INTRODUCTION
1. INTRODUCTION

The oral route of drug administration has been most commonly used and preferred route for drug administration of many diseases from decades. Thus, the design of an oral dosage form is the main choice for most of the new chemical entities which are introduced into the market. Nevertheless, the oral delivery of approximately 50% of the drugs is hampered due to various reasons such as, a) poor drug solubility in the gastrointestinal (GI) fluids, b) poor drug permeability across the GI epithelium, c) pronounced hepatic first-pass extraction of the drug, d) intestinal P-glycoprotein (PGP) efflux or pre-systemic elimination of the drug, e) drug complexation, and f) insufficient stability of the drug in some segments of the GI tract due to enzymatic and non-enzymatic degradation.

Since 50% to 60% of drugs are lipophilic in nature and demonstrate hydrophobic behavior, formulation development of these drugs would be a challenging task, in order to overcome this problem. The clinical efficacy of these highly lipophilic drugs is being inhibited, particularly when they are designed for oral administrations by their low aqueous solubility which further leads to their poor absorption and permeation. In addition to solubility, PGP-efflux is another factor which is responsible for the poor oral drug bioavailability. Drugs which are PGP-substrates have relatively good permeability, but are expelled out of the GI tract by PGP-efflux mechanism, which results in low bioavailability. In addition, few drugs have sufficient solubility and permeability across GI, but have poor bioavailability due to their extensive hepatic-first pass metabolism. Apart from this, it’s a well-established fact that the pharmaceutical industry faces more difficulties in formulating and developing novel drug delivery systems of new chemical entities. Thus, a great collection of poorly water soluble, PGP-substrates, poorly permeable and highly metabolized drugs offers a rigorous demand to its successful formulation and clinical application.

1.1 Bioavailability

According to the code of Federal Regulations (CFR 21.320.1) in USA, bioavailability (F) is the rate and extent (fraction or the percentage of the dose) to which the active drug ingredient or therapeutic moiety delivered is absorbed from a drug product into the general circulation and becomes available at the site of drug action (Lobenberg and Amidon, 2000). Drug bioavailability is clinically important because the pharmacological and toxic effects of the drug are proportional to both the dose and its University Institute of Pharmaceutical Sciences, Panjab University.
bioavailability. Nevertheless, it is difficult to measure the drug concentration directly at the site of action. Thus, the most bioavailability studies involve the measurement of drug concentration in the blood. This is done on the basis that the drug at the site of action is in equilibrium with the drug present in the blood.

The effective exchange of a drug from an oral dosage form into the general circulation can be described as four step process i.e.; a) disintegration of the drug product (if the drug product or formulation is in the form of a solid dosage form), b) dissolution of the drug in the GI fluids at the absorption site, c) movement of the dissolved drug molecules through or across the membranes of the GI tract, and d) movement of the drug molecules away from the site of absorption into the general circulation or at the site of action (Aungst, 1993). Bioavailability may be determined by administering a dosage form to an animal or human subject and measuring the concentration of unchanged active ingredient present in the bloodstream over time. Both the rate and extent of the drug absorption determines the shape of the curve of a drug concentration versus time plot. The area under the curve obtained from this plot is directly proportional to the total amount of unchanged drug present in the systemic circulation and is the most reliable measure of bioavailability. Absorption rate of a drug is defined as the time at which the maximum systemic unchanged drug concentration \((C_{\text{max}})\) occurs which indicates the extent of drug absorption.

1.1.1 Poor and variable drug bioavailability

Poor and variable drug bioavailability by the oral route can be due to the poor drug solubility, drug degradation in the GI lumen, poor drug membrane permeation, first-pass metabolism and pre-systemic elimination of the drug. Figure 1 depicts some of the major issues that lead to poor and variable oral drug bioavailability. According to the Biopharmaceutical Classification System (BCS), the drugs are classified into four groups according to their solubility and permeability properties; Class 1: high permeability and high solubility drugs; Class 2: high permeability and low solubility drugs; Class 3: low permeability and high solubility drugs; and Class 4: low permeability and low solubility drugs (Amidon et al., 1995; Waterbeemd, 2000). Class I drugs do not show any problem in the absorption (nevertheless, its systemic availability may be low due to extensive hepatic first-pass metabolism). The BCS as Class II and IV drugs have poor aqueous solubility while former have high permeability and later shows poor drug permeability.

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Poor drug bioavailability is often associated with the oral dosage forms having low drug solubility, low drug permeability, or both.

Figure 1: Factors that lead to poor oral drug bioavailability such as, 1) poor drug dissolution or solubilization in the GI tract; 2) limited absorption window of some drugs; 3) unstirred water layer which hinders drug permeation of lipophilic drugs; 4) PGP-efflux pumps; 5) some drugs undergo intra-enterocyte metabolism; and 6) extensive hepatic first-pass metabolism (Dhan and Hoffman, 2006a; 2008).

The poor water solubility of the drug is one among the major issues leading to their limited oral bioavailability. Because of their poor water solubility, when they are orally administered, the dissolution rate in the GI tract is considered to be the rate-limiting step. Poor water solubility complementarily hampers the permeation and thus the quantity of drug absorbed leads to low and unpredictable oral bioavailability (Amidon et al., 1995). In general, the solubility of weak base is high at acidic conditions (stomach), low at intestinal conditions and they can be converted into uncharged species at neutral conditions. As a result, it dissolves completely in the stomach however then precipitates in the intestine due to rapid pH increase and/ or extensive dilution of excipients. However, it is essential to avoid precipitation and maintain the dispersed and dissolved state of the basic drug compounds in neutral media, which recovers the absorption of basic drugs having poor solubility (Patel and Sawant, 2007; Pouton, 2006; Sinha and Ghai, 2010; Wagner et al., 2003).

Another major issue to low and erratic bioavailability is that most of the orally administered drugs have little chance to permeate across the GI membrane (Ruan et al., University Institute of Pharmaceutical Sciences, Panjab University.
Even though they penetrate across the GI membrane, few transport processes mediated by transporters and pumps play an important role in the absorption and permeation. These transporters and pumps limit the absorption of nutrients from the intestine into the blood circulation. A number of transporters that facilitate transport of drug in the opposite direction i.e. from the interior of enterocytes to back into the intestinal lumen called as pre-systemic elimination. This phenomenon is also referred to as intestinal drug efflux. The best-known efflux pump in the human intestine is the P-glycoprotein (PGP). PGP efflux the drug molecules back into the GI lumen to be excreted without showing their therapeutic effect. This intestinal secretion phenomenon results in a decreased oral drug bioavailability for PGP-substrates.

1.1.2 Methods to enhance oral drug bioavailability

Any of the formulation approaches, which can modify drug solubility, permeability, metabolism and pre-systemic elimination, should help in the improvement of oral bioavailability of drugs. Various important approaches used to improve the oral bioavailability of drug are shown in Figure 2.

**Figure 2: Formulation approaches to improve the oral drug bioavailability.**

Among these approaches, modification of the physico-chemical properties of drugs such as salt formation, particle size reduction, change in crystal form etc. are used to improve the dissolution rate and absorption of drugs. However, these methods have their own limitations. For instance, salt formation of neutral compounds is not feasible.

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and the synthesis of weak acids and weak base salt may not always be practical. Moreover, the salts that are formed may convert back to their original acid or base forms and lead to aggregation in the GI tract. Apart from this, the particle size reduction of the drug may not be desirable in situations where poor wettability and handling difficulties are experienced for very fine powders (Serajuddin, 1999). In order to overcome these drawbacks various other formulation strategies have been adopted including the use of cyclodextrins, nanoparticles, solid dispersions, permeation enhancers etc. (Aungst, 1993; Robinson, 1996). However, only in some selected cases, these approaches have been successful.

The dosage form of an active ingredient can have a great effect on its solubility and permeability, thereby affecting oral drug bioavailability. When approaches in regard to solubility and permeability are considered, efforts are made to change the properties of Class II, III and IV drugs. These efforts are made with respect to improve the dissolution and permeability of these classes of drugs in order to resemble them with Class I drugs. Table 1 presents some of the most common causes of poor drug bioavailability and their possible solutions.

Table 1: Formulation approaches to solve the oral drug bioavailability problems.

<table>
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<tr>
<th>Problem(s)</th>
<th>Test method(s)</th>
<th>Possible solutions</th>
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<tr>
<td>Poor drug solubility or dissolution rate.</td>
<td>Determination of aqueous drug solubility.</td>
<td>Particle size reduction.</td>
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<td></td>
<td>Bioavailability with alternative solid forms.</td>
<td>Solubilization or wetting agents.</td>
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<td></td>
<td>Bioavailability with non-aqueous solutions.</td>
<td>Alternative solid forms.</td>
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<tr>
<td>Drug degradation or metabolism in the GI lumen.</td>
<td>Drug stability in the gastric and intestinal fluids.</td>
<td>Non-aqueous vehicles.</td>
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<td>Poor partitioning.</td>
<td>Octanol/buffer distribution.</td>
<td>Lipid based formulations.</td>
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<td>Low diffusivity.</td>
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<tr>
<td>Pre-systemic elimination by brush border and intestinal cells (PGP).</td>
<td>Intestinal homogenate.</td>
<td>Efflux and metabolism inhibitors.</td>
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<td>Bioavailability after portal dosing.</td>
<td>Lipid based formulations.</td>
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Further PGP efflux that serves to protect the body from xenotoxins (Zhang and Bent, 2001) is among the factors, which inhibit most of the drugs permeability across the GI membrane. It limits the bioavailability of many orally administered drugs, by transporting them back into the intestinal lumen following their absorption by the enterocytes. Therefore, it is essential to inhibit these mechanisms so as to keep the drug molecules in the absorbed state and in the systemic circulation for enhanced oral bioavailability. Such inhibitors may have a significant role to play in the enhancement of drug intestinal absorption (Barthe et al., 1998; Buckingham et al., 1995; Carreño-Gómez and Duncan, 2000; Cornaire et al., 2004; Lin et al., 2007; Rege, et al., 2002; Takahashi et al., 2002). Such phenomena should be addressed for in the design of oral dosage forms for PGP substrates. The absorption of drug through lymphatic transport can bypass the hepatic first-pass metabolism. The use of long-chain triglycerides (LCT) which led to increased lymphatic transport is one of the approaches that can be used to enhance oral drug bioavailability. This approach acts as one of the alternatives to the oral route of drug administration, particularly for those drugs that undergo first-pass effect and lead to poor drug bioavailability (Lespinea et al., 2006).

Bioavailability of drugs from a lipid solution is expected to be good because in the intestine, the triglycerides are rapidly digested to free fatty acids and 2-mono-glycerides. These products are further solubilized to form a colloidal dispersion within bile salt-lectithin mixed micelles. So, a hydrophobic drug is likely to be solubilized in the mixed micelles if administered with lipid, resulting in the reservoir of drug in colloidal solutions from which it can partition, allowing efficient passive or transcellular absorption (Pouton, 2000). These lipid formulations can also enhance the drug bioavailability by other mechanisms such as inhibition of PGP-mediated drug efflux and preabsortptive metabolism by gut membrane-bound cytochrome enzymes, promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing GI membrane permeability by opening intracellular spacing (Hauss, 2007).

In recent years, self-emulsifying drug delivery systems (SEDDS) as lipid and surfactant based formulations encompass a practical achievement in improving the oral bioavailability of poorly water-soluble drug by presenting and maintaining the drug in a dissolved state, at molecular level, in small droplets of oil, all over its transit through the GI tract (Araya et al., 2005a; Ghai and Sinha, 2011; Holm et al., 2003; Pouton, 2000; University Institute of Pharmaceutical Sciences, Panjab University.)
Pouton, 2006). Nanoemulsion/SEDDS as lipid based drug delivery systems are considered to be a promising pharmaceutical solutions for enhanced oral drug bioavailability, improved reproducibility of plasma profiles and reduced inter and intra subject variability. Nanoemulsions are biphasic, thermodynamically and kinetically stable systems with globule size in the nano-range. These systems behave as a super-solvent for the drug. They possess higher solubilization capacity than simple miceller solutions and their thermodynamic and kinetic stability offers advantages over unstable dispersions, such as emulsions and suspensions. These can be manufactured with little or no input of energy i.e. heat or mixing, and has a long shelf-life, thus can be manufactured easily at industrial scale. SEDDS are physically stable formulations that are easy to manufacture and scale up.

SEDDS are isotropic liquid mixtures of oil, surfactant and co-surfactant and they are capable of forming thermodynamically and kinetically stable oil-in-water nano-sized emulsion upon moderate stir provided by the stomach and the upper small intestine (Charman et al., 1992; Craig et al., 1993; Devani et al., 2004; Shah et al., 1994; Wakerly et al., 1986). SEDDS are mixtures of natural or synthetic oils, solid or liquid surfactants or, alternatively, one or more hydrophilic solvents and co-surfactants/solvents. SEDDS characteristically produce emulsions which are with a droplet size of below 200 nm. Thus, for lipophilic drugs that exhibit dissolution rate-limited absorption, SEDDS/nanoemulsions may improve the rate and extent of absorption and result in more reproducible plasma-time profile. The improved drug absorption provided by these systems is dependent upon the maintenance of the drug in the solubilized state and essential stability until the drug can be absorbed from the GI tract. The permeation and absorption of drugs from these systems are subject to: a) the rate of dispersion, b) extent of emulsification, c) droplet size of resulted emulsion, and d) solubilization and precipitation of drug from the formulation upon dispersion (Erkko et al., 1997; Keng et al., 2004; Porter and Charman, 2001; Pouton, 2000).

The primary mechanism of action, which leads to improved bioavailability, is usually avoidance or partial avoidance of the slow dissolution (rate-limiting) process. Ideally, the SEEDS/nanoemulsion formulation allows the drug to remain in the dissolved state throughout its transit through the GI tract (Pouton, 2000). SEDDS can be administered in soft or hard gelatin capsules and will produce fine oil droplets or micelle dispersion upon disintegration and aqueous dilution of capsule. It has been reported that
the self-emulsifying formulations spread readily into the GI tract, while the motility of the stomach and intestine provides the agitation necessary for their self-emulsification process (Pouton, 2000).

Nanoemulsion/SEDDS offer an improvement in the rate and extent of absorption for lipophilic drugs, which display dissolution rate-limited absorption. Drugs like talinolol that have poor solubility and permeability and tizanidine that undergoes extensive first-pass metabolism, possess limited bioavailability and thus are suitable candidates for the study.

Talinolol is a cardio-selective β₁-blocker drug which lack intrinsic sympathomimetic activity. The therapeutic doses of talinolol are 25 to 300 mg/day. It is an anti-hypertensive and anti-arrhythmic agent which does not undergo first-pass metabolism (Oertel and Richter, 1995; Oertel et al., 1994; Sinegubova et al., 2000; Tubic et al., 2006a, b). It is also increasingly used in the treatment of heart failure along with other beta-blockers. Due to its incomplete and erratic absorption, talinolol has a poor bioavailability of <50%. This is due to its poor water solubility and high affinity to the PGP-efflux (Doppenschmitt et al., 1999; Tamilvanan, 2009; Tubic et al., 2006a, b). Among the different innovative formulation approaches for enhancing the oral bioavailability and efficacy of talinolol, nanoemulsion/SEDDS provides promising pharmaceutical solution.

Another drug used in the study is tizanidine, which is an orally administered centrally acting imidazoline α₂ adrenoceptor agonist, a skeletal muscle relaxant extensively used in the treatment of spasticity associated with multiple sclerosis or spinal cord disorders (Henney et al., 2008; Henney and Shah, 2007; Mutalik et al., 2009; Shah et al., 2006). Tizanidine undergoes rapid and extensive hepatic first-pass metabolism with an estimated absolute bioavailability of <21% on repeated dosing and with a problem of dose adjustment. Therefore, the need was to develop a system that protects tizanidine from metabolism and helps to improve its oral bioavailability. SEDDS/nanoemulsion composed of long-chain triglycerides (LCT) are capable of bypassing the hepatic metabolism up to some extent as it has been reported that nano-sized particles can be absorbed directly into the blood through paracellular pathway and enter the blood circulation, thus demonstrating a systemic drug effect. Further, LCTs have a higher affinity towards lymphatic drug transport. Thus formulating SEDDS/nanoemulsion formulations offers a promising approach to enhance the bioavailability of tizanidine.
The present work comprises of formulation, development and evaluation of nanoemulsion/SEDDS formulations of talinolol and tizanidine to increase their oral bioavailability. Developed formulations were characterized for thermodynamic and kinetic stability, self-emulsification ability, robustness to dilution, droplet size, polydispersity, zeta potential, transmission electron microscopy, pH, drug content, percentage transmittance, refractive index, conductivity and viscosity. In-vitro release, ex-vivo permeation and in-vivo bioavailability evaluation of developed formulations were also done in comparison with conventional formulations.