INTRODUCTION

Camptothecin (CPT) naturally occurring quinolone alkaloids shows a significant anticancer activity with a broad spectrum of human malignancies. CPT is an inhibitor of the DNA-replicating enzyme topoisomerase I which is believed to act by stabilizing a topoisomerase I-induced single strand break in the phosphodiester backbone of DNA, thereby preventing relegation [1]. Despite of its promising activity, the clinical applications are hampered by its poor water solubility, low stability in physiological medium, severe systemic toxicity, and low antineoplastic activity [2]. Accordingly, a novel drug delivery system is imperative to overcome the internal defects. In recent years, nanostructured materials such as nanoparticles have been considered as potential carriers for hydrophobic drug delivery that may resolve the aforementioned problems [3].

A key goal in the pharmaceutical development of dosage forms is a good understanding of the in vitro and in vivo performance of the dosage forms. One of the challenges of biopharmaceutical research is correlating in vitro drug release information of drug formulations to the in vivo drug profiles [4]. In vitro - in vivo correlations (IVIVC) play an important role in reducing the drug development time and optimization of the formulation. A good correlation is a tool for predicting in vivo results based on in vitro data [5]. The IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development [6].

The IVIVC is a mathematical relationship between in vitro properties of a dosage form with its in vivo performance. For oral dosage forms, the in vivo release is usually measured and considered as dissolution rate. The relationship between the in vivo and in vitro characteristics can be expressed mathematically by a linear or nonlinear correlation [7]. However, the plasma concentration cannot be directly correlated to the in vitro release rate; it has to be converted to the in vivo release or absorption data, either by pharmacokinetic compartment model analysis or by linear system analysis [6].

IVIVC definitions

Food and Drug Administration (FDA) definition of IVIVC (FDA, 1997)

The IVIVC has been defined by the FDA as “a predictive mathematical model describing the relationship between an in vitro property of a dosage form and an in vivo response.”

In general, the in vitro property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed [8]. However, the correlation between the in vitro dissolution rate and in vivo absorption rate does not always exist. Nevertheless, making an accurate prediction of in vivo performance based on in vitro dissolution is not a straightforward process and many traps could inadvertently bias the predictions. Thus, the main objective of the IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers [9].

Levels of IVIVC

There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C. The concept of correlation level is based on the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form [10].

Level A correlation

The IVIVC that correlates the relationship between the entire in vitro and in vivo profiles for its regulatory relevance and is called the level A correlation. This level of correlation is generally linear and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form [6,11].

ABSTRACT

Objectives: The aim of this study was to develop an in vitro - in vivo correlation (IVIVC) for the prepared Camptothecin (CPT)-loaded polymeric nanoformulation.

Methods: In this study, CPT-loaded polymeric nanoformulation was prepared by nanoprecipitation method using containing poly (methacrylic acid-co-methyl methacrylate) (polymer), poloxamer 188 (non-ionic surfactant), and β-cyclodextrin (stabilizer). In vitro release rate data were obtained from prepared polymeric nanoformulation using the USP apparatus type 2. A single-dose, crossover pharmacokinetic study for the nanoformulation was carried out in six albino rats. These data were used as the basis for the IVIVC model development.

Results: The plasma concentration of CPT was estimated by high-performance liquid chromatography. The pharmacokinetic parameters were calculated from the plasma concentration of CPT and time data. Furthermore, the deconvolution of the in vivo concentration-time data was performed using Wagner–Nelson method to estimate the in vivo drug release profile.

Conclusion: Therefore, a level A IVIVC was developed for CPT-loaded polymeric nanoformulation between dissolution percentage and intestinal absorption in rats. The simplest way to demonstrate a correlation is to plot the percentage absorbed in vivo versus the percentage released in vitro at the same time.

Keywords: Camptothecin, In vitro - in vivo correlation, Wagner–Nelson, Dissolution, Pharmacokinetics.
The purpose of level A correlation is to define a direct relationship between in vivo data such that measurement of in vitro dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form. In this context, the model refers to the relationship between the in vitro dissolution of an extended release dosage form and an in vivo response such as plasma drug concentration or amount of drug absorbed.

**Level B correlation**

A level B IVIVC is based on the principles of statistical moment analysis. In this level of correlation, the mean in vitro dissolution time of the product is compared to either mean in vivo residence time or the mean in vivo dissolution time. The level B correlation does not uniquely reflect the actual in vivo plasma level curves because a number of different in vivo curves will produce similar mean residence time values [6,11].

**Level C correlation**

A Level C correlation relates a single dissolution time point \( (t_{\text{max}}, t_{\text{50\%}} \text{, etc.}) \) to a pharmacokinetic parameter such as area under the curve \( (AUC) \), \( t_{\text{max}} \) or \( C_{\text{max}} \). This is the weakest level of correlation relationship between absorption, and dissolution is established since it does not reflect the complete shape of plasma drug concentration-time curve, which is the critical factor that defines the performance of a drug product [6,11].

**Multiple level C correlations**

This level refers to the relationship between one or more pharmacokinetic parameters of interest \( (t_{\text{max}}, AUC, \text{or any other suitable parameters}) \) and the amount of drug dissolved at several time point of dissolution profile. Multiple point level C correlation may be used to justify biowaivers provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest [6,11] (Table 1).

**METHODS**

**Formulation of CPT-loaded polymeric nanoparticles**

About 100 mg of poly (methylacrylic acid-co-methyl-methacrylate) polymer with 10 mg of CPT were dissolved in 10 ml of dimethyl sulfoxide. The prepared organic phase was transferred at once into 500 ml beaker containing 50 mg of β-cyclodextrin, 100 mg of poloxamer 188, and 20 ml of distilled water under mechanical stirring (Remi, India) at 500 rpm. Polymeric nanoparticles were formed spontaneously, but the stirring process is continued for 50 minutes to aid the size reduction and to evaporate the residual solvents (Table 2). The fabrication experiments were performed in triplicate.

**In vitro evaluation**

In vitro drug release of CPT from polymeric nanoparticles was evaluated by dialysis bag diffusion technique. The prepared CPT-loaded polymeric nanoformulation (weight equivalent to 10 mg of drug) was placed in a cellulose dialysis bag (cutoff 12 000; HIMEDIA, Mumbai, Maharashtra, India) and sealed at both ends. The dialysis bag was immersed in the cellulose dialysis bag (cutoff 12 000; HIMEDIA, Mumbai, Maharashtra, India) and sealed at both ends. The dialysis bag was immersed in the process is continued for 50 minutes to aid the size reduction and to evaporate the residual solvents (Table 2). The fabrication experiments were performed in triplicate.

**IVIVC**

The level A IVIVC, the point-to-point relationship between in vitro dissolution and the in vivo input rate, was studied. The procedure of developing an IVIVC consisted of the following steps: Calculation of cumulative in vitro dissolution rate, calculation of cumulative in vivo absorption rate from concentration-time data obtained by Wagner–Nelson method and modeling the relationship between in vivo absorption rate and in vitro dissolution rate.

Wagner–Nelson is a mass equation which allows calculation of the absorption in the case of the one compartment model as stated in guidelines [8,16]. This equation uses observed concentrations \( (C(t)) \), AUC, and apparent elimination rate constant determined from the data \( (k_e) \) as presented in Equation 1.

\[
\text{AUC} = \frac{C(t) + K_e \times \text{AUC}_0}{K_e \times \text{AUC}_0} \times 100
\]

This equation exhibits a domain from 0% to 100%. In some cases, when the Wagner–Nelson equation is used, a flip-flop model could exist, especially in case of sustained release formulations where the absorption rate is much lower than the elimination rate. In this case, the terminal decreasing of the plasma concentration curve, which normally reflects the elimination rate \( (k_e) \), becomes a reflection of actual absorption rate \( (k_a) \), while the initial increasing part of the curve, which normally reflects the absorption rate \( (k_a) \), is the actual representation of the elimination rate \( (k_e) \).
RESUL TS AND DISCUSSION

Fabrication of plain and CPT-loaded polymeric nanoparticles

CPT-loaded poly (methacrylic acid-co-methyl-methacrylate) nanoparticles were prepared based on the principle of nanoprecipitation under the influence of stirring. In nanoprecipitation method, the solvent stream contains CPT and poly (methacrylic acid-co-methyl-methacrylate) in water miscible organic solvent dimethyl sulfoxide, and antisolvent stream contains poloxamer 188 as a surfactant and β-cyclodextrin as a stabilizer in water. Addition of solvent stream into the antisolvent stream results in the miscibility of dimethyl sulfoxide with water, which leads to the increase in the polarity of dimethyl sulfoxide, which in turn decreases the solubility of the polymer. However, nucleation of polymer gets initiated when the equilibrium concentration surpasses the solubility threshold of the polymer. Stirring process aid the size reduction of polymer at the initial stage but in the later stages, anionic nature of polymer provided anionic charge to the nanoparticle surface and higher number of likely charged nanoparticles repels each other and creates an electrostatic repulsive force and maintains the nanoparticles in Brownian motion, which is expected to overcome the Van der Waals attractive force arising from induced dipole-dipole interaction between nanoparticles and gravitational force, thereby stabilize the nanoformulation by preventing the aggregation.

In vitro dissolution profiles of the prepared CPT-loaded polymeric nanoparticles are presented in Fig. 1. The prepared CPT-loaded polymeric nanoparticles showed 98.22 % of drug release. In vitro drug release from the drug-loaded polymeric nanoparticles was assessed in simulated gastrointestinal conditions. The pH condition used was pH 1.2 for 2 hrs (stomach), pH 4.5 for 2 hrs (duodenum) followed by pH 7.4 (distal ileum and colon) for the remaining period of the study using a USP dissolution test apparatus (Apparatus type 2). The drug release was found to be less than 5% up to 4 hrs and the drug release increased when the pH of the medium was adjusted to 7.4.

The plasma concentration-time profiles for the CPT-loaded polymeric nanoparticles are presented in Table 3. The pharmacokinetic parameters of the mean concentration-time profile of the prepared CPT-loaded polymeric nanoparticles were estimated.

The pharmacokinetic profiles of the prepared CPT-loaded polymeric nanoparticles showed a significant difference from the pharmacokinetic profiles of free CPT suspension. The AUC of CPT in rats treated with nanoparticles was 1826.52±76 ng/h/ml, which was significantly improved (***p<0.001) compared with that of free CPT suspension (187.80±58 ng/h/ml). The improved AUC of CPT nanoparticles is due to more uptake of CPT in the intestine from the nanoformulation.

The feasibility of developing the level A correlation for CPT-loaded polymeric nanoparticles is due to more uptake of CPT in the intestine from the nanoformulation. Nominal values are represented as mean±standard deviation (n=6).
correlation ($r^2>0.965$) was observed between in vitro and in vivo profiles. The correlation quality depends solely on the quality of the data. The proposed method demonstrates a schema for developing IVIVC using data from biosudies conducted during formulation development.

CONCLUSION

As the objective of the study is to develop the IVIVC mathematical model to describe the relationship between the in vitro fraction dissolved and in vivo fraction absorbed, the level A IVIVC was developed and showed a best-fit relationship between in vitro dissolution and in vivo absorption data for CPT-loaded polymeric nanoparticles formulation.

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