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PREPARATION AND CHARACTRIZATION OF PROPRANOLOL-LOADED SOLID LIPID NANOPARTICLES FOR TOPICAL DELIVERY

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ABSTRACT

It was aimed to develop solid lipid nanoparticles (SLN) of propranolol using cold homogenization technique for topical delivery and to study its physicochemical properties. SLNs were prepared by different surfactants, different amounts of lipids and different concentrations of drug. Tween 20+Span 80, 1:1, or Lecithin were used as surfactants, cholesterol and oleic acid or compritol® and oleic acid were used as lipid phase. In vitro characterization of SLNs such as particle size and distribution, Differential Scanning Calorimetry study, drug entrapped efficiency and finally kinetic model of release of drug from formulations using phosphate buffer (pH=7.4) through cellulose acetate membrane were carried out. The results showed that the size of particles ranged from 15 to 84 nm which this allows drug-loaded particles to penetrate into skin easily and significantly. Drug entrapment efficiency for all formulations was 41 – 70% which is an acceptable range for propranolol as a hydophilic drug. Release of drug was less than 22% within the first 2 hours and more than 75% after 72 hours for the majority of formulations. DSC thermograms showed a phase transitional peak for each formulation in range of -11.5 to -47° C in cooling program and range of 60 – 117° C in heating program. Based on results, propranolol may be a candidate to be prepared as a topical solid lipid nanoparticles.

KEY WORDS: Propranolol hydrochloride, Solid Lipid Nanoparticle, Topical drug delivery.

1. INTRODUCTION

This study was undertaken to develop solid lipid nanoparticles (SLNs) of propranolol using cold homogenization technique and study the physicochemical properties.

Propranolol hydrochloride is a sympatholytic non-selective beta blocker which is used to treat and control many cardiovascular problems. Researchers have been reported that beta blockers have a benefit effect on diabetic ulcers, burns and injuries because of increasing angiogenesis.

On the other hand, since transdermal drug delivery specially prepared with nanotechnology could prolong the residence time of the dosage form at the absorption site, consequently increasing its bioavailability and therefore reduce systemic adverse effects, it was aimed to develop topical solid lipid nanoparticles (SLNs) of propranolol and study the physicochemical properties and drug release profile.

Solid lipid nanoparticles (SLNs) consist of nanosized solid lipids dispersed in an aqueous medium, a new nanoparticle-based drug-delivery system with particles that range in diameter from 10 to 1000 nm, have attracted considerable attention. SLNs have the advantages and avoid the disadvantages of other colloidal carrier systems such as liposomes and polymer nanoparticles.[1-3] SLN formulations are adhesive, and they could prolong the residence time of the dosage form at the absorption site, consequently increasing its bioavailability.[4] Controlled drug delivery, enhanced bioavailability of entrapped drugs[5], and/or improved tissue distribution, good tolerability, and drug targeting have been attributed to SLN formulations.[6] However, these systems generally exhibit a low drug pay-load capacity and drug expulsion during storage due to the transition of highly ordered lipid particles.[7,8] These disadvantages can be remedied by using structured lipid matrices in SLN formulation and surface modification of the particle.[9]

Transdermal delivery has many advantages over conventional methods of drug administration, because it avoids hepatic first-pass metabolism, potentially decreases side effects and improves patient compliance. Propranolol, a beta-adrenergic blocking agent used in the treatment of hypertension, is reportedly subjected to an extensive and highly variable hepatic first-pass metabolism following oral administration.[10,11] Propranolol hydrochloride requires multiple daily drug dosage in order to maintain adequate plasma concentration. Controlled administration of propranolol via transdermal delivery system could improve its

systemic bioavailability and therapeutic efficacy by avoiding first-pass effect, as well as decreasing the dosing frequency required for treatment.

Therefore, the aim of the present study was to prepare SLNs for the topical delivery of propranolol. Such formulation could have a potential to be tested ex-vivo and in-vivo in the next.

2. MATERIALS AND METHODS

2.1. Materials

Propranolol hydrochloride powder was purchased from Tolid Daru company(IR Iran). Tween 80, PG, oleic acid, Span20, cholesterol, lecithin were obtained from Merk (Germany), Comperitol® (Glycerol behenate) was gift from Gattefosse Pharmaceuticals (France). Cellulose acetate membrane was purchased from Tooba company(IR Iran).

All chemicals and solvents were of analytical grade. Fresh double distilled water was used in the experiments. Minitab15 software was used for experimental design and the evaluation of the effects of variables on responses.

2.2. Methods

2.2.1. Preparation of propranolol-loaded SLNs

SLNs were prepared using an emulsion- congealing technique with cold high pressure homogenization. The lipid phase consisted of lipid (cholesterol and Oleic acid or Compritol and oleic acid) and surfactant (tween 80 and span 20 or lecithin alone), and the aqueous phase consisted of water and propranolol. The two phases were heated separately to 65 ℃. The oil phase was added to the aqueous phase and mixed for 3 minutes at 65 ℃. Afterward the mixture was further treated by sonication (90W for 2 min.). The emulsion then was diluted with mixture of 4℃ water and propylene glycol solution (4:1) to a final volume of 50 ml. Finally, the suspension was passed through a homogenizer (Avestin, Emulsiflex-C3, Canada)) for 3 cycles (20s for each cycle) at 1000-2000 bars.[12] Table 1 shows the contents and their amounts of formulations.

Table 1: Independent variables and levels

Independent variable	Levels	
	+	-
Type of lipid	cholesterol+ oleic acid (90:10)	comperitol+ oleic acid (90:10)
Amount of lipid	90%	80%
Amount of drug	100mg	30mg
Type of surfactants	tween + span (1:1)	lecithin

2.2.2. Assaying the drug

To assay the drug, it was needed to prepare a standard curve. To do this, six predetermined concentrations of propranolol were prepared and read absorbances at 290 nm using UV spectroscopy instrument (CECIL, CE250, England). Determination the amount of drug loaded and released was performed using the standard curve.

2.2.3. Particle size determination

Particle size and polydispersity index (PDI) of the SLNs were done by particle size analyzer (Malvern, Master SIZER 2000, England) after dilution with a determined double distilled water. All determinations were made in triplicate.[13]

2.2.4. Differential Scanning Calorimetry (DSC) of nanoparticles

To study thermal behavior of nanoparticles, DSC (METTLER, Switzerland) was used through two programs including *heating* in range of 10 to 120^{0}C and *cooling* in range of -30 to -60^{0}C and phase transitional temperature and enthalpy of each peak was calculated and effect of independent variables on them were analyzed.

2.2.5. Entrapment efficiency percentage (%EE)

5 ml of each formulation centrifuged (vision, VS-550, Korea) at speed of 12000 rpm for 15 minutes, isolated the aqueous phase, rinsed particles with waters for three times, gathered with aqueous phase, and finally determined the concentration of drug as unloaded part. The following equation was used to calculate the drug loaded in particles.[14]

$EE\ (\%) = [(C_T - C_F) / C_T] \times 100$

Where C_T is the total concentration of drug existing in 5 ml of formulation and C_F is the concentration of free (unloaded) drug in aqueous phase.

2.2.6. Drug release study

Franz static Diffusion Cell System (Malek Teb, IR Iran) was used over 72 hours at temperature of 37 ± 1 °C. The diffusion barrier was a cellulose acetate membrane. In each case, 5 g of the formulation was introduced into the donor compartment and the open ends of the apparatus sealed with Parafilm to prevent evaporation. Phosphate buffer (pH= 7.4) was provided as the receiver compartment and stirred with a magnetic stirrer at 600 rpm during the experiment. At intervals of 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, and 72 h, 2 mL sample from receiver unit was removed for determination of drug released and immediately replaced with an equal volume of fresh receptor solution (phosphate buffer). Samples were analyzed by UV visible spectrophotometer at 290 nm. Sink condition was kept throughout the release period. Data obtained in triplicate were plotted versus time. Cumulative amount of released drug was calculated considering respective times, then release kinetic profile was specified through fitting with kinetic models like Higuchi, zero-order, first order and so on. Also, effects of independent variables on release kinetic were analyzed.[15]

2.2.7. Estimation of Sample Size and Sampling Method

For this purpose, factorial design was applied for estimation of the quantity of formulations. To do so, considering number of variables, 4 variables, each of which analyzed in two levels, 2^4= 16 formulas was provided. Each formula was repeated three times and, finally, 48 formulas was provided and analyzed.

2.2.8. Statistical Methods of Analyzing Results

The One-Way Analysis of Variance (ANOVA) statistical test has been used to compare fabricated products and, pursuant to it, Compared Means Test-Tukey was used for pairwise comparison of them. In addition, for study of the relationship between variables and responses, concurrent multiple regression was used. Maximum p value of 0.05 was applied when required.

3. RESULTS AND DISCUSSION

3.1. Standard Curve

Fig. 1 shows standard curve for five predetermined concentrations of propranolol in phosphate buffer (pH=7.4) and the maximum wavelength of 290 nm UV spectrophotometry.

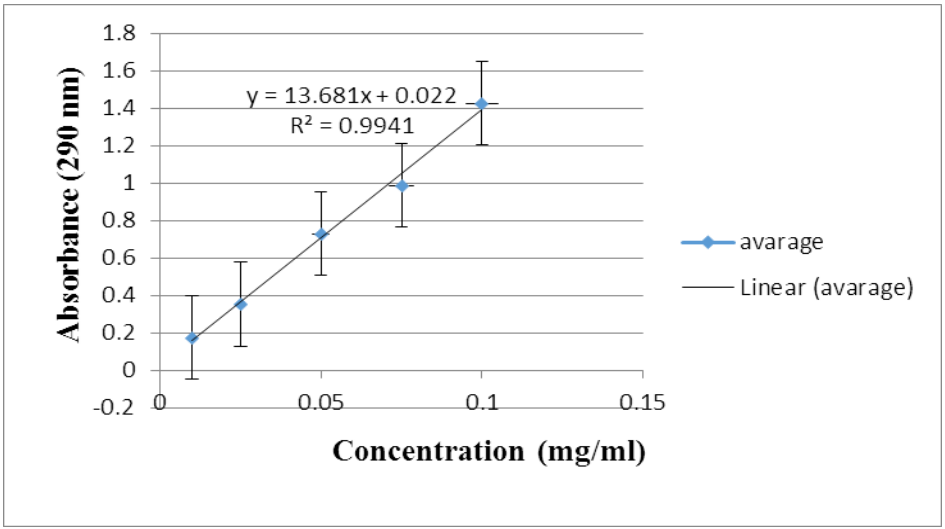


Fig 1: Standard Curve of assaying propranolol

The assaying method showed a linearity with correlation coefficient of 0.9941 within range of 0.01-0.1mg/ ml propranolol (p= 0.0001).

3.2. Particle size

The particle size of different formulations and their PDI are represented in Table 3.

Table 2: Particle size and polydispersity index (Mean±SD, n=3)

Formulation		Particle size(nm)	PDI
1		29.8±4.63	0.37
2		52±4.3	1.01
3		66.8±9.26	0.38
4		83.03±4.80	0.23
5		66±8.52	0.24
6		44.63±7.45	0.31
7		19.1±0.35	0.97
8		25.97±7.27	0.28
9		51.07±4.36	0.46
10		17.93±1.23	0.96
11		38.23±3.91	0.34
12		36.83±2.85	0.27
13		28.97±2.7	0.31
14		23.24±1.88	0.33
15		15.69±3.32	0.22
16		21.7±3.15	0.39

As it shown, the particle size ranges from 15 – 84 nm for all formulations which is less than 100 nm. The minimum size was 15.69 nm for formulation 15 and the maximum 83.03 nm for formulation 4. Furthermore, PDI was less than 0.5 for all formulations except for formulations 2, 7 and 10. These mean that the technique and materials used to prepare SLNs were properly selected and particle size was mainly affected by lipid content. Compritol® could lead to smaller particles which seems it is due to its lower melting point and good lubricating effect followed by better fusion with medium.

Allawadi et al prepared nanospheres loaded with Propranolol Hydrochloride by using solvent evaporation technique with different concentration of Eudragit RS100 and Eudragit RL100 polymers. The range of particle size was 220 – 900 nm mainly depending on ratio of drug : polymer.[16] In comparison with this study, it could be judged that lipid carrier is more efficient than polymeric one to produce nano- size particles which is more effective to penetrate into the skin.

3.3. Differential Scanning Calorimetery (DSC)

Table 3 presents the DSC results of all formulations and Figure 2 (a, b, c, and d) show DSC thermograms for formulations No. 7 and No. 12 as models.

Table3: DSC results of formulations

	Cooling		Heating	
Formulations	**Phase transitional temperature**	**ΔH**	**Phase transitional temperature**	**ΔH**
1	-35	116.2	117	-584.1
2	-47	321.5	100	-872.1
3	-30	316.2	90	-131.7
4	-28	337.4	100	-644.9
5	-11.5	108.2	100	-471.3
6	-26	101.6	98	-248.0
7	-25	85.8	94	-693.4
8	-28	232.2	80	-431.3
9	-26	112.3	66	-351.6
10	-26	38.6	82	-374.3
11	-30	121.8	60	-367.4
12	-25	105.8	90	-746.2
13	-32	109.7	86	-824.7

14	-22	313.8	90	-853.8
15	-22	141.8	80	-765.3
16	-31	272.9	82	-941.7

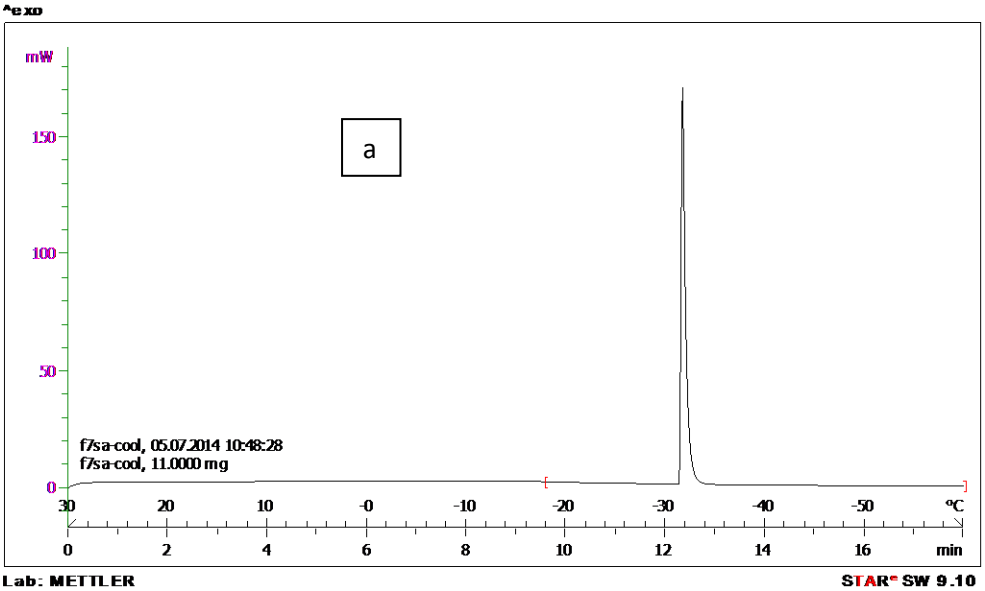
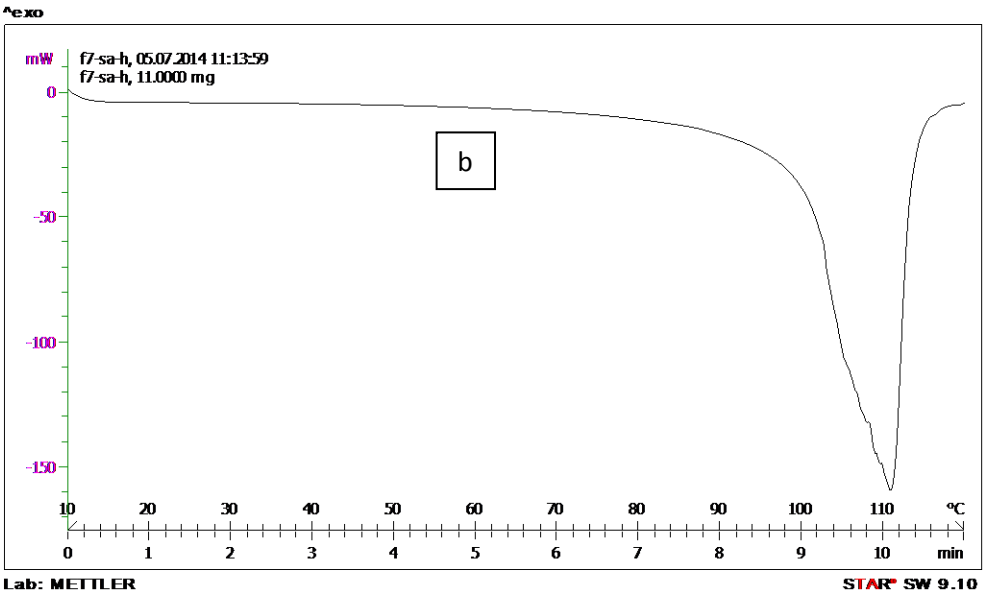
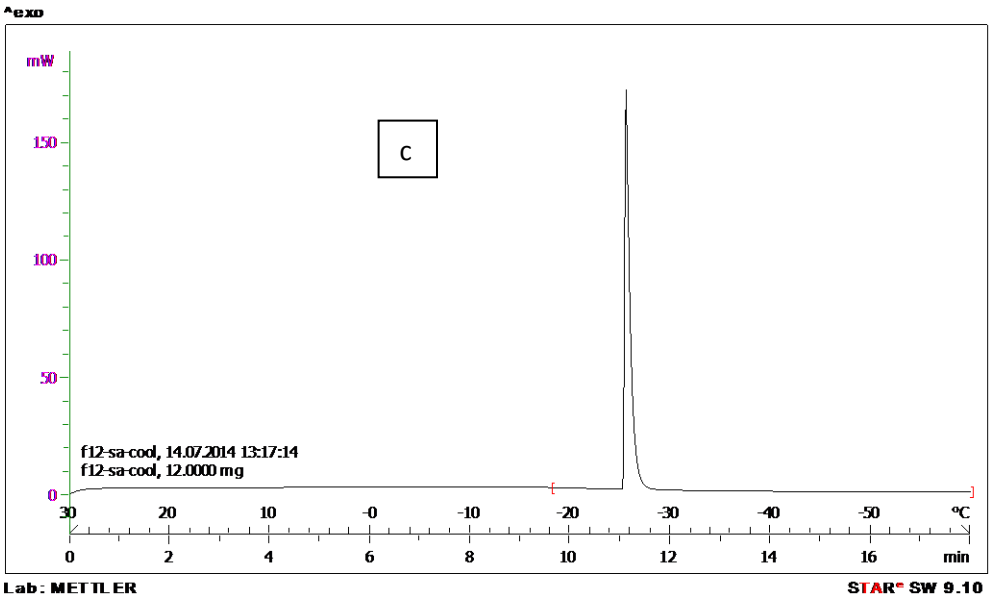
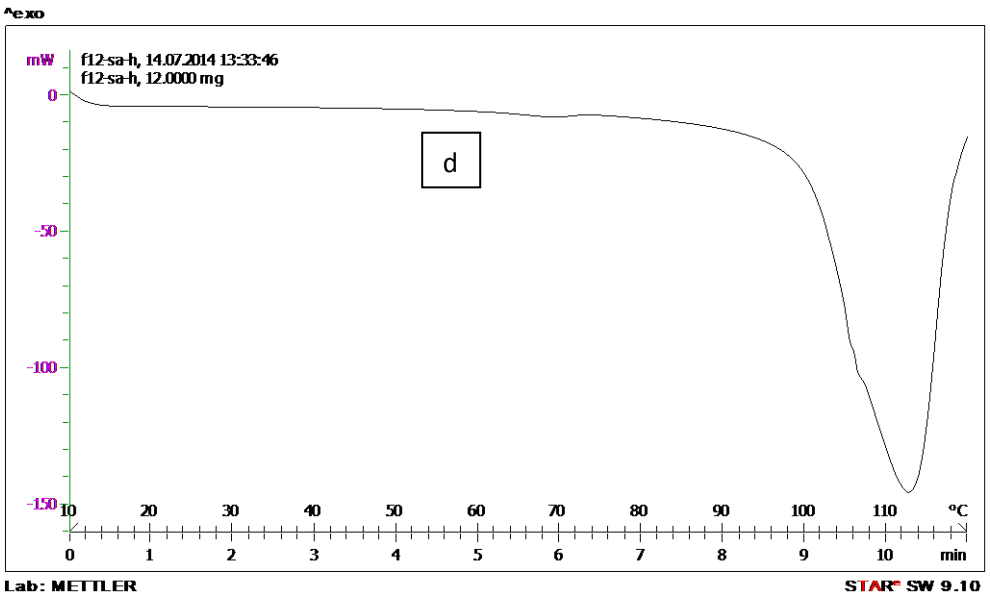


Fig 2: DSC Thermograms (a: cooling F.7, b: heating F.7, c: cooling F.12, and d: heating F.12)







A phase transitional peak for each formulation is seen in range of -11.5 to -47° C in cooling program and range of 60 – 117° C in heating program which is related to bound water in nanoparticles. Regression analysis of results from cooling program showed no significant correlation between variables and phase transitional temperatures and enthalpies ($p>0.05$). This means there was no considerable interaction between components and water existing in formulations and no effects on frozen water. Regression analysis of results obtained from heating program gave a significant correlation between lipid type and phase transitional temperature ($p=0.007$). This relationship was the way in which by changing the solid lipid from Compritol® to cholesterol resulted in increase of phase transitional temperature.

3.4. Loading of drug

Table 4 represents the drug loading in formulations. It is clear that the percentage of drug loaded in particles is varied from 40.95 to 69.66 which is an acceptable entrapment for propranolol as a relatively hydrophilic drug. Analysis of the effects of variables and their interactions on responses showed %EE was affected by lipid type, amount of lipid, and type of surfactant. The p values were 0.001, 0.021, and 0.002 respectively. By using of Compritol® mixed with oleic acid as lipid phase, in low level, and mixture of Tween 80 and Span 20 as surfactant resulted in increase of entrapment efficiency significantly. Considering hydrophilicity of propranplol (log p = -0.45), decrease in lipid content resulted in increased EE%.

Table 4: percentage drug Entrapment Efficiency (%EE) of SLN formulations.

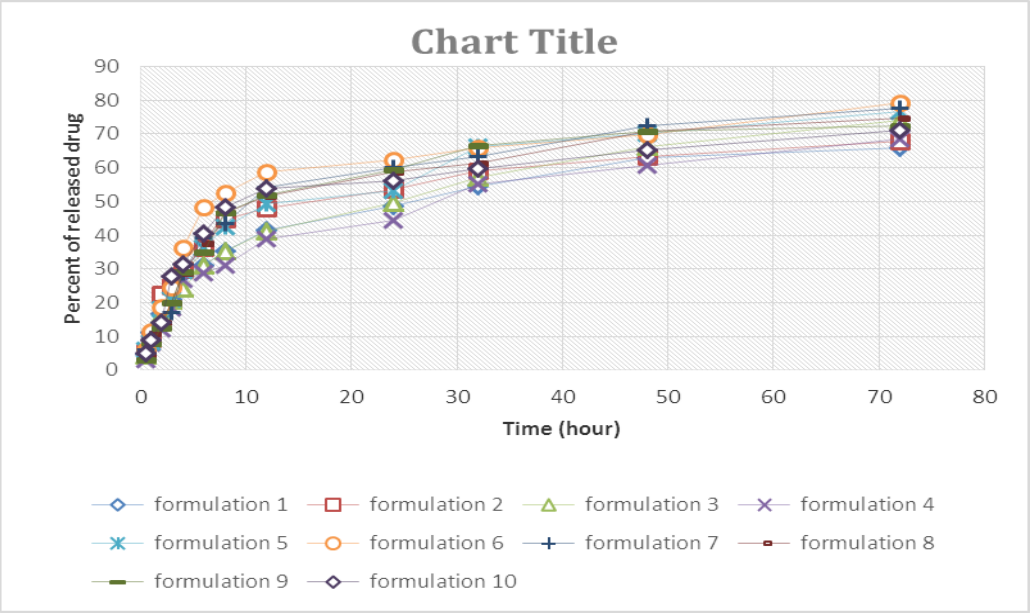
Formulations	(%EE)
1	46.77
2	49.56
3	43.59
4	40.98
5	48.74
6	49.93
7	43.97
8	47.55
9	65.13
10	61.26
11	52.85
12	54.68
13	68.04
14	69.66
15	57.51
16	56.51

3.5. Release of drug from nanoparticles

The results of drug released from all formulations within 72 hours are shown in Table 5 and Fig.2 shows profile of release.

Table 5: Percentage of drug released

Formulation No.	%Release 2h	%Release 8h	%Release 48h	%Release 72h
1	16.63	35.28	62.92	65.88
2	22.45	44.82	63.44	67.75
3	16.35	35.42	66.07	73.93
4	12.33	31.13	60.52	68.63
5	17.99	42.38	70.17	76.78
6	18.73	52.41	69.74	79.26
7	13.41	43.32	72.44	77.71
8	14.25	46.71	70.76	74.8
9	12.55	46.53	70.92	72.4
10	14.27	48.47	65.32	70.94
11	11.17	45.32	64.27	69.46
12	18.98	40	60.67	64.21
13	16.98	54.26	71.24	77.29
14	20.82	51.55	77.27	82.98
15	13.18	42.97	65.59	68.24
16	12.48	39.59	62.01	65.14



Considering %R2h and %R72h as rapid and slow release respectively, the majority of formulations have released less than 22% of entrapped drug within 2 hours and more than 75% after 72 hours which means the technique and materials used to prepare SLNs had been able to provide a slow- release profile. Analysis of regression of results for %R2h and %R72h showed a significant direct correlation (p=0.034) between surfactant type and %R2h and an

indirect significant relationship (p= 0.015) between lipid percent and %R72h. Increase in lipid content resulted in increase of the drug maintained in particles. It seems the particles had been loaded homogeneously of drug, so the drug has been released in sustained model. By fitting the results of release to different kinetic models, it was determined that the log wagner was the best and strongest model to analyze the results.

4. CONCLUSION

It could be concluded that propranolol hydrochloride has a potential to be loaded and delivered by solid lipid nanoparticles for topical administration. Selection of lipids and surfactants is a very important step of preparation. However, to complete the study, it is needed to optimize formulation and study the skin permeation properties in ex-vivo and in-vivo conditions and consequently to apply on wounds.

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