Design, development and characterization of Naringenin loaded Solid Lipid Nanoparticles to enhance It's Oral Delivery

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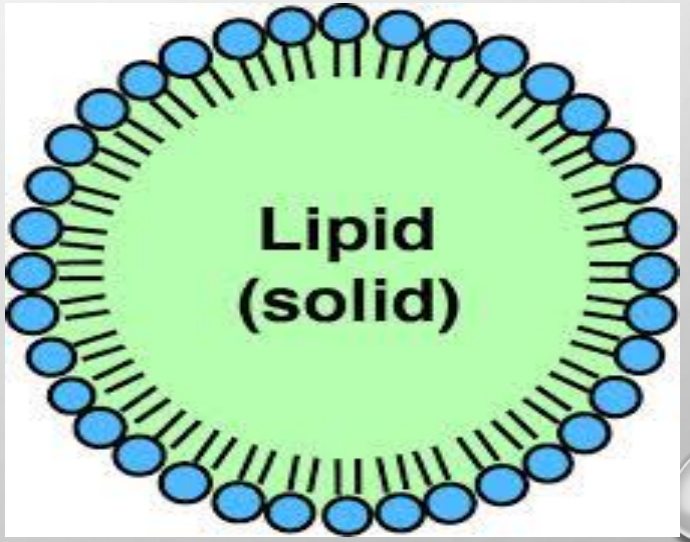
SLNs

- **Definition:** SLNs are sub-micron colloidal carrier (50-1000 nm)composed of physiological lipid, dispersed in water or in an aqueous surfactant solution; in which liquid lipid is replace by Soild Lipid.

Advantages:

- High & enhanced drug content (compared to other carriers).
- Controlled and targeted drug release.
- Feasible for carrying both lipophilic & hydrophilic drug.
- Easy to scale up and sterilize. Long term stability.



Componants :

Solid Lipid
Emulsifier
Water

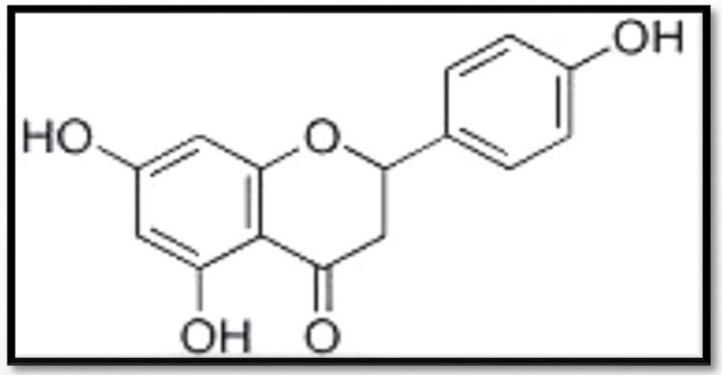
Aim & Objective

- **Aim**
- Main problem associated with NAR is its bioavailability 8% due to extensive first pass metabolism and low water solubility.
- Its bioavailability could consequently be improving its solubility by preparing lipid based formulation. SLNs are considered particularly a useful approach to improve the absorption and thus the oral bioavailability of poorly water soluble drug.

- **Objective:**
- The main objective is to design, develop and characterize an optimal SLN of NAR to enhance the solubility, dissolution rate and thereby bioavailability of Naringenin.
- To avoid extensive first pass metabolism of Naringenin.

Drug Profile

Naringenin:



Chemical Structure of Naringenin

Parameter	Description
Drug name	Naringenin (NAR)
IUPAC name	4’, 5, 7-trihydroxyflavanone
Chemical formula	$C_{15}H_{12}O_5$

Parameter	Description
Molecular weight	272.26 g/mol
Melting point	247 - 250 ºC
BCS class	II
Category	Estrogen antagonist, Anti-ulcer, Antioxidant
Solubility	Insoluble in water, soluble in methanol, ether
Log P/ Hydrophobicity	2.52
Half life	2.6 h
Bioavailability	8 %

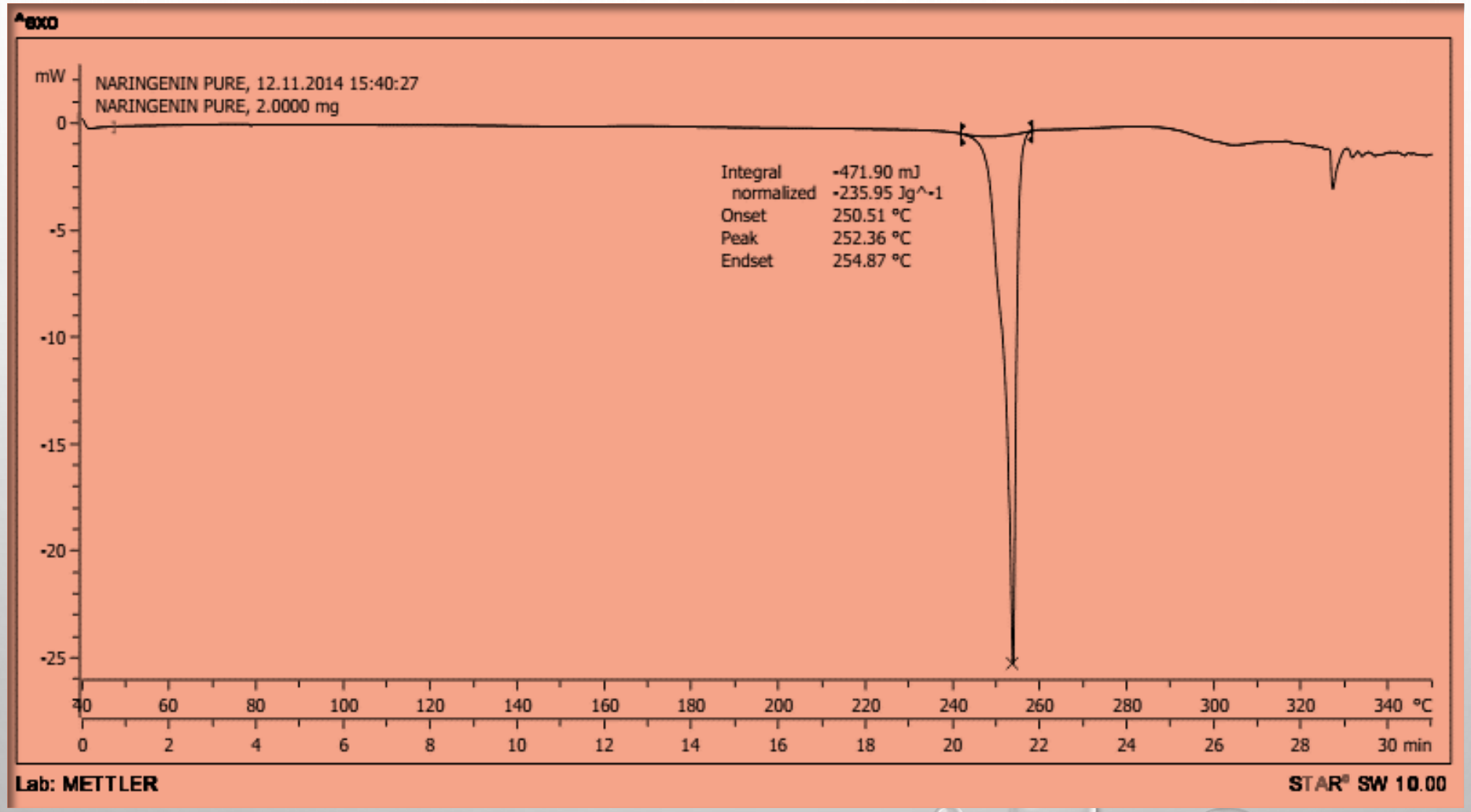
Preformulation Study

1. **Identification and confirmation of drug:**
   a) Melting point by melting point apparatus
   b) Melting point by DSC
   c) UV method
   d) FTIR
   e) HPLC
2. **Solubility study of drug in different solvents**
3. **Lipid screening**
4. **Surfactant screening**
5. **Standard Calibration Curve**
6. **Drug excipient compatibility study**

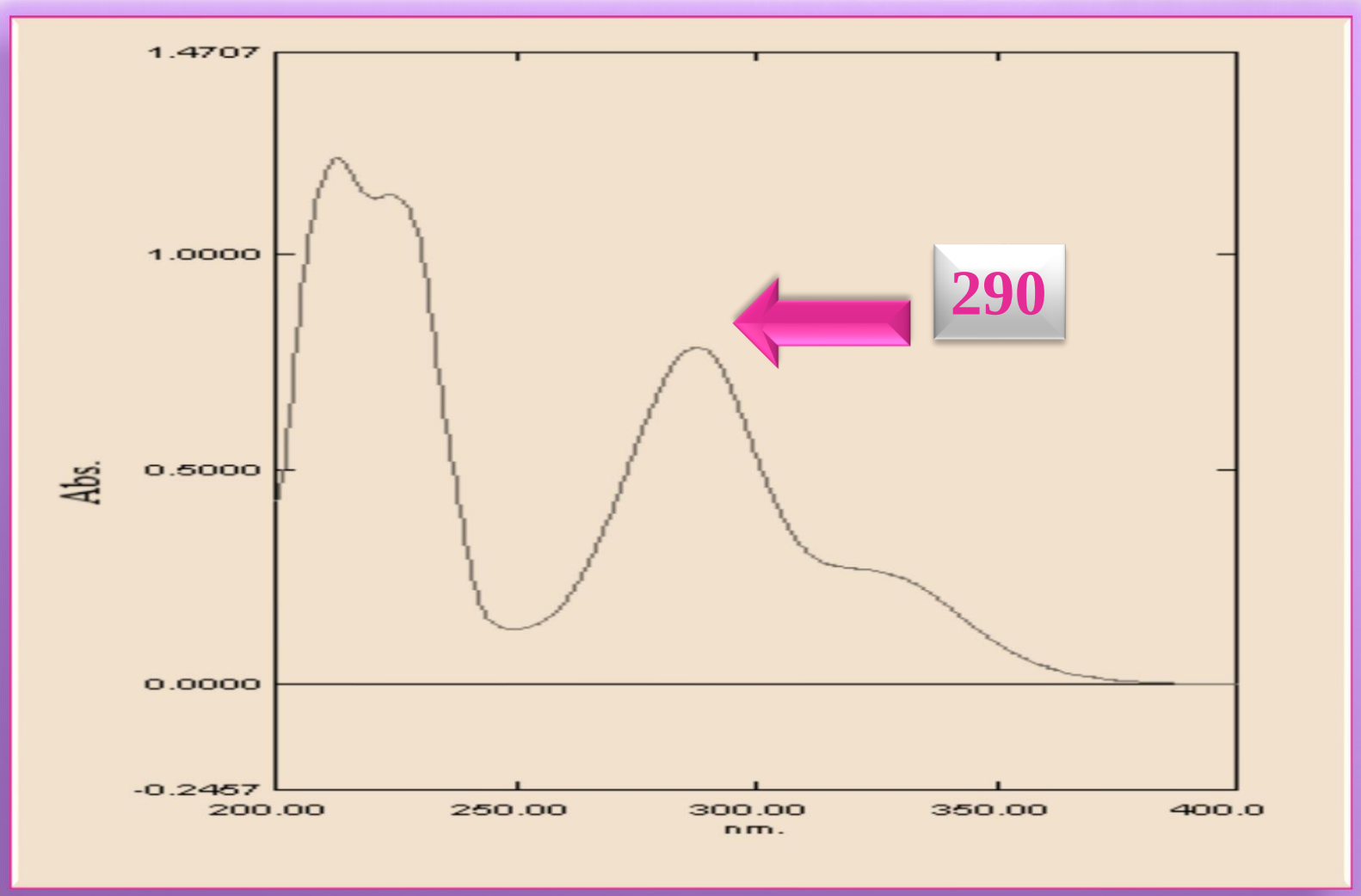
1. a) Melting point

Standard MP	247-250º C
Observed MP	249-251º C

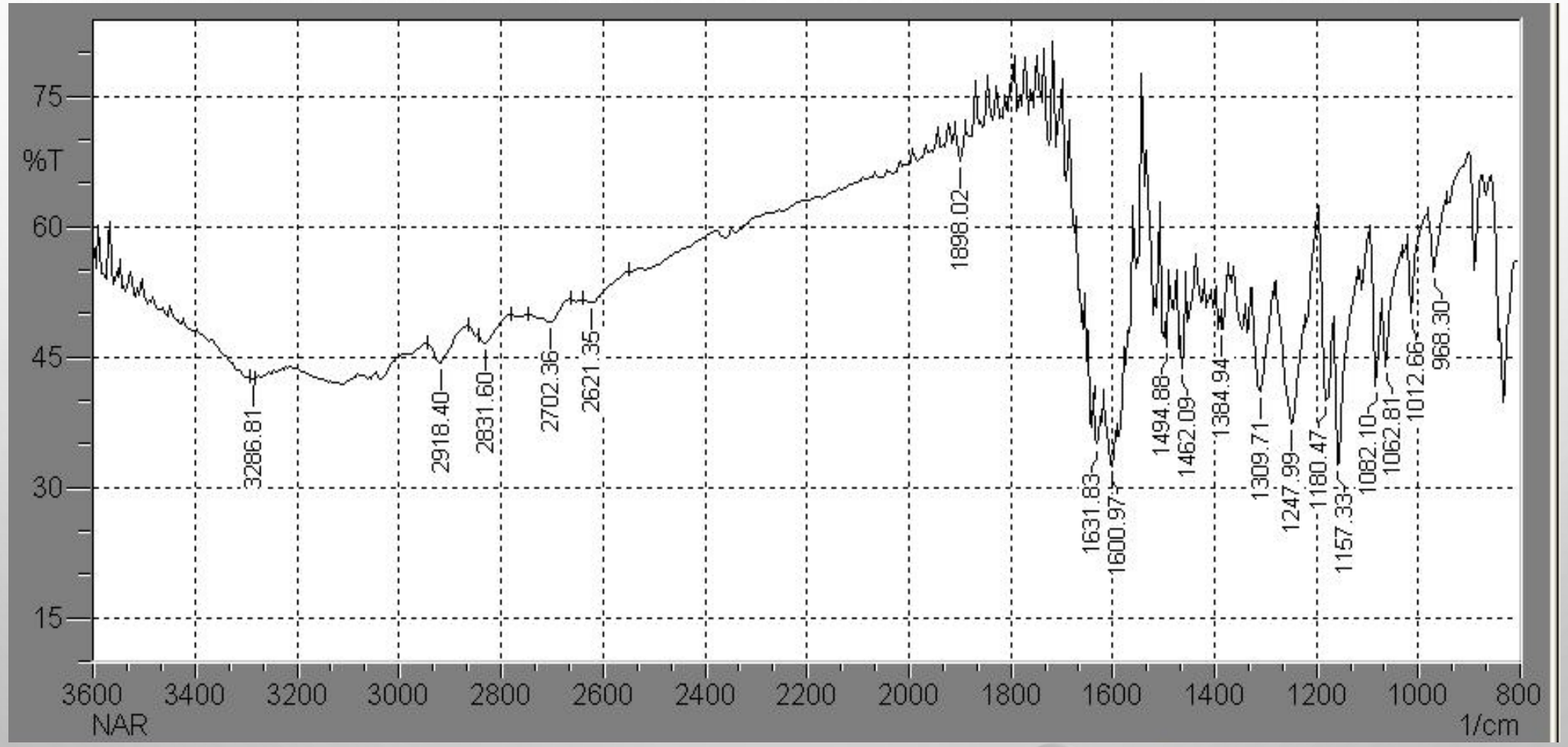
b) Melting point by DSC



c) U.V. Spectroscopic Method



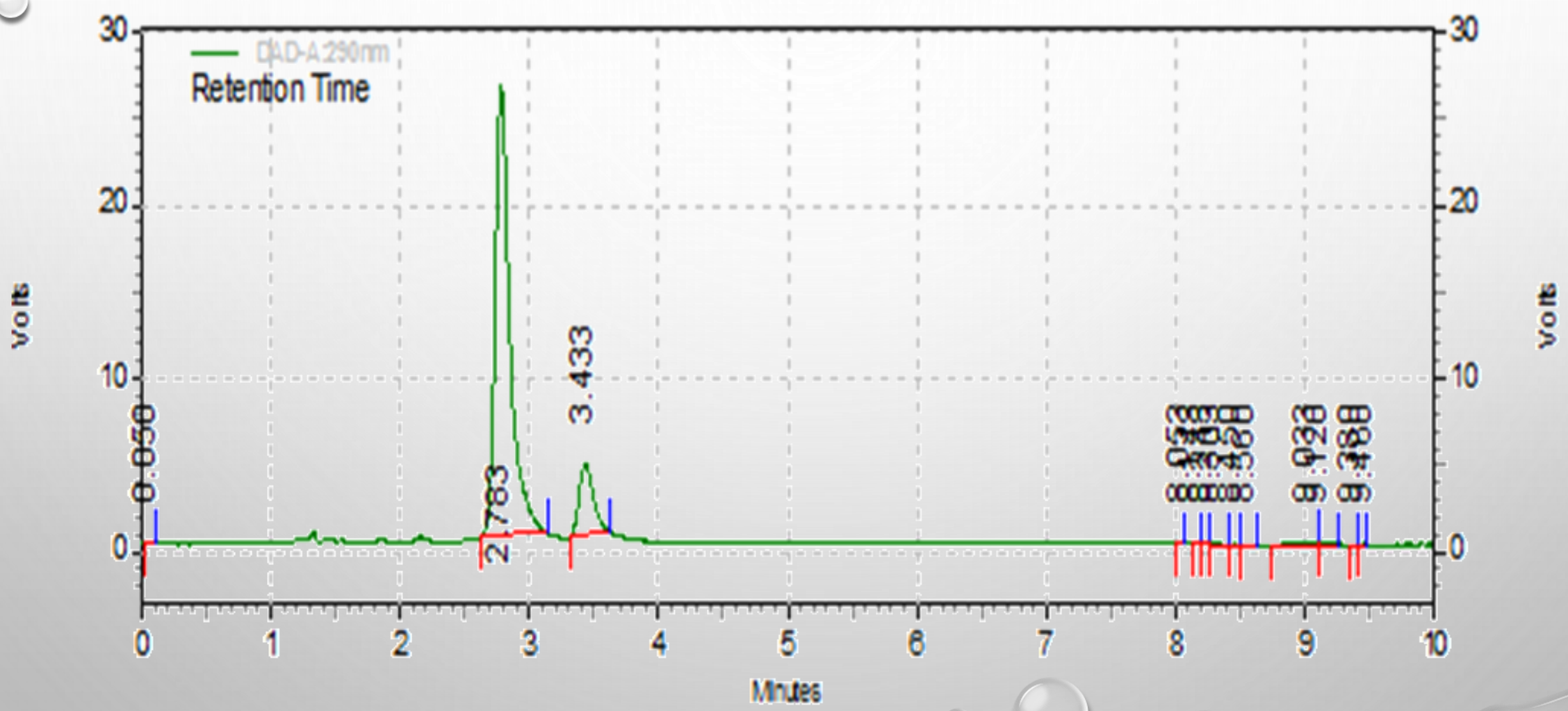
d) FT-IR


75
%T
60
45
30
15
3286.81
2918.40
2831.60
2702.36
2621.35
1898.02
1631.83
1600.97
1494.88
1462.09
1384.94
1309.71
1247.99
1180.47
1157.33
1082.10
1062.81
1012.66
968.30
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
NAR
1/cm

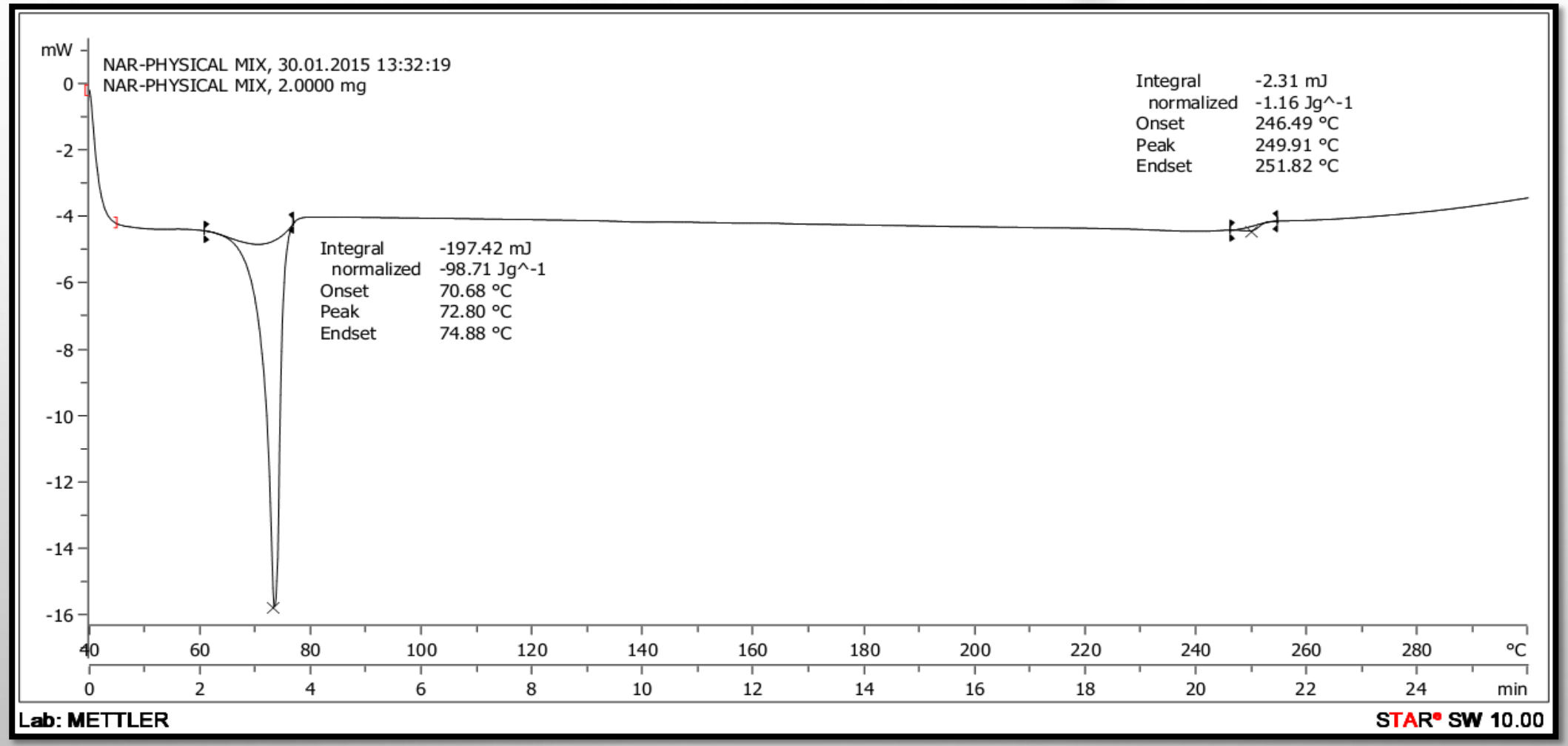
FT-IR Interpretation

Sr. No.	Functional Group	Standard Peak (cm^{-1})	Observed Peak (cm^{-1})
1	**Ar. C-H (Stretch.)**	3150-3050	**2918.40**
2	**Ar. C-H (Bend.)**	900-690	**759.98**
3	**Al. C-H (Stretch.)**	2800-2700	**2702.36**
4	**Al. C-H (Bend.)**	1450 & 1375	**1384.94**
5	**C=C**	1600 & 1475	**1600.97**
7	**C=O**	1680-1630	**1631.83**
8	**C-O**	1300-1000	**1157.33**
12	**O-H**	3307	**3286.81**

e) HPLC


DAD-A:280nm
Retention Time
2.763
3.433
8.928
9.388
Volts
Minutes

DSC Thermogram

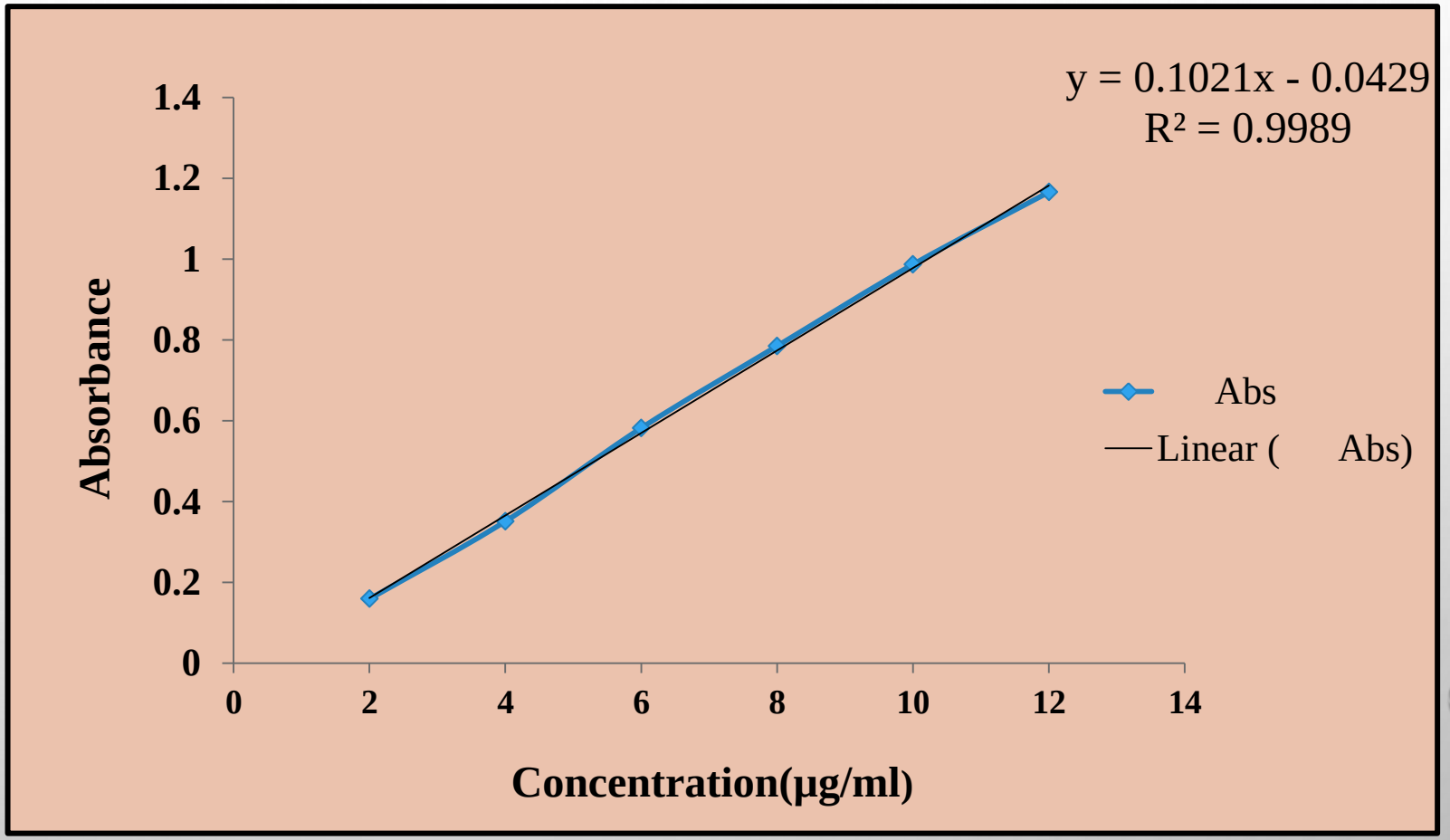


DSC thermogram of physical mixture of (drug +Polymer)

2. Standard Calibration Curve

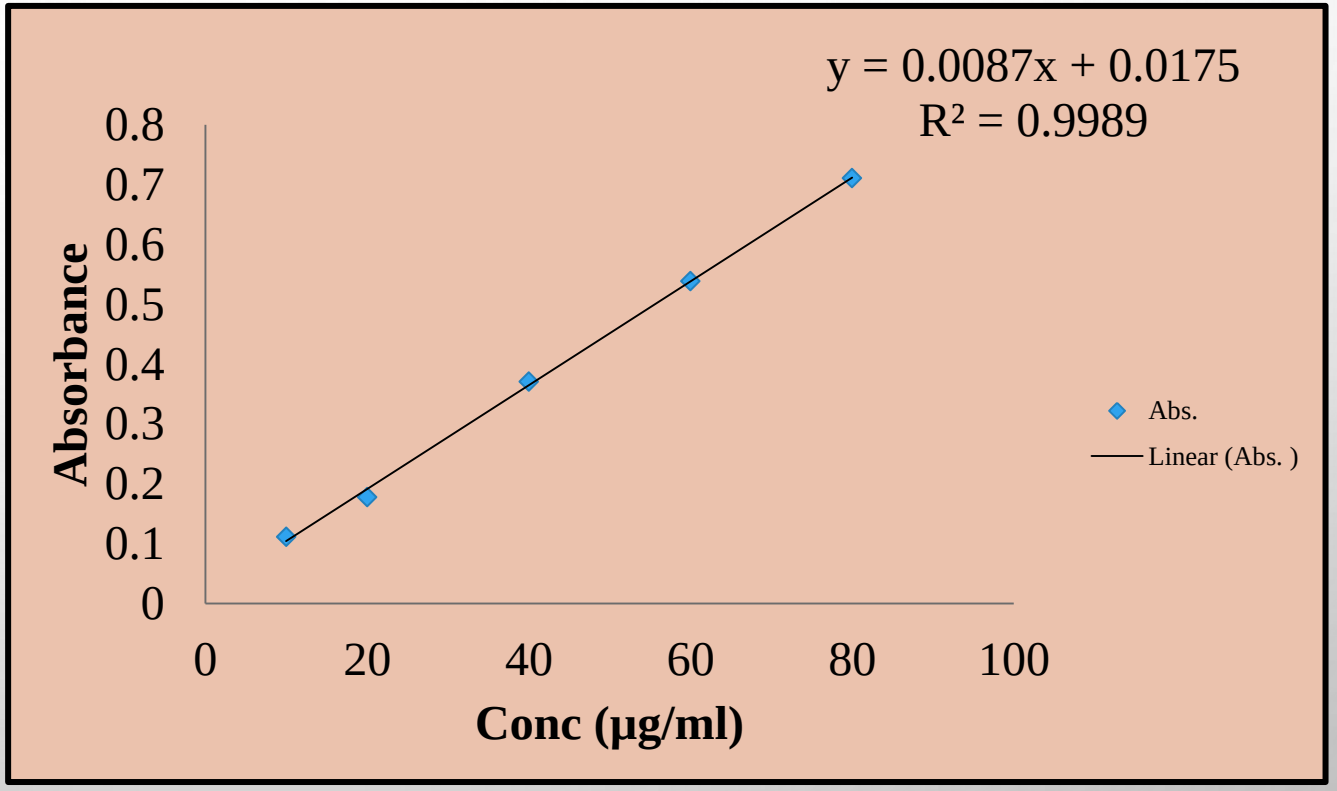
A) In Methanol:

Conc.	Abs.
2	0.159
4	0.351
6	0.582
8	0.785
10	0.987
12	1.116



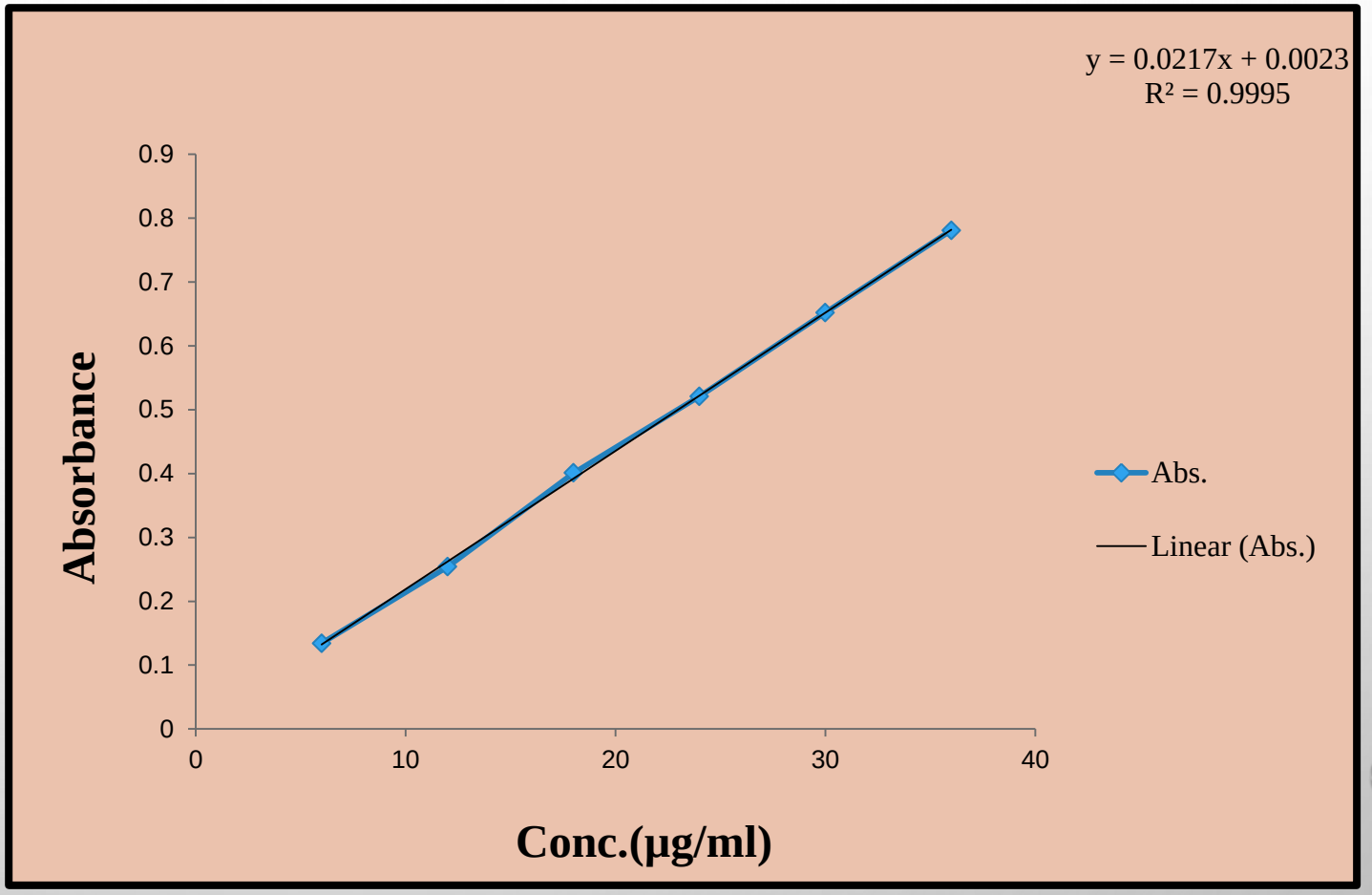
B) In Buffer pH 1.2:

Conc.	Abs.
0	0
20	0.112
30	0.178
40	0.371
50	0.539
60	0.711
70	0.891



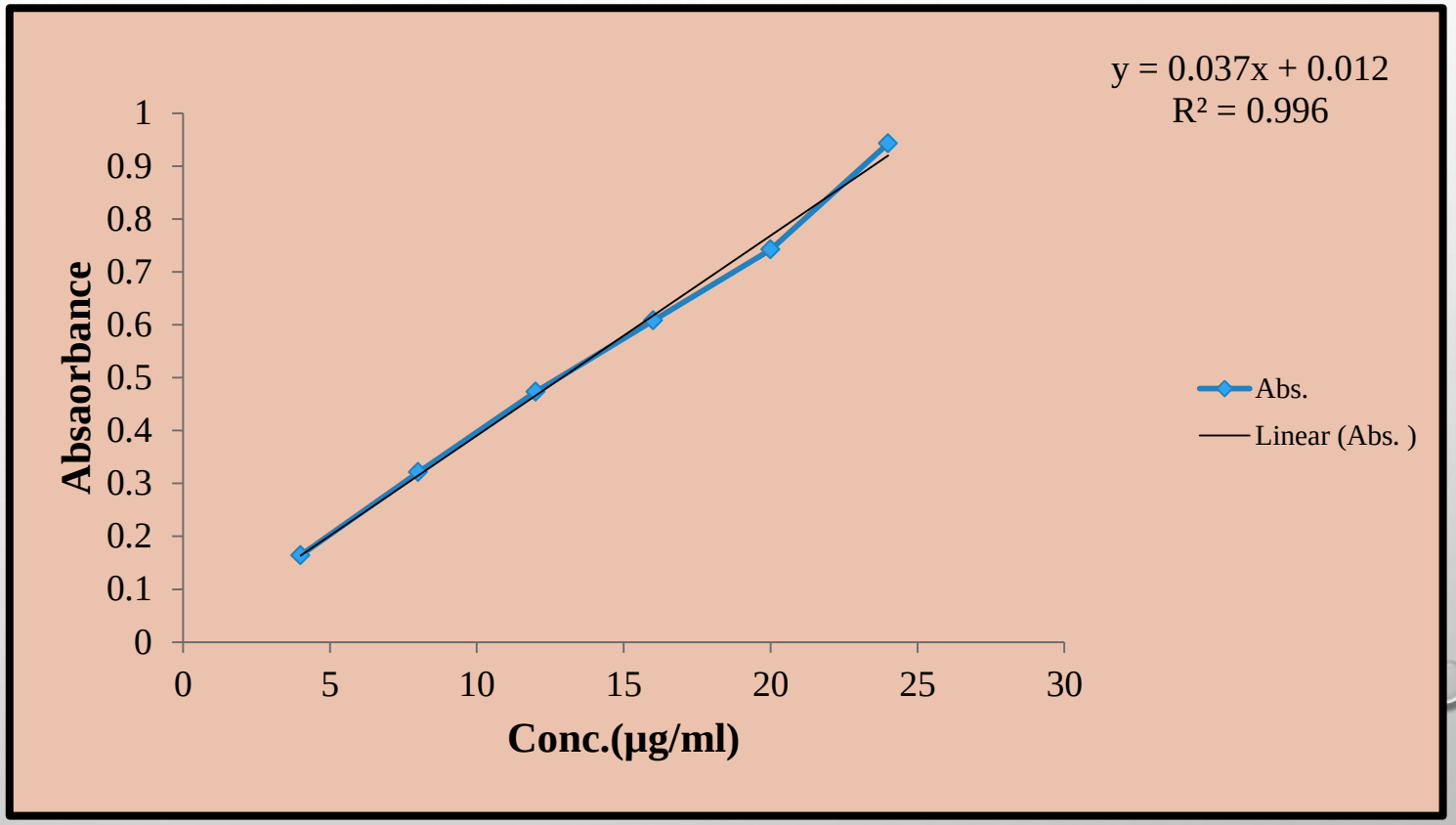
C) In PBS pH 7.4:

Conc. (µg/ml)	Abs.
6	0.134
12	0.254
18	0.401
24	0.521
30	0.652
36	0.781



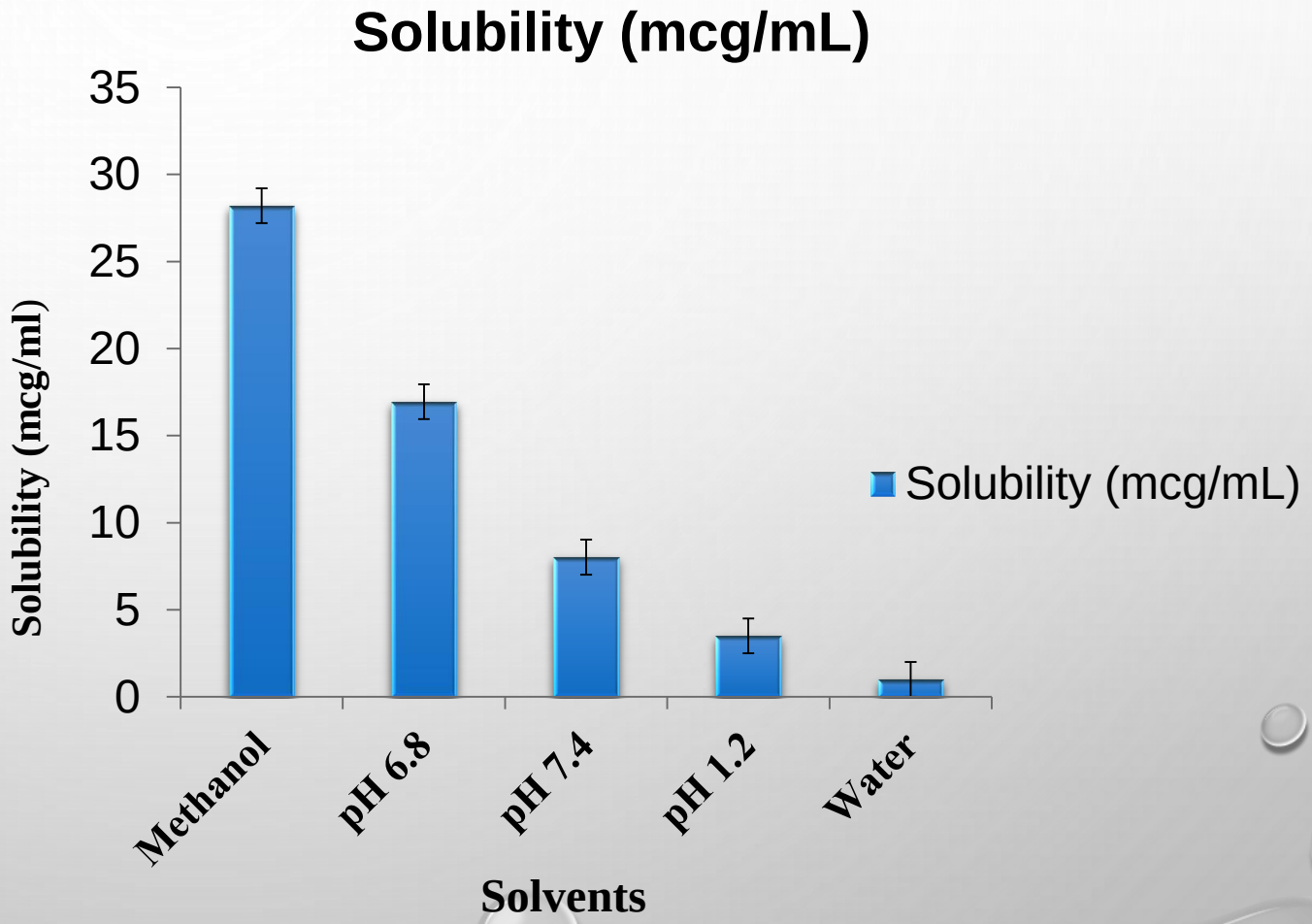
D) In PBS pH 6.8:

Conc.	Abs.
4	0.164
8	0.321
12	0.473
16	0.608
20	0.742
24	0.943



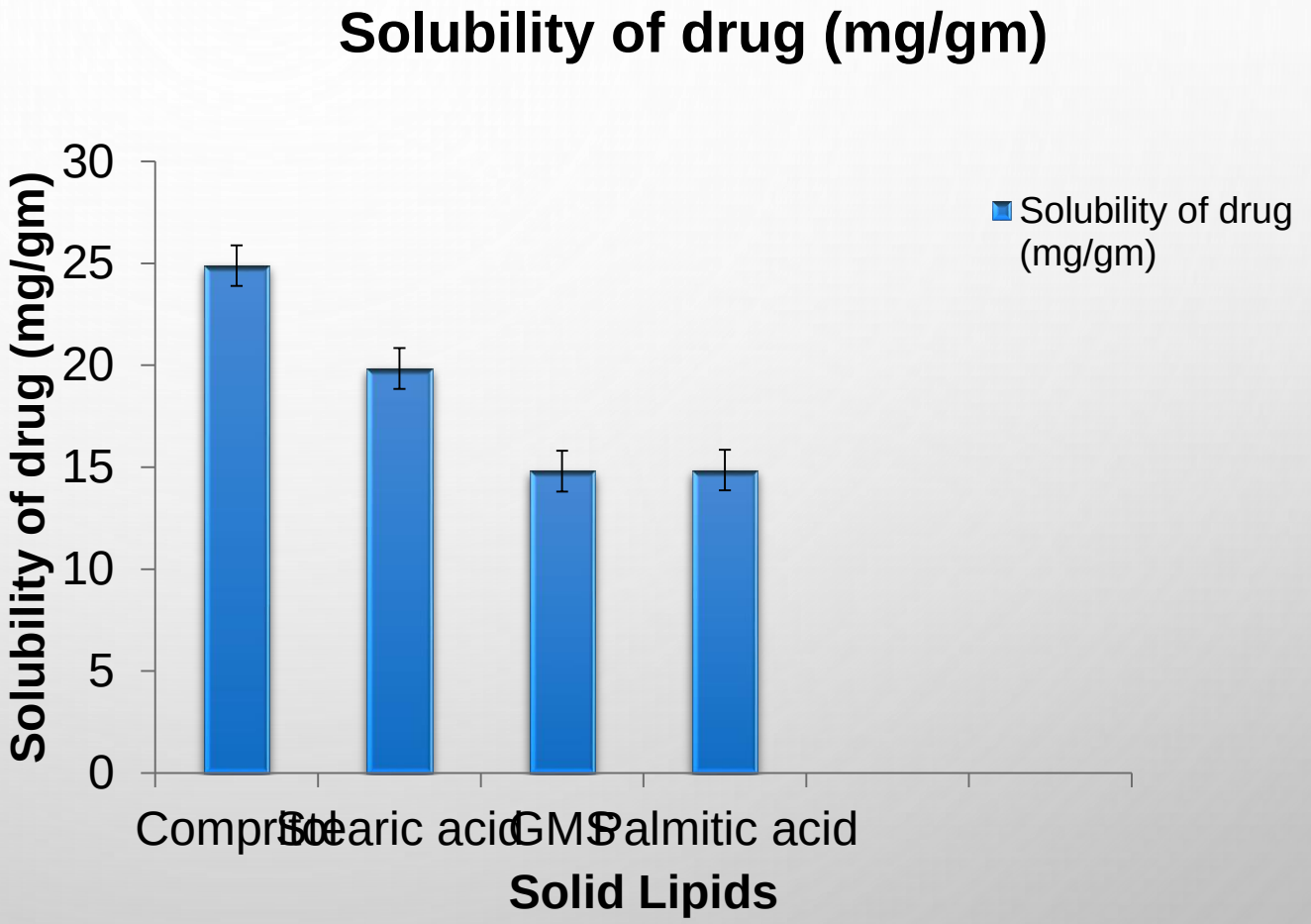
3. Solubility study

Solvent	Solubility (mcg/mL) (n=3)
Methanol	28.2 ± 0.05
pH 6.8	16.95 ± 0.03
pH 7.4	8.02 ± 0.09
pH 1.2	3.5 ± 0.04
Water	1.0 ± 0.02



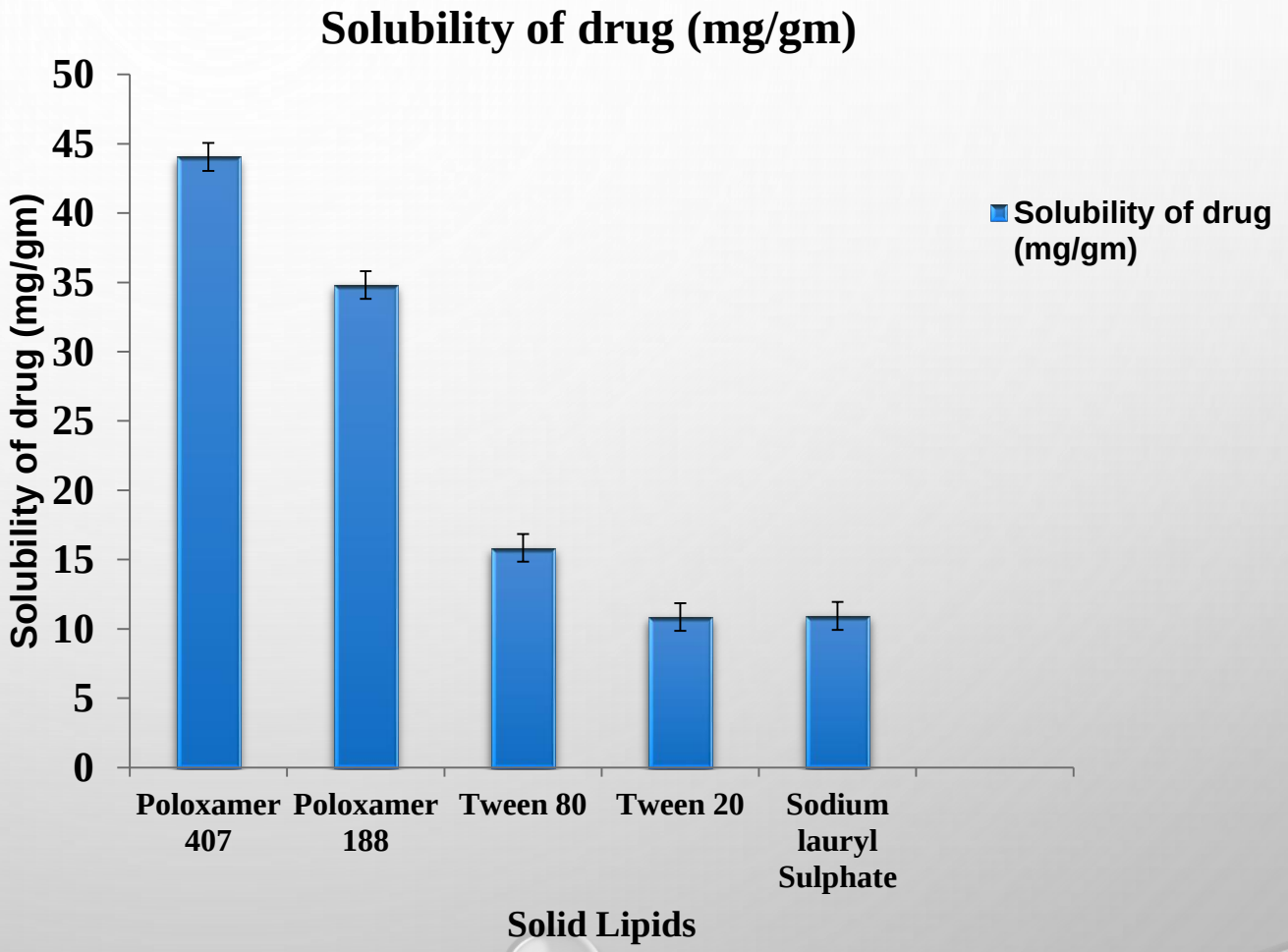
4. Lipid screening

Solid Lipids	Amount of drug dissolved in 1 gm solid lipid (mg/gm)
Compritol	24.886 ± 0.059
GMS	14.805 ± 0.043
Stearic acid	19.847 ± 0.063
Palmitic acid	14.861 ± 0.032

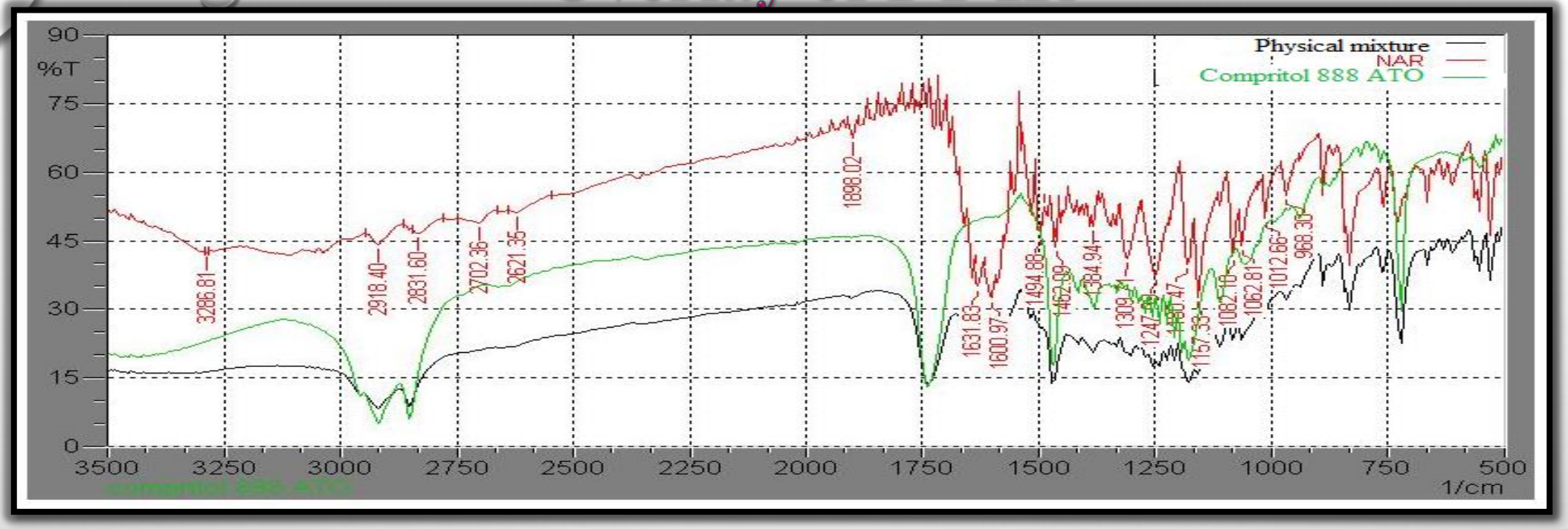


5. Surfactant screening

Surfactant	Amount of drug dissolved in 1 gm surfactant(mg/gm)
Poloxamer 407	44.05 ± 0.48
Poloxamer 188	34.805 ± 0.062
Tween 80	15.847 ± 0.054
Tween 20	10.861 ± 0.032
Sodium lauryl Sulphate	10.936 ± 0.059



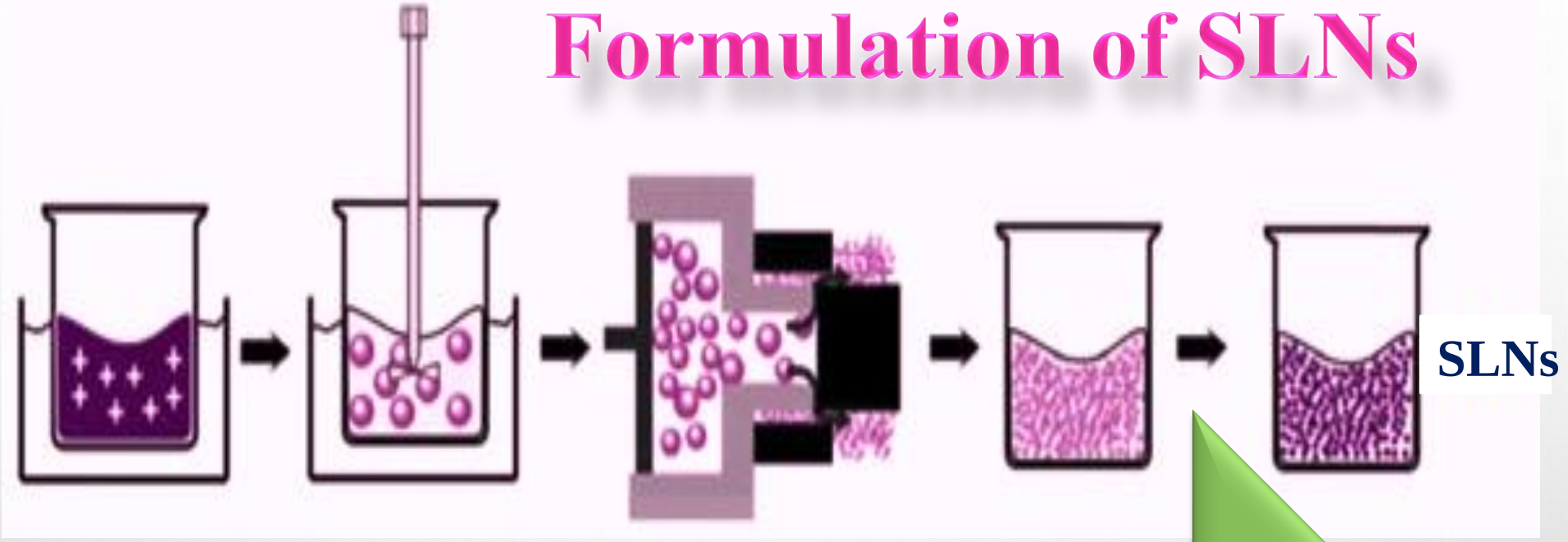
Overlay of FT-IR



Drug-Polymer	Functional groups
Compritol 888 ATO	1737.92, 1178.55, 1467, 2916.47, 2848.96
Naringenin	2918.40, 759.58, 2702.36, 1384.94, 1600.97, 1631.33, 1157.33, 3286.81
Physical Mixture	1157.33, 1600.97, 1384.94,1732.13, 2752.5, 2916.47, 3286.81, 2848.96

There was no interaction between drug and polymer by checking functional groups in overlay

Formulation of SLNs



Melting lipids and disperse drug into lipid solution

Disperse lipid solution in surfactant solution and prepare pre-emulsion

Hot High pressure homogenization

Hot O/W Nano-emulsion

Solidification by cooling down to room temperature

Experimental Design

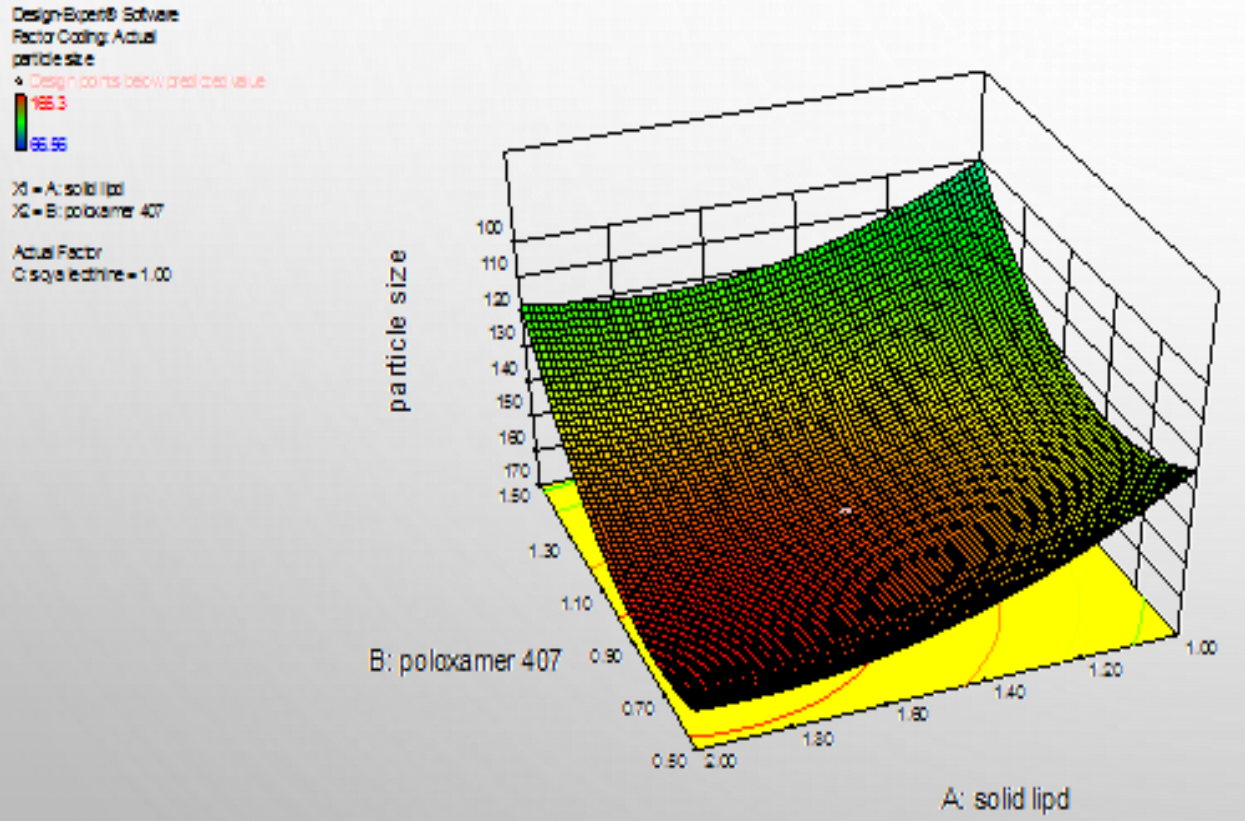
The coded and actual values of the variables used in CCRSM of NAR-SLNs preparation.

Independent variable	Actual values			
	Low (-1)	High (+1)	-alpha	+alpha
X_1= Solid lipid concentration in gm	1	2	0.6591	2.3409
X_2= Pluronic f 127 concentration in gm	0.5	1.5	0.159104	1.8409
X_3= (Soya lecithin) co-surfactant concentration in gm	0.5	1.5	0.5	0.5

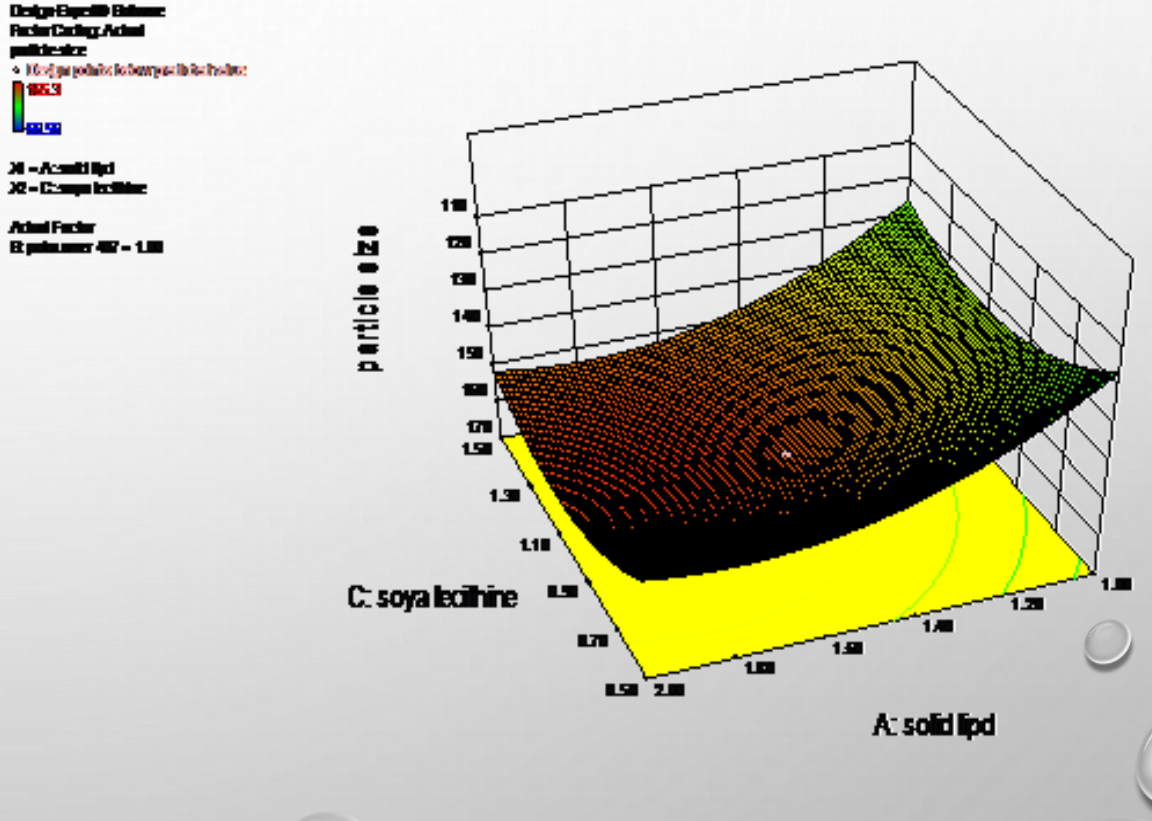
Run	X_1 (gm)	X_2 (gm)	X_3 (gm)	Particle size (nm)	% EE	Zeta potential (mV)
1	1	0.5	0.5	118.2±11	85.37 ± 2.1	-20.36 ± 2.1
2	1.5	1	1.84	120.1±13	67.25 ± 0.8	-15.9 ± 2.2
3	1.5	1	0.16	222.9.5±06	68.87 ± 0.9	-13 ± 2.0
4	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ± 1.2
5	1.50	0.16	1	119.6±12	92.08 ± 2.9	-21.24 ± 1.2
6	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ± 1.2
7	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ±1.2
8	2	0.5	1.5	165.3±04	62.5 ± 0.4	-15.3 ± 1.8
9	2	1.5	0.5	98.66±09	84.13 ± 2.0	-11.4 ± 2.0
10	1	0.5	1.5	115.2±10	80.25 ± 1.9	-19.72 ± 1.4
11	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ± 1.2
12	1	1.5	0.5	66.56±06	93.75 ± 2.9	-12.8 ± 1.9
13	2	0.5	0.5	123.5±05	89.15 ± 2.2	-23.7 ± 1.3
14	2	1.5	1.5	95.12±07	85.78 ± 2.1	-14.59 ± 1.5
15	2.34	1	1	158.8±04	91.27 ± 1.7	-16.25 ± 1.5
16	1.5	1.84	1	79.23±06	91.37 ± 1.4	-15.77 ± 2.4
17	0.66	1	1	96.28±08	90.5 ± 2.4	-17.23 ± 1.7
18	1	1.5	1.5	110±12	94.05 ± 2.1	-19.8 ± 1.8
19	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ± 1.2
20	1.5	1	1	156.4±08	89.38 ± 2.3	-13.4 ±1.2

A) Response surface plot showing the effect of the concentration of solid lipid and surfactant on particle size

B) Response surface plot showing the effect of the concentration of solid lipid and co-surfactant on particle size.



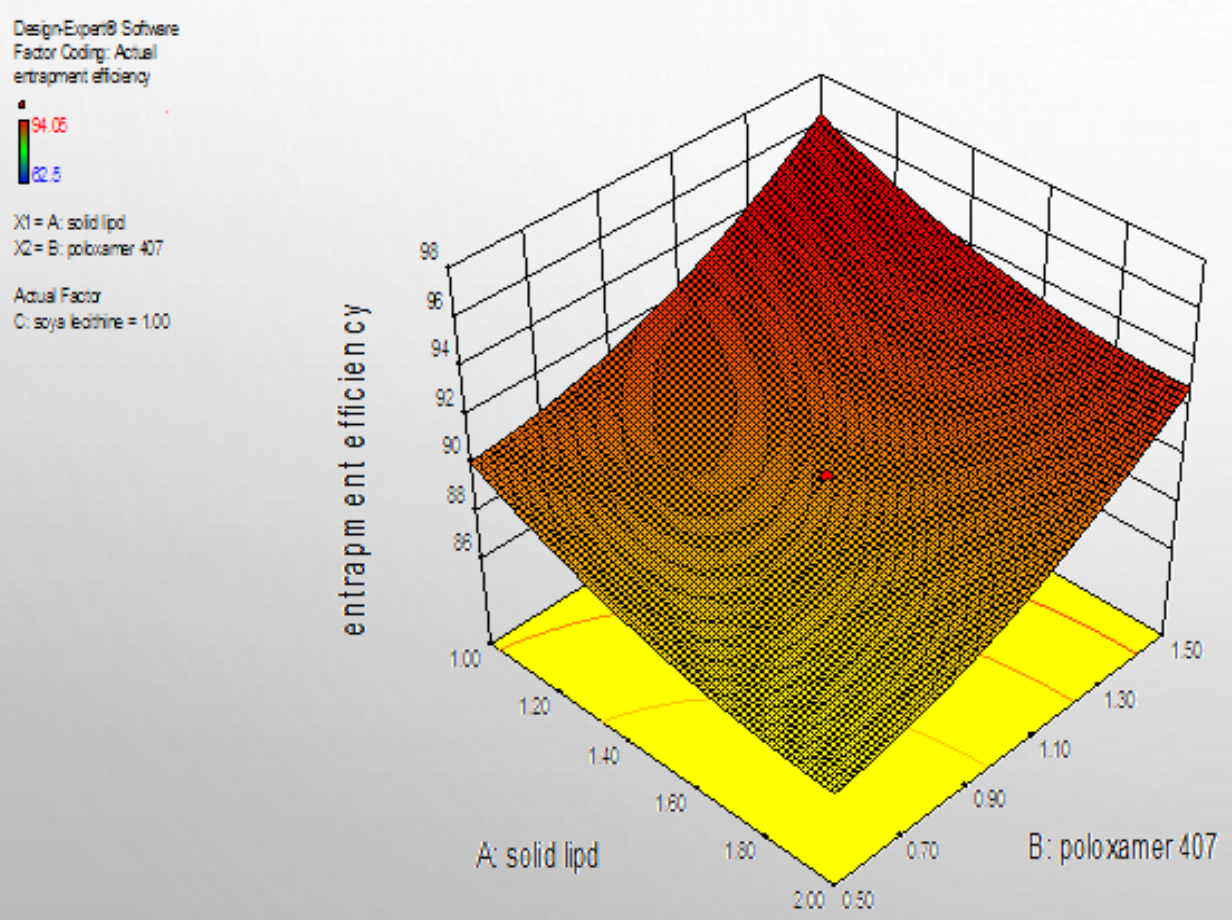
A



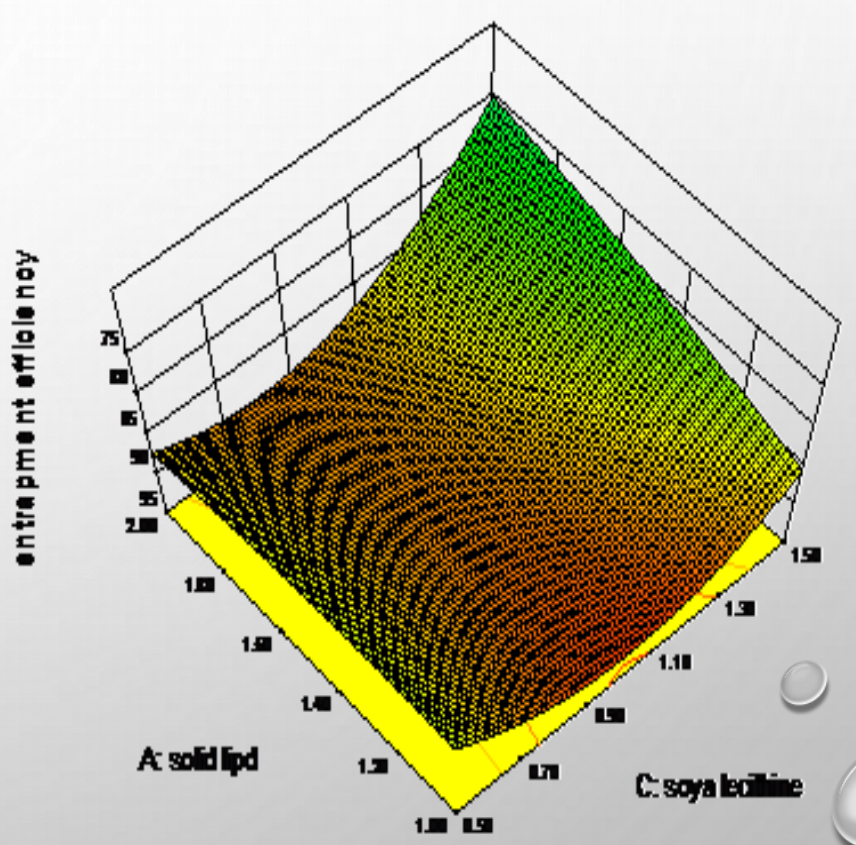
B

A) Response surface plot showing the effect of the concentration of solid lipid and surfactant on entrapment efficiency.

B) Response surface plot showing the effect of the concentration of solid lipid and co-surfactant on entrapment efficiency.



A



B

Summary of results of regression analysis for responses $Y_1$and Y_2 and analysis of variance for particle size and EE

Parameter	DF	SS	MS	*F*	P-value	R^2	SD	CV%
Particle size (Y_1)								
Model	9	14826.26	1647.36	7.49	**0.0021 significant**	0.7546	14.82	11.68
Residual	10	2198.63	219.86	-	-	-		
Total	19	17024.89	-	-	-	-		
% EE (Y_2)								
Model	9	1299.99	144.44	7.48	**0.0021 significant**	0.834	5.07	5.90
Residual	10	257.55	25.75	-	-	-		
Total	19	1557.55	203.14	-	-	-		

For Particle size

Y_1=156.52+13.07A-1609+4.11C

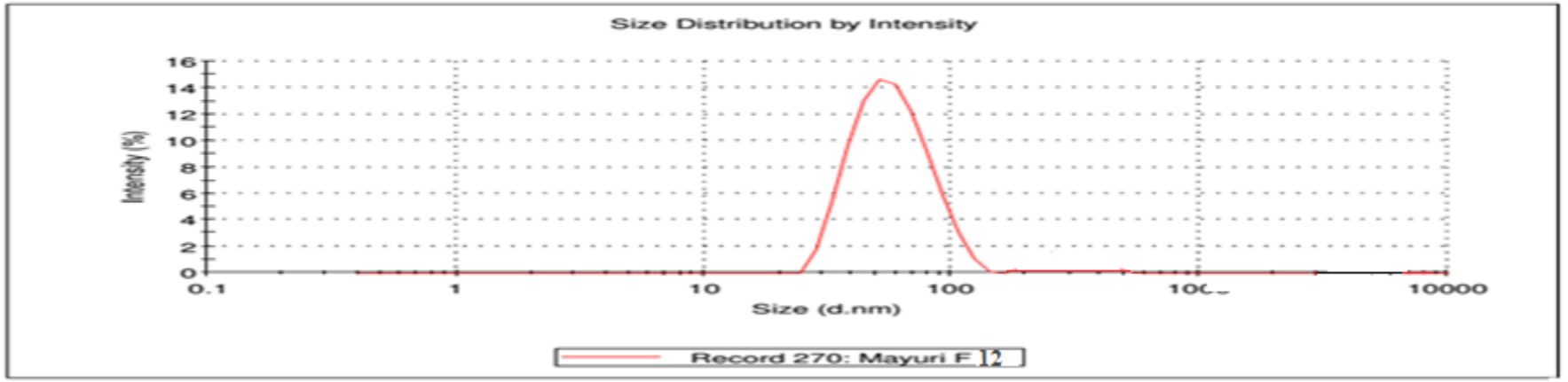
For % EE

Y_2=89.32-1.89A+3.24B-2.75C

Particle Size and Zeta Potential of Optimized Batch

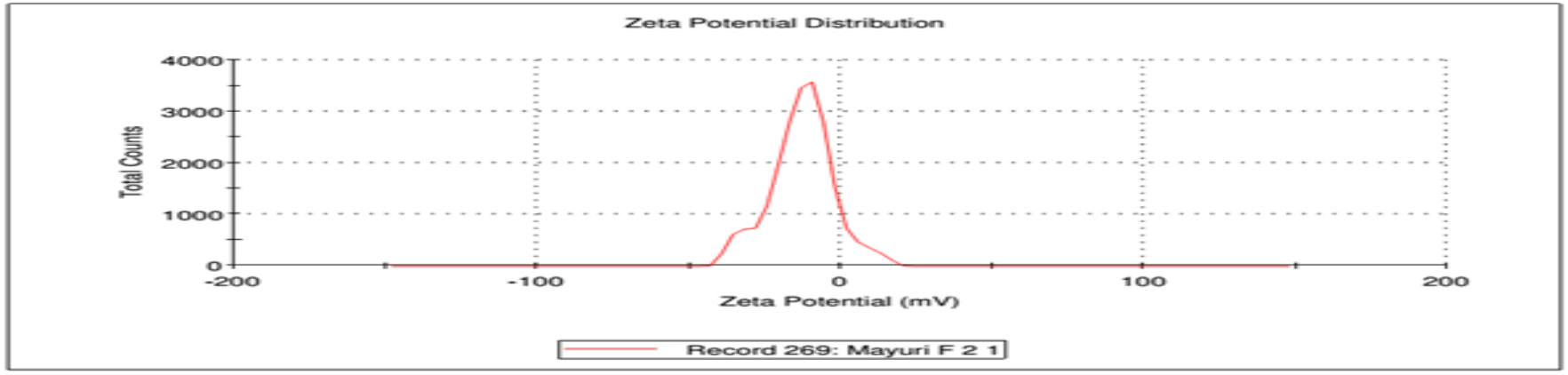
Particle size

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 66.56	Peak 1:	58.84	90.3	20.09
PdI: 0.318	Peak 2:	301.8	7.9	71.18
Intercept: 0.974	Peak 3:	5418	1.8	295.4
Result quality : Good				



Zeta potential

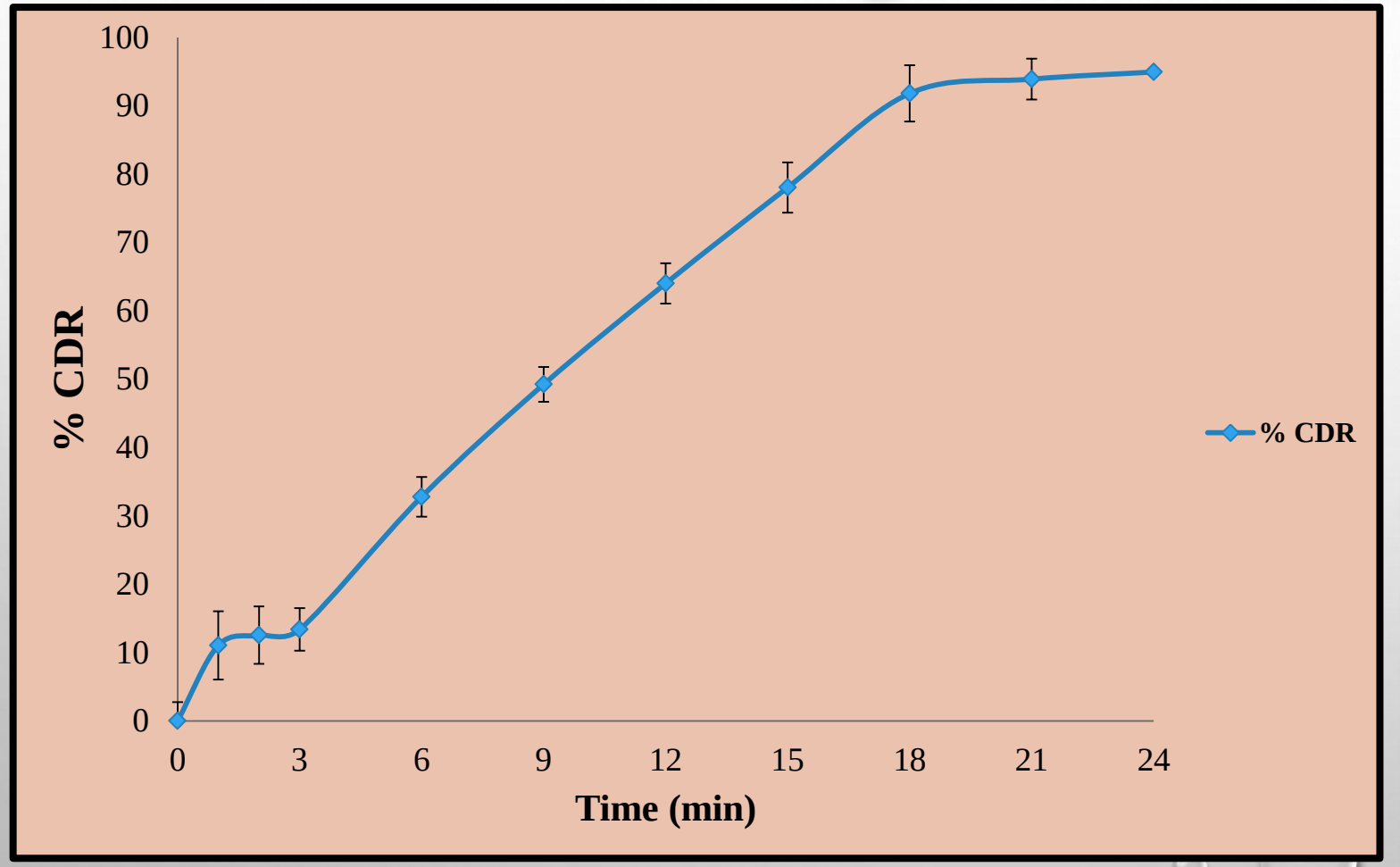
		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -12.8	Peak 1:	-12.8	100.0	10.5
Zeta Deviation (mV): 10.5	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.525	Peak 3:	0.00	0.0	0.00
Result quality : Good				



Lyophilization of SLNs

Lyophilization was carried out for optimised batch at -75°C with 5% mannitol as a cryoprotectant. The role of cryoprotectant is to decrease nanoparticle aggregation during the process of freeze-drying. The obtained lyophilised powder was found to be dry, porous and friable after 72 h. The vacuum was maintained at 76 mTorr.

In vitro dissolution profile of Optimized Batch



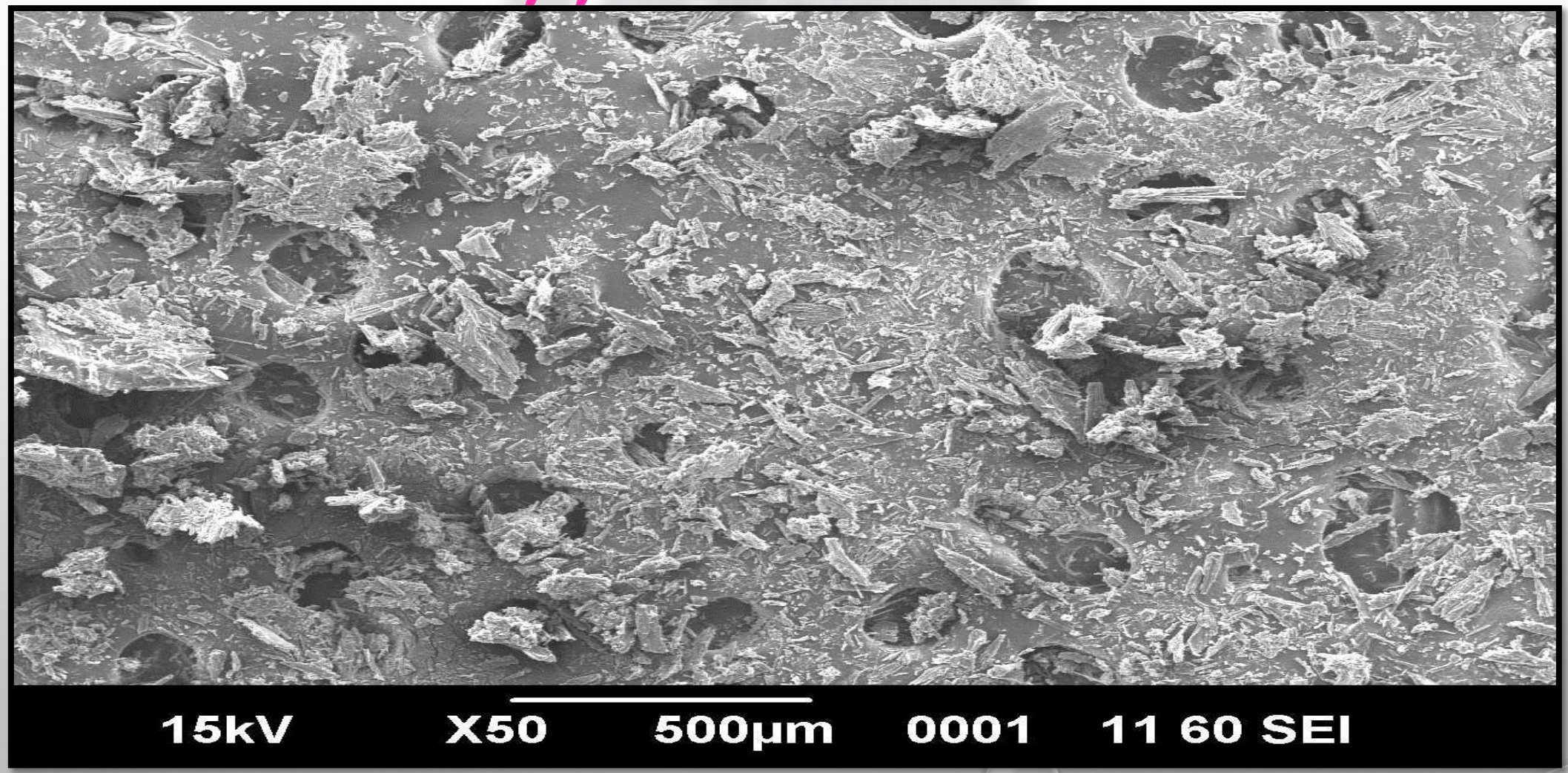
Characterization of Solid State SLN

A. Scanning Electron Microscopic Photographs (SEM)

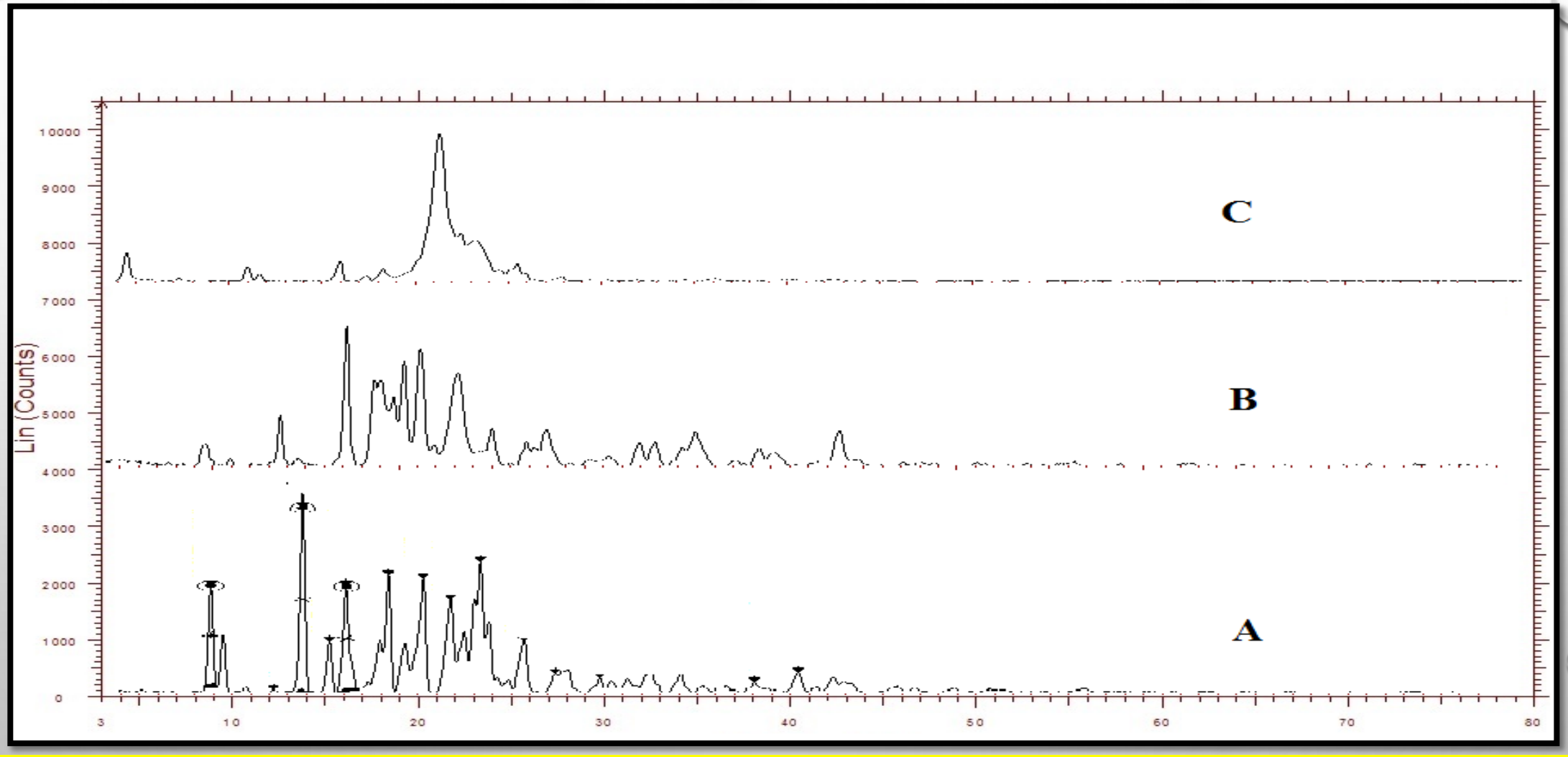
B. X-ray Diffraction Study (XRD)

C. DSC study

A. Scanning electron photomicrographs of formulation

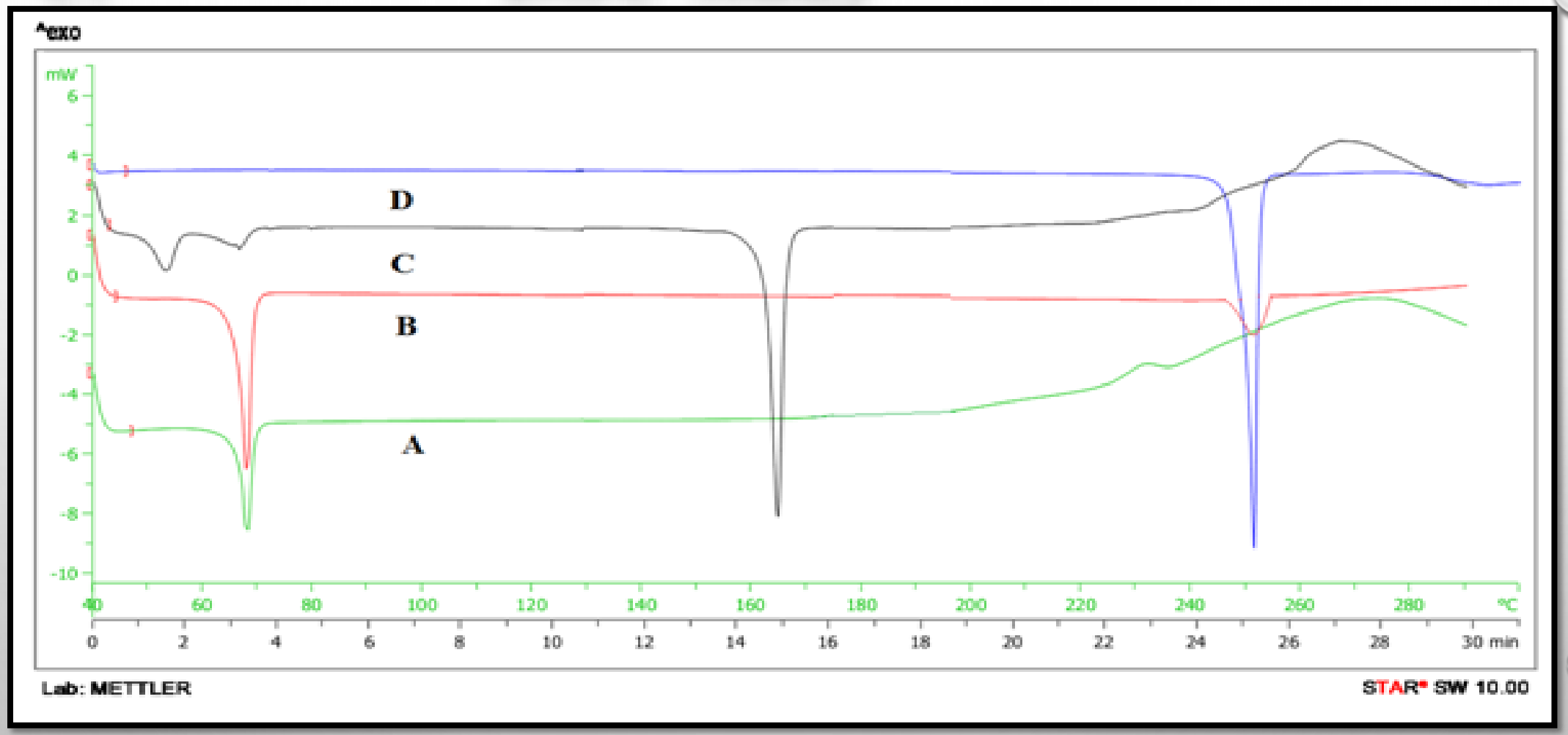


B. X-ray Diffraction study



XRD Pattern of A) Pure drug, B) placebo lyophilized formulation, C) drug-loaded lyophilized formulation.

C. DSC study



Where A-Solid lipid, B- Physical mixture C-Lyophilized NAR-SLNs and D-Drug

Accelerated stability study

Stability Parameters		Test periods		
	0 month	1 month	2 month	3 month
Particle size	66.56 ± 11	69.24 ± 10.4	76.93 ± 0.08	78.56 ± 8.80
PDI	0.310 ± 0.11	0.312 ±0.10	0.313 ± 0.10	0.315 ± 0.02
EE	94.05 ± 2.2	93.99 ± 2.0	93.89 ± 1.5	93.85 ±1.4

Conclusion

❖ Naringenin was successfully incorporated in to SLNs by HPH method by using Central Composite Design and Response Surface Methodology (CCRSM).

❖optimized formulation was selected on the basis of MPS and % EE. Drug release studies were found be precise and followed zero order release kinetic model with Fickian release mechanism and DSC and XRD studies proved the compatibility of drug with used lipids.

❖Accelerated stability study was conducted for 3 monthsit was found stable.

❖NAR-SLNs can be demonstrated as a potential carrier to improve oral bioavailability of NAR.

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Thank You...

My Guide –Dr. S J. Surana

Dr. A.U.Tatiya

Omic Group and Organizers

Audience and Judges