Studies on β-CD Complexation of a Poorly Soluble Drug

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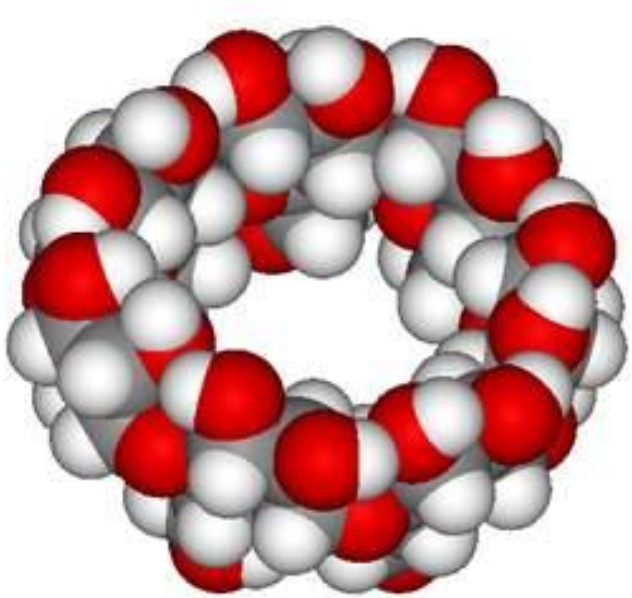
Cyclodextrins are physically chemically stable macromolecules produced by enzymatic degradation of starch with a hydrophilic outer surface and lipophilic central cavity.

Three types of naturally occurring CDs.

- α-
- β-
- γ-

Here in this study β-CD was used for complexation



Why β-CD

- **Most accessible**
- **The lowest priced**
- **Orally very small amounts get absorbed in the upper intestinal tract (1-2%)**
- **Metabolism resistant in the upper intestinal tract**
- **Metabolized by bacteria in the caecum and colon**
- **The best studied CD in humans**
- **Listed in a number of Pharmacopeias including US Pharmacopeia , European Pharmacopeia and National Formulary**

MARKETED PREPARATIONS

Rofizgel	**Tab Rofecoxib**	**Wockhard India**
Betahist	**Tab Betahistidine**	**Genopharm India**
Mesulide fast	**Tab Nimusilide**	**Novartis Europe**
Prostomom E	**Sublingual tab PGE2**	**Ono Japan**
Nicorette	**Sublingual tab nicotine**	**Pfizer**
Flogene	**Suppository piroxicam**	**Ache , Brazil**
Meiact	**Tablet cephalosporin**	**M Seiko Japan**
Ombeta	**Tablet omeperazole**	**Beta form, Europe**
		(Agarwal R, et al., 2012)

β- CD COMPLEXATION OF FEW ANTI CANCER DRUGS

Methotrexate	**Melanoma.**	**Enhancement of aqueous solubility and bioavailability**
Exmestane	**Breast cancer**	**Increase in solubility, improvement in bioavailability and dissolution.**
Imitinab.	**Chronic leukemia**	**Enhancement of solubility**
Flutamide	**Prostatic carcinoma**	**Enhancement of solubility dissolution.**
Oridonin	**Esophageal and cardiac cancer**	**Enhancement of bioavailability**
		(Gidwani Bina ,et al.,2015 BioMed research International)

OBJECTIVE OF THE STUDY

- **To improve solubility and dissolution properties of Clarithromycin by inclusion complex formation with β-CD.**
- **CLARITHROMYCIN** : A practically water insoluble drug / an oral semi synthetic macrolide antibiotic effective against *Mycobacterium avium complex* (MAC) also used to treat gastrointestinal *Helicobacter pylori. (Howden CW et al., 1998)* and treatment/maintenance therapy of toxoplasmosis. *(Essential Medicines and Health Products Information Portal)*

Reported solubility = 0.33mg/ l *(Martin A, 2003)*

Bioavailability = 50%

(Essential Medicines and Health Products Information Portal)

PLAN OF WORK

- Characterization of drug
- Analytical methodology

  Preparation of calibration curve
- Preparation of drug-βCD complexes in different ratios using different methods
- Evaluation of inclusion complexes by

  Saturation solubility study

  Dissolution study

  Phase solubility study
- Interaction studies

  Using FTIR

  Using UV spectroscopy
- Surface morphology studies

  using SEM (Scanning electron microscope)

EXPERIMENTAL WORK

RESEARCH DESIGN AND METHODOLOGY

Characterization of drug

By physical characters

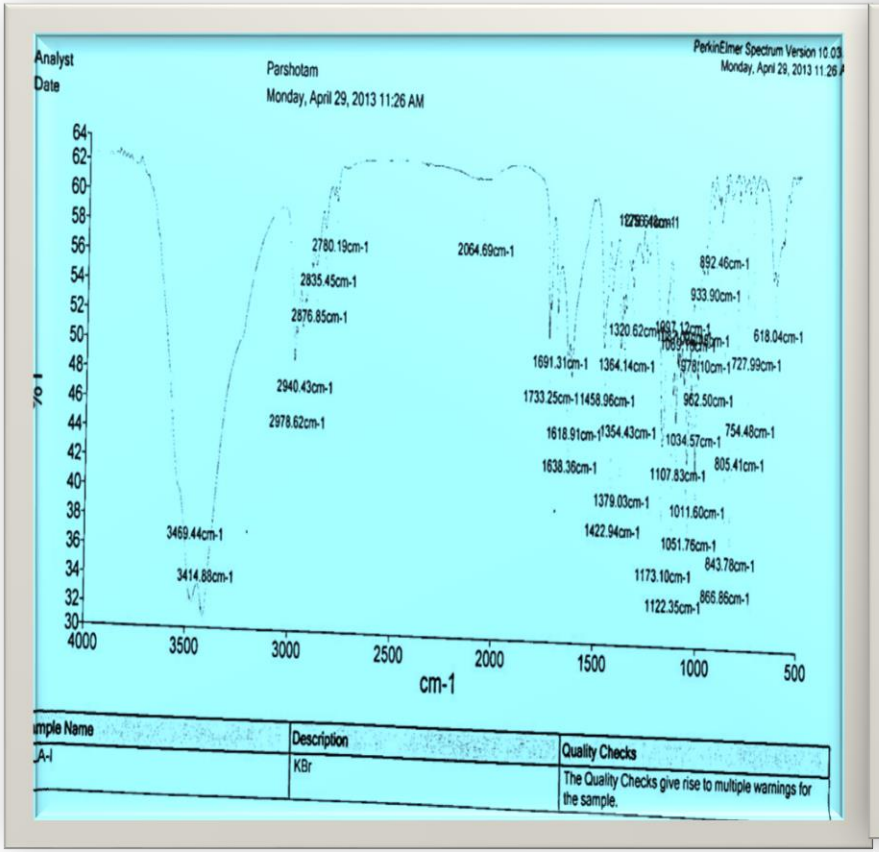
Nature	Crystalline
Color	white
Odor	odorless
Melting point	(218^{0}C) in conformity with the range of 217-220^{0}C (using Remi MP1, 2/95)
Taste	Bitter
Solubility	Solubility(freely soluble in acetone chloroform and ether, sparingly soluble in alcohol, Soluble in 0.1 N HCl and practically insoluble in water)
FTIR spectral study	
UV study	

Characterization of Drug by FTIR

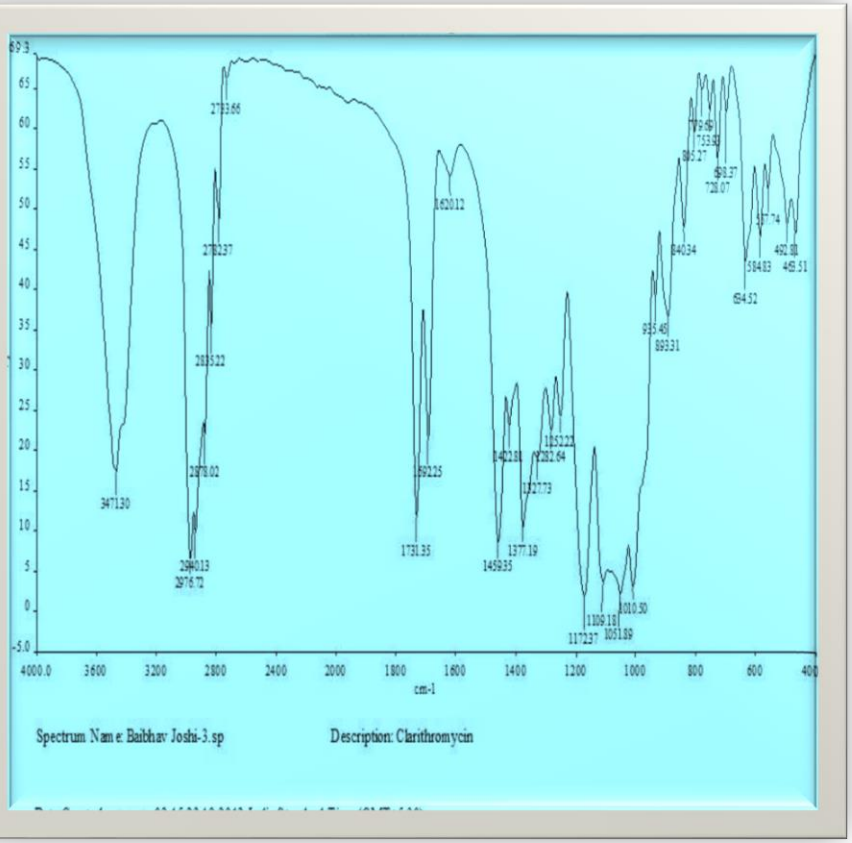
Reference wave number of CLA (cm^{-1})	Observed wave number of CLA (cm^{-1})	Assignment of functional group
1692	1691	C=O
1732	1733.25	Lactone carbonyl
1422	1422.94	N-CH_3
2780-3000	2780.19- 2978.62	Alkane stretching peaks
3450	3469.44	Hydrogen bonds between OH groups
1000-1200	1011.60-1275	C-O-C stretches
1340-1400	1364.14- 1422.94	CH_2 groups

Characterization of Drug by FTIR

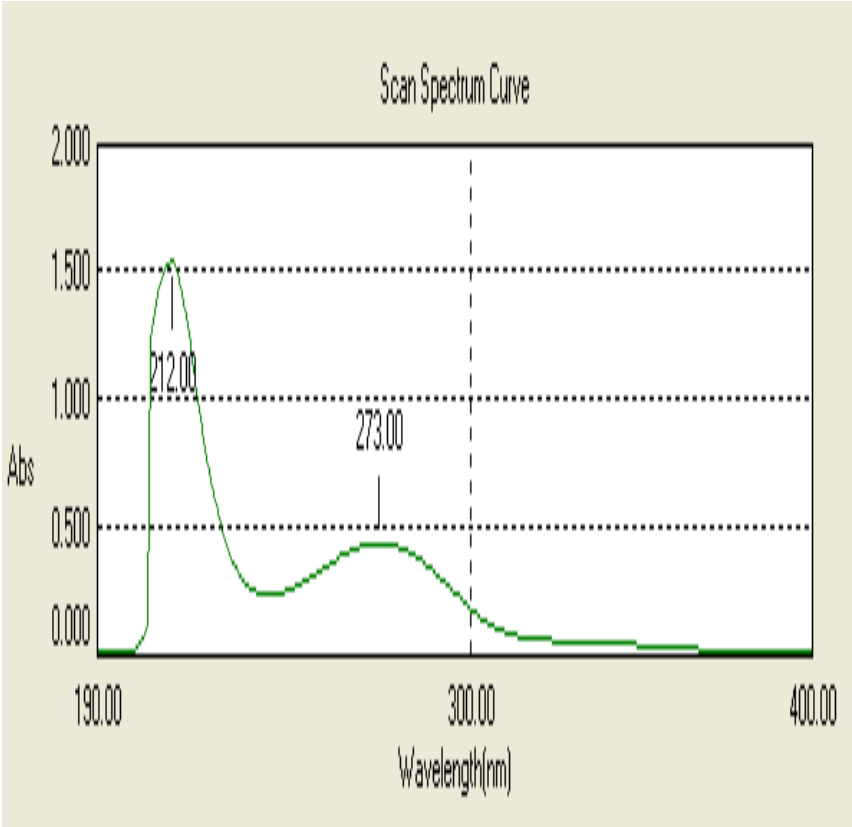
Spectra of pure CLA



Reference spectra of CLA

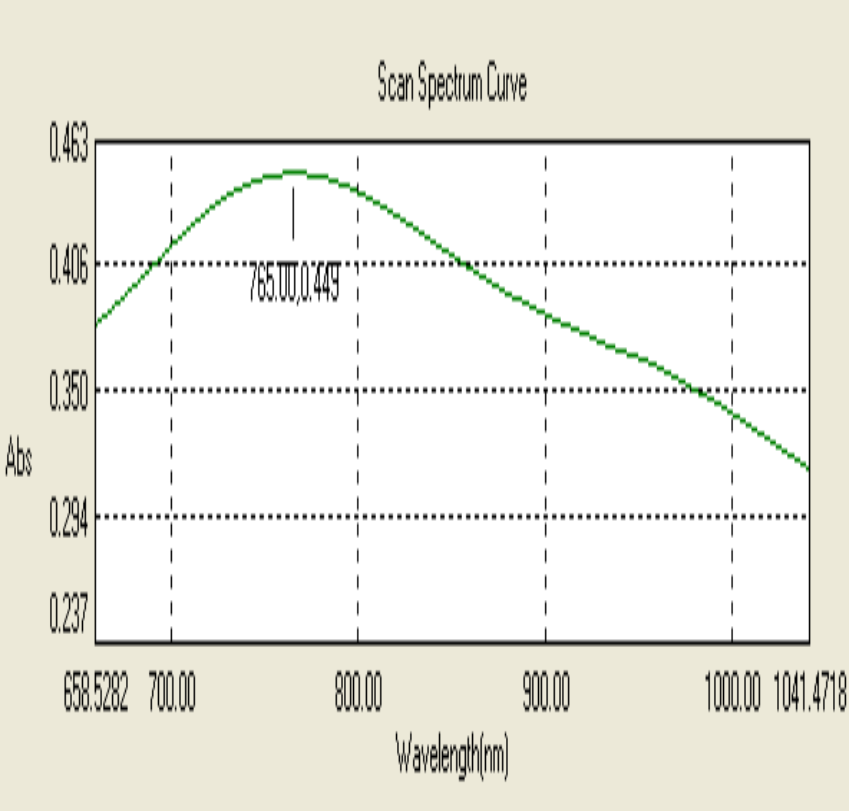


Characterization of Drug by UV spectral analysis



(Spectra in 0.1 N HCl λ max 273nm)

Spectra in 0.1 N HCl with FCP reagent (λ max 765 nm)



OBSERVATIONS

Observed λ max in 0.1 N HCl	**273 nm**
Reported λ max in 0.1N HCl	**273 nm** ***(Bhopal A et al., 2012***
Observed λ max in 0.1 N HCl using FCP reagent	765 nm
Reported λ max in 0.1N HCl	**765 nm** (*Nangude S et al.,2013)*
These observations confirm true identity of the drug Clarithromycin	

ANALYTICAL METHODOLOGY

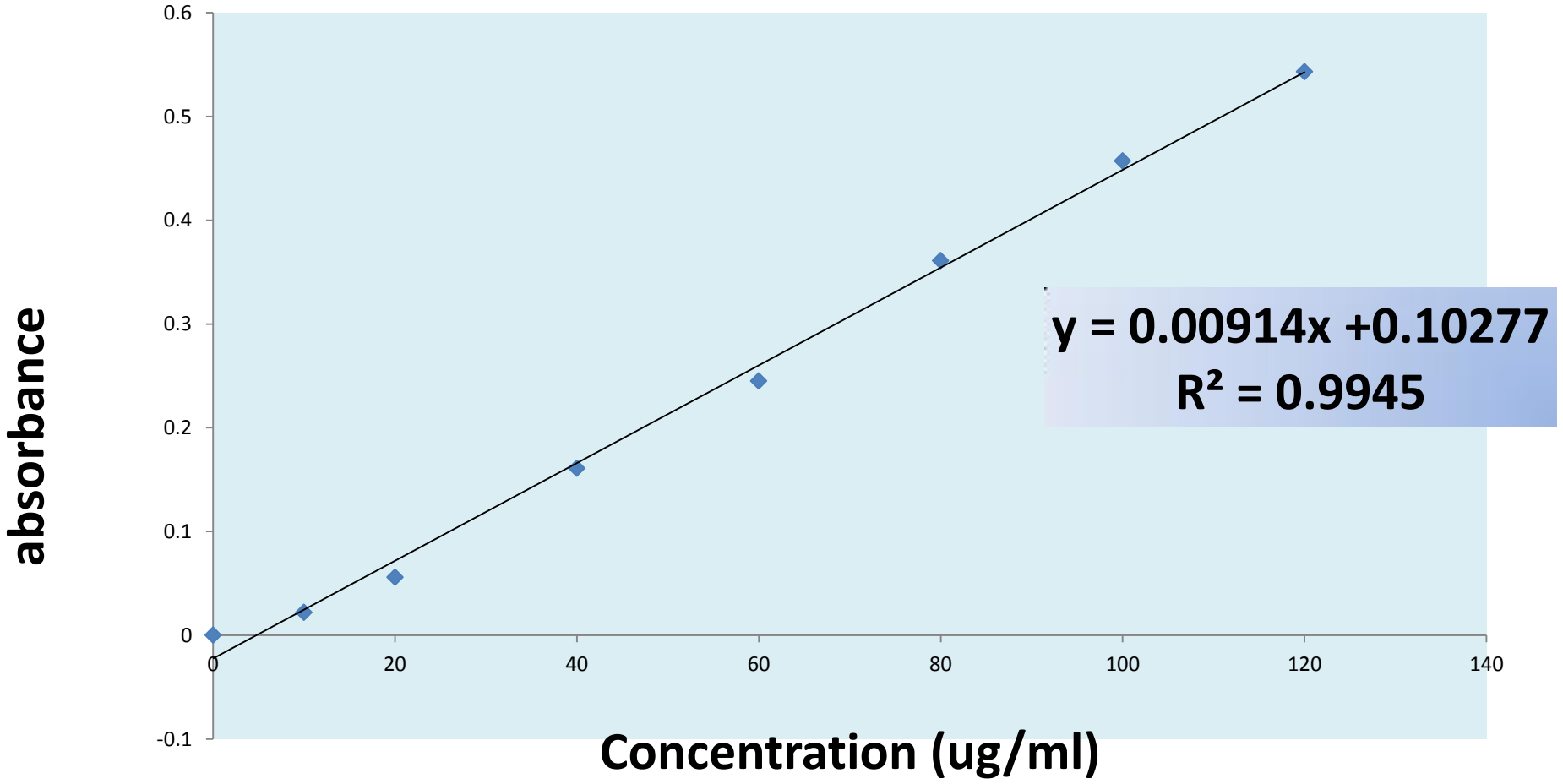
Preparation of calibration curve of Clarithromycin using 0.1N HCl and FCP reagent

- To 10ml of 2mg/ml solution of Clarithromycin in 0.1N HCl, 20 ml of FCP reagent and 20 ml of sodium bicarbonate (25% w/v) was added.
- The flask containing the above solution was immersed in hot water for 15-20 minutes. Dilutions in the range of 10-120 (µg/ml) were made with 0.1N HCl and subjected to UV analysis at 765 nm.

Table 1: CALIBRATION CURVE OF CLA IN 0.1N HCl USING FCP REAGENT

Conc (µg/ml)	Mean absorbance at 765 nm (n=3) (± SD)	Regressed absorbance
10	0.02 ± 0.017	0.023
20	0.056± 0.043	0.054
40	0.161± 0.035	0.162
60	0.245± 0.056	0.242
80	0.36± 0.046	0.360
100	0.457± 0.004	0.458
120	0.543± 0.043	0.541

Fig. 1: Calibration curve in 0.1N HCl using FCP reagent



PREPARATION OF DRUG β-CD COMPLEXES

METHOD OF PREPARATION	RATIOS TAKEN	FORMULATION CODES
KNEADING	1:1	K1
	1:2	K2
SOLVENT BLEND METHOD	1:1	SB1
	1:2	SB2
CO SOLVENT EVAPORATION METHOD	1:1	CSE1
	1:2	CSE2

EVALUATION OF DRUG β-CD COMPLEXES

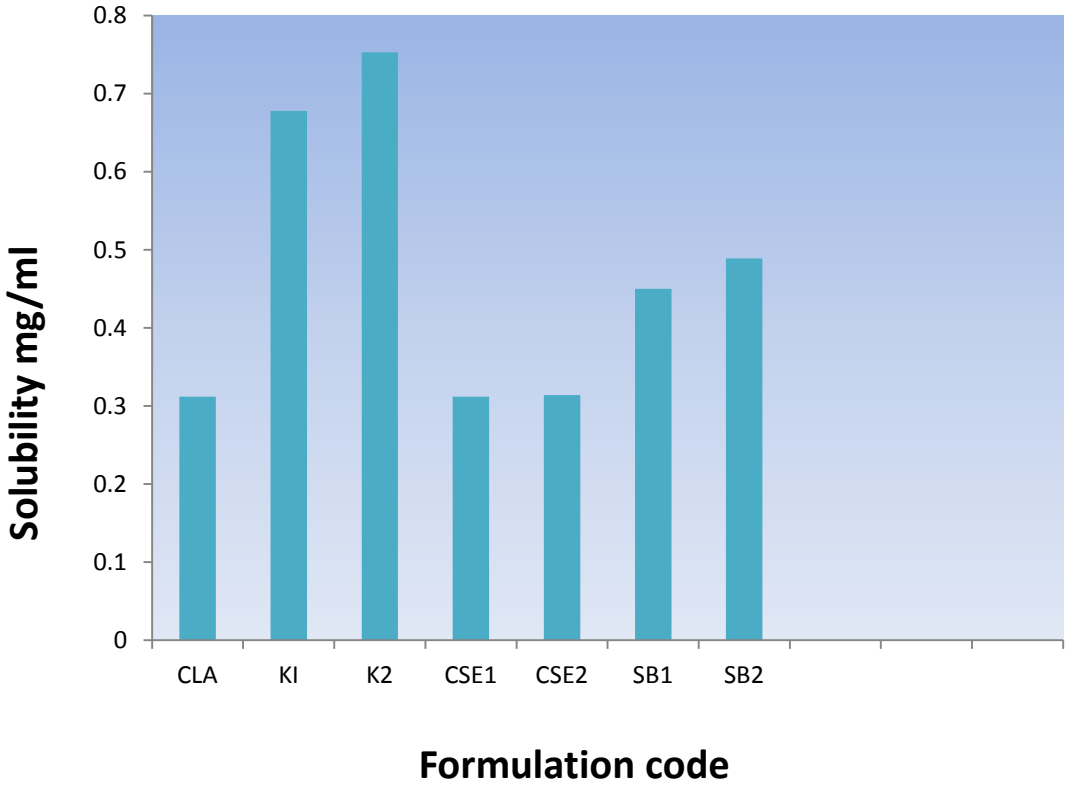
SATURATION SOLUBILITY STUDIES *(Ibrahim A et al., 2010).*

- Excess of pure drug and prepared drug β-CD complexes were separately taken in 25 ml of conical flasks, to which 15 ml of distilled water was added .
- Flasks were shaken for 48 hours at 37^0 C $\pm$ 0.5^0 C in orbital shaker at 90 RPM
- Samples were kept overnight and then filtered using Whatmans Filter.
- Absorbance of the filtrate of the pure drug and prepared CD complexes were measured at 765 nm using FCP reagent.

DISSOLUTION STUDIES *(Jill B et al., 2010)*

- 125 mg of the pure drug and 125 mg equivalent weight of the CLA β- CD complexes were taken in empty gelatin caps (00) and subjected to dissolution in 900 ml of 0.1N HCl at a temperature of $37^0 \pm 5^0$C using USP type II dissolution apparatus at 75 RPM.
- At fixed time intervals, 5 ml of sample was withdrawn, filtered and assayed for drug content at 765 nm using FCP reagent.
- Volume of the dissolution medium was adjusted to 900 ml by replacing each 5ml aliquot withdrawn with 5 ml of fresh 0.1 N HCl.

Saturation solubility study



Formulation code	Solubility mg/ml
CLA	0.312
K1	0.678
K2	0.753
CSE1	0.312
CSE2	0.314
SB1	0.450
SB2	0.489

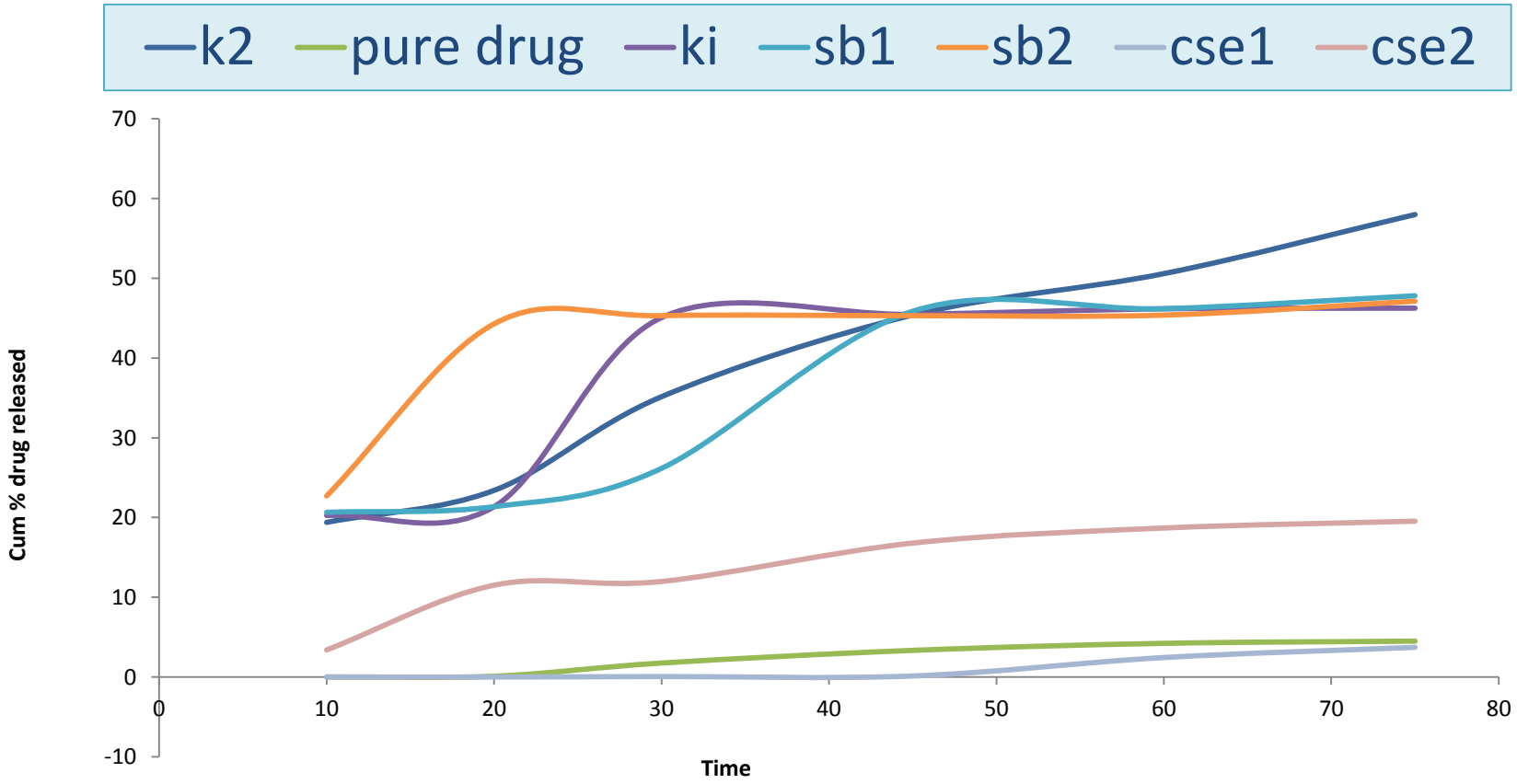
OBSERVATIONS

- **Promising increase in solubility was observed in case of K1 and K2 followed by SB2 and SB1 method.**
- **Co solvent evaporation method showed no significant improvement in solubility**

CUMULATIVE %DRUG RELEASE OF CLA AND DIFFERENT β- CD COMPLEXES IN DISSOLUTION STUDY

TIME	PURE DRUG	K1	K2	SB1	SB2	CSE1	CSE2
10	NO REL	20.25	19.39	20.65	22.70	NO REL	3.39
20	0.096	21.36	23.4	21.36	44.28	NO REL	11.51
30	1.75	45.07	35.15	26.16	45.31	0.56	11.98
45	3.34	45.47	45.39	45.86	45.31	0.127	16.79
60	4.22	46.17	50.58	46.18	45.38	2.44	18.68
75	4.50	46.25	57.99	47.80	47.12	3.72	19.54

DISSOLUTION STUDIES



Cumulative % drug release at different time intervals for pure CLA and different β-CD complexes

OBSERVATIONS

- The inclusion complexes of K_1, K_2, SB_1 and SB_2 show almost same pattern of drug release i:e from 19.39 – 22.7 % in first ten minutes of dissolution study.
- The drug release was increased up to 57.99 % in case of K_2 in 75 minutes which is the highest release. K_1 method showed a release of 46.25 %.
- The cumulative % drug release pattern can be shown as follows:

$$K_2 > SB_2 > SB_1 > K_1 > CSE_2 > CSE_1 > CLA$$

PHASE SOLUBILITY STUDIES

- An excess amount of CLA was added to aqueous solution, containing increasing β-CD concentration (0.25-2.0 mM) in stoppered conical flasks.
- Samples were shaken at room temperature in an orbital shaker at 100 RPM for seven days.
- After shaking the solutions were filtered
- Absorbance was measured at 765 nm using FCP reagent
- The apparent stability constant K_C of drug and β-CD complex was calculated according to the following equation (Higuchi and Connors).

$$KC = \frac{Slope}{S0\ (1 - Slope)}$$

y=0.9867x-0.1309,

R^2=0.9412,Slope=0.9867, Intercept=0.1309,

Kc=2768.20

Where,

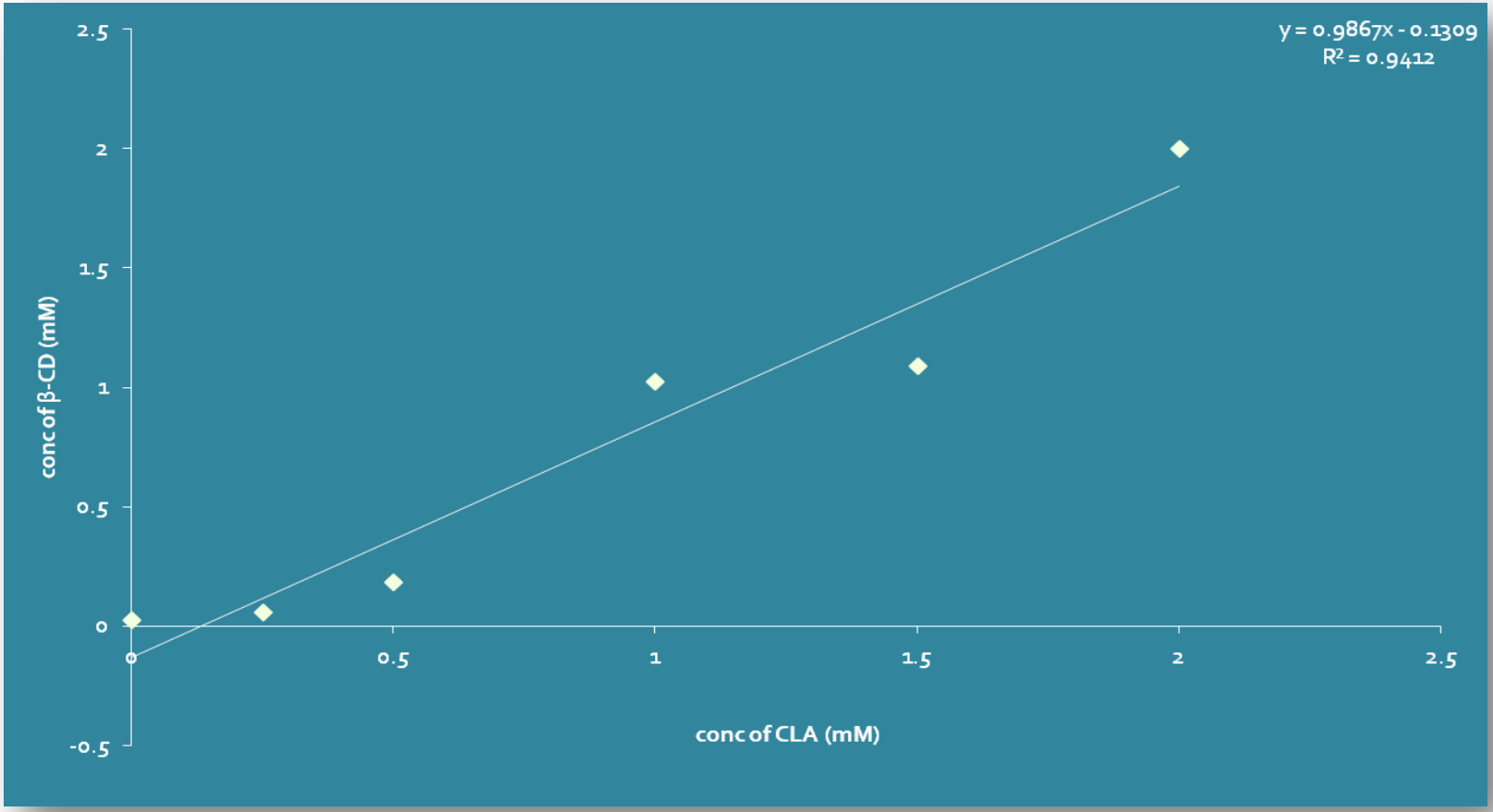
- K_C = Apparent Stability constant
- S_0 = Intrinsic solubility
- Slope is obtained from linear portion of phase solubility diagram *(Rajshree S et al., 2009. Liu X et al., 2003).*

PHASE SOLUBILITY STUDY

VIAL NO	β-CD	CLA (Conc. in mM)
1	0.00	0.0268
2	0.25	0.0614
3	0.50	0.187
4	1.00	1.0280
5	1.50	1.0900
6	2.00	2.0013

Phase solubility diagram of CLA and β-CD (0-2.0mm) aqueous solution

K_{C}= 2768.20



PHASE SOLUBILITY STUDY

R^2=0.9422, Slope >1

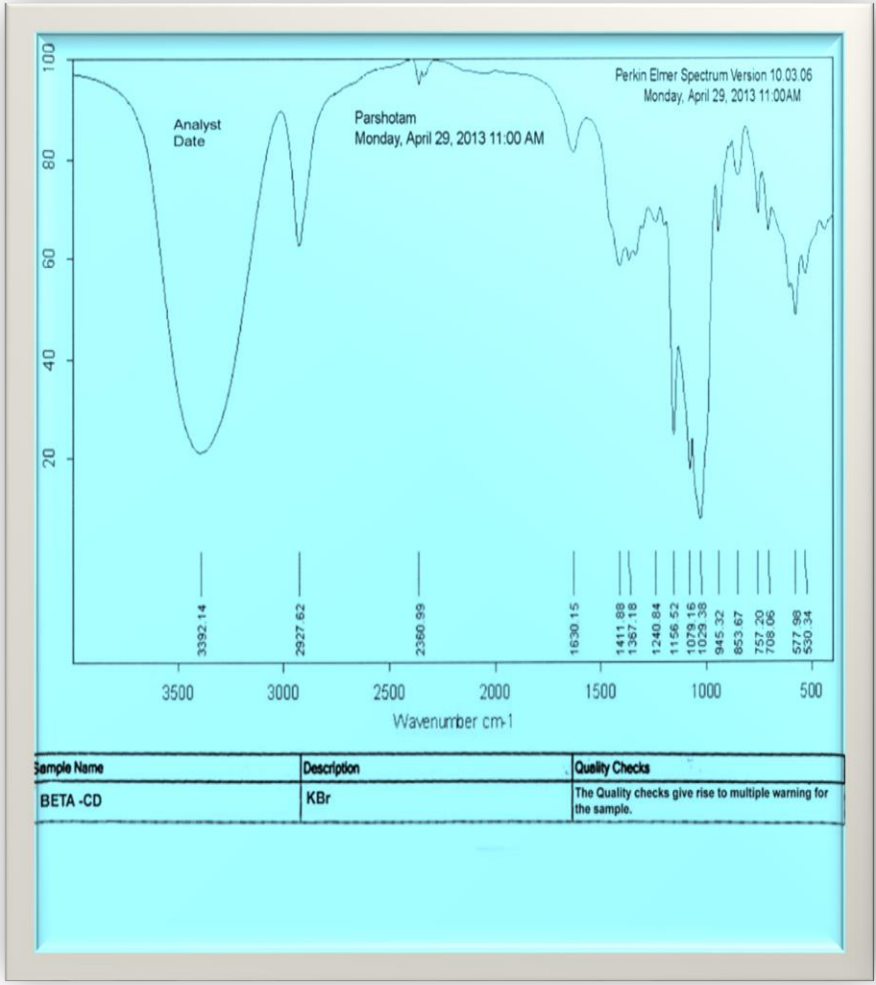
OBSERVATIONS

$$KC = \frac{\text{Slope}}{S0\ (1 - \text{Slope})}$$

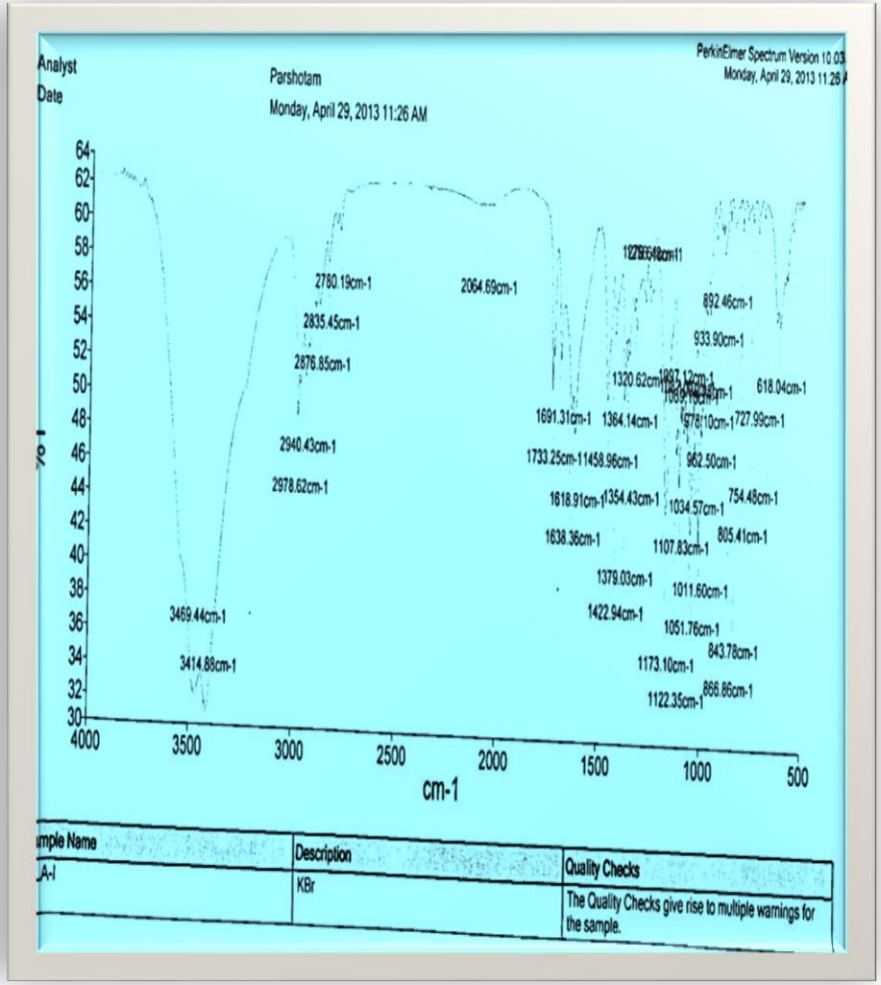
- **K_C = 2768.20** indicates that the inclusion complex of CLA and β-CD is quite stable and moderate.
- Phase solubility curve here can be classified as type **$A_{L.}$** Value of slope which is less than one (0.9867) indicates formation of 1:1 complexes but at the same time does not exclude the formation of higher order complexes *(Martin DVM, 2004Takahashi I Andrea et al., 2012)*

FTIR PATTERNS

β- CD

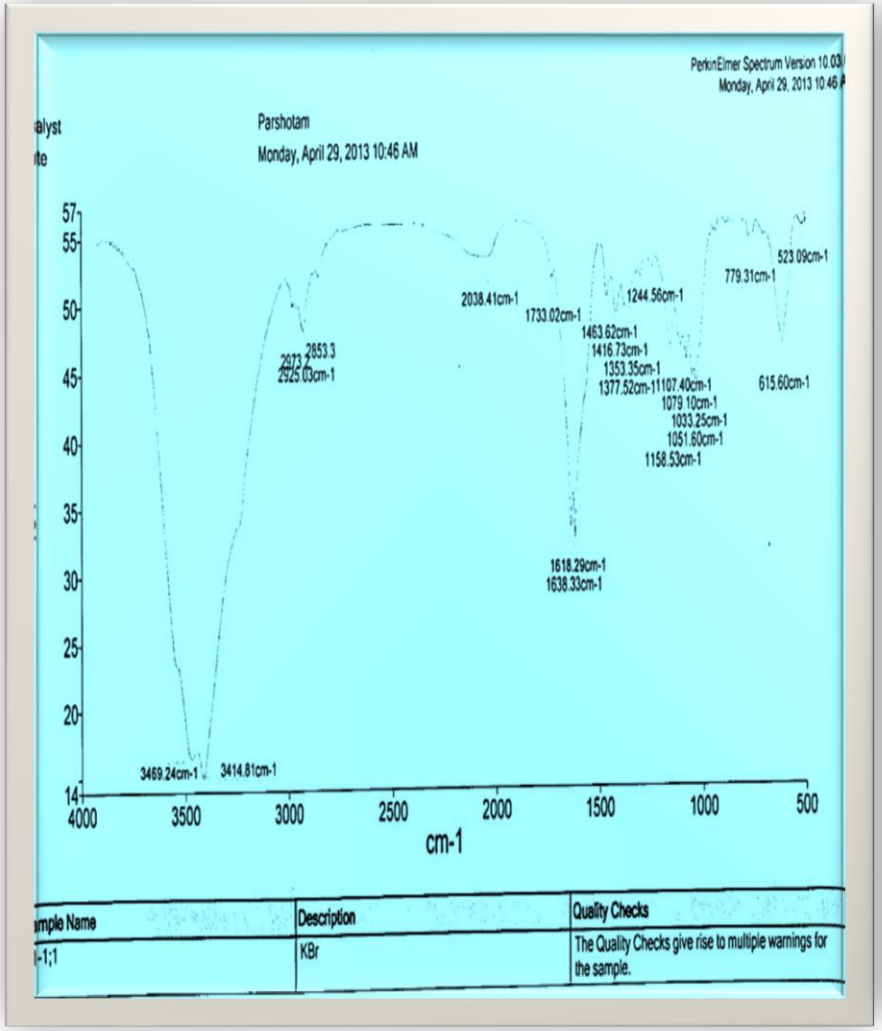


CLA

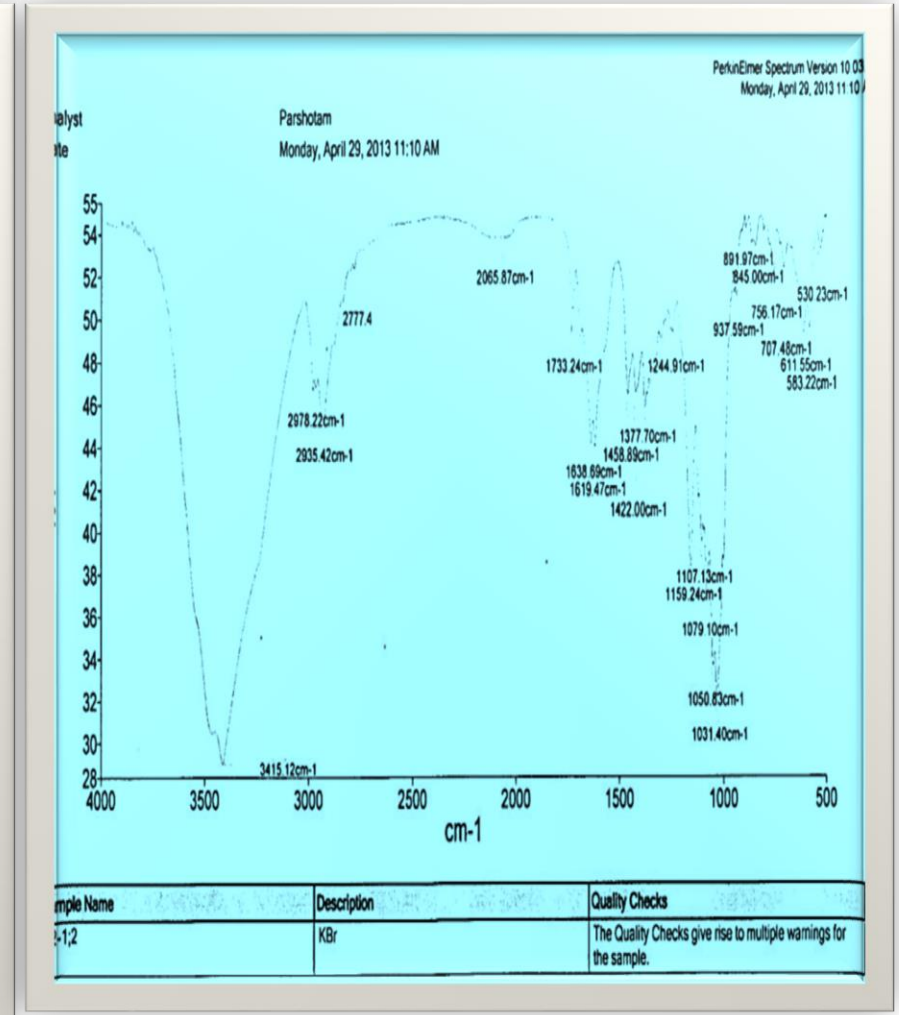


FTIR PATTERNS OF K1 AND K2

K1

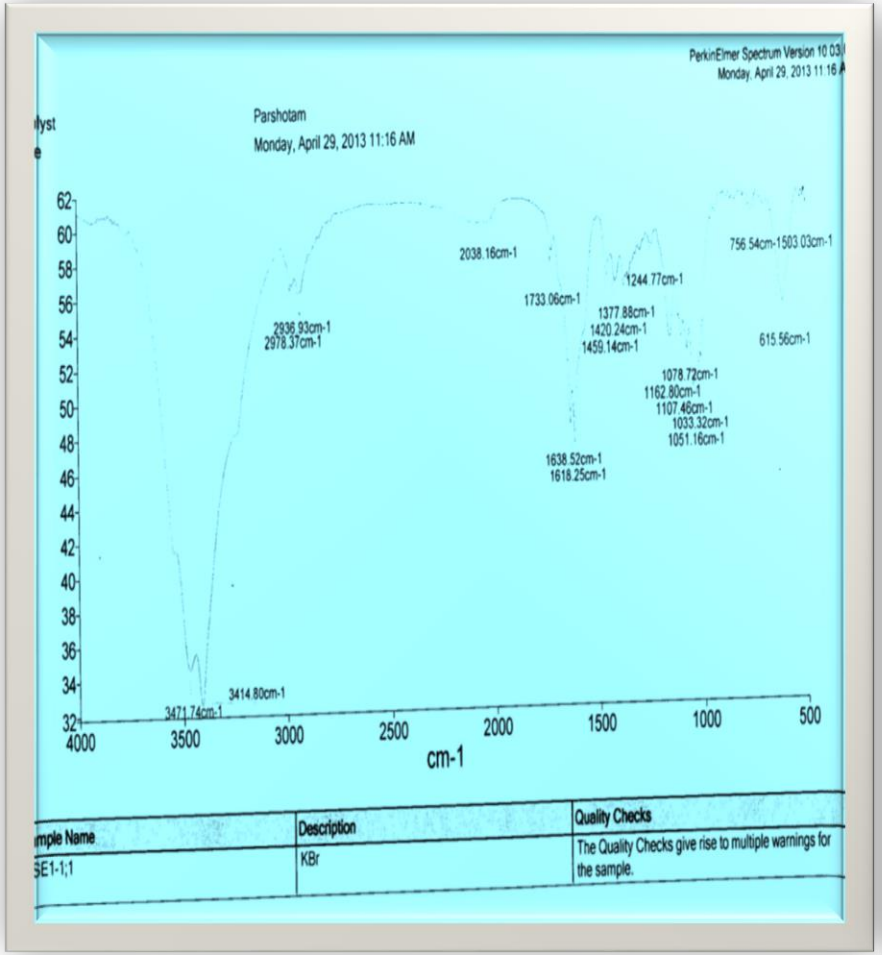


K2

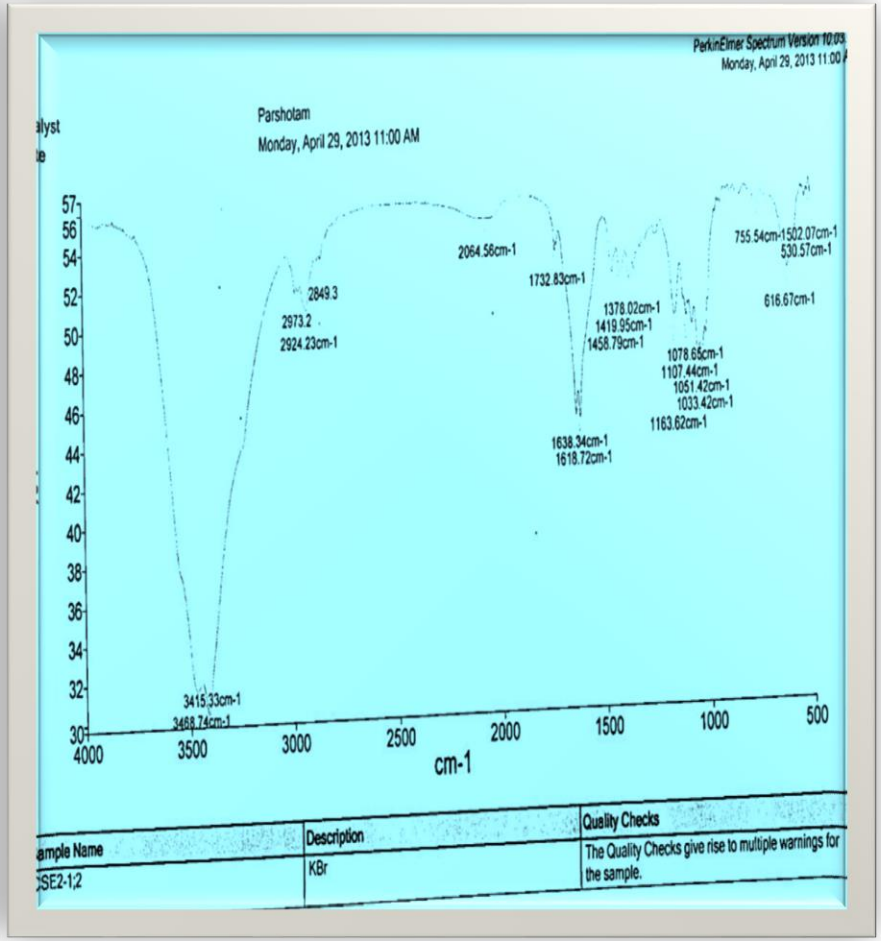


FTIR PATERNS OF CSE1 and CSE2

CSE1

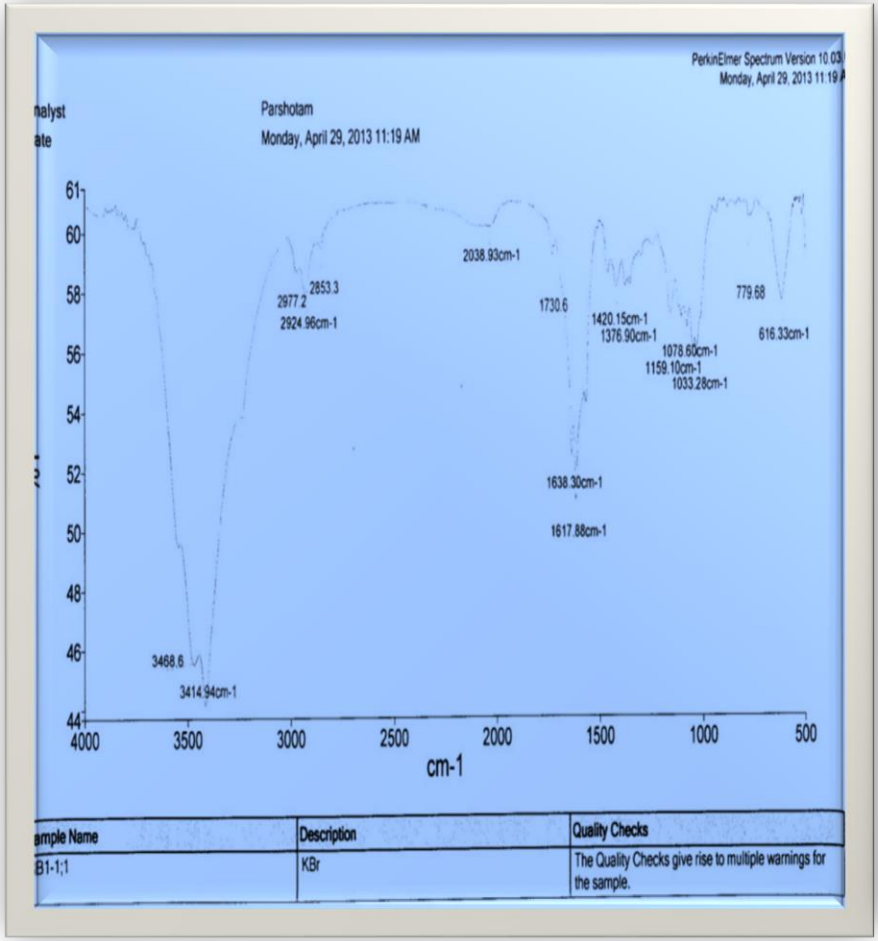


CSE2

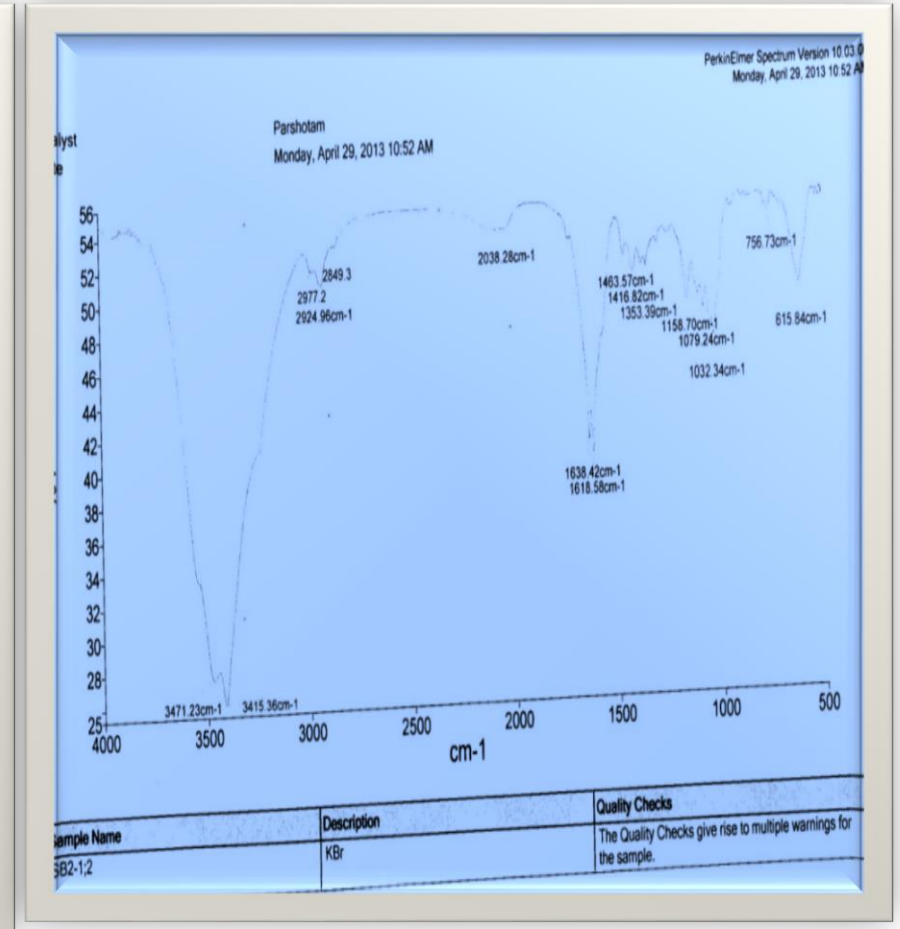


FTIR PATTERNS OF SB1 and SB2

SB1



SB2



OBSERVATIONS (K1)

- **Significant difference in -OH vibrations were found. Peaks got shifted and changed after complexation.**
- **The broad peak of –OH at 3392.14 of β-CD became sharp and intense after complexation.**
- **In K1 it was observed that alkane stretching bands became visible at 2853.3, 2952.03 and 2973.3 cm^{-1}**
- **C-O- C stretches 1011.60- 1275 of CLA became sharp and narrowed down to 1033.25- 1244.66 cm^{-1}**
- **The signal for functional group N-CH_3 in CLA became prominent at 1422.94. In K1 complex the signal got down shifted to 1416.73 cm^{-1}**
- **The functional group signal for C= O at 1691 in CLA got disappeared in FTIR after complexation**

OBSERVATIONS (K2)

- **K2 showed complete disappearance of band 1691 for C=O**
- **Alkane stretching band of K2 showed clear and obvious change from 2777.14 to 2978.32**
- **The peaks 2780.19, 2835.45, 2876.85, 2940.43 cm^{-1} got disappeared in K2.**
- **Complete disappearance of 3469.44 peak of Clarithromycin which stands for hydrogen bonds between OH groups in K2.**
- **C-O-C stretch band resulted in band shortening**
- **The band for CH_2 groups 1364.14-1422 was modified to single peak in K2.**

OBSERVATIONS (SB1)

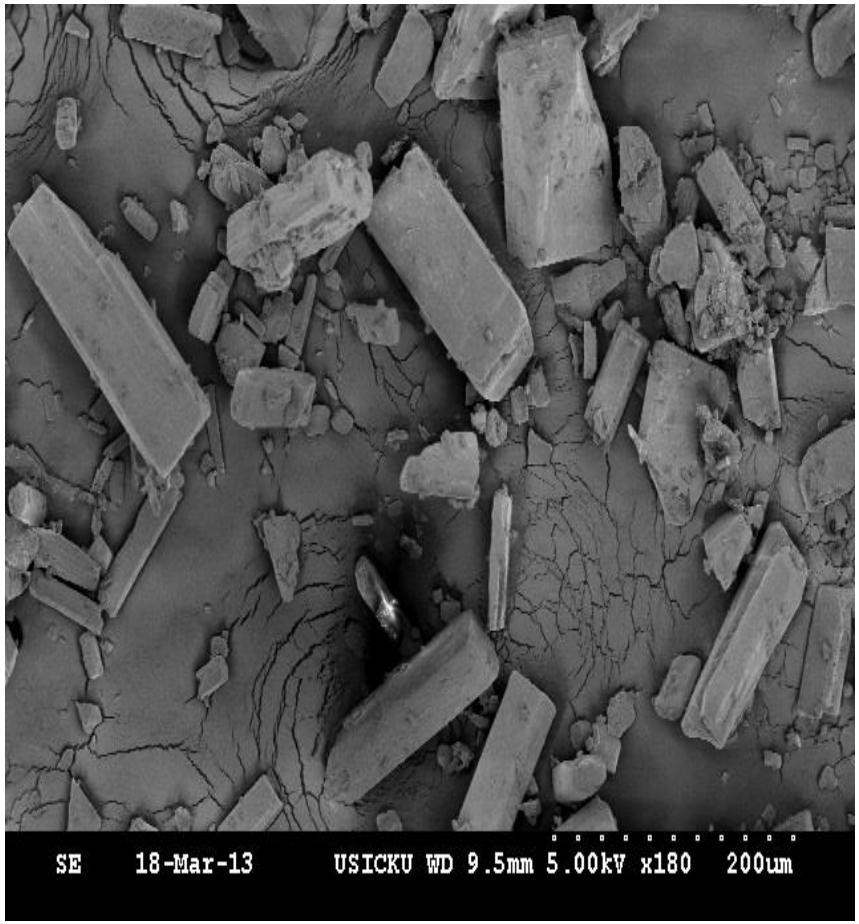
- **C=O band 1691.31 cm^{-1} got disappeared while as lactonyl carbonyl group 1733.25 showed slight down shifting to 1730.6 cm^{-1}**
- **Alkane stretching band of CLA ranging from 2780.19 – 2978.62 showed modification to 2853.3- 2977.00.**
- **C-O-C stretches at 1011.60-1275 showed band narrowing to 1033.28 – 1159.10**
- **CH_2 functional group band 1364.14 - 1422.94 again narrowed down to 1376.90 – 1420.15**

OBSERVATIONS (SB2)

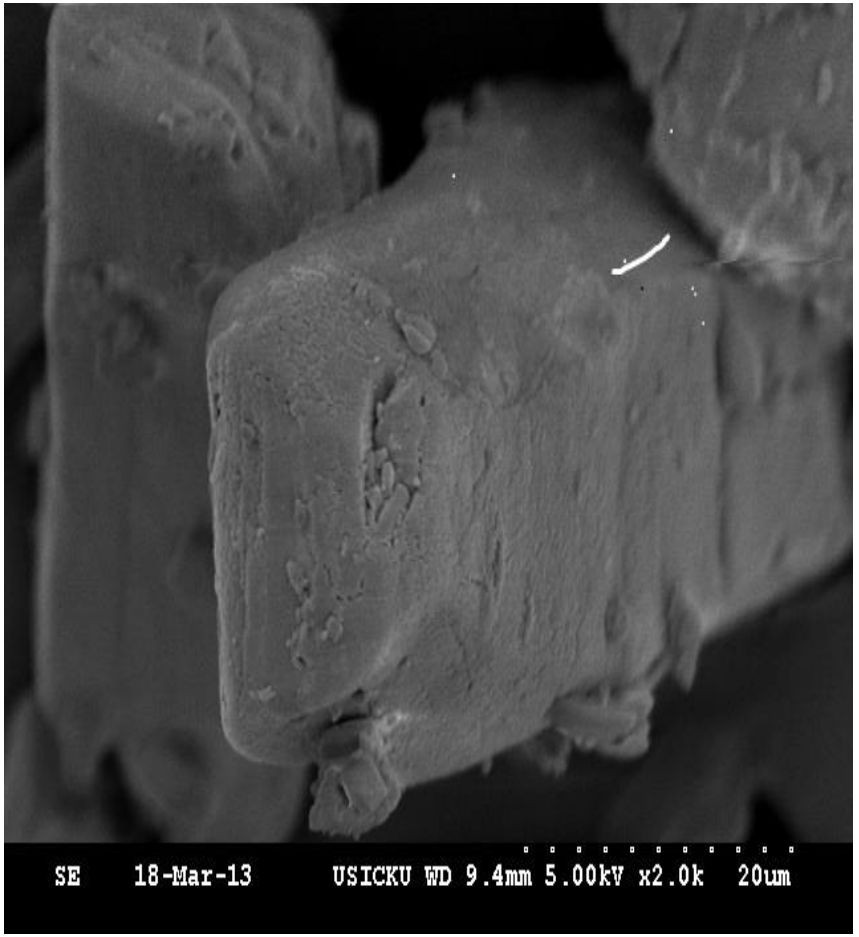
- **Disappearance of C=O 1691 band is observed in spectra of FTIR of SB 1:2.**
- **Disappearance of lactonyl carbonyl C=O.**
- **Down shifting of N- CH_3 1422.94 to 1416.82 cm^{-1}**
- **Alkane stretching band showed up shifting from 2780.19 to 2849.3 and from 3469.44 to 3471.23**

SURFACE MORPHOLOGY STUDY (SEM)

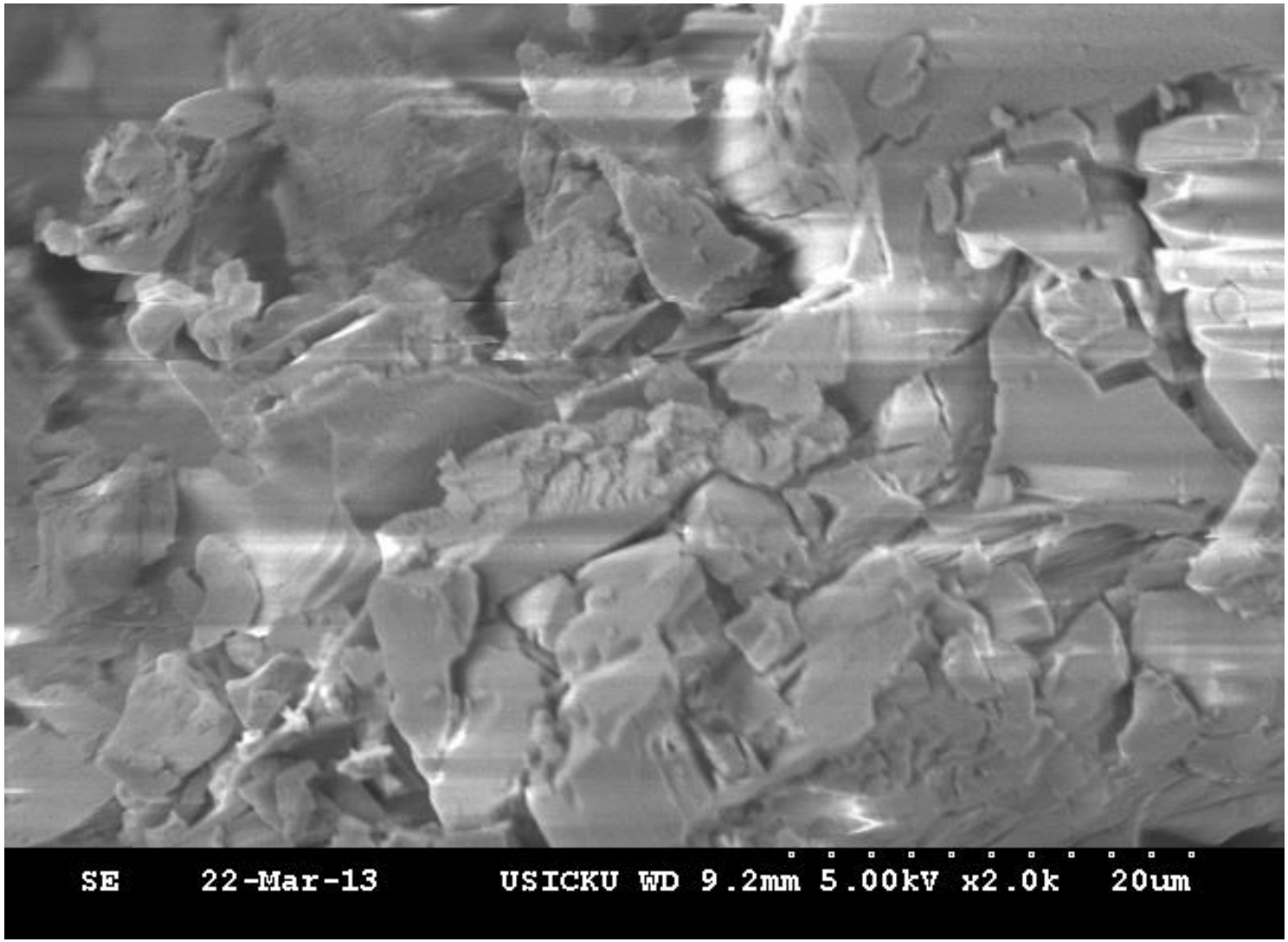
Clarithromycin (5.00x180)



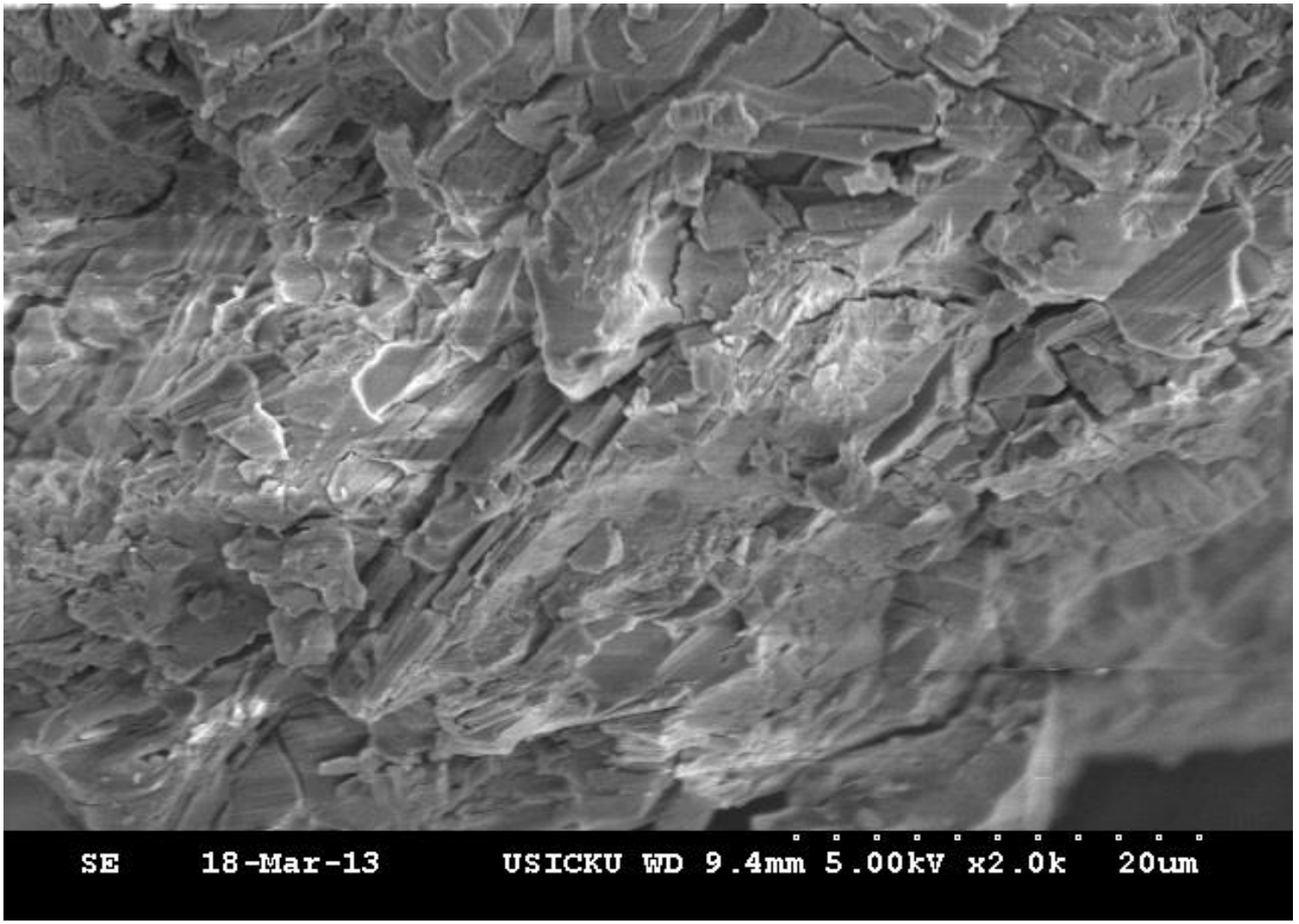
Clarithromycin (5.00x2.0)



K1

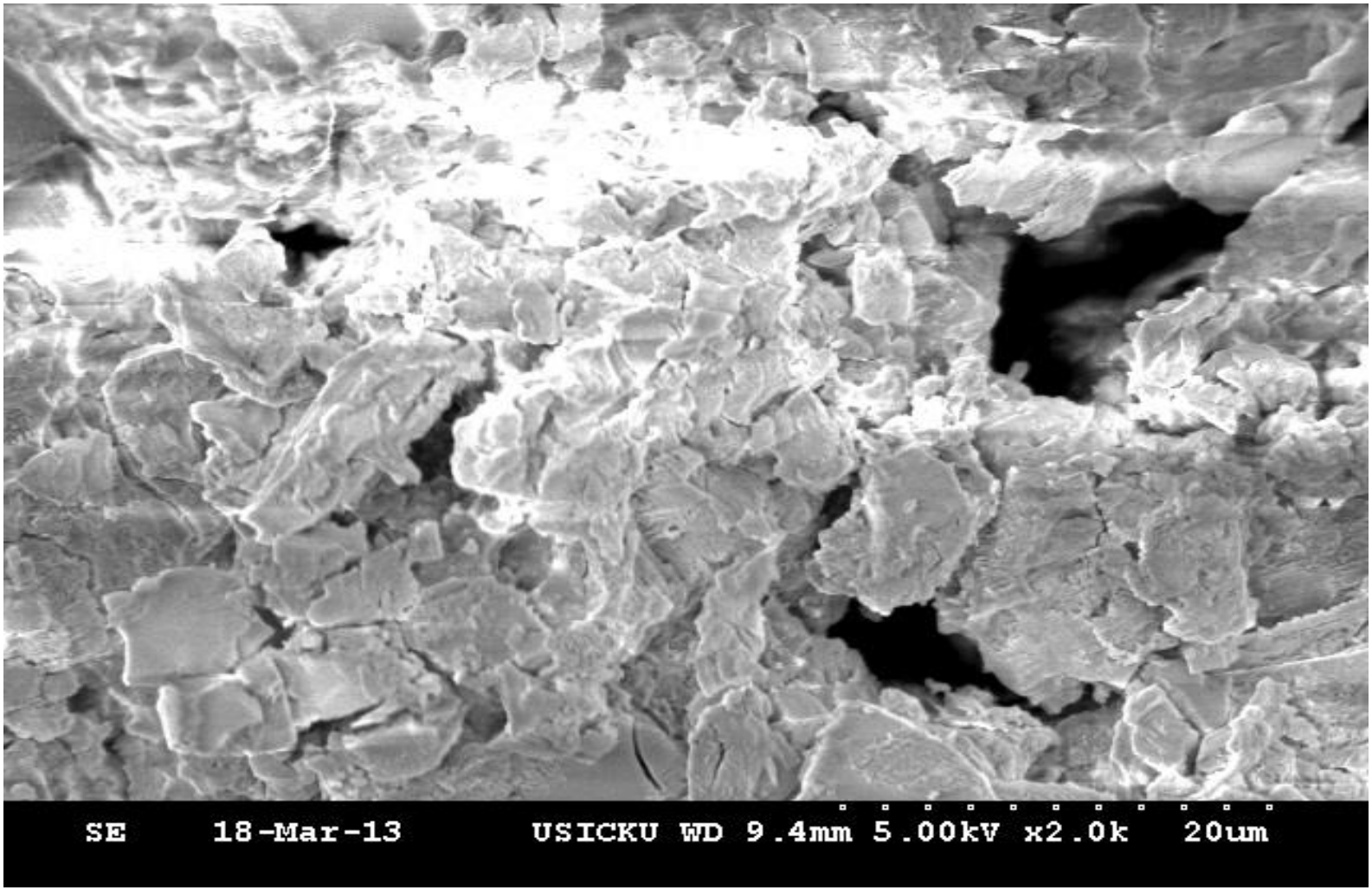


SB1

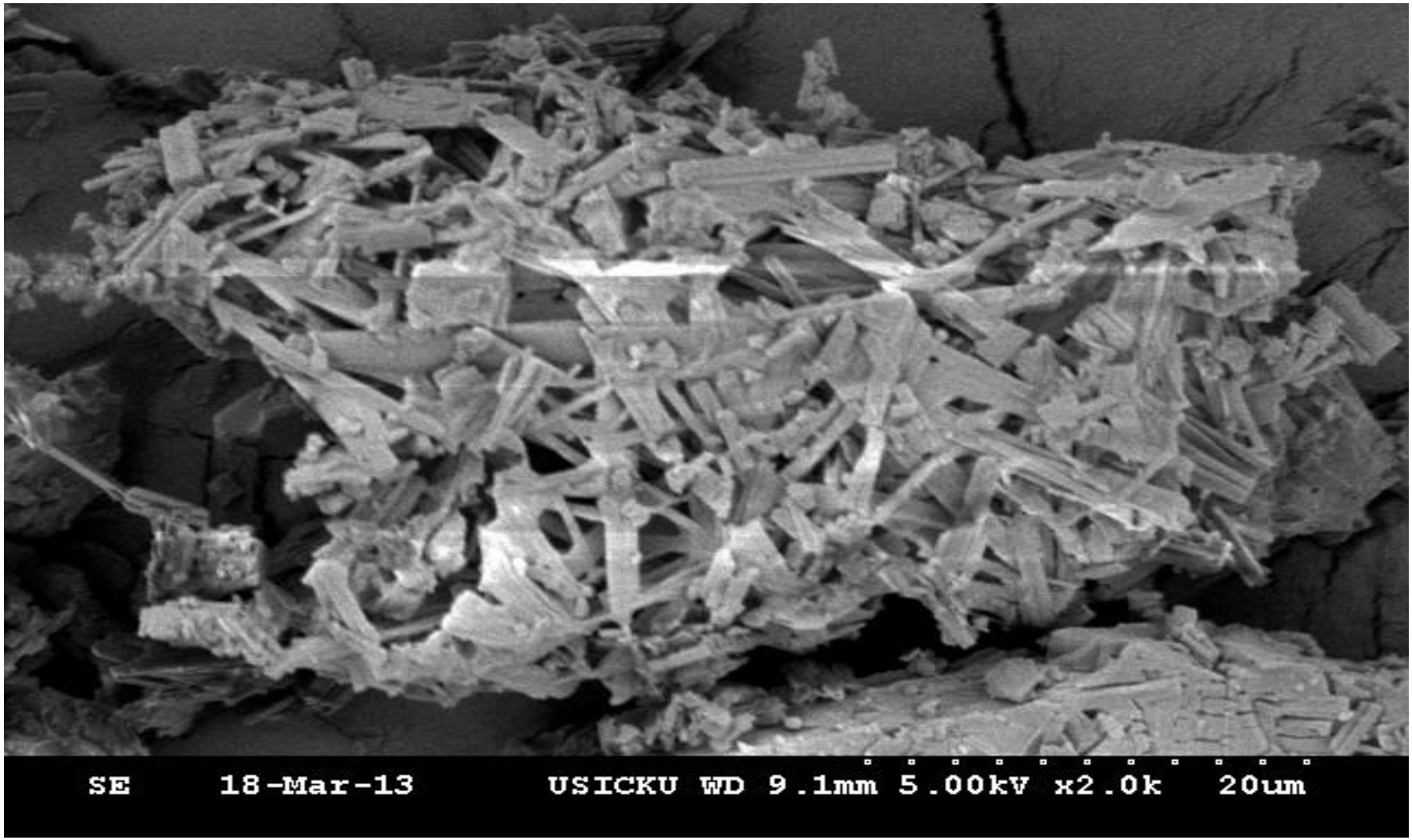


CSE1

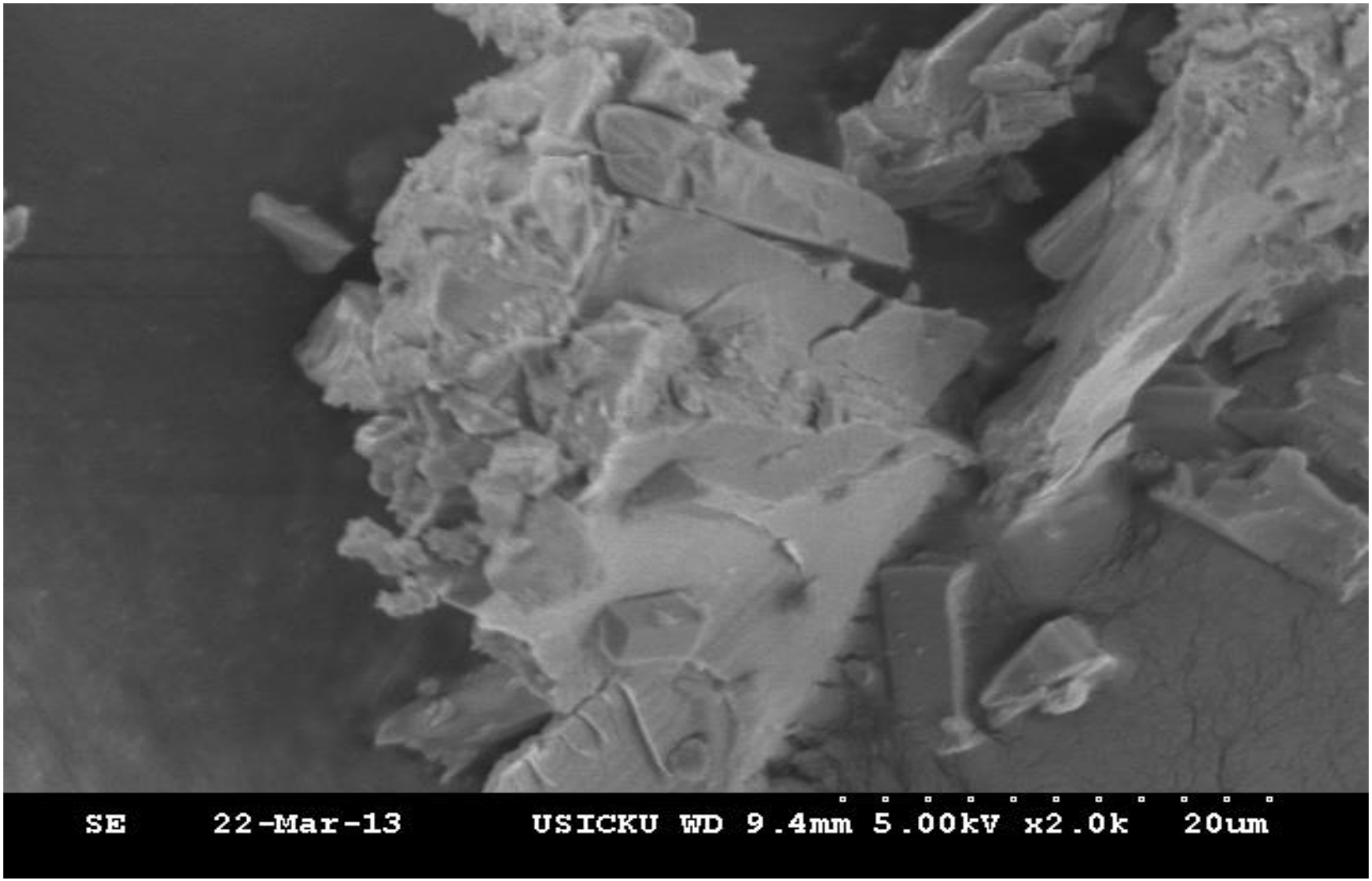
K2



SB2



CSE2

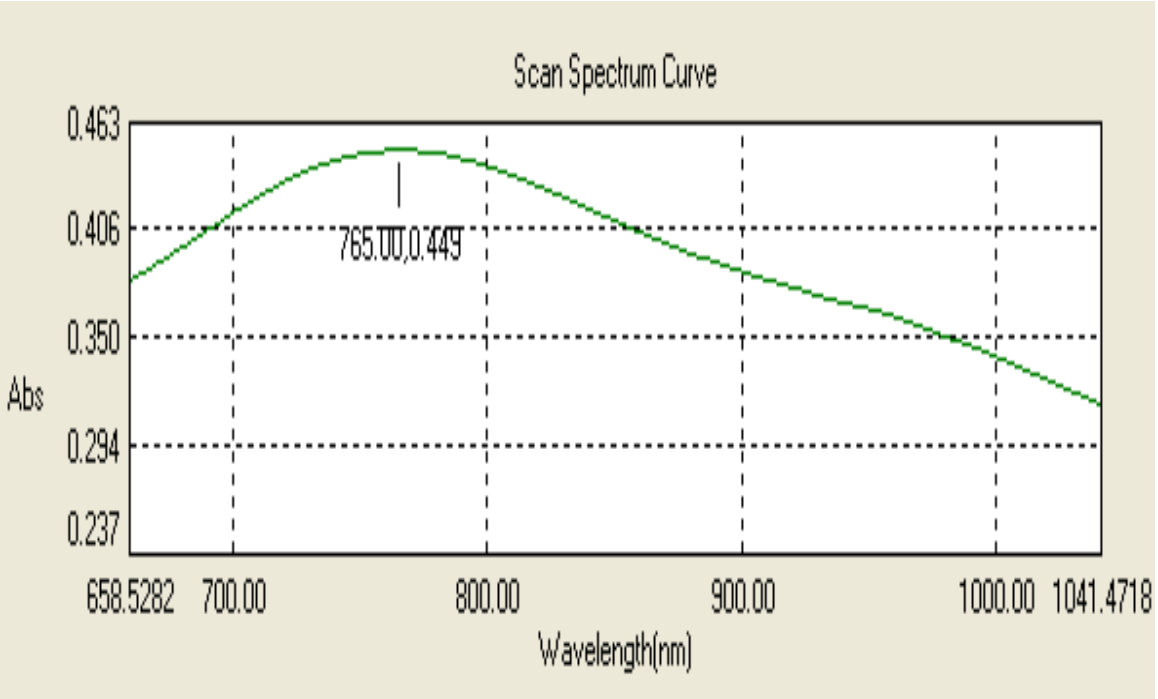


OBSERVATIONS

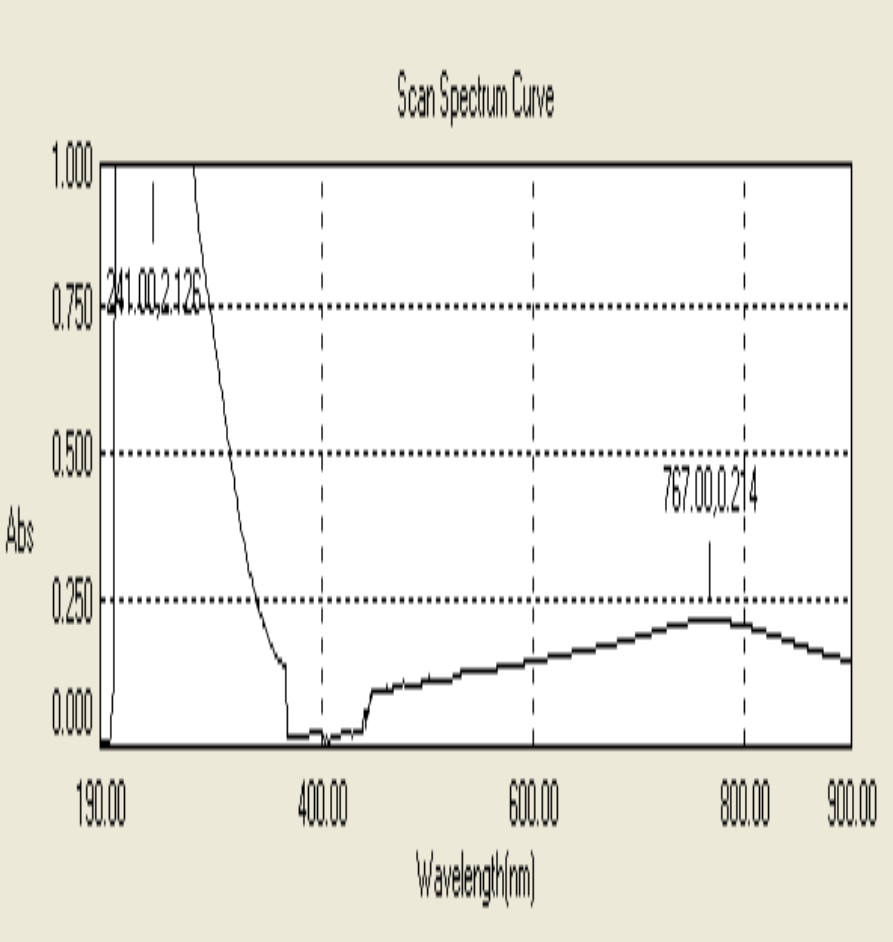
- Crystalline structure of CLA is observed as parallelograms
- SEM microphotographs of K1 and K2 at a magnification level of 5.00 kv X 2.0 show homogenous agglomerates with an uneven porous face and lack crystalline structure as shown by the pure drug CLA.
- The appearance of amorphous agglomerates is seen in SB1 and SB2 also, while as in CSE1 and CSE2 such amorphous agglomerates are absent.

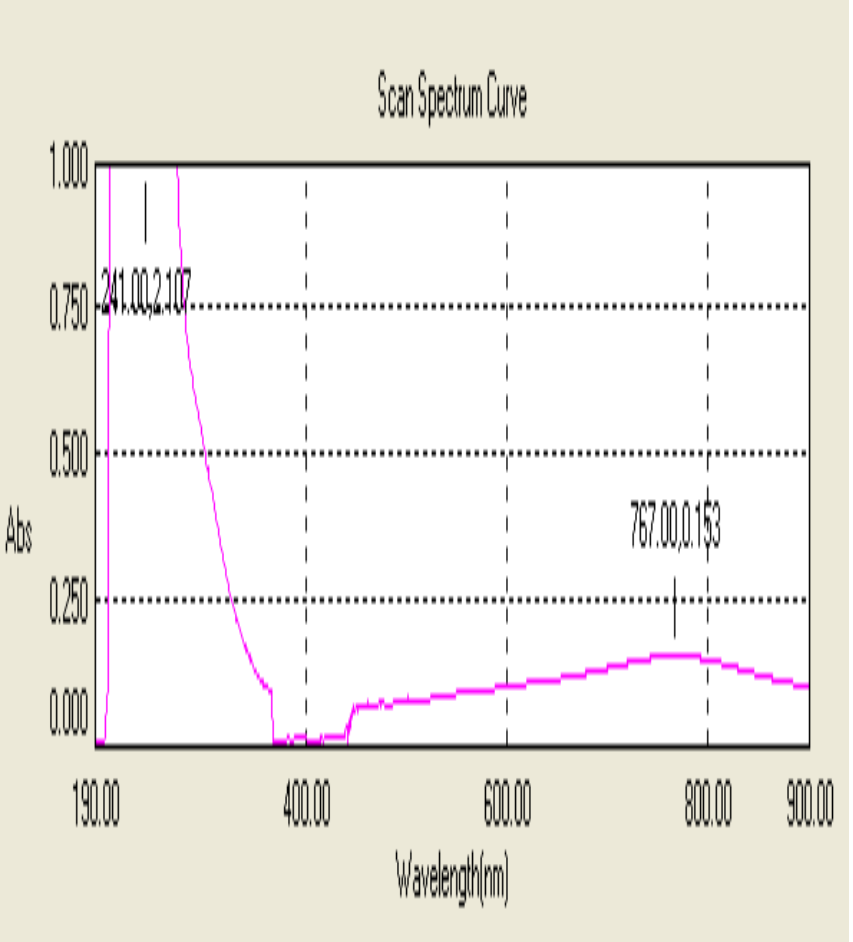
UV SPECTRAL STUDIES

UV spectra of pure drug CLA in 0.1 N HCl using FCP reagent



UV SPECTRA OF K1 & K2



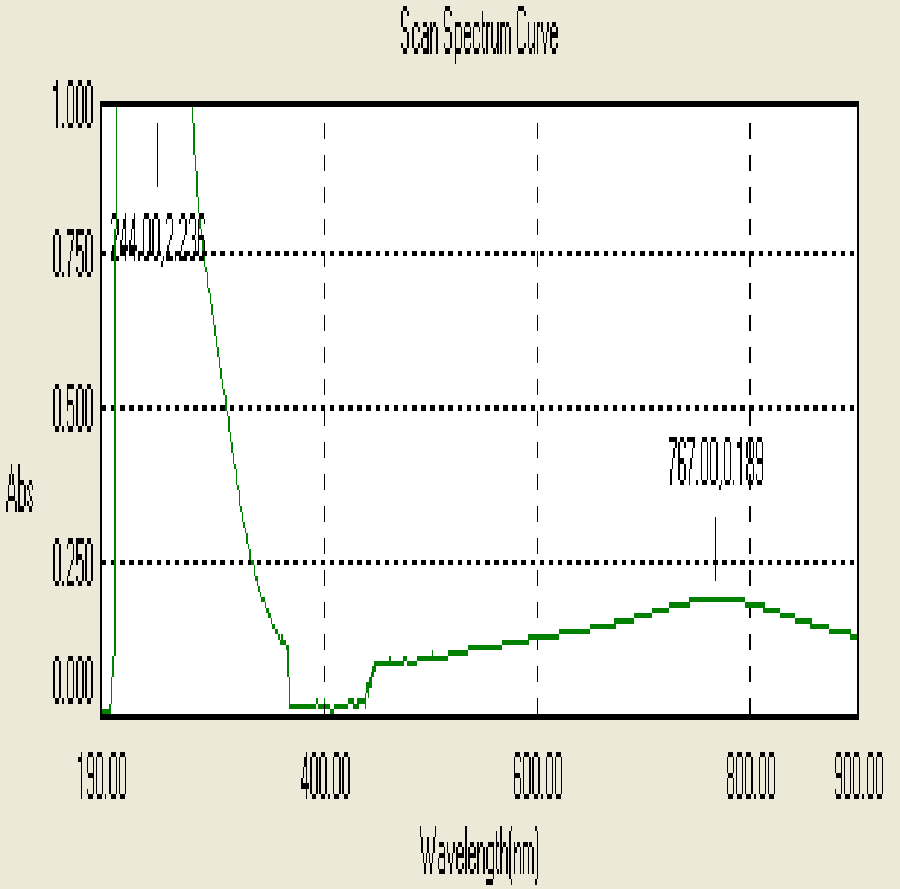


Λ max shifted to 767 nm

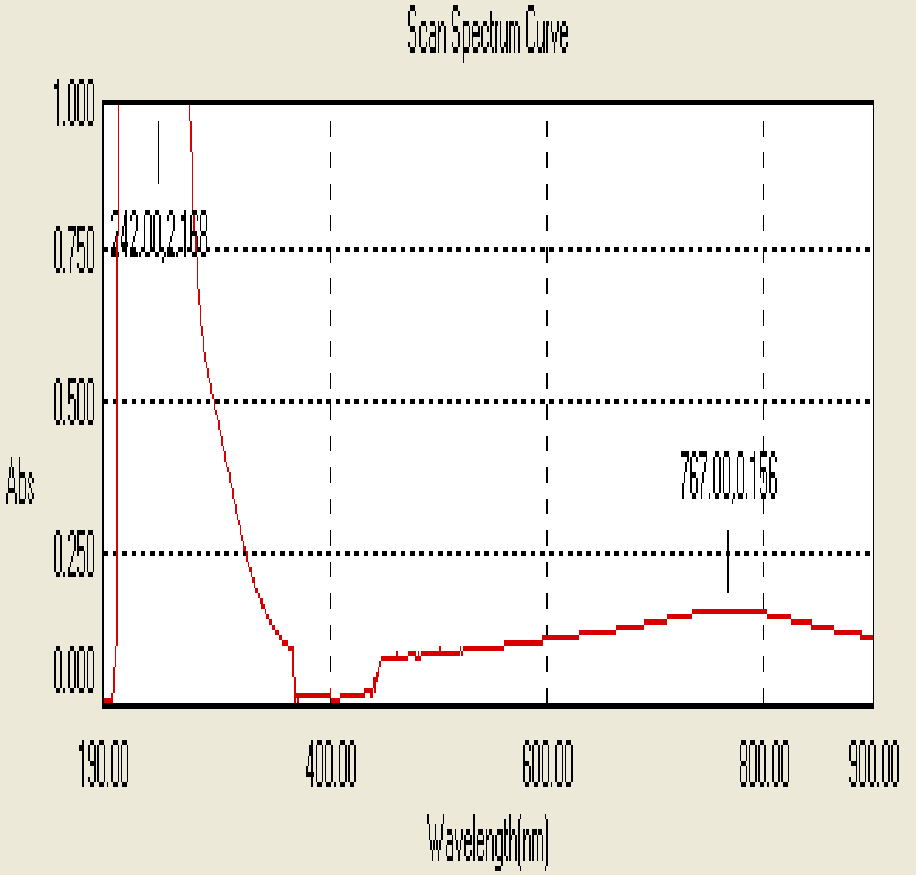
Λ max shifted to 767 nm

UV SPECTRA OF SB1 AND SB2

Λ max shifted to 767 nm

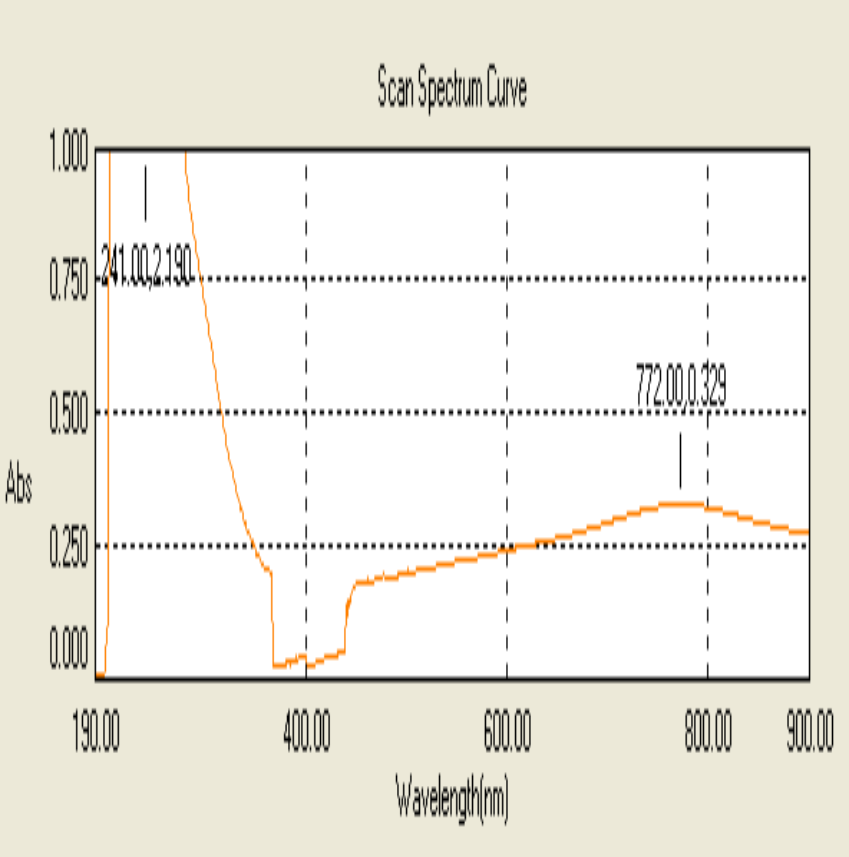


Λ max shifted to 767 nm

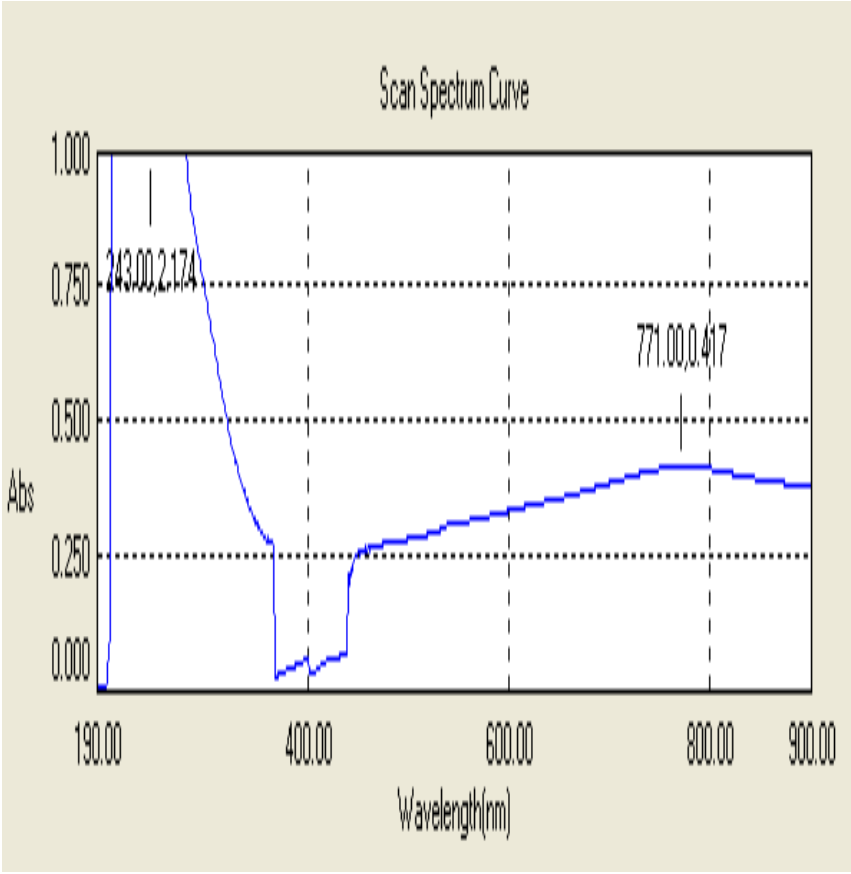


UV SPECTRA OF CSE1 & CSE2

Λ max shifted to 772 nm



Λ max shifted to 771 nm



OBSERVATION

FORMULATION	Λ max(with FCP reagent)
Pure drug	765nm
K_1	767 nm
K_2	767 nm
CSE_1	772 nm
CSE_2	771 nm
SB_1	767 nm
SB_2	767 nm

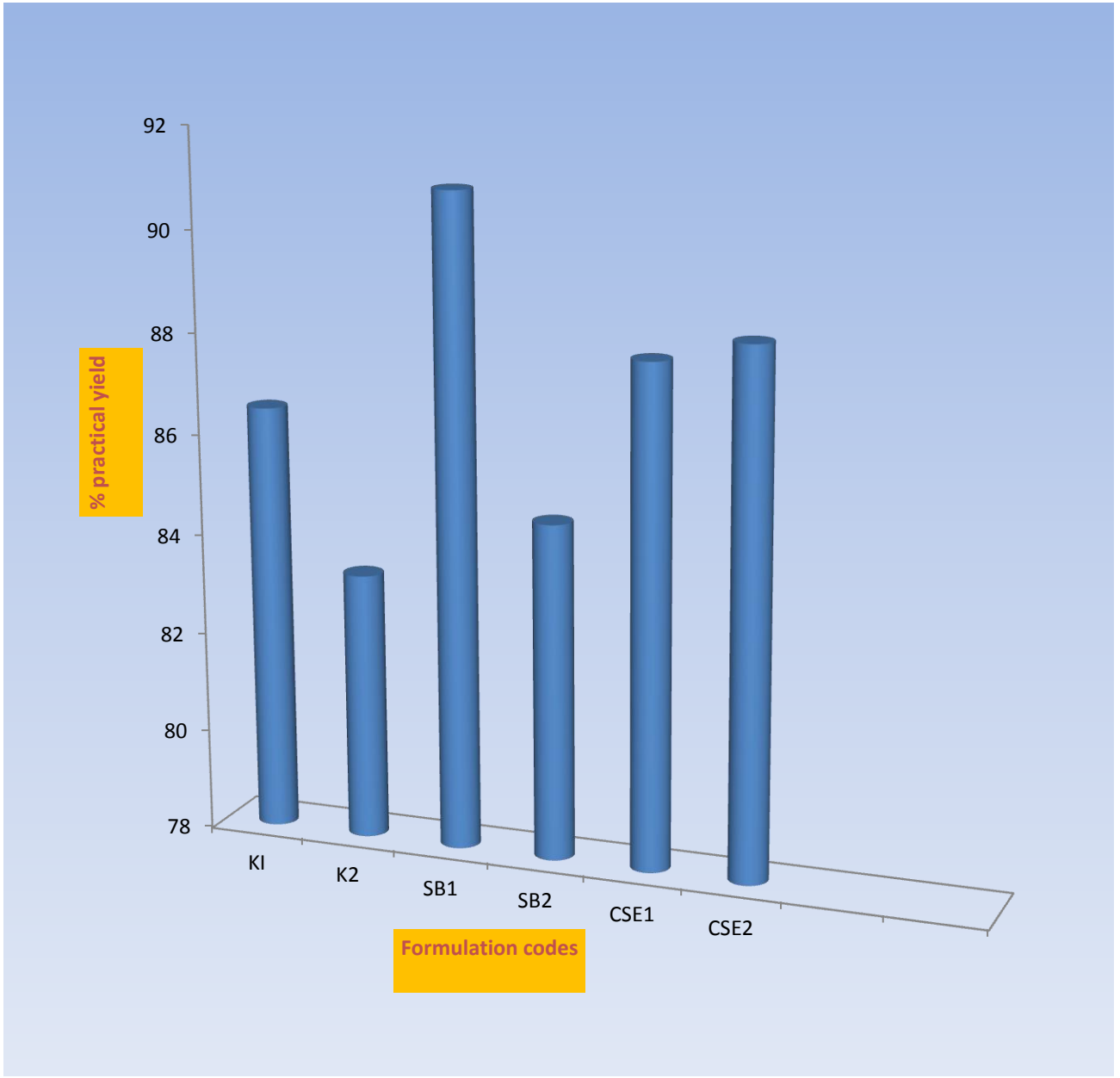
OBSERVATIONS

- Change in spectrum is observed which is approximately 2nm in case of K1, K2, SB1 and SB2.

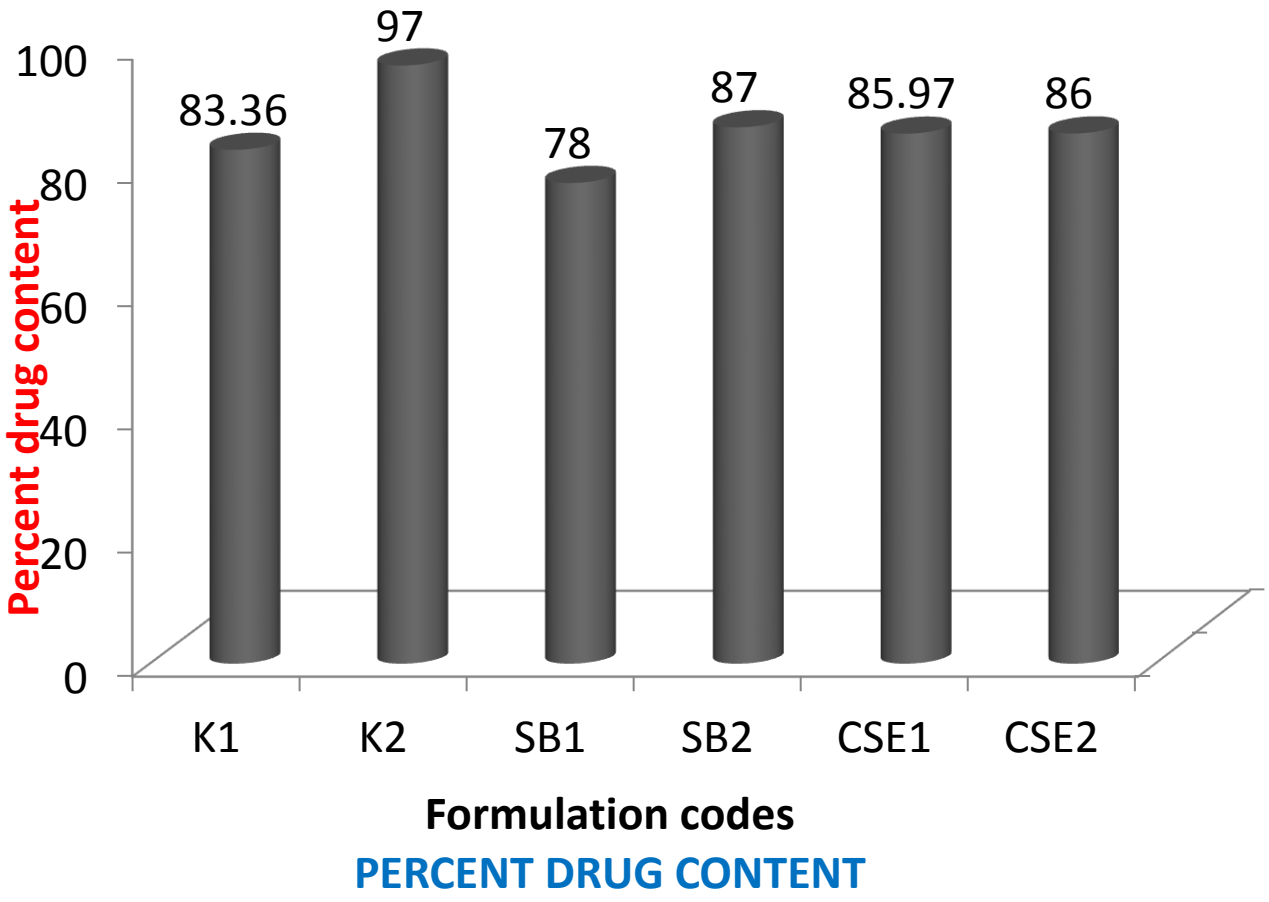
- More than 2nm in case CSE1 and CSE2.

Percent practical yield

FORMULATION CODE	% PRACTICAL YIELD
K1	86.5
K2	83.33
SB1	**90.95**
SB2	84.72
CSE1	88.0
CSE2	88.47



FORMULATION CODE	% DRUG CONTENT
K1	83.36
K2	97.0
SB1	78.0
SB2	87.0
CSE1	85.97
CSE2	86.0



RESULTS AND DISCUSSION

- **Saturation solubility** showed promising increase in solubility of K1, K2, SB1 and SB2 method while CSE did not give any positive results.
- **Phase solubility study** A_L type of curve obtained and Kc value obtained are 2768.20 falling in the range of 1000-5000, indicating moderate bond strength between the host and the guest and reveal that aqueous solubility increased linearly as a function of carrier concentration.
- Complexation efficiency was calculated by using equation CE = Slope/(1-Slope) = 74.0

RESULTS AND DISCUSSION

- **DISSOLUTION STUDY** showed drug release pattern in this way **K_2 >SB_2>SB_1>K_1 >CSE_2 > CSE_1** indicating highest release of drug in K2 and lowest in CSE.
- Dissolution study confirmed that there is **12** times enhancement of drug release in K2, **10** times enhancement in case of K1, **10** times enhancement in case of SB1 and **10** times in case of SB2.

RESULTS AND DISCUSSION

- Change in **FTIR** can be attributed to insertion of part of CLA molecule into the electron rich cavity of β-CD.
- The increase in the density of electron cloud leads to the increase in frequency.
- The decrease in the frequency between the inclusion complex and its constituent molecule can be attributed to the changes in the micro environment which lead to the formation of hydrogen bonding and the presence of Vander Walls forces during their interaction to form the inclusion complexes.

RESULTS AND DISCUSSION

- Disappearance of C=O(1691) band in all the inclusion complexes can be attributed to overlapping with the β-CD molecule.
- Disappearance can be due to complete or partial insertion of Clarithromycin molecule inside the hollow cavity of β-CD.
- Thus the FTIR spectra significantly proves the formation of inclusion complexes

(Jhon Coates, 2000 p.12. Elgindy N et al., 2009. Elenora Mariani et al., 2011. Farasac A et al., 2006. Sapkal NP et al 2010. Sharma MC et al., 2011. Sapkal NP et al., 2007. Bhise DS et al., 2011).(Li J et al., 2007. Li W et al ., 2010. Li j et al ., 2003. Rusa CC et al ., 2001.Tang B et al ., 2006. Hamidi H et al., 2010. Bradea o et al., 2012.Sarasija Set al., 2011).

RESULTS AND DISCUSSION

- The band broadening and a small shift in λ max in **UV** can be due to electron density change of the guest trapped inside CD molecule which partially shields the electrons of the guest.
- The small spectral shift was interpreted as an indication of inclusion complex formation.
- The shift of the UV absorption max upon complex formation may be explained by a particular shielding of the excitable electrons in the cavity.

(Szejtli UV et al., 1980. Patel P et al., 2011, Abderrazak D, Cyclodextrin Materials vol 1, 2006, p 124. Flohr k., 1975. Martinie J., 1975. Schenider HJ., 1998.Harata K., 1981. Shimizu H., 1979. Sata H., 1979. Havata K., 1980. Lambert JB., 1998. Singh Pooja. 2011. Hirlekar R et al., 2009. Singh P et al., 2011.Sharma MC et al ., 2010. Rao KS et al., 2012).

RESULT AND DISCUSSION

- In **SEM** study the microphotographs of pure CLA and various drug β-CD complexes under different magnifications taken clearly show change of the particle shape in K1, K2, SB1, SB2 complexes indicating the presence of new solid phase as a result of reduction in crystalanity.
- Numerous cracks and fissures indicate formation of amorphous system.
- Regular parallelogram crystals of Clarithromycin got disappeared, and it is no longer possible to differentiate the crystals of CLA which reveals an apparent interaction between the drug and β- CD.

(Zarief MS et al., 2012. Riberio A et al., 2008. Chadha R et al ., 2004. Farasc A et al ., 2006.irlekar R et al., 2009. Sarasija S et al., 2011. Singh P et al., 2011).

CONCLUSION

Therefore from above studies it can be concluded that β-CD complexation of CLA has effectively improved the solubility and dissolution rate of the drug.

FUTURE PROSPECTS

After successful in vitro studies which demonstrated profound increase in solubility and dissolution rate of β-CD complexed CLA, there is enough scope for in vivo studies to be carried out in animals and human models to ascertain the improvement of bio availability of drug as well.

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Thank you

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