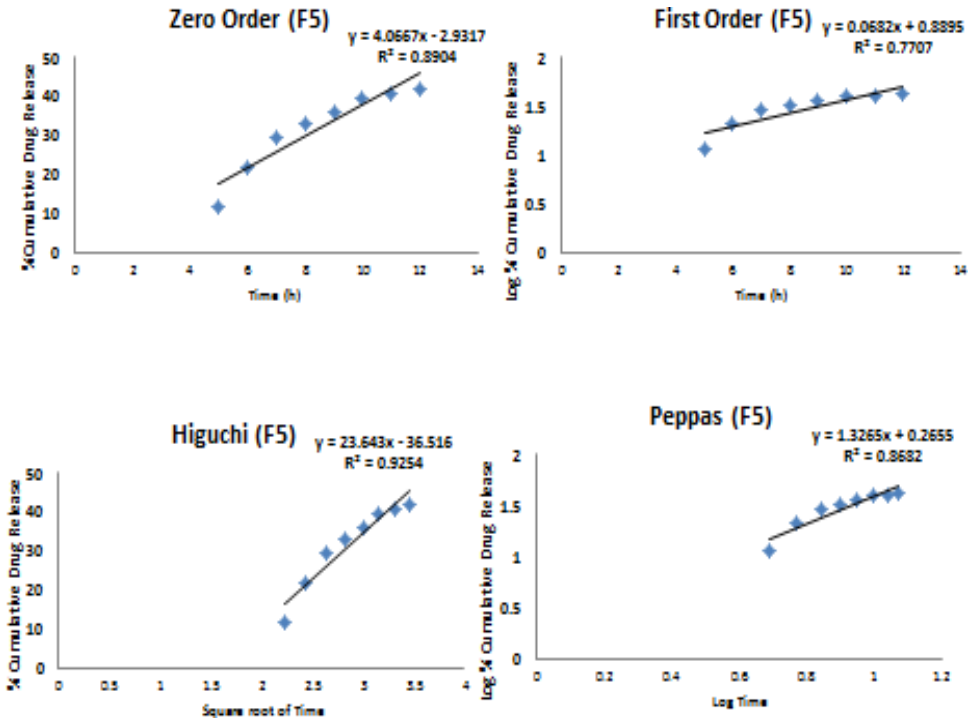

Fig. 1: Kinetic Profile for *in-vitro* release of F5 formulation

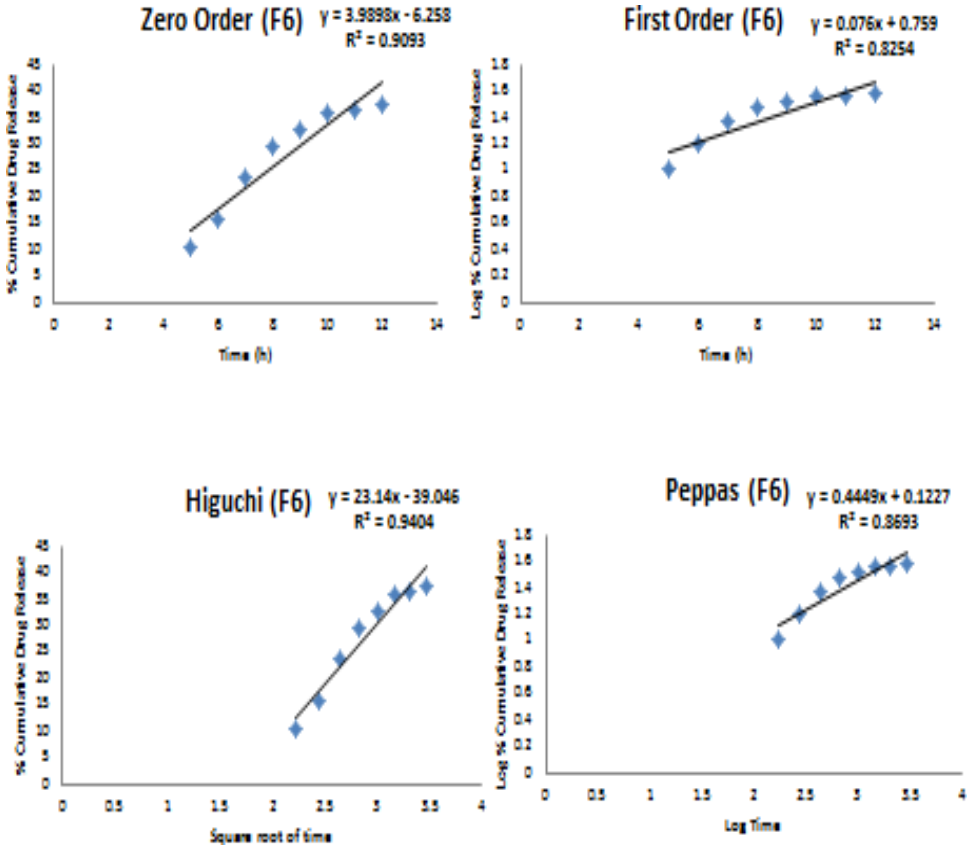

Fig. 2: Kinetic Profile for *in-vitro* release of F6 formulation

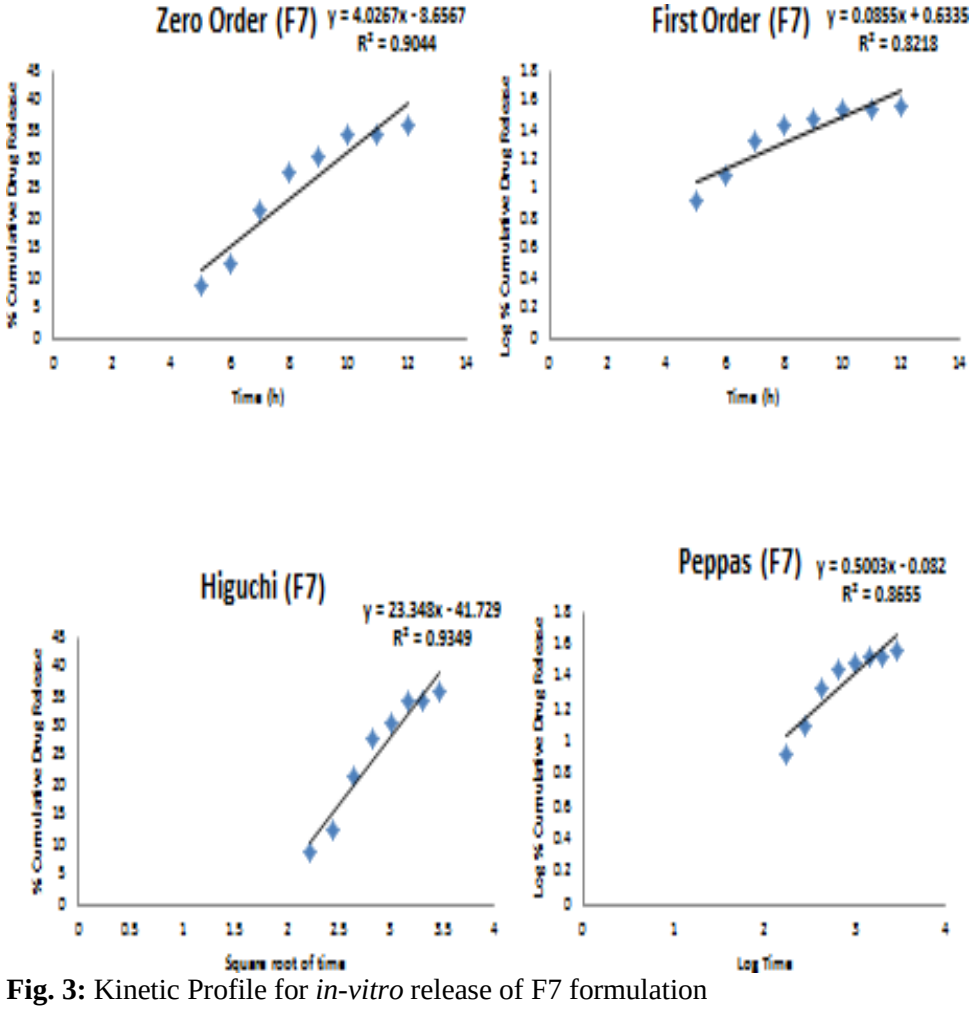

Fig. 3: Kinetic Profile for *in-vitro* release of F7 formulation

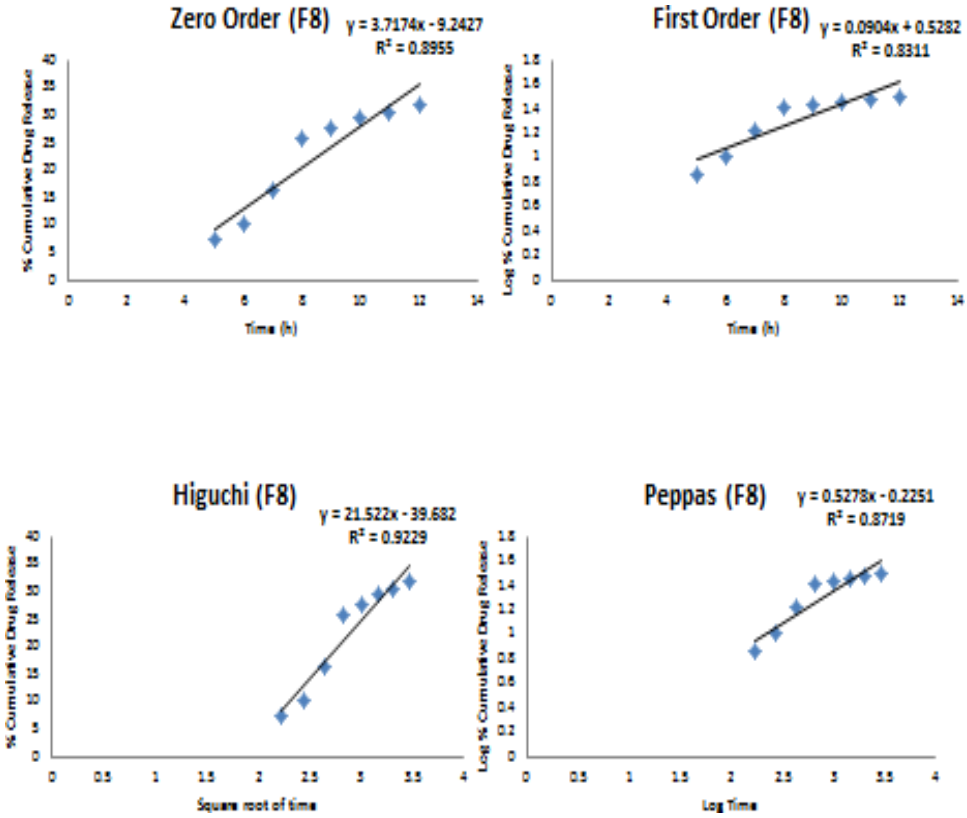

Fig. 4: Kinetic Profile for *in-vitro* release of F8 formulation

Thin Layer Chromatography

Thin Layer Chromatography of Curcumin (SEDDS) was performed using mobile phase Chloroform: Methanol [9:1] and the result of Rf value is shown in Table 2. The Rf value was found to be 0.81±0.05.

Table 2: Rf value of Curcumin (SEDDS)

Thin layer Chromatography	Rf value (Observed)
Curcumin (SEDDS)	0.81 ± 0.05

(n=6**),** mean± S.D.

IR spectroscopy

IR spectra of curcumin and its metal complex are shown in Fig 5 and 6. The 1629 and 1603 cm^{-1} bands correspond to the mixtures of stretching vibrations of υ(C=C) and υ(C=O) in curcumin and they are red shifted to 1626 and 1588 cm^{-1} in the Curcumin (SEDDS) respectively.

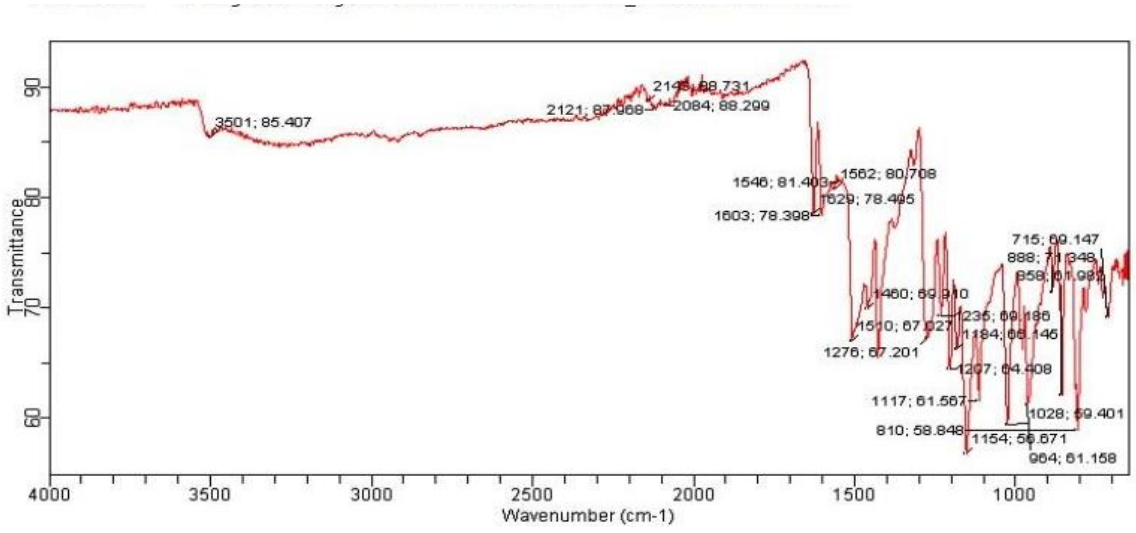

Fig. 5: IR spectra of Curcumin

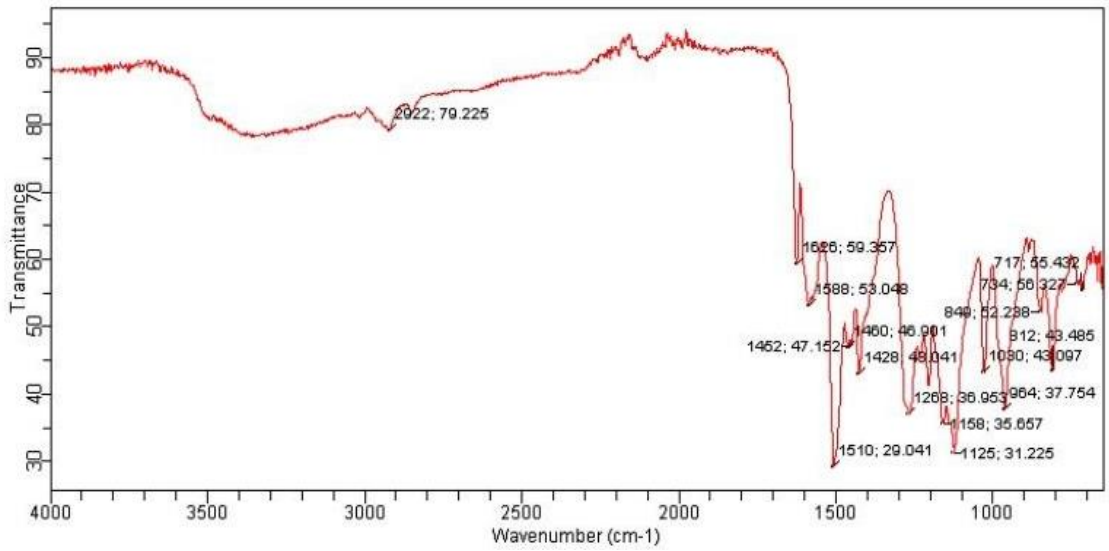

Fig. 5: IR spectra of Curcumin (SEDDS)

UV spectroscopy

UV spectra of curcumin and its metal complex are shown in Fig. 6 and 7. Their spectra aredeconvoluted with absorption band at 432 nm for curcumin, at 466.4 nm for curcumin (SEDDS) the formation of complex.

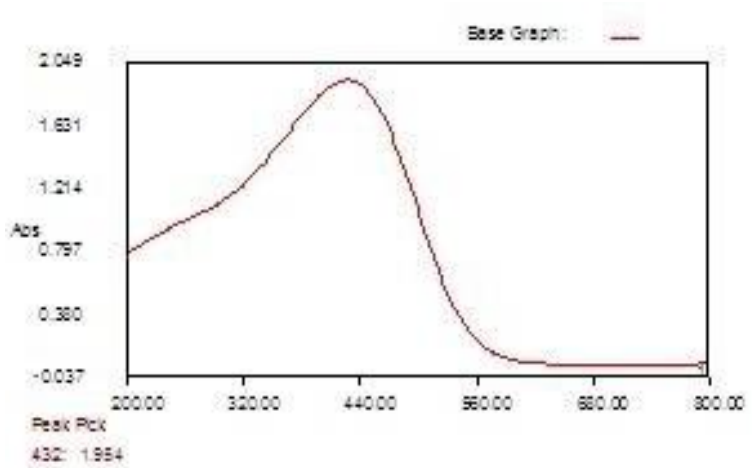

Fig. 6: UV spectra of Curcumin

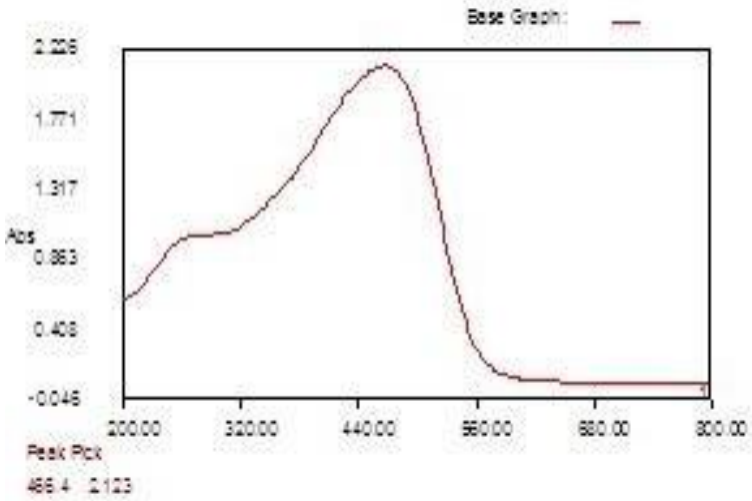

Fig. 7: UV spectra of Curcumin (SEDDS)

^{1}H NMR spectroscopy

^{1}H NMR spectrum of curcumin is shown in Fig. 8, and the spectra of Curcumin (SEDDS) is shown in Fig. 9. The detailed chemical shift assignments of ^{1}H NMR spectra are summarized in Table 10. It is clearly showing by upfieldshift of H_a and H_b (Fig11) due to diamagnetic isotropic effect because of the presence of zinc ion in close vicinity

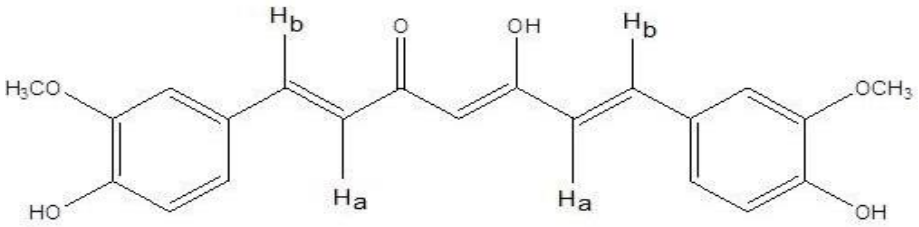

Fig. 8: Structure of Curcumin

Table 3: Chemical shifts of ^{1}H NMR spectra of Curcumin and Curcumin (SEDDS)

Peak	Pure Curcumin*		Curcumin (SEDDS) *	
	Δ value	J value (Hz)	Δ value	J value (Hz)
H_a	6.741(s)	16.1	6.70(s)	16.1
H_b	7.563(s)	16.1	7.56(s)	16.1
H-2	7.33(s)	-	7.272	-
H-5	6.83(d)	8.1	6.79(d)	8.1
H-6	7.157(d)	8.1	7.08(d)	8.1
OCH_3C-3	3.954(s)	-	3.840(s)	-
CH_2	2.6(s)	-	2.508(s)	-

*The chemical shift values are with reference to TMS = 0

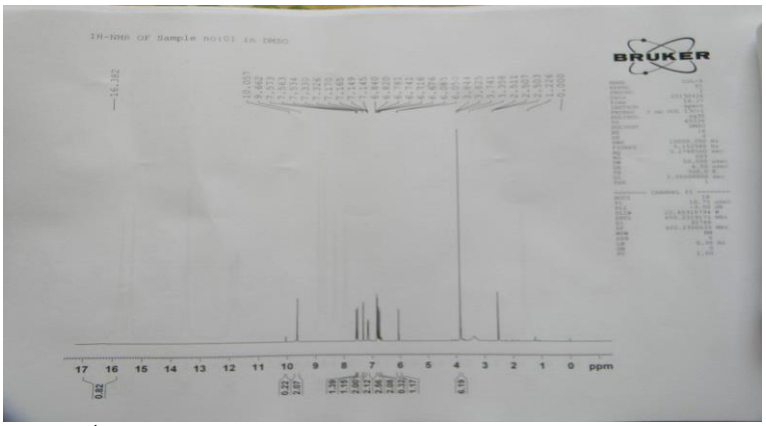

Fig. 9: ^{1}H NMR spectra of Curcumin in DMSO d_6

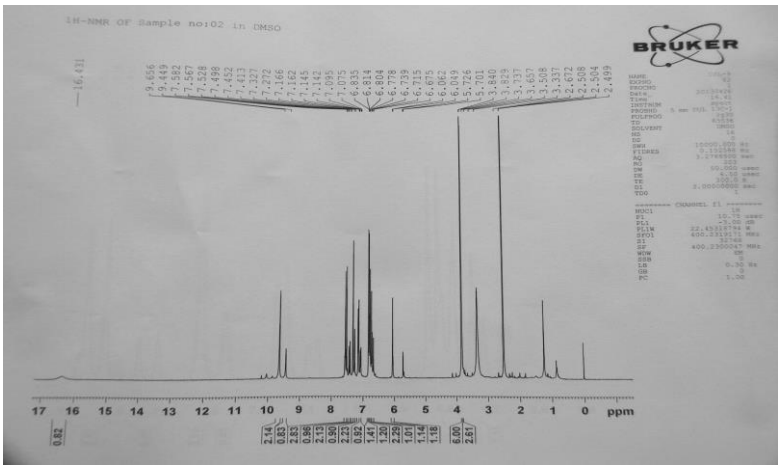

Fig. 10: ^{1}H NMR spectra of Curcumin (SEDDS) in DMSO d_6

Calibration Curve

The calibration curve of Curcumin-(SEDDS) in 0.1N HCl (pH 1.2), Phosphate buffer (pH 6.8) and Phosphate buffer (pH 7.4) at 466 nm has been shown in Fig. 3, 4, 5 and its absorption values are given in Table 4. The calibration curve of Curcumin (SEDDS) using HPLC at 254 nm has been shown in Fig. 3 and its observation table is in Table 4.

Chromatographic parameters of Curcumin (SEDDS) have been shown in Table 5. HPLC Chromatogram of Curcumin (SEDDS) and HPLC Chromatogram of Curcumin (SEDDS) in plasma has been shown in Fig 11.

Table 4: Observation table for standard curve of Curcumin (SEDDS) by UVSpectrophotometer

Sr. No.	Concentration (µg/mL)	Absorbance Phosphate buffer pH 7.4	Absorbance Phosphate buffer pH 6.8	Absorbance0.1N HCl pH 1.2
1	5	0.273±0.0015	0.242±0.0007	0.229±0.0012
2	10	0.439±0.0024	0.412±0.0019	0.398±0.0005
3	15	0.625±0.0004	0.587±0.0025	0.551±0.0028
4	20	0.815±0.0011	0.751±0.0014	0.709±0.0034
5	25	1.025±0.0037	0.927±0.0028	0.865±0.0017
6	30	1.203±0.0042	1.081±0.0036	0.999±0.0028

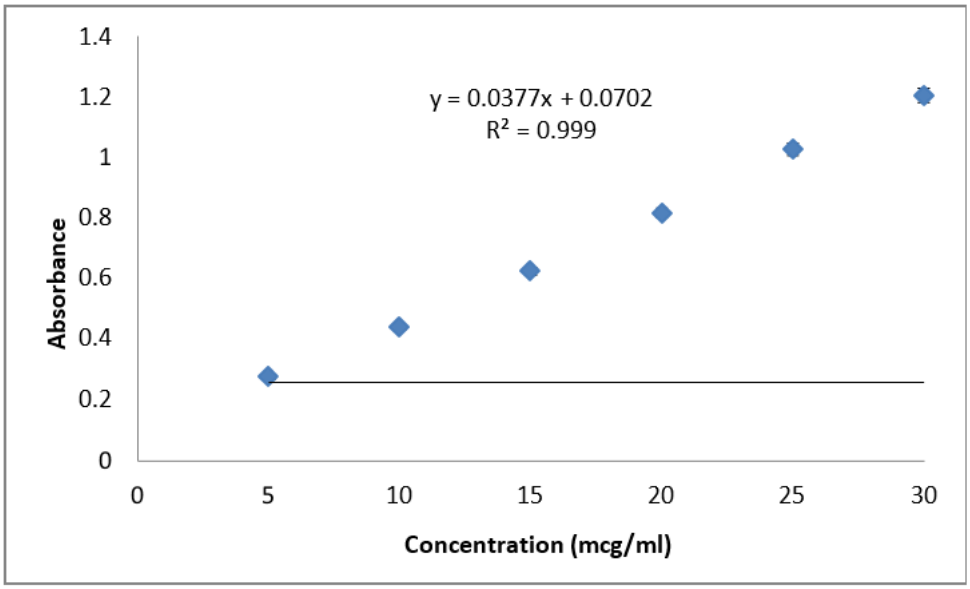

Fig. 11: Standard plot of Curcumin (SEDDS) in phosphate buffer (pH 7.4) by UVspectrophotometer at 466 nm

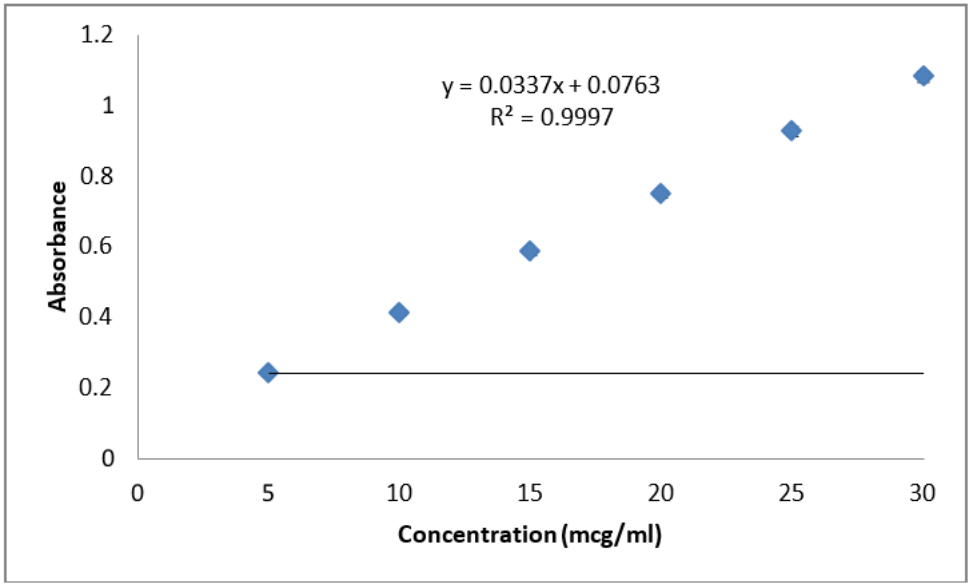

Fig. 12: Standard plot of Curcumin (SEDDS) in phosphate buffer (pH 6.8) by UVspectrophotometer at 466 nm

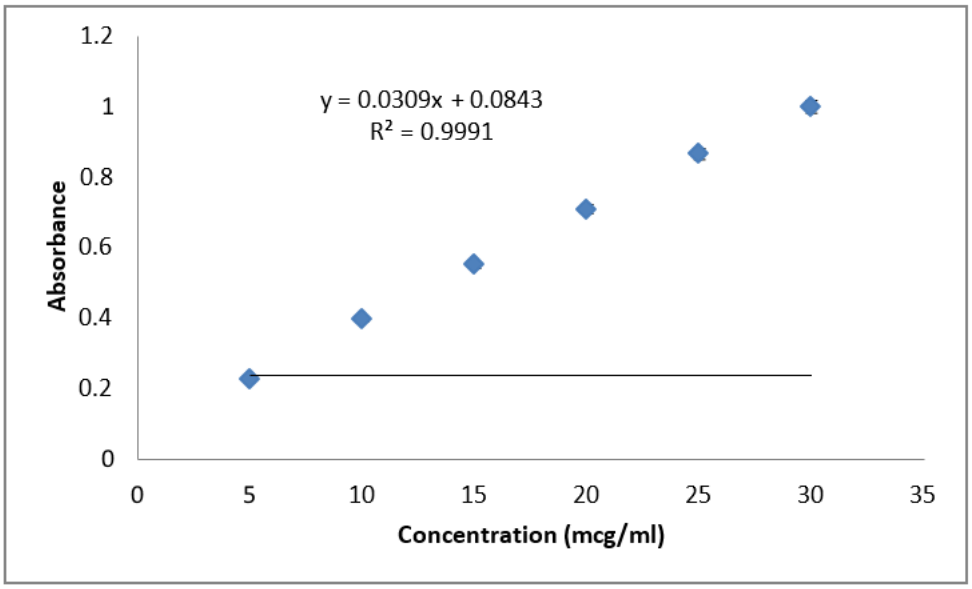

Fig. 13: Standard plot of Curcumin (SEDDS) in HCl (pH 1.2) by UV spectrophotometer at466 nm

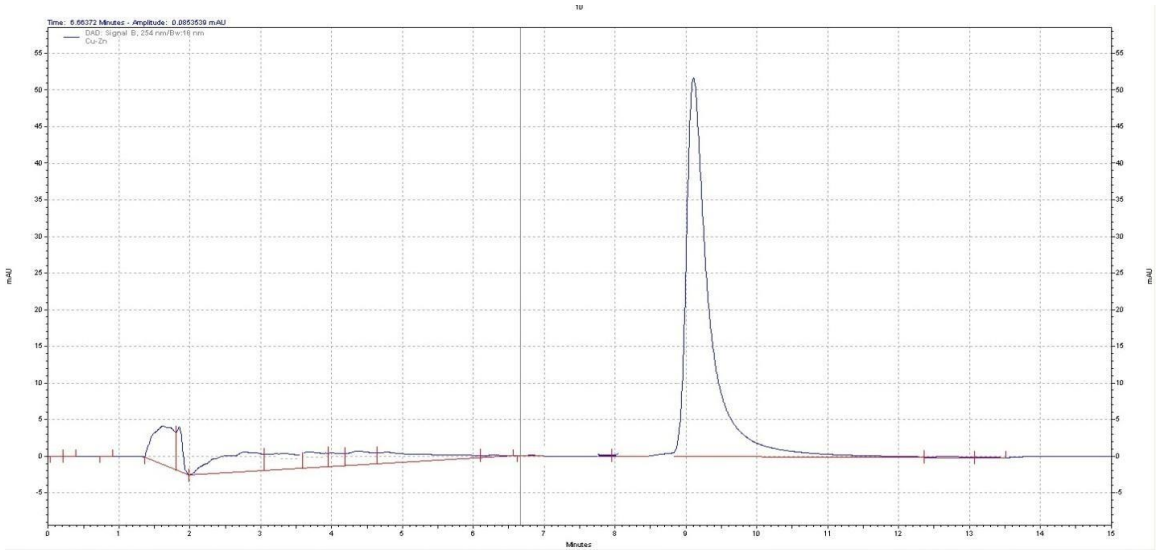

Fig. 14: HPLC Chromatogram of Curcumin (SEDDS)

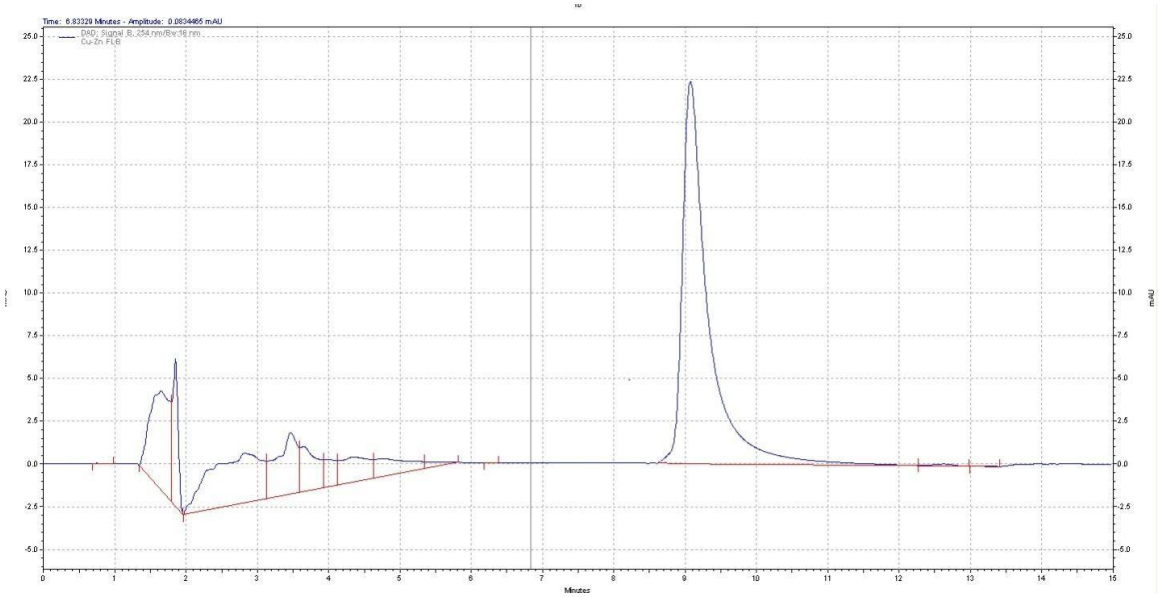

Fig. 15: HPLC Chromatogram of Curcumin (SEDDS) in Plasma

Table 5: Observation table for standard curve of Curcumin (SEDDS) by HPLC

Sr. No.	Concentration(µg/mL)	Area
1	2	36606
2	4	78576
3	6	120248
4	8	158957
5	10	202479
	Slope	20606
	Intercept	-4264.9
	Corr. Coeff.	0.9998

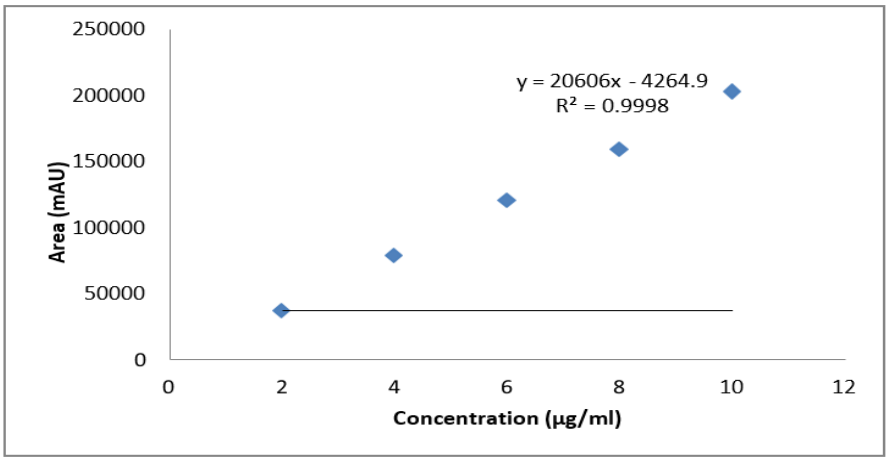

Fig. 16: Standard plot of Curcumin (SEDDS) by HPLC

Table 6: Chromatographic Parameters of Curcumin (SEDDS)

Sr. No.	Validation Parameter	Results	
1	Linearity range	2-10 µg/mL	
2	Regression equation	Slope	20606
		Intercept	-4264.9
3	Regression coefficient	0.9998	
4	Precision	%RSD first day	0.29947
		%RSD second day	1.549
5	Accuracy (%recovery)	SD	1.347
		%RSD	1.33
		Mean	101.49
6	LOD	0.058 µg/mL	
7	LOQ	0.1752 µg/mL	

Stability Evaluation

Stability study of curcumin and Curcumin (SEDDS) performed to analyze the kinetic degradation in phosphate buffer (pH 7.4) at 37º C. The results are shown in Fig. 18. The resultsof kinetic stability studies showed that curcumin degraded extensively with in 12 h while its metal complex with zinc exhibited good stability in the similar conditions (Table 17).

Table 17: Kinetic stability of Curcumin (SEDDS) compared to Curcumin

Sr. No.	Time (h)	% Residual	
		Curcumin	**Curcumin (SEDDS)**
1	0	100	100
2	2	82.44±1.23	98.23±0.52
3	4	70.55±0.69	97.23±0.36
4	6	62.45±2.45	95.45±0.21
5	8	54.19±0.75	94.78±1.08
6	10	50.48±1.16	92.58±0.83
7	12	44.89±0.42	92.41±0.41

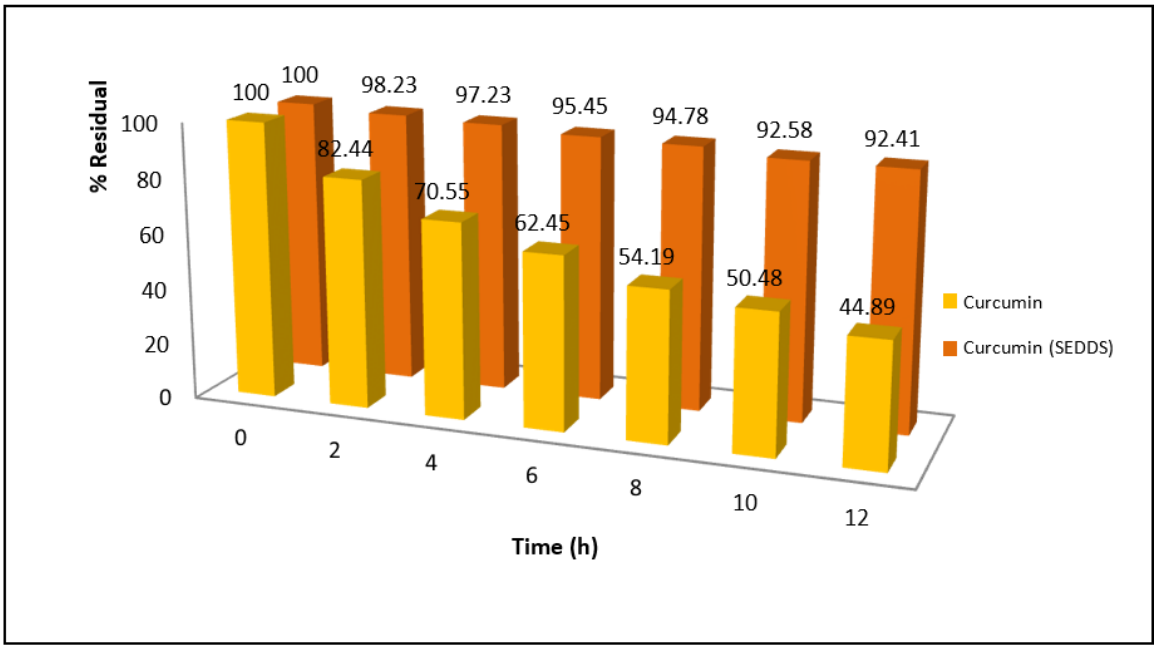

Fig. 18: Kinetic stability of Curcumin (SEDDS) compared to Curcumin alone inPhosphate buffer pH 7.4 at 37ºC

Conclusion

On the basis of best correlation co-efficient the *in-vitro* release data seem to fit better with Higuchi model, thus suggesting diffusion as the main mechanism for drug release. Among the various Eudragit coated microsphere formulations the F14 exhibited best pattern of release with highest value of r^2 (0.9963) and the release exponent of Peppas model revealed super case II diffusion kinetics.

In-vivo organ distribution study of optimized formulation (F14) was carried out in order to analyze its targeting potential in the colon and results suggested protection of microspheres in upper GIT and the drug was released after reaching to colon due to solubilization of Eudragit coating and microbial degradation of chitosan.

From in vivo results obtained it indicates that the pharmacokinetic parameters of Irinotecan hydrochloride conjugated nanoparticle capsule are better than Irinotecan hydrochloride unconjugated nanoparticles capsule and Irinotecan hydrochloride conjugated nanoparticle tablets are better than Irinotecan hydrochloride capsule.

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