RESEARCH METHODOLOGY
LEC. 1

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Starting with the laboratory work as pre-formulation followed by formulation

Preparation or demonstration of calibration curve:

1. Beers-Lamber Law(a direct relationship exists between the concentration and absorbance at a specific wavelength).

2. The stock solution was prepared by dissolving API in a suitable solvent (in which the API is highly soluble), e.g., methanol, ethanol, DMSO4,…

Sometimes, these solvents were used as co-solvents (use 5 or 10 ml), and then the volume was completed with buffer, 0.1 HCl, or deionized water, according to the experiment's requirements.

3. Sometimes, **SLS** or **Tween 80** was used to dissolve the drug and then complete the volume with the required media.

4. Volumetric flasks were used in all ways to prepare the calibration curve.

5. According to the drug's properties and relative absorbtivity in certain media (absorbance intensity), we prepared two stock solutions: the first stock solution and the second stock, which was prepared from the first one using the dilution equation C1V1=C2V2.

e.g.:

Weight 1g of drug in 100ml of the media (in which the drug is highly soluble)-------- to get a concentration of 0.01g per ml = **10mg/ml**

Upon reading, the absorbance is very high, and after dilution with various ratios, it stayed very high and near the border value **of 0.9-1**

Solution:

- **Take 10 ml from stock 1------- dilute with the required media to 50 ml to obtain stock 2. Different volumes are drawn using a bulb pipette or micro pipette, put in volumetric flasks, and then the volume is completed to 10 ml or as required with the same media used for preparing the stock.**

Calibration curve of drug X in phosphate buffer at pH 7.4:

1. Weigh 0.025 g of drug X → put in volumetric flask → add 10 ml of methanol ensure completely dissolved → complete the volume with PBS to 50 ml →

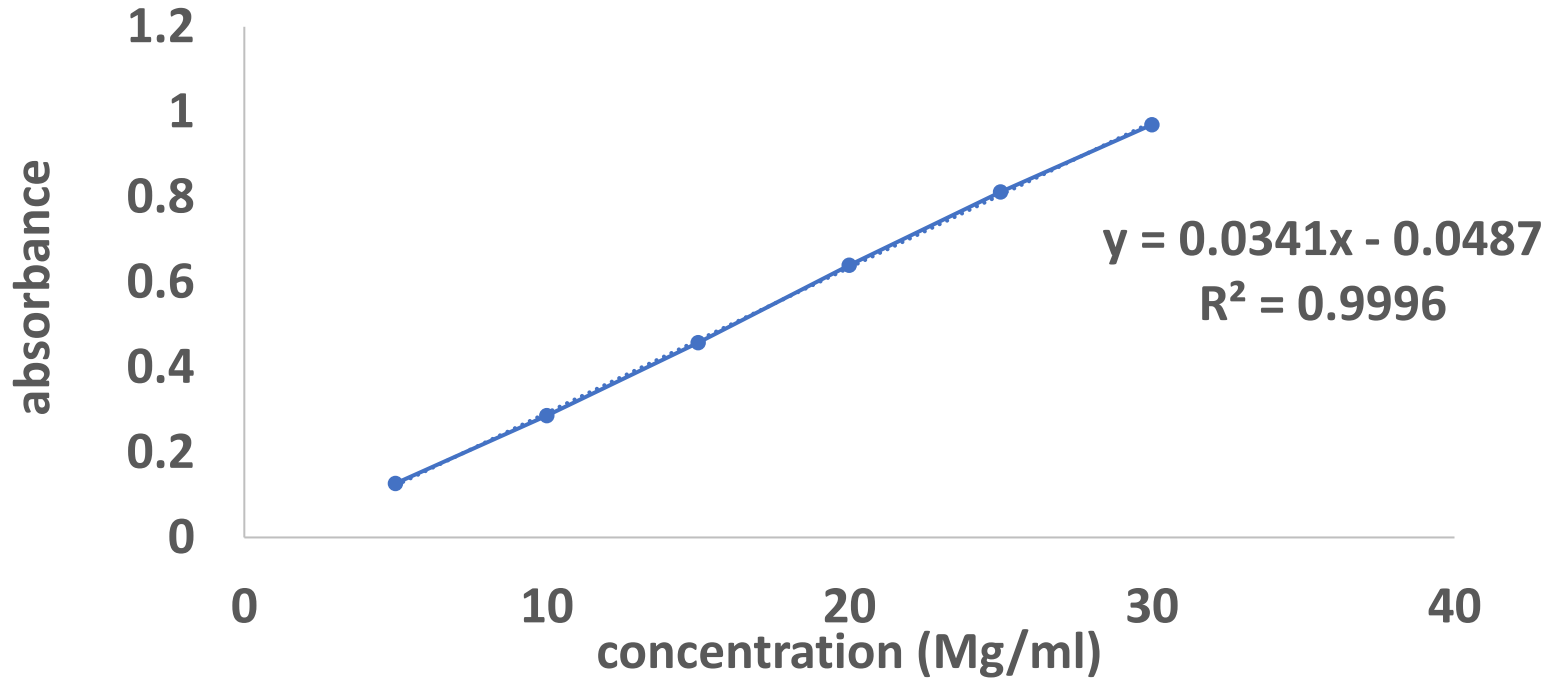
500Mg/ml(conc. of stock solution).

1. According to the table below:

Volume taken from stock(ml)	Concentration (Mg/ml)	Absorbance
0.1	5	0.127
0.2	10	0.286
0.3	15	0.458
0.4	20	0.665
0.5	25	0.812
0.6	30	0.962

- Take each volume seperately from stock --------put in 10 ml volumetric flask ---------complete the volume with BPS pH7.4.
- C1V1=C2V2------ 500x 0.1= C2 x10 ---------C2= 500X0.1/10 =**5Mg/ml**

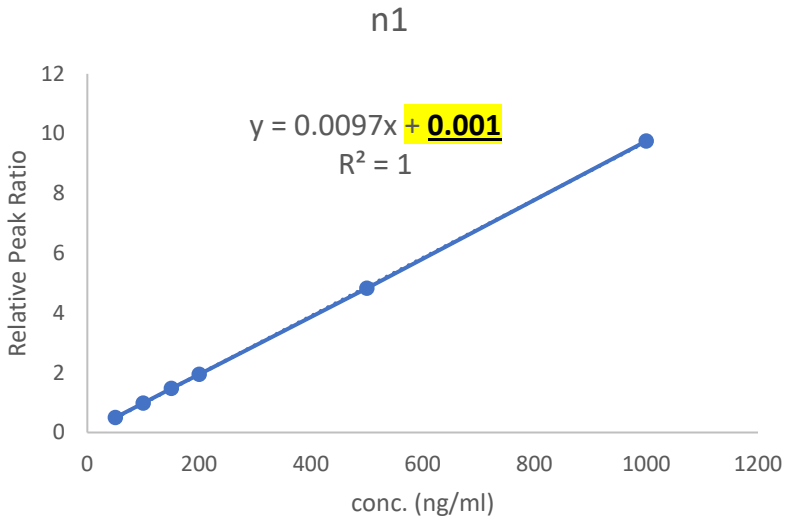
Draw the calibration curve using the Excel program or prism.

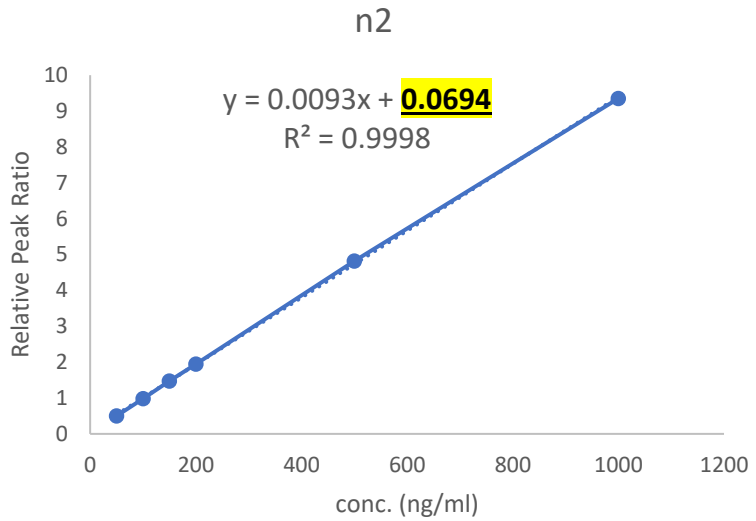


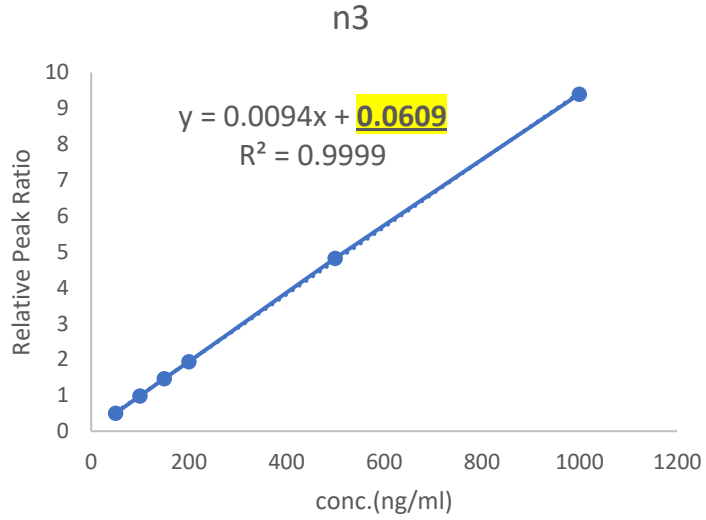
NOTE: sometimes due to the solubility of certain drugs especially class 2 and 4
In the beginning, start with the first stock in an organic solvent that dissolves the drug, e.g, methanol
Then, prepare the second stock with a buffer using the dilution equation.
Finally, from the second stock, we prepare a series of standard solutions

Validation of UV or HPLC method depending on the calibration curve

N1(AUC stan./AUC int.stan)	concentration (ng/ml)	N2(2AUC stan./AUC int.stan)	concentration (ng/ml)	N3(AUC stan./AUC int.stan)	concentration (ng/ml)
0.4997	50	0.4997	50	0.4997	50
0.982	100	0.982	100	0.982	100
1.469	150	1.469	150	1.469	150
1.942	200	1.942	200	1.942	200
4.8197	500	4.8197	500	4.8197	500
9.75	1000	9.35	1000	9.4	1000







intercept 1(c1)	c2	c3	sd of c	sd/b		
0.001	0.0694	0.0609	0.037280066	3.883340226	11.7	**LOD(3x sd/b)**
					38.8	**LOQ(10 x sd/b)**

Solubility study or scan:

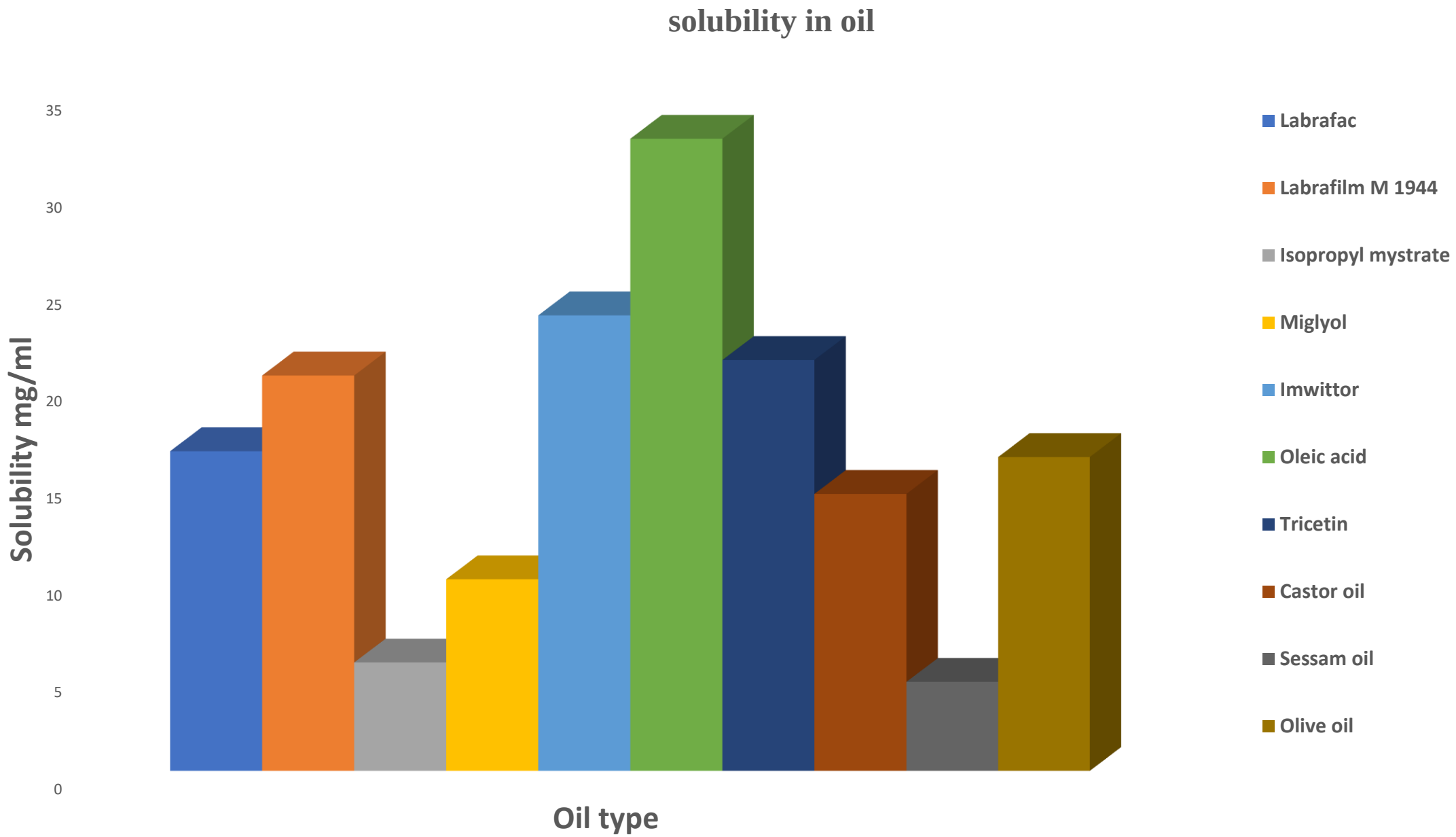
- For most advanced drug delivery systems that use oils, surfactants, and co-surfactants, it's very important to start with a solubility study using the traditional shake flask method:

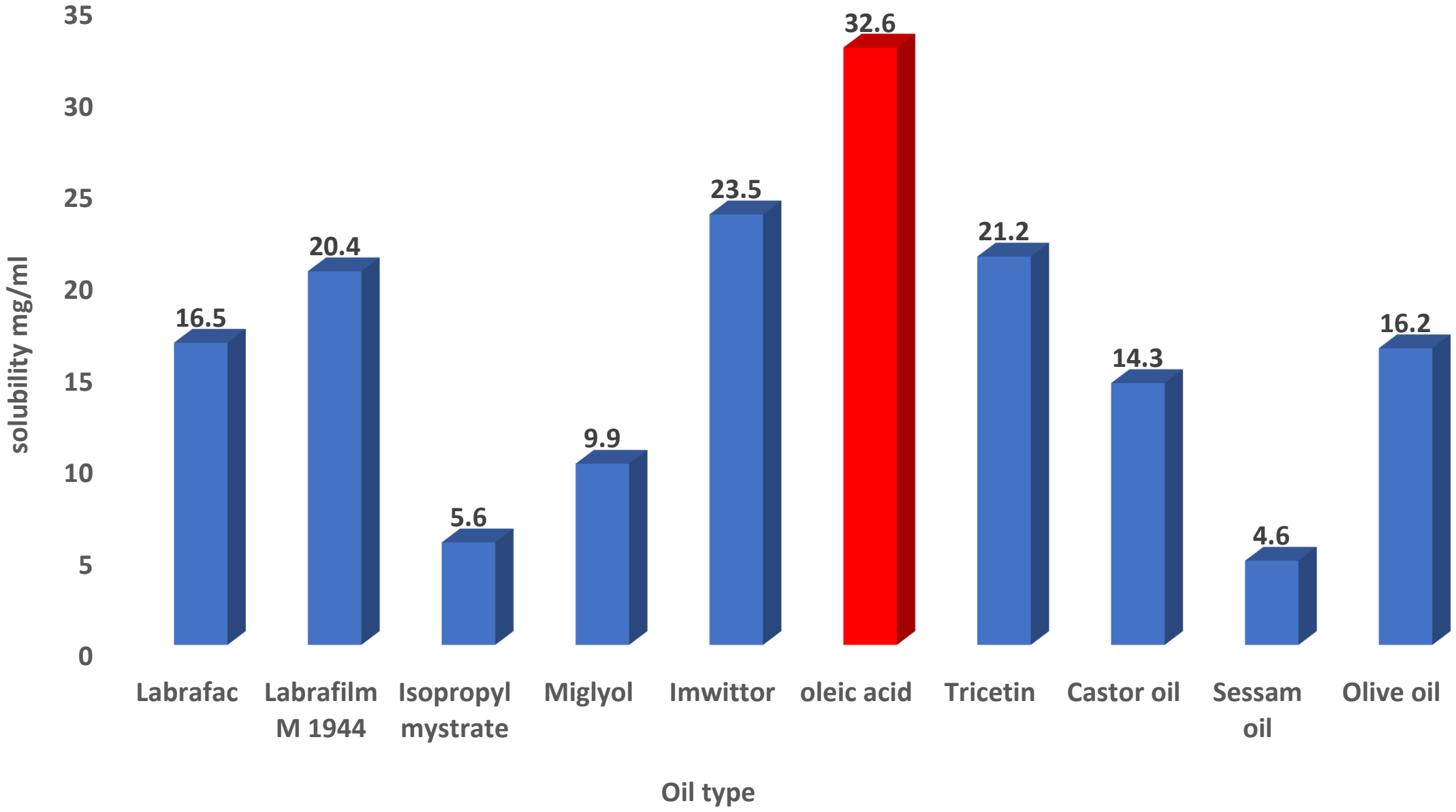
1. Using test tub +2ml of (oil or surfactant or co surfactant) then add excess drug with shaking till the ppt. of drug begins to increase to form non compressible layer----------stoppered the test tube
2. Put in shaking water bath for 72 hours at 25°C----------then centrifuge for 20 min at 3000rpm.

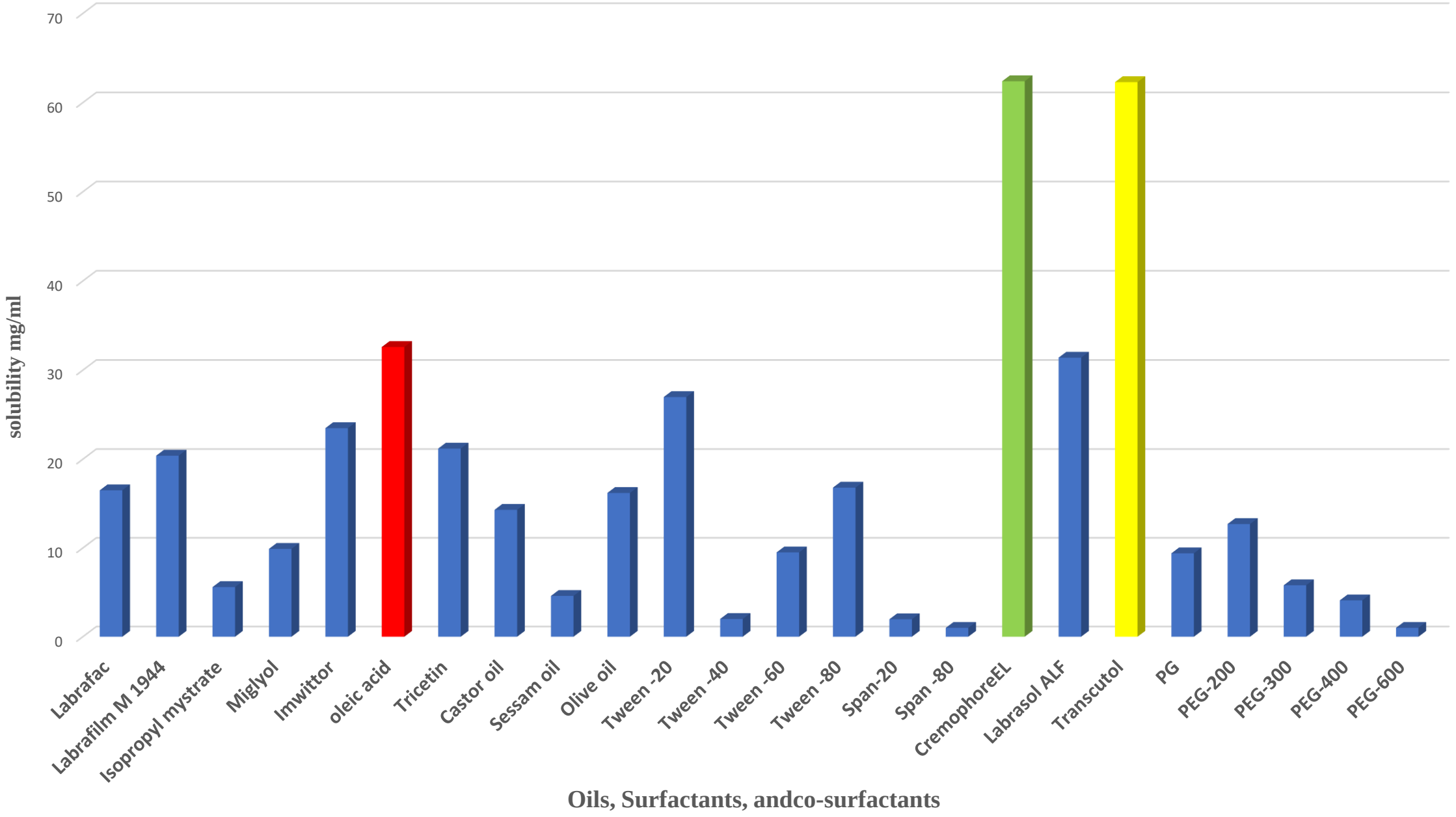
3. For the dilution of a specific volume of supernatant (which should be the same for all media used), methanol was utilized.

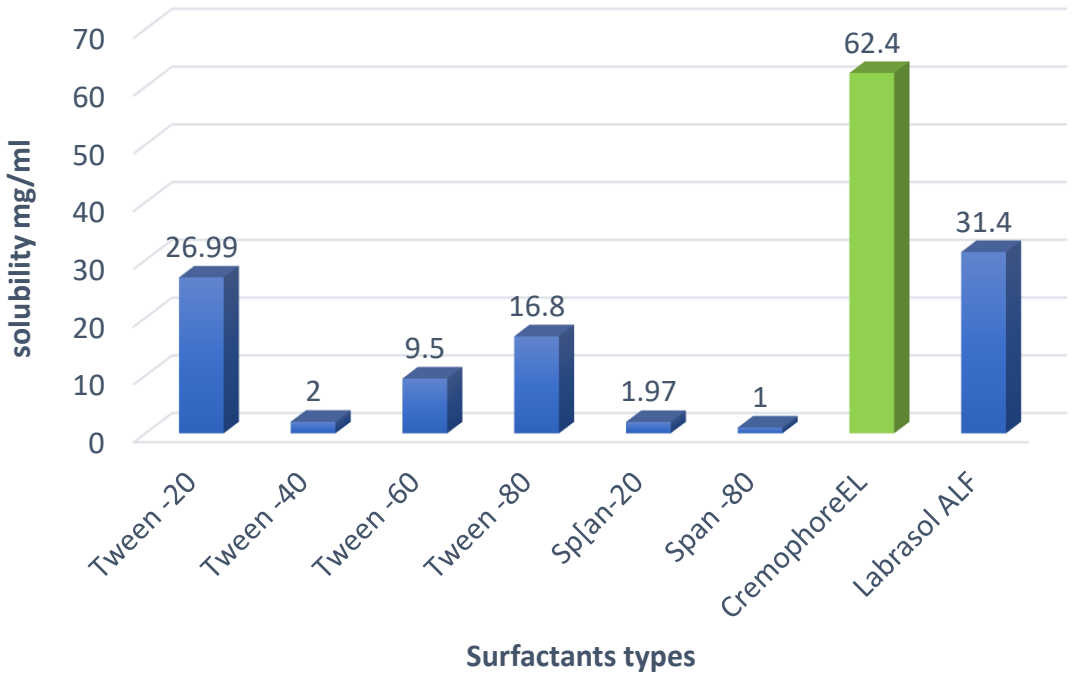
4. At specified λ, the dug concentration was measured spectrophotometrically.

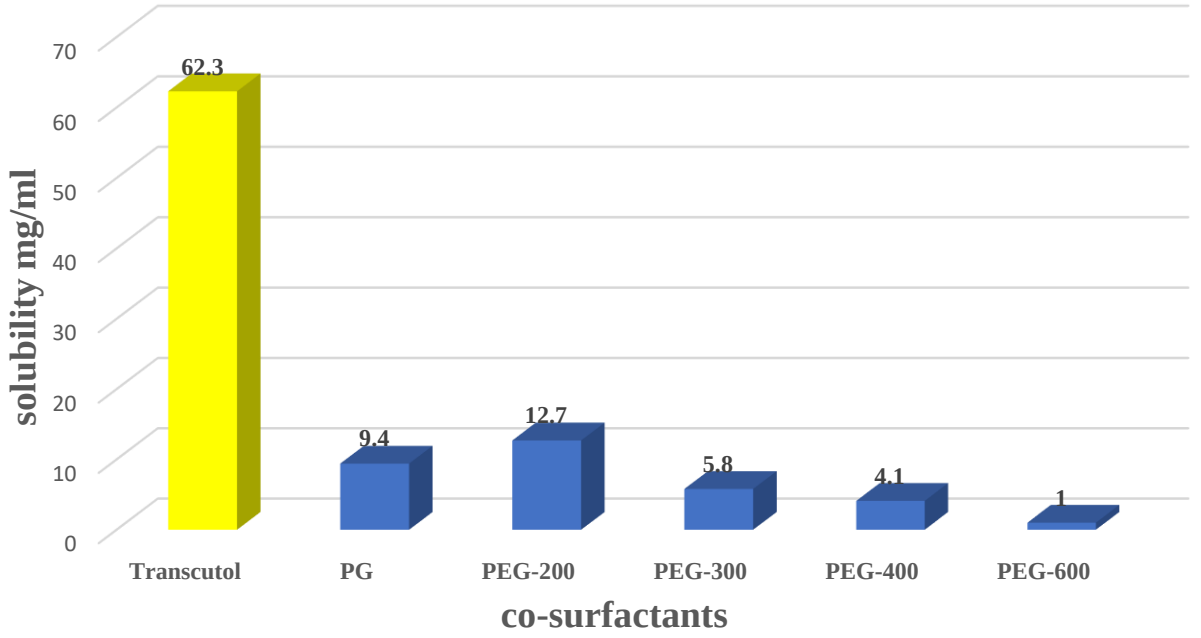
5. The solubility behavior is always explained as below

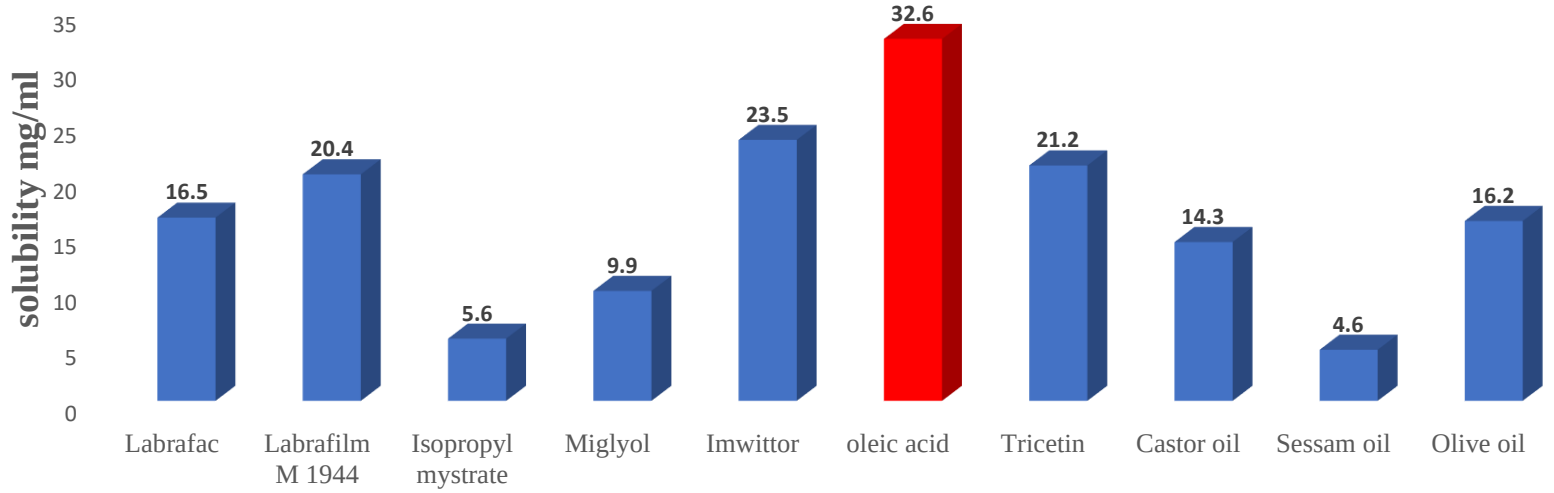

solubility in oil
Solubility mg/ml
35
30
25
20
15
10
5
0
Oil type
Labrafac
Labrafilm M 1944
Isopropyl mystrate
Miglyol
Imwittor
Oleic acid
Tricetin
Castor oil
Sessam oil
Olive oil

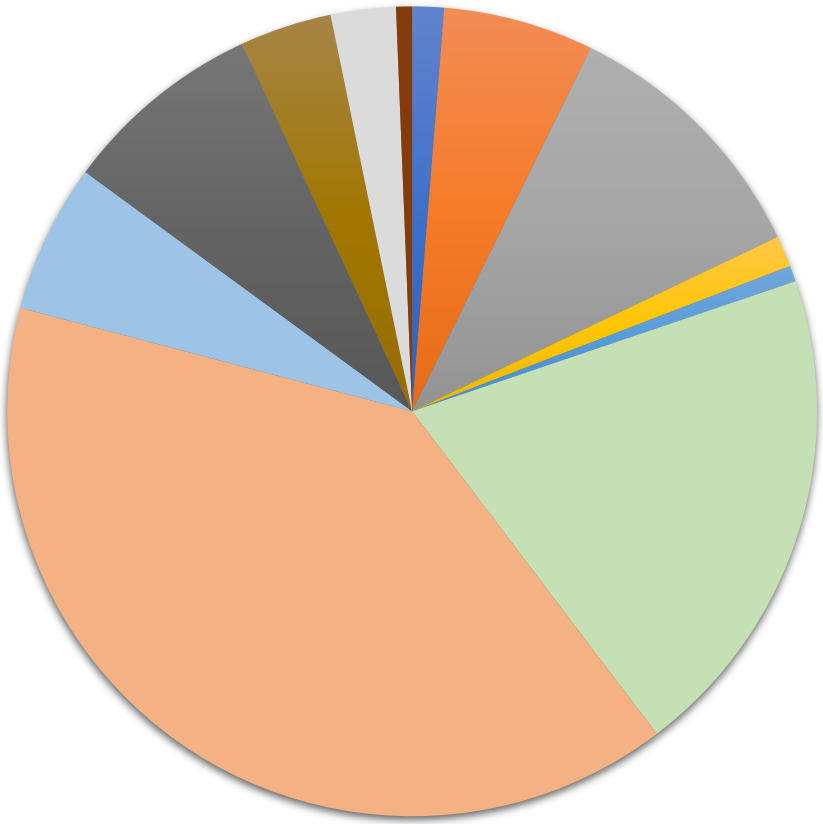

35
30
25
20
15
10
5
0
solubility mg/ml
16.5
20.4
5.6
9.9
23.5
32.6
21.2
14.3
4.6
16.2
Labrafac
Labrafilm M 1944
Isopropyl mystrate
Miglyol
Imwittor
oleic acid
Tricetin
Castor oil
Sessam oil
Olive oil
Oil type


70
60
50
40
30
20
10
0
solubility mg/ml
Labrafac
Labrafilm M 1944
Isopropyl mystrate
Miglyol
Imwittor
oleic acid
Tricetin
Castor oil
Sessam oil
Olive oil
Tween -20
Tween -40
Tween -60
Tween -80
Span-20
Span -80
CremophoreEL
Labrasol ALF
Transcutol
PG
PEG-200
PEG-300
PEG-400
PEG-600
Oils, Surfactants, andco-surfactants

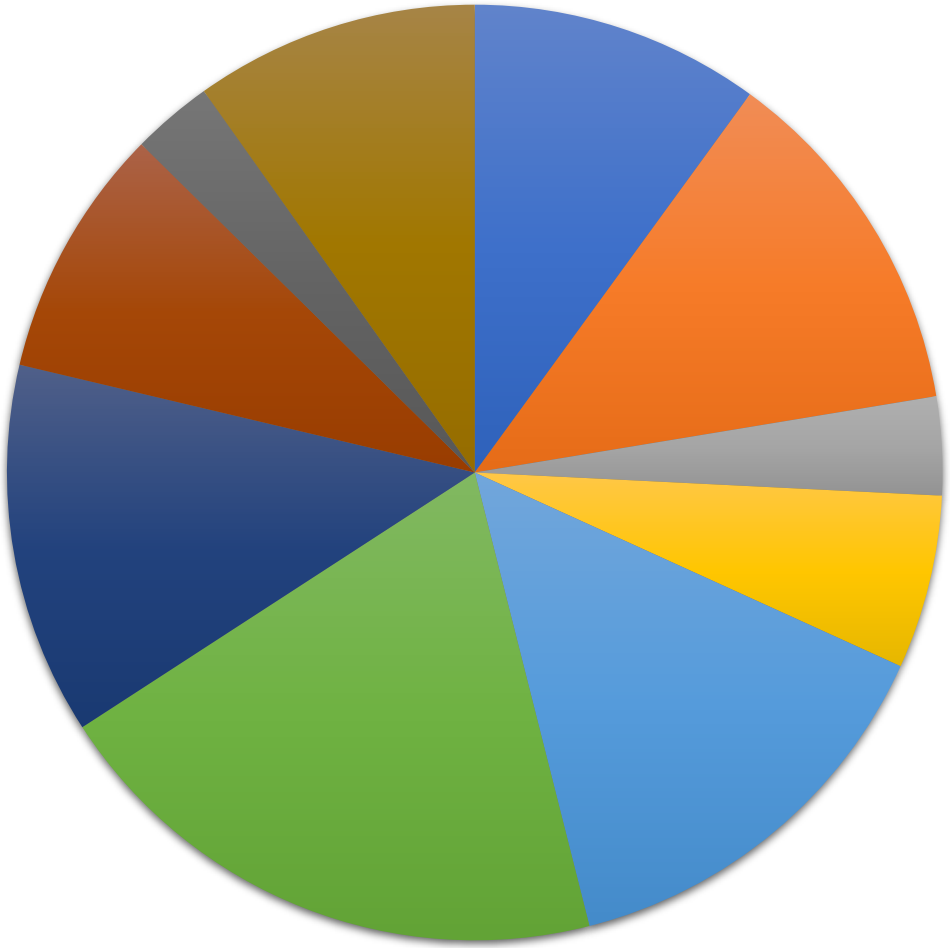

solubility mg/ml
70
60
50
40
30
20
10
0
26.99
2
9.5
16.8
1.97
1
62.4
31.4
Tween -20
Tween -40
Tween -60
Tween -80
Sp[an-20
Span -80
CremophoreEL
Labrasol ALF
Surfactants types


solubility mg/ml
70
60
50
40
30
20
10
0
62.3
9.4
12.7
5.8
4.1
1
Transcutol
PG
PEG-200
PEG-300
PEG-400
PEG-600
co-surfactants


solubility mg/ml
35
30
25
20
15
10
5
0
16.5
20.4
5.6
9.9
23.5
32.6
21.2
14.3
4.6
16.2
Labrafac
Labrafilm M 1944
Isopropyl mystrate
Miglyol
Imwittor
oleic acid
Tricetin
Castor oil
Sessam oil
Olive oil
Oil type



Tween -40
Tween -60
Tween -80
Span-20
Span -80
Labrasol ALF
Transcutol
PG
PEG-200
PEG-300
PEG-400
PEG-600



Labrafac
Labrafilm M 1944
Isopropyl mystrate
Miglyol
Imwittor
oleic acid
Tricetin
Castor oil
Sessam oil
Olive oil

Dissolution and Release:

- The dissolution process means going into solution, and it’s covered by
- Noys Whitny equation.

The release means the drug should be released from its delivery system to be absorbed, so it’s covered by many mechanisms like

ZERO ORDER	**F=F0+k0*t**
FIRST ORDER	**F=Fmax*[1-Exp(-k1*t)]**
HIGUCHI	**F=F0+kH*t^0.5**
HIGUCHI	**F=kH*t^0.5**
Korsmeyer-Peppas	**F=kKP*t^n**

By using the DD SOLVER –Program to predict the release mechanism

F1	F7	F14	F15	F18	F19	F24	F31	F34	DRUG- LAS	time (min)
2	2	30	35	22	20	10	20	35	1	2
5	4	65	45	35	40	30	40	65	3	4
10	8	85	65	55	60	50	65	80	5	5
20	12	89	85	80	75	65	75	90	12	10
35.3	27	96	100	90	79	75	85	100	24	15
85.7	69	99	100	96	84	85	95.8	100	28	20
95	79	100	100	100	86	90	100	100	32	25
100	100	100	100	100	95	100	100	100	36	35
100	100	100	100	100	100	100	100	100	48	40
									51.6	50
									59	60
									60.8	75
									89	90
										120

F1	F7	F14	F15	F18	F19	F24	F31	F34		
0.9002	0.9217	0.5199	0.6714	0.7353	0.7254	0.8243	0.7321	0.5562	ZERO ORDER	**F=F0+k0*t**
0.9092	**0.9483**	**0.9335**	**0.9769**	**0.9839**	**0.9596**	**0.9687**	**0.9599**	**0.9759**	**FIRST ORDER**	**F=Fmax*[1-Exp(-k1*t)]**
0.9127	0.9276	0.6338	0.7707	0.8310	0.8627	0.9148	0.8284	0.6695	HIGUCHI	**F=F0+kH*t^0.5**
0.7082	0.6783	0.4970	0.9253	0.9266	0.7851	0.9146	0.9021	0.8194	HIGUCHI	**F=kH*t^0.5**
0.8896	0.9362	0.7592	0.9408	0.9561	0.8971	0.8768	0.8361	0.8508	Korsmeyer-Peppas	**F=kKP*t^n**
0.913	1.094	0.491	0.565	0.772	0.672	0.821	0.649	0.470	n value	

Practical work:

1. Measure the saturated solubility of the drug in dissolution media by the shake flask method at 37°C, which is very important for predicting the sink condition.
2. For example: saturated solubility =**13.3 mg/ml**

Dose = **1 ml** of the Dosage form contains the equivalent of **40mg**

❑**If the dissolution media is 100ml**

40 mg in 100ml------ 0.4 mg/ml (100% released)

13.3 mg/ml	100%
x	10%

x = **1.33 mg/ml** (10% of saturated solubility)

So 0.4mg/ml << 1.33mg/ml

So less than 10 % of saturated solubility.

The dissolution or release processes were done with in-sink conditions.

➢For the release procedure, we depend on specific research or BP, USP for the Design of the release procedure relating to the dissolution media (type & volume), stirring speed, and sampling periods.

➢So we should fix the following before the beginning of dissolution:

1. Type of dissolution apparatus I or II.

1. Volume of the dissolution media, 900 ml, 500ml, 200ml,100ml…..

2. The stirring speed may be 200,150.100,50 rpm.

3. The temperature was always fixed at 37 °C; if there is any difference sometimes at 32°C or 34°C, we should fix the reference before starting the work.

4. The amount of drug (weighed or a specific volume of the newly prepared dosage form),

Sometimes, the dose is put in the media if it is within the sink condition, or half of the dose may be used.

6. Make a protocol for sampling that should be as early and frequent:

5, 10,15,20,30,45,60,90,120 min….

7. For each period, withdraw either 5 or 3 ml from the supernatant, filter through a 0.45 Millipore filter, then read at the specified wavelength, if it passes over 1.0 absorbance, so dilution is necessary with the same dissolution media.

(Take 1 ml diluted to 10 ml, then read at the specified wavelength).

NOTE: The dilution factor (DF) is calculated as follows:

Final volume/initial volume ---------- 10/1= 10

NOTE: After each with drawing, an equal volume of fresh media should be added.

8. For calculation, the cumulative percentage released was plotted versus time as follows:

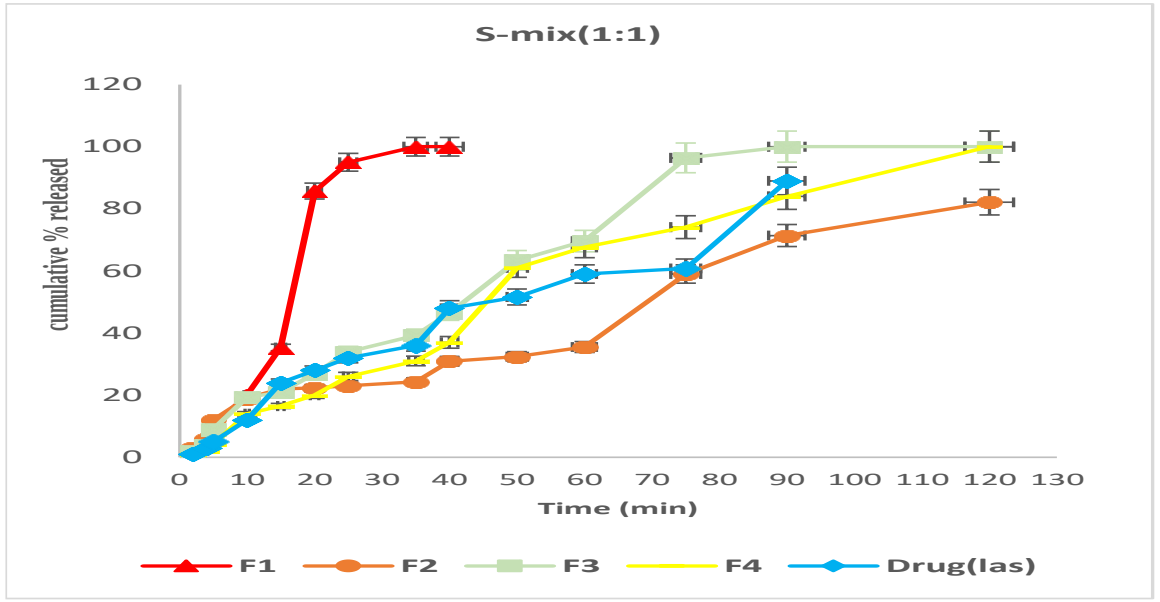
Absorbance (Ab) substituted at the calibration curve equation → Concentration (Mg or mg/ml) → multiplied by the dissolution media volume → Amount released → then calculated as a percentage [amount/dose (put in the media)] x 100 → Cumulative percentage released, which was plotted versus time to get the release profile.

OR

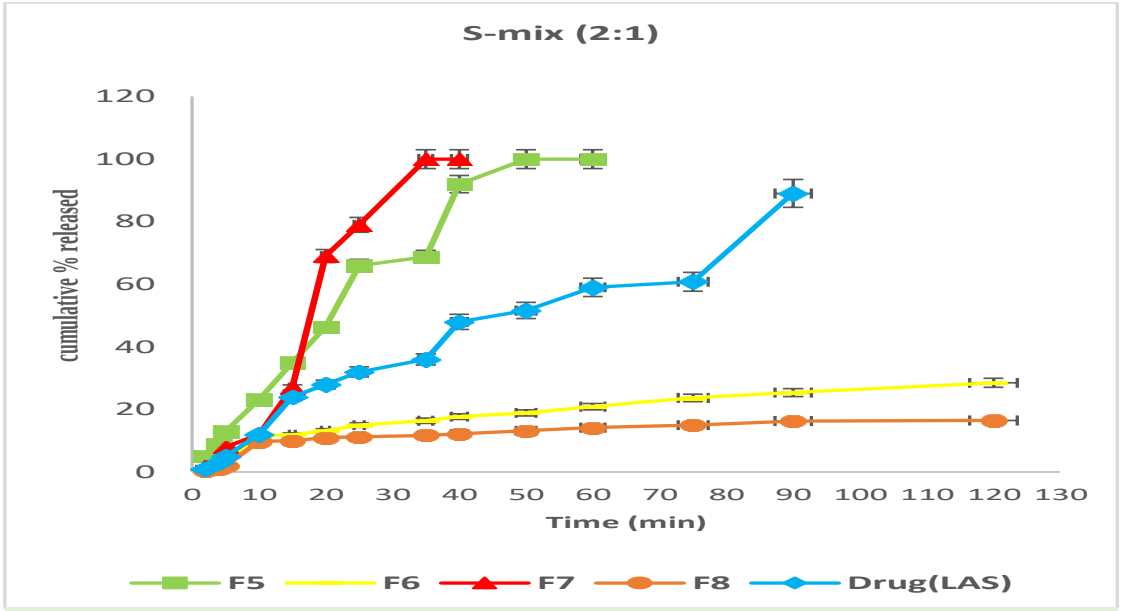
We can put an equal amount of drug present in the formula -----put in the jar with the same condition take ` only sample at the end of the experiment, which predict 100% released and the calculation will be as follow:

[Calculated conc. at each time/concentration of final sample reading of drug only]x 100= Cumulative percentage released plotted versus time.

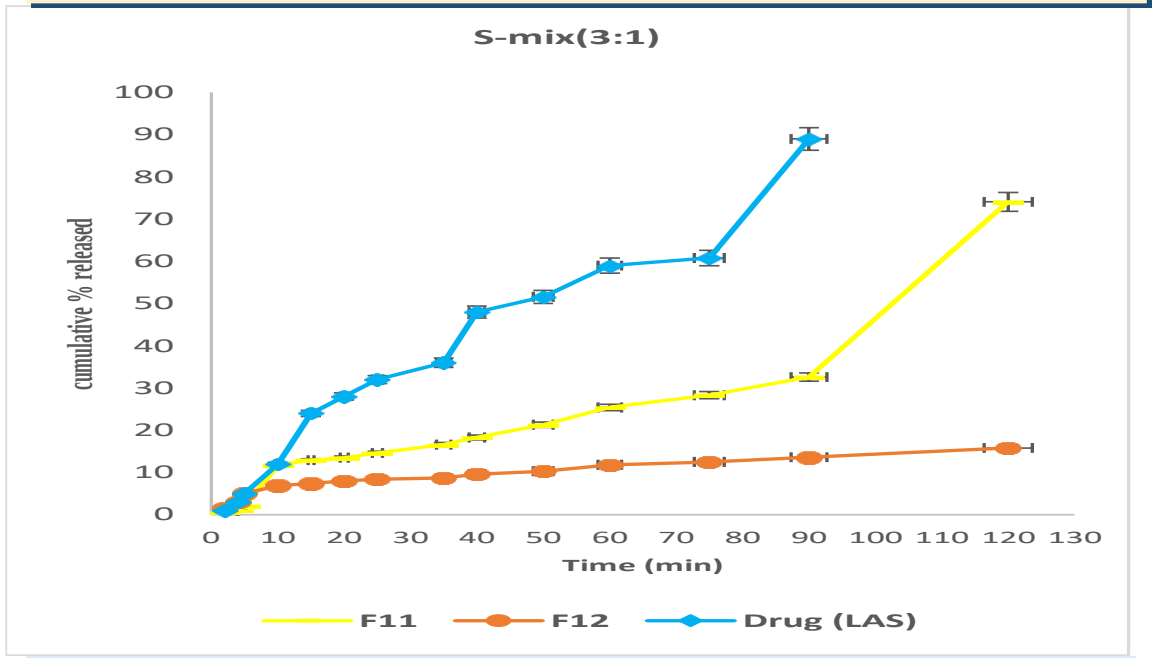
❑We can also depend on the Absorbance calculated rather than concentration.



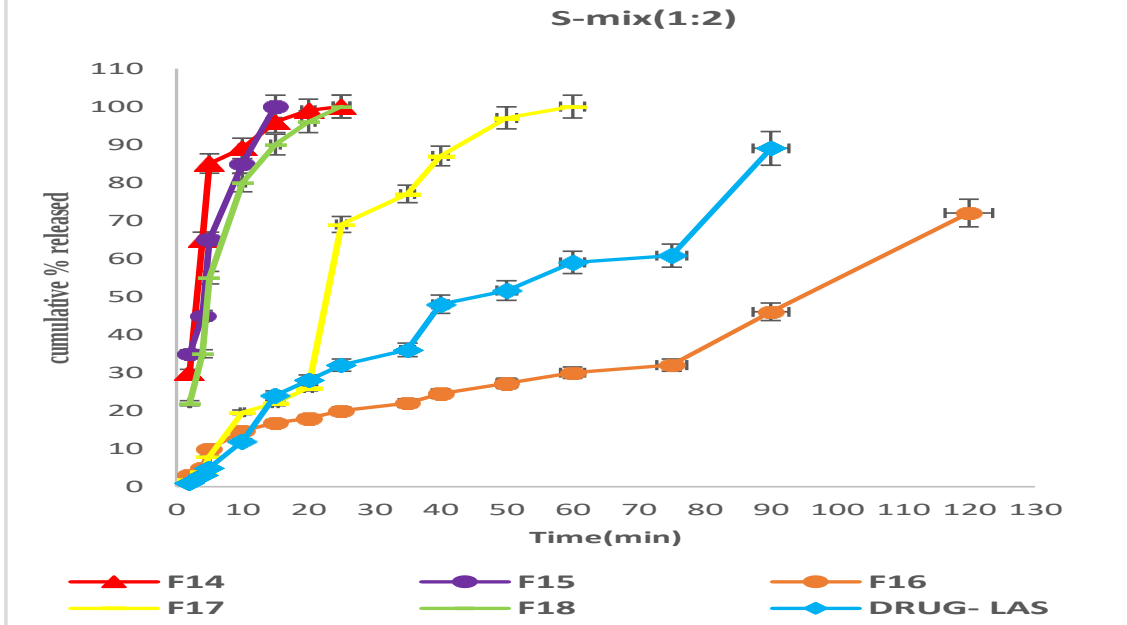
F1(7%), F2(10%), F3(20%), F4(25%), O.A %



F5(7%), F6(15%), F7(20%), F8(25%) O.A%



F11 (15%), F12 (20%)
O.A%



F14(7%), F15(10%), F16(15%), F17(20%), F18(25%)
OA%