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Propranolol Micro Particle Production by Spray Drying Technique and Evaluation of the *In Vitro* and *In Vivo* Lung Deposition

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Abstract

The goal of this study is to prepare the inhalable micro particles from propranolol by spray drying method. Prescription of propranolol by the oral rout of administration suffers from the hepatic first pass metabolism effect. This phenomenon caused to decrease the oral bioavailability of the propranolol. It is possible to eliminate the hepatic first pass metabolism effect by prescription of the propranolol inhalable micro particles via the pulmonary system.

The different solvent as the spray drying vehicle such as water and the mixture of the water and ethanol were applied for preparation of the propranolol inhalable micro particles during the spray drying process. The physical characteristics of the micro particles such as true and bulk density, size, shape and aerodynamic behavior of the particles were evaluated by the *in vitro* test. In the in vivo tests the inhalable microparticles were insufflated to the lung of the rats. The plasma samples 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 hours after insufflation of the drug to the lung of the animal, intravenous and oral administration of drug were collected. The concentration of propranolol in the plasma samples were measured by the HPLC method. The pharmacokinetic parameters of the drug such as AUC_{0-60}, T_{max}, C_{max}, $T_{1/2}$, K_e, K_a, V_d and absolute bioavailability of drug were calculated.

The results of the *in vitro* tests showed that the type of the spray drying vehicle has the significant effect on the physical characteristics and the aerodynamic behavior of the propranolol inhalable micro particles. The presence of 75% ethanol in the spray drying vehicle caused to increase in fine particle fraction. The value of the fine particle fraction for these micro particles was ±35.771.

The results of the *in vivo* tests showed that the rate and extent of the pulmonary absorption of propranolol is more than the oral rout of administration. The presences of the different excipients in the spray drying vehicle and the type of the spray drying vehicle have the significant effect on the pulmonary absorption of the drug. It is possible to reach 0.69+0.27 as the value of the absolute bioavailability by prescribing the propranolol via the pulmonary system.

Keywords: Fine particle fraction; Aerodynamic behavior; Propranolol; Ethanol; Pulmonary absorption-propranolol-inhalable microparticles-absolute bioavailability

Introduction

The advantages of lung as the respiratory organ such as high level of the blood flow rate, the permeability of the capillary, the wide surface for absorption of drugs and the low level of peptidase enzyme in compare with the gastrointestinal system make it the suitable organ for delivering of drugs to the systemic circulation [1,2]. Respiratory drug delivery has many advantages include eliminating the hepatic first pass metabolism effect and delivering of the impermeable macromolecules such as amino glycosides to the blood circulation [3]. These advantages cause to apply from this rout as the alternative for the oral and the parenteral rout of administration for peptide and proteins [4-8]. For this reason the drug delivery via the respiratory system is more considerable process in the recent decades.

There are three types of inhalable dosage forms include nebulizer solutions, pressurized metered dose inhalers (PMDI) and dry powder inhalers (DPI) for respiratory drug delivery [9-11]. Among these types of dosage forms DPIs as the solid pharmaceutical dosage forms are more stable than the others. The formulation of the DPIs is not the complicated process [12]. As a matter of fact in the formulation of the DPIs the micro particles of drug as the active ingredients is surrounded by the solid particles of the inert materials that plays the role of vehicle in the formulation. The different inert material can play the role of the DPIs vehicle. Lactose is applied as the vehicle in most of the DPI formulations [13,14].

The physicochemical characteristic of the active ingredient and the DPI formulations is the effective factor on the lung deposition of the drugs [15]. The stability of drugs and the

absence of propellant in the formulation of DPIs proposed them as a good alternative to MDIs [16]. DPI formulations consist of drug with an aerodynamic particle size ideally smaller than 5 m [17]. The flow and dispersion properties of these small particles are influence by inter particle forces, including electrostatic, Van der Waals, capillary and mechanical forces. The intensity of these forces is affected by several physicochemical properties of particles, including size distribution, morphology, density and surface composition [18]. A study has suggested a more important role for powder formulation than the inhaler design [19]. There are several methods for production of respirable microparticles such as supercritical fluid technique [20].

Spray drying is another technique that has been widely used to manipulate the physical properties of pharmaceutical materials [21]. Also micronized but spherical particles can be prepared by spray drying method. The amorphous particles are characterized by a low area of contact and a smaller and more homogenous particle size distribution resulting in a higher respirable fraction than mechanically micronized drugs [22]. Spray drying also allows a control over particle shape, morphology and density dependent on the spray drying conditions [23].

The oral rout of administration of propranolol suffers from the hepatic first pass metabolism effect [24]. This effect cause to decrease the oral bioavailability of the propranolol. The oral absolute bioavailability of the propranolol as the unselective B blocker was reported in the text 0.3 is the value of the oral bioavailability of propranolol that has been reported in the text [25]. In this study the deposition profiles of propranolol micro particles using spray drying procedures as a dry powder inhalation was investigated by the *in vitro* methods and the pulmonary absorption of these formulations in rats was measured by the *in vivo* method.

Materials and Methods

Materials

Propranolol powder was supplied by ABIDI Company (Iran, Tehran). Lactose monohydrate was purchased from DMV (Amsterdam, the Netherlands). All solvents which were used supplied by Merck (Frankfurt, Germany) and were at least analytical grade.

Methods

Spray drying: Solutions (1 g/50 ml) of propranolol in different Water:Ethanol ratios **(Table 1)** were spray dried using a lab scale spray drier (Buchi 191, Buchi, Switzerland).

Preparation of blends: Powder formulations containing propranolol and lactose with ratio 1:1 were prepared. In each mixing process 0.5 gram of spray dried propranolol sample was blended with 0.5 gram of lactose in a turbula mixer (Dorsa Iran) at 46 Rev/min for 30 min.

Analytical method: The concentration of propranolol in plasma samples was measured by HPLC method [26].

Table 1 Spray drying vehicle with different solvent ratios.

Solvents		
Water%	**Ethanol%**	**Code**
100	0	E:W(0:100)
75	25	E:W(25:75)
50	50	E:W(50:50)
25	75	E:W(75:25)

Experimental

Particle size analysis

The particle size of samples were determined by laser light scattering (Malvern mastersizer x, Malvern, UK). Approximately 20 mg of sample was suspended in water and sonicated at 25°C for 4 min. A few drop of each sample was poured into the small volume cell of the instrument to obtain an obscuration of sample between 18 and 20%. The analysis was carried out in triplicate for each sample.

Scanning electron microscopy

Morphology of each sample was examined by scanning electron microscopy (SEM) (Philips XL 30 scanning microscope, Philips, The Netherlands) at 25 Kev. Samples were gold coated prior to analysis (SCD005 Sputter coater, Bal-Tec, Germany).

Particle density

The bulk density of the samples was determined by measurement of the volume of a known mass of the material that had been poured in to a 25 ml graduated cylinder. The true density was also determined using a helium pycnometer (Multipycnometer, Quantachrome, USA). Each sample was analyzed in triplicate.

Drug assay determination

Quantification of propranolol-lactose blend content uniformity and *in vitro* lung deposition was by UV-VIS spectrophotometer at 290 nm. Linearity was confirmed between 2 and 500 µg/ml. Lactose did not interfere with the propranolol response.

In vitro deposition

One capsule, containing 10 mg of propranolol was introduced to an Andersen cascade impactor via a Spinhaler (dahlia, India). After aerosolization of the powders for 4sec at a flow rate of 60 l/min, the inhaler, capsule shell, throat, preseparator, the seven stages and plates and filter were washed with dichloromethane as the solvent. The aerodynamic characteristics of propranolol in each sample

were determined as follows: Fine particle dose (FPD) was determined as the amount of drug deposited on stage 1 to the filter. The effective cut-off diameter of stage 1 of Anderson cascade impactor at 60 l/min was reported to be <6.18 µm [27].

Fine Particle Fraction (FPF) was calculated as the percentage of the ratio of the FPD to the total amount of the drug recovered per capsule. The emitted dose (ED) was defined as the total drug recovered from throat, preseparator, seven stages and plates and filter. The percentage emitted was calculated as the ratio of ED to the total drug which recovered per capsule and expressed as percentage. Dispersibility was defined as the ratio of FPF per ED percentage.

In vivo studies

Male Wister rats (The Pasteur institute, Iran), weighing 250-300 g, were anaesthetized with an intraperitoneal injection of ketamin (50 mg/kg) and xylene (10 mg/kg). All the animals were fasted for 16 h before the experiments; they were allowed free access to water.

Drug administration

3 mg of drug as the powder was introduced into the lung through the obtuse syringe which was connected through the tracheal cannula to a depth of 2.5 cm below the tracheal incision. The tip of the syringe was located 1-2 mm above the bifurcation of the trachea. The powder was introduced over a period of 1-2 sec, to the rat which was maintained at an angle of 80°. Then, the tubing was withdrawn completely and 45 sec after administration of the powder the animal was positioned to an angle of 10°.

Propranolol solution in PBS (3 mg/0.2 ml) was intravenously administered into the caudal vein by bolus injection.

Absorption studies

Absorption of propranolol from rat lung was investigated by the reported method [28]. All animals were fasted for 16 h before the experiments but had free access to water. After the animal was secured on its back on animal board, the trachea was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was then cut transversely, halfway through, between the fourth and fifth tracheal rings caudal to the thyroid cartilage.

For determination of the drug concentrations in plasma, 250 µl blood samples were taken from jugular vein 0.25,0.5,0.75,1,1.5,2,4,6,8 after dosing, centrifuged at 1800 g for 10 min, and the plasma was separated and stored at 30°C until analysis.

Pharmacokinetic parameters

The pharmacokinetic parameters, C_{max} (maximum plasma concentration) and T_{max} (the peak plasma concentration time) were obtained from the plasma concentration-time curve [18]. AUC_{0-t} and $AUC_{t-\infty}$ were calculated by numerical integration using a linear trapezoidal formula. The mean residence time (MRT) was determined using AUMC/AUC. The K_a (absorption rate constant) was determined using the residual method, K_e was calculated from terminal section of the plasma concentration-time curve and $T_{1/2}$ was calculated from $0.693/k_e$.

Statistical analysis

The T test as the Statistical Analysis test was apply in this study. The P<0.05 was consider as the significance.

Results and Discussion

Physical characterization

Table 2 shows the particle size distribution data for all of the samples. The commercial propranolol was shown to have a volume median diameter (d 50%) of 37.5 with a mode at 50.99. The SEM photograph is shown in **Figure 1** suggests a columnar shape for the commercial propranolol crystal with a particle size predominantly smaller than 400 **(Figure 1).** This sample had not a suitable particle distribution for inhalation.

The spray drying process produced micro particles with different particle size distribution pattern and densities depending on the nature of the vehicle, which had been used in the preparation of the feed. All of the spray dried propranolol micro particle were shown to have a monomodal particle size distribution with a particle size smaller than 5.

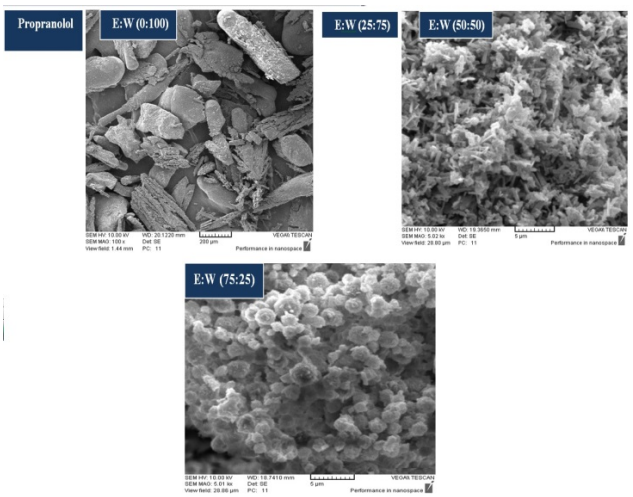

Figure 1 SEM photograph of the sample: Propranolol before spry drying-), E:W(25:75), E:W(75:25).

According to **Figure 1** increasing the percentage of ethanol in the spray drying vehicle caused to formation of the amorphous and spherical shape propranolol micro particles. The number of the crystal shaped propranolol micro particles will be increased by increasing in the percentage of water in the spray drying vehicle.

According to **Table 2** both the true density value and the bulk density value of the commercial powder were different to that of the spray dried samples.

Table 2 Particle size distribution and densities of the samples (Mean, n=3).

Particle size (µm)			Density g/ml	
Sample	d 50%	Mode(s)	Bulk	TRUE
Propranolol before Spray drying	37.5 ± 1.96	50.99 ± 2.46	0.673 ± 0.03	1.125 ± 0.007
E:W(0:100)	2.24 ± 0.075	4.35 ± 0.22	0.202 ± 0.002	1.065 ± 0.007
E:W(25:75)	2.23 ± 0.072	3.47 ± 0.02	0.383 ± 0.04	1.055 ± 0.007
E:W(50:50)	2.07 ± 0.085	3.52 ± 0.02	0.281 ± 0.041	1.075 ± 0.007
E:W(75:25)	2.16 ± 0.125	3.51 ± 0.02	0.254 ± 0.002	1.02 ± 0.014

In vitro deposition

Deposition data for each micronized propranolol powder after aerosolization of the samples at 60 L/min through a spinhaler ®, using an Andersen cascade impactor are presented in **Figure 2** and **Table 3**. The amount of propranolol deposited on various stages of the Andersen cascade impactor varied for different samples.

Table 3 *In vitro* deposition data.

Remained in capsule %	ED%	FPF%	Sample
2.32 ± 51.6	5.46 ± 45.7	1.11 ± 7.61	Propranolol
1.53 ± 41.67	0.4 ±5 7.33	1 ± 19.47	E:W(0:100)
1 ± 46	0.58 ± 53.57	4.57 ± 20.66	E:W(25:75)
2.38 ± 39.57	1.91 ± 59.8	6.54 ± 28.37	E:W(50:50)
0.92 ± 25.2	0.73 ± 74.5	1.15 ± 35.77	E:W(75:25)

These results suggested different aerodynamic properties of the drug particles aerosolized from commercial and spray dried samples ($p<0.05$). The comparison of the effect of the type of spray drying vehicle on the physicochemical properties of propranolol indicated many changes in characteristics of samples such as particle size distribution and *in vitro* deposition profile.

According to **Table 3** Spray dried samples processed from 75% ethanol and 25% water solution produced significantly ($p<0.05$) higher percentage emission and higher FPF than the other spray dried samples and commercial propranolol powder.

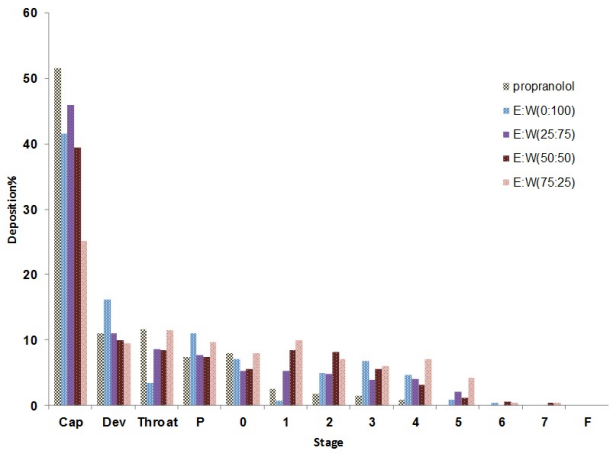

Figure 2 *In vitro* deposition profile: Propranolol befor spray drying, E:W(0:100), E:W(25:75), E:W(50:50), E:W(75:25).

In order to improve the emitted dose (ED) there are two options, namely, reducing the amount adhering to the surface of the inside wall of a capsule and improving the flow ability of the powder [29,30]. As far as the first option is concerned, the material of the capsule shell could influence drug adhesion [31]. In the DPI formulation with the Spinhaler®, a conventional gelatin capsule was adopted as the container for the unit dose. There are some reports describing the relationship between the adhesive force and the moisture content of a polymer film, whereby a higher moisture content results in a higher amount of adhering particles [32,33]. The particles captured in stages 1-7 and the filter, were expected to deposited in the lung lobe or trachea after inhalation. This was defined as the fine particle dose (FPD). A hard gelatin capsules usually contains 13%-15% water as a weight ratio and it is hard to reduce the water below 10% because this makes the capsules very brittle. The amount of drug remained in the capsules for each samples was shown in **Table 3**. Increasing in the percentage of ethanol in the spray drying vehicle resulted in the production of micro particles with lower affinity to remain in the capsule shell. The affinity of micro particles spray dried from 50% water and 50% ethanol for remaining in the capsule is more than the others.

The remained drug in the capsule shell is dependent to the material of the capsule shell and the water content of the conventional gelatin capsule, there are several reports that confirm this phenomenon [31-33].

In vivo pulmonary absorption

The pulmonary absorption of drugs was generally influenced by various physicochemical and biological factors. The physicochemical factors include molecular size of drugs [34], lipophilicity of drugs [35], pH in drug solution [36], various additives [37,38] etc. We examined the effect of various spray drying propranolol single dose 3 mg was administered via intra tracheal, intravenous and oral rout to healthy rats and the plasma concentration of propranolol was measured. The peak

concentration achieved via intra tracheal rout was between 30-45 min depending on the type of the spray drying vehicle. The concentration time profiles of propranolol after intra tracheal, intravenous and oral administration of propranolol dry powder inhaler were presented in **Figure 3**. The pharmacokinetic parameters of propranolol were summarized in **Tables 4 and Table 5**.

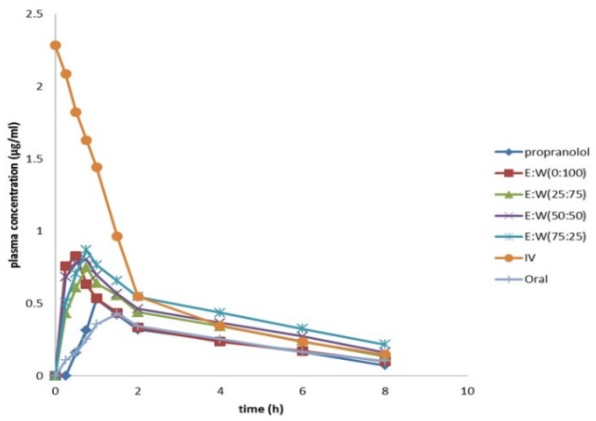

Figure 3 Mean plasma concentration time profiles after administration of 3 mg propranolol.

AUC increased when the percentage of ethanol was increased in the spray drying vehicle. E: W (50:50) sample showed the highest value of the AUC and Propranolol showed the lowest value of the AUC when the powder was administered via the intra tracheal rout. According to **Table 4** the values of T_{max} and K_a showed that the rate of drug absorption from the respiratory system is more than the oral rout of administration ($p<0.05$).

According to **Tables 4 and 5** when the powder administered by oral and intra tracheal rout there was not any difference between the pharmacokinetics parameters such as K_e, $t_{1/2}$, MRT and Cl related to the elimination phase of propranolol from the animal body.

Table 4 Pharmacokinetic parameters for the samples after administration of 3 mg of propranolol.

Sample	Tmax (h)	Cmax (µg/ml)	Ka (1/h)	Ke (1/h)	T1/2 (h)
Propranolol	1	0.53 ± 0.081	2.39 ± 0.791	0.42 ± 0.219	1.96 ± 1.021
E:W(0:100)	0.5	0.83 ± 0.111	5.76 ± 0.367	0.31 ± 0.096	2.38 ± 0.7962
E:W(25:75)	0.75	0.75 ± 0.108	3.60 ± 0.204	0.34 ± 6.798E-17	1.99 ± 0.1221
E:W(50:50)	0.75	0.80 ± 0.077	2.48 ± 0.200	0.24 ± 0.101	3.23 ± 1.442
E:W(75:25)	0.75	0.87 ± 0.08	3.05 ± 0.507	0.34 ± 6.798E-17	1.99 ± 0.1231
Oral	1.5	0.53 ± 0.086	1.20 ± 0.546	0.28 ± 0.111	2.83 ± 1.45
IV	0	2.28 ± 0.145	------	0.25 ± 0.070	2.83 ± 0.885

Table 5 Pharmacokinetic parameters for the samples after administration of 3 mg of propranolol.

Sample	MRT (h)	AUC0-8 h (µgh/ml)	Cl (ml/h)	Vd (ml)	F Bioavailability
Propranolol	3.20 ± 0.189	1.84 ± 0.538	439.5 ± 8.987	4274.87 ± 425.7	0.30 ± 0.0931
E:W(0:100)	2.70 ± 0.326	2.31 ± 0.738	439.52 ± 4.019	3128.85 ± 311	0.396 ± 0.161
E:W(25:75)	3.07 ± 0.126	2.81 ± 0.637	439.52 ± 9.8	3301.76 ± 391	0.46 ± 0.114
E:W(50:50)	3.19 ± 0.224	3.59 ± 0.850	439.52 ± 2.22	2924.57 ± 178.5	0.69 ± 0.272
E:W(75:25)	3.21 ± 0.201	3.56 ± 0.768	439.52 ± 5.6	2954.71 ± 131.8	0.61 ± 0.154
Oral	3.37 ± 0.254	1.85 ± 0.456	439.52 ± 6.8E	5374.11 ± 2213	0.33 ± 0.068
IV	2.18 ± 0.229	4.69 ± 0.779	581.23 ± 130.	1317.54 ± 86.53	1

It is clear that intra tracheal administration of spray dried propranolol micro particles caused to 2 fold increases in absolute bioavailability in compare with the oral rout of administration (E:W(50:50) sample). This phenomenon confirms the suitability of administration of propranolol micro particle via the intra tracheal rout which can easily absorbed from the pulmonary system.

Conclusion

The first advantage of pulmonary rout of administration of drugs is by passing the first pass metabolism effect when the drug is administered via the pulmonary rout in comparison with the oral rout. The second advantage of pulmonary rout of administration of drugs is that the food cannot interact with the absorption of drug when the drug is administered via the pulmonary rout in comparison with the oral rout in addition the different pH related to the different site of gastrointestinal tract cannot have effect on the absorption of drug when the patient use the pulmonary rout instead of oral rout. The pharmacological effect of drug appears faster than the oral rout.

The results of the study showed that it is possible to prepare the inhalable propranolol micro particle by spray drying

method. The type of the spray drying vehicle is the effective factor on the physicochemical properties and aerodynamic behavior of the micro particles. It is important effective factor on the rate and extent of the pulmonary absorption of drug.

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