1. Introduction

Drug delivery through oral route is the most preferable route of administration, especially to treat chronic diseases. Many drugs fail to meet their required therapeutic actions owing to poor bioavailability which is usually due to low solubility, low permeability and/or high metabolism. Oral bioavailability of drugs majorly depends on their rate and extent of dissolution from the dosage form, their solubility in gastro intestinal fluid, in vivo stability and permeability of the drug. The absorption of drugs administered orally occurs majorly in the small intestinal region through various mechanisms such as passive transcellular diffusion, paracellular transport, carrier mediated transport and endocytosis. Usually, passive diffusion mechanism is responsible for absorption of lipophilic drugs from intestinal region while carrier mediated transport mechanism is responsible for absorption of hydrophilic drugs. It has been found that several transporters are also involved in drug absorption process (Kim, 2006; Kunta and Sinko, 2004). Till date, the physiological, pathological and pharmaceutical functions of approximately 350 transporters have been extensively studied. Some of these transporters are peptides, amino acids, organic cations and anions, bicarbonates, glucose, neurotransmitters, ion exchangers, bile salts, carboxylates, urea, amines, folates, fatty acids, nucleosides, phosphates, nucleoside-sugar and ATP binding Cassettes (ABC). It has been

recognized that ABC transporters are involved in the active efflux of certain drugs to outside the cell which critically affect the drug absorption, disposition and elimination processes in the body. Many clinically used drugs, for example, ACE inhibitors, anti cancer and anti viral agents are substrate of the ABC transporters and thus, the efflux system operated by the transporters should be considered to optimize the oral bioavailability and decrease variability at the site of absorption (Ayrton and Morgan, 2001; Zhang *et al*., 2006).

Phosphoglycoprotein (P-gp) which is an ABC transporter has been identified as the one responsible for active efflux of drugs which are used in treatment of various chronic diseases (Fardel *et al*., 1996). P- gp, a plasma membrane glycol protein of 170 kDa, is abundant in columnar epithelial cells (enterocytes) in lower gastro intestinal tract, canalicular surface of hepatocytes, apical surface of proximal tubules, capillary endothelial cells of brain, testis and in pregnant uterus (Ambudkar *et al*., 2006; Loo and Clarke, 2006). Usually, P-gp does not play any important role in the vital body functions but may be considered on the basis of its interaction with the foreign body, to play a defensive role in the body, such as active transport of cytotoxic compounds and endotoxins from intracellular to extracellular level (Thiebaut *et al*., 1987; Fromm, 2004). The exact mechanism of efflux system operation is still not clear. Substances which suffer with P-gp efflux are called as P-gp substrates.

Drugs which are P-gp substrate with affinity towards P-gp have the problem in its absorption due to P-gp efflux and may finally effect the treatment or therapy (Mouly and Paine, 2003). This can also lead to build up of multi drug resistance (MDR) for a particular disease due to over expression of P-gp. The drugs which lack affinity towards P-gp are not effluxed and may not show problem in its absorption (Varma *et al*., 2003; Varma *et al*., 2005). Based on the above discussion it may be concluded that inhibiting the function of P-gp at various over expression sites of the body can help to increase the rate and extent of absorption and permeation, and thus improve the bioavailability of drugs which are potential substrate of P-gp efflux (Chiou *et al*., 2001; Breedveld *et al*., 2006; Varma *et al*., 2005 a). It may be done by using one of the following methods (Bansal *et al.,* 2009; Bansal *et al.,* 2009 a).

1. Development of novel non P-gp substrates
2. Administration of P-gp inhibitors with drug
3. Designing formulations that allow the drug to bypass P-gp efflux

Among the above methods, first method is quite tedious and may consume great deal of time and money for synthesis of a new chemical entity. The second and third methods are relatively feasible due to advances in polymer science technology which has resulted in development of novel excipients for pharmaceutical applications (Lo, 2003; Cornaire *et al*., 2004; Bernkop-Schnürch and Grabovac, 2006;

Batrakova and Kabanov, 2008). Certain pharmaceutical excipients such as polyethylene glycols (PEG), poly propylene oxanes (PPO) and vitamin E TPGS have been studied which have the ability to inhibit the P-gp efflux (Shen et al., 2006). Interestingly, some lipid excipients either in solid or liquid state such as Peceol, Gelucire 44/14 and Cremophor EL also exhibit similar property (Sachs-Barrable *et al*., 2007). However, the exact mechanism of P-gp inhibition by these unique excipients is still unclear. Therefore, co-administration of drugs with suitable pharmaceutical excipients (P-gp inhibitors) in optimized concentration can help to improve the bioavailability and the possibility of drug delivery at targeted site.

Thus, a solution to the above problem may be the development of a dosage form wherein the drug is encapsulated or coated with certain inactive and biodegradable pharmaceutical excipients having capability of P-gp efflux inhibition. These encapsulated or coated intermediates may then be administered as nanoparticulate systems i.e. nanoparticles (Nassar *et al*., 2009). Nanoparticulate delivery system was first developed around 1970 and is currently of great interest because of its colloidal nature and small size, ranging from 1 to 1000 nm at least in one dimension. Advantages of nanoparticles like increased bioavailability, prevention of first pass metabolism, ability to modify the biodistribution of drugs, targeting capability, sustained drug release, decrease toxicity

and improved stability have made this technology in more demand in drug delivery (Couvreur *et al*., 1986; Kreuter *et al*., 1994). Nanoparticulate drug delivery system has the unique characteristic of submicron sized drug encapsulated particles which can evade recognition by P-gp at the intestinal ileum region or tissue membrane. This approach would lead to enhanced systemic uptake or delivery at targeted site with minimized drug loss as compared to the conventional dosage form (Nassar *et al.,* 2009). It has been reported that delivery of drugs which are P-gp substrates in nano-encapsulation form could be helpful for improvement of bioavailability through circumvention of P-gp efflux system. Recently, nanoparticles of cyclosporin A (also a P-gp substrate) were fabricated by using glyceryl monooleate/poloxamer 407. In vitro and in vivo studies (in Beagle dogs) were carried out using Neoral (cyclosporin A) as a reference product. Interestingly, the pharmacokinetic evaluation exhibited higher C_{max} (1371.18 ± 37.34 vs 969.68 ± 176.3 ng/ml), higher $AUC_{0\text{-}t}$ (7757.21 ± 1093.64 vs 4739.52 ± 806.30 ngh/ml), and $AUC_{0\text{-}infinity}$ (9004.77 ± 1090.38 vs 5462.31 ± 930.76 ngh/ml). The relative oral bioavailability was 178% compared with the reference product clearly indicating the role of P-gp inhibitors and nanoparticle formulations in improving drug utilization, thereby improving its therapeutic efficacy (Lai *et al*., 2010). Thus, it may be concluded that

nanoparticulate drug delivery has the potential for delivery of P-gp substrates.

Recently, many research based pharmaceutical companies are investing relatively more time and money on development of herbal rather than synthetic drug formulations. This is because treatment of chronic diseases requires long term drug therapy and using synthetic drugs in such cases may cause adverse effects. Due to the above mentioned reason, even the WHO encourages use of traditional medicines of natural origin. Among all, plant extracts are the most widely used natural medicine due to their ease of availability and comparatively low production cost. The use of plant products as medicine in treatment of various chronic diseases is supported by Ayurveda, a system of traditional medicine, native to the Indian Subcontinent and also practiced in other parts of the world (Patwardhan and Hopper, 1992). Berberine (BBR) is a plant alkaloid with a long traditional history and is used both in Ayurveda and Chinese medicine. This alkaloid is present in many plants, including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Tinospora cordifolia, Arcangelisia flava*, *Cortex rhellodendri, Rhizoma coptidis, Coptis japonica, Thalictrum* minus, *Berberis wilsonae* and *Berberis aristata* (tree turmeric). This phytochemical constituent can be found in the root, rhizome and stem bark of the plants.

As a drug, it is traditionally used for its antimicrobial and antiprotozoal properties in Ayurveda, Chinese and Middle-East folk medicine. Specifically, Ayurveda describes BBR extracts and decoctions to have significant antimicrobial activity against a variety of organisms including bacteria, virus, fungi, protozoa, helminthes and Chlamydia (Arayne *et al*., 2007). Recently, BBR also found to be beneficial in treatment of chronic diseases including diabetes, cancer, depression, hypertension and hypercholesterolemia (Imanshahidi and Hosseinzadeh, 2008). Thus, the drug has emerged as a medicinal agent with multispectral activities. In spite of having superior therapeutic activities, the drug is not efficiently utilized in the treatment of diseases due to problem associated in its delivery. BBR has poor bioavailability (less than 5%) as its uptake is inhibited by P-gp efflux (Pan *et al*., 2002). Moreover, the determination of BBR in rat blood, liver and bile fluid carried out in a pharmacokinetic study also suggests that BBR is metabolized in the liver and also undergoes hepatobiliary excretion (Tsai and Tsai, 2002). Therefore, there is a need to overcome the bioavailability issue of the BBR to improve its therapeutic efficacy. Literature search indicates that earlier an attempt has been made to improve the bioavailability of BBR by administering with Cyclosporine A which is a recognized P-gp inhibitor. As cyclosporine A by itself is an antibiotic with several side effects, this approach is not suitable to enhance the bioavailability of the drug (Xin *et al.,* 2002).

Another attempt has been made by preparing a micro emulsion using PEG and Tween-80 as a polymer and surfactant, respectively. The absorption behavior of the formulation in rat intestine was investigated. It was observed that the absorption of BBR from the microemulsion at the ileum region of intestine was significantly higher than that of pure BBR (Gui *et al.,* 2008)). Recently, few of the researchers have also developed various formulations such as tablets, complex membranes, β cyclodextrin complexes, anhydrous reverse micelle and nanoparticles for BBR (Ke *et al*., 2009; Liu *et al*., 2009; Battu *et al*., 2010; Lv *et al*., 2010; Wang *et al*., 2011; Chen *et al.*, 2011; Khemani *et al.,* 2012). In spite of improving of bioavailability of BBR, the authors have not reported significance of P-gp inhibition and toxicity profile of developed formulations. Furthermore, the basic issue of P-gp efflux associated with BBR still need to be addressed and is a challenge for pharmaceutical researchers.

Based on the above facts and discussions, nanoformulation approach is selected as a platform technology for the development of an effective dosage form for P-gp substrate; BBR, thereby, helping in achieving basic goals like maximum bioavailability, minimum toxicity and reduced dose. Therefore, in development of nanoparticulate formulation of BBR, Poly(ε) caprolactone (PCL); United States Food and Drug Administration (USFDA) approved biocompatible and biodegradable polymer, was selected as drug carrier based on its potential biomedical and drug

delivery applications (Dash and Konkimalla, 2012). D-alpha-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS); a PEG derivative is selected as surface coating agent (Zhang *et al*., 2012; Vijayakumar *et al*., 2013). It is water soluble derivative of natural vitamin E (non-ionic amphilic copolymer) which facilitates increased cellular uptake, prolonged blood circulation and enhanced bioavailability. Specifically, it enhances drug permeability and absorption of drugs by inhibiting the P-glycoprotein. It has been also reported as a stabilizer and surface coater in drug delivery for different types of cancer treatment. However, it would also be important to consider the extensive distribution of P-gp due to which administration of developed formulations containing P-gp inhibitors may cause partial or reversible disturbance in the physiological system of the body. Therefore, a detailed toxicological evaluation is also needed for such developed drug formulations.

Since past two decades nanotechnology has been tremendously employed in pharmaceutical research field for the development of novel dosage forms (Chavhan *et al*., 2011; Singh and Nalwa, 2011). As a result, various nanomedicine products are available in market for treatment of various complex and chronic diseases (Bhavna *et al.,* 2008; Shegokar and Muller, 2010). The majority of marketed nanomedicines are meant for parentral route administration rather than oral route consumption. In fact, orally administered nanomedicnes would be highly appropriate

particularly when the therapy is prolonged. Despite of availability of various robust validated techniques for production of nanoparticles, the major limitation such as uncertainty of nanoparticles stability in stomach environment creates hindrance for further exploration of nanoparticles intended for oral administration (Merisko-Liversidge *et al*., 2003; Galindo-Rodriguez *et al*., 2005; Iqbal *et al*., 2012; Khayata *et al*., 2012; Carbone et al., 2013). Few attempts such as applying of external gastric resistant enteric coating on nanoparticles, using of homing carrier systems, filling of lyophilized or freeze dried nanoparticles in capsules and compression as conventional tablet have been made towards over coming of above said limitation (Murakami *et al.,* 2000; Chakraborty *et al*., 2010). However, these attempts have not given satisfied assurance for preservation of stability of nanoparticles on their oral administration. Further, it increases the manufacturing steps involved in the production of oral nanoparticle dosage forms. Ultimately, it may lead to increase the cost burden on manufacturers as well as patients.

The above facts indicate that there is emerging need for development of suitable technology which is capable of overcoming the above said limitations. Therefore, we have developed and evaluated smart technology "Naplet" for efficient oral delivery of BBR nanoparticles as a compact solid dosage form.

The final product and apparatus used is termed as Naplet and Naplet device, respectively for simple understanding of readers. Naplet was evaluated as per standards of conventional solid oral dosage forms which are mentioned in United States Pharmacopeia. No such technology existed at the time of conceptualization of this work.

Thus, in view of the above the discussion the present research work aims to develop polymeric nanoparticles of BBR for enhancement of oral bioavailability. Surface coating of BBR nanoparticles were also proposed for circumvention of P-gp efflux. Histopathological and biochemical estimations studies were planned for evaluation of toxicity of developed nanoformulations. Further, Naplet technology was explored for efficient delivery of BBR nanoparticles by oral route.