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Modulation of Pharmacokinetic Processes for Enhancing Bioavailability of Carvedilol

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Abstract

Aim and Background: Oral bioavailability is increased when P-glycoprotein inhibitors reverse P-glycoprotein-mediated efflux, which increases the effectiveness of drug transport across the epithelia. Using curcumin and piperine as P-gp inhibitors, the current study aims to enhance the pharmacokinetic pattern of carvedilol by inhibiting P-glycoprotein (P-gp).

Materials and Methods: Using curcumin and piperine, carvedilol composites were made using this technique. Using the direct compression technique, the prepared carvedilol composite was compacted into a tablet. Fourier transform-infrared (FTIR) spectroscopy, X-ray diffractometry, permeability research, and in-vitro drug release were performed on the produced carvedilol composite. A cell viability investigation was conducted using the modified MTT test.

Results: By performing FTIR and DSC analyses, the three components' chemical compatibility has been verified. PXRD graph of the formulation was compared to that of the pure medication, low intensity peaks were seen, indicating the formulation's stability. The two P-gp inhibitors' interaction with the targeted protein P-gp has been verified by docking studies, which have also revealed a variety of hydrogen bond, van der Waals, and hydrophobic interactions that enable piperine and curcumin's robust binding to the P-gp protein.

Conclusion: Based on the concentration of drug absorption by the cells, the carvedilol composite tablet demonstrates the augmentation of carvedilol's bioavailability by 28% to that of 10% in carvedilol alone. This method would therefore offer a workable way to formulate carvingilol once daily, maybe with a lower dosage.

Keywords: Carvedilol; P-glycoprotein inhibitors; Bioavailability; Docking; MTT assay.

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INTRODUCTION

The percentage of an administered dosage of an unmodified medication that enters the systemic circulation is referred to as bioavailability, one of the main pharmacokinetic features of drugs [1]. A medication's bioavailability is 100% when it is injected, by definition [2].]. A medication's bioavailability is 100% when it is injected, by definition[2]. On the other hand, a drug's bioavailability is reduced when taken in other ways, such orally (because of insufficient absorption or first-pass metabolism). The pace and degree at which the active pharmaceutical component is absorbed from the drug product and becomes accessible at the site of action is indirectly indicated by measurements of the medication's plasma concentration taken at regular intervals [3]. One of the most important pharmacokinetics tools is bioavailability, which is taken into account when determining doses for non-intravenous modes of administration. It is expressed as either absolute or relative bioavailability[4, 5].

P-glycoprotein inhibitors that reverse P-glycoprotein-mediated efflux have been shown in a number of studies to be a potential tool for increasing oral bioavailability by increasing the effectiveness of drug transport across the epithelia. In the process of modifying pharmacokinetics, P-glycoprotein inhibitors may also have an impact on the distribution, metabolism, absorption, and excretion of P-glycoprotein substrates[6]. Recent research has revealed that P-glycoprotein and other drug efflux pumps significantly influence how different medicines behave pharmacokinetically. P-glycoprotein has been proposed to have a crucial physiological function in the absorption, distribution, and excretion of xenobiotics because of its selective distribution at the port of drug entrance and departure[7]. P-glycoprotein inhibitors that reverse P-glycoprotein-mediated efflux have been shown in a number of studies to be a potential tool for increasing oral bioavailability by increasing the effectiveness of drug transport across the epithelia In the process of modifying pharmacokinetics, P-glycoprotein inhibitors may also have an impact on the distribution, metabolism, absorption, and excretion of P-glycoprotein substrates. P-glycoprotein inhibitors that reverse P-glycoprotein-mediated efflux have been shown in a number of studies to be a potential tool for increasing oral bioavailability by increasing the effectiveness of drug transport across the epithelia [8].

In its most basic form, drug design is the creative process of creating small molecules that are complementary in shape and charge to the bimolecular target with which they interact and will therefore bind to it. A drug, also known as ligand design, is an organic small molecule that activates or inhibits the function of a biomolecule, such as a protein, resulting in a therapeutic benefit to the patient [9]. Finally, the process of developing novel drugs based on knowledge of the target biomolecule's three-dimensional structure is known as "structure-based drug design." Pharmaceutical formulation with well defined ways to address bioavailability concerns may be advantageous for excipients. Excipients are safe, do not absorb from the stomach or intestines, and are widely used in the pharmaceutical industry. As such, they have a modest history of being added to parenteral and external formulations as solubilizing and stabilizing agents [10]. Besides, in the present scenario, there is increasing development of novel drug delivery systems (NDDS) like microspheres, nanoparticles[11] and liposomes, all of which have inherent P-gp evading activity[12]. It is understood that by saturating the P-gp carrier and

reversing the P-gp efflux, the nanosystems transfer concentrated drug levels across the plasma membrane[13]. Through endocytic vesicular transport, it was shown that surfactant polymer nanoparticles overcome P-gp-mediated efflux [14]. As a result, a hybrid approach employing both the NDDS and P-gp inhibiting excipients might be a more potent P-gp inhibitor [15]. In this work, we produced a composite of carvedilol and used the direct compression technique to compress it into a tablet. Fourier transform-infrared (FTIR) spectroscopy, X-ray diffractometry, permeability investigation, and in-vitro DR were performed on the produced carvedilol composite. Based on the concentration of drug absorption by the cells, the carvedilol composite tablet demonstrates the augmentation of carvedilol's bioavailability by 28% to that of 10% in carvedilol alone. This method would therefore offer a workable way to formulate carvingilol once daily, maybe with a lower dosage.

MATERIAL AND METHOD

MATERIALS

The active carvedilol was received as gift sample from Titan laboratory, Mumbai, India. Curcumin and Piperine was obtained as a gift sample from Meron lab, Kerala, India, and all other chemicals were of analytical grade and used as provided.

METHOD

Preparation of Carvedilol composite by direct compression method

The proportions listed in Table 1 were followed in the preparation of the Carvedilol composites using the direct compression technique. Before combining, the raw materials were run through a 40 mesh filter. Other excipients were used with the medication. After ten minutes of mixing, talc and magnesium stearate were added. With an 8mm punch, a tablet compression machine was used to compress the final powder blend that had been made. The weight of the pill was changed to 200 mg [16, 17].

Table 1. Composition of Carvedilol Composites

INGREDIENTS	A1 (mg)	A2 (mg)	A3 (mg)
Carvedilol	12.5	12.5	12.5
Curcumin	0.36	1.84	3.68
Piperine	2.85	14.26	71.33
Cross Carmellose Sodium	20	20	20
Mannitol	40	40	40
Micro Crystalline Cellulose	36	36	36
Talc	2.5	2.5	2.5
Magnesium Stearate	1	1	1
Lactose	q.s	q.s	q.s

CHARACTERISATION

Fourier Transform-Infrared Spectroscopy (FTIR)

The fundamental peaks of an FTIR spectrum are indicative of the chemical makeup of the medicine and its excipients. To ascertain any potential interactions between the medication and the excipients in the finished formulation, FTIR experiments were conducted. An FTIR spectrophotometer (Jasco-4100) was used to perform the analysis. In summary, a 2 mg sample was evenly mixed with previously dried KBr at 120ºC for 30 minutes after being properly powdered. The combination was then stored in a sample container, and spectra were taken over the 400–4000 cm^{-1} wave number [18].

Differential Scanning Calorimetry

Studies utilizing differential scanning calorimetry (DSC) were conducted with the Mettle-Toledo DSC 821 apparatus. The pure medication carvedilol and the optimized batch of carvedilol composites were heated at a steady rate of 10°C/min throughout a temperature range of 25–400°C in aluminum crucibles that were hermetically sealed. The nitrogen gas was purged at a rate of 50 milliliters per minute to maintain an inert environment.

X-Ray Diffraction Studies (PXRD)

Using an X-ray diffractometer (PW 1729, Philips, Netherlands), the XRD patterns were captured. After being exposed to monochromatized Cu-Ka radiation (1.542A^), the samples were examined between 10 and 900 degrees. The applied voltage was 30 kV, while the applied current was 30 mA. The measurement of the total scattering and the scattering from the crystalline area of formulations served as the foundation for the X-ray diffraction technique, which was used to determine the degree of crystallinity in the formulation [19].

Dissolution studies

The USP dissolve Test Apparatus Type II was utilized to conduct a dissolve test on the composites from formulations (A1-A3). Phosphate buffer pH 6.8 was the in-vitro dissolving medium that was employed. The formulation batch A1-A3 mouth dissolving composites were put into 900ml of dissolution media that was kept at 37±0.5°C. The mixture was then agitated at a predetermined speed of 50 rpm. At intervals of 5, 10, 15, 30, 45, and 60 minutes, 5 ml aliquots of the dissolving medium were removed and replaced with 5 ml of new dissolving media that was maintained at 37±0.5°C. A UV-visible double beam spectrophotometer was used to filter the extracted materials via 0.45 micron millipore filters, dilute them, and then measure their absorbance at 241 and 420 nm [20].

Selection of optimized formula

Optimized formulation was selected by comparing in-vitro drug release study of formulation A1 to A3. The selected formulation was used for further processing.

Drug Permeation studies of formulation

The Franz Diffusion Model was used to conduct the In-vitro Dissolution research of the carvedilol composites. Phosphate Buffer pH 6.8 was the in vitro dissolving medium that was utilized. The oral dissolving composites from Formulation batch (A3) were added to 60 milliliters of dissolution media that was kept at 37±0.5°C. The mixture was then agitated at a specific speed of 50 revolutions per minute. Five milliliter aliquots of dissolution medium were taken out of one end of the Franz Diffusion Model at intervals of five, ten, fifteen, thirty, forty, and sixty minutes. These were then replaced with five milliliters of brand-new dissolution medium that was maintained at 37±0.5°C. A UV-visible double beam spectrophotometer was used to filter the extracted materials via 0.45 micron millipore filters, dilute them, and measure the results at 241 nm [21].

Molecular Docking Study

In order to estimate the affinity and activity of a small molecule, docking is often utilized to predict the binding orientation of small molecule therapeutic candidates to their protein targets. Docking is therefore crucial to the logical design of pharmaceuticals. Considerable attention has been focused on refining docking prediction techniques due to the biological and medicinal importance of molecular docking. In this work, we've tried to dock piperine and curcumin molecules with the P-gp protein in order to provide a computer-generated picture that would corroborate the data pertaining to P-gp's interaction with piperine and curcumin [22-24].

Pharmacokinetic Evaluation using In-vitro Models

The pharmacokinetic evaluation of drug has been correlated with cell transportability of the carvedilol drug composites and pure carvedilol in human colonic adreno carcinoma cell line (Caco-2).

Cell Viability Studies

The cells were cultured in plastic cell culture flasks using Dulbecco's Modified Eagle's Medium, which was supplemented with 3,7 g/l NaHCO3, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% (v/v) non-essential amino acids solution, 1% (v/v) l-glutamine, and 730 nM (about 0.4 μg/ml) of the antibacterial puromycin (3′-[α-Amino-p-methoxy hydro cinnamamido]-3′-deoxy-N, N-dimethyladenosine dihydrochloride) at 37ºC in a 5% CO2 atmosphere. After three days of incubation, trypsinized Caco-2 cells were planted at a density of 5 × 105 cells in 75 cm2 flasks with complete media, covering 80–90% of the flask. Passaging of the cells was done on a regular basis. Once Caco-2 cells attain a high cell density of >0.5 x 105 cells/cm2, they are passaged into 24-well cell HTS plates including 0.4 μm Polycarbonate membrane inserts after 5 to 6 days. Cells were employed in assays involving cytotoxicity and transport between passage numbers 20 and 40. Following seeding at a density of either 225000 or 600000 cells/cm2, the cells were grown in puromycin-containing media for six days. Every other day, the culture media was refreshed [25-28].

RESULTS AND DISCUSSION

FTIR analysis

An overlain spectrum of pure carvedilol, curcumin, piperine and optimized formulation of carvedilol is shown in Figure 1. The spectrum of carvedilol shows spectrum at 3425 cm^{-1} (N-H), 3002 cm^{-1} (C-H, Sp 2), and 2836 cm^{-1} (C-H stretching vibrations). The spectrum of piperine shows 1580 cm-1(-CO-NH2), also indicates the presence of aromatic and aliphatic 2957, 2885 cm-1 (C–H), 2902 (NCH2), 1499 (C=O), 1479 (aromatic C=C). Spectrum of curcumin shows characteristic peaks at 3001 cm^{-1} (O-H stretching), 1714 cm^{-1}(C=O ring), 1242 cm^{-1} (C-O), and 1098, (C-O-C stretching). The optimized batch formulation showed important bands at 2943 cm^{-1} (C H),1643 cm^{-1} (C=O), 3291 cm^{-1} (-OH) and 1584 cm^{-1} (R-NH). According to the IR overlain spectra, the peaks of pure drug carvedilol were retained in the peaks of the optimized formulation. Hence, pure drug does not react chemically with excipients present in the formulation, thus compatible with excipients resulting into stable formulation hence it does not indicate any chemical interaction.

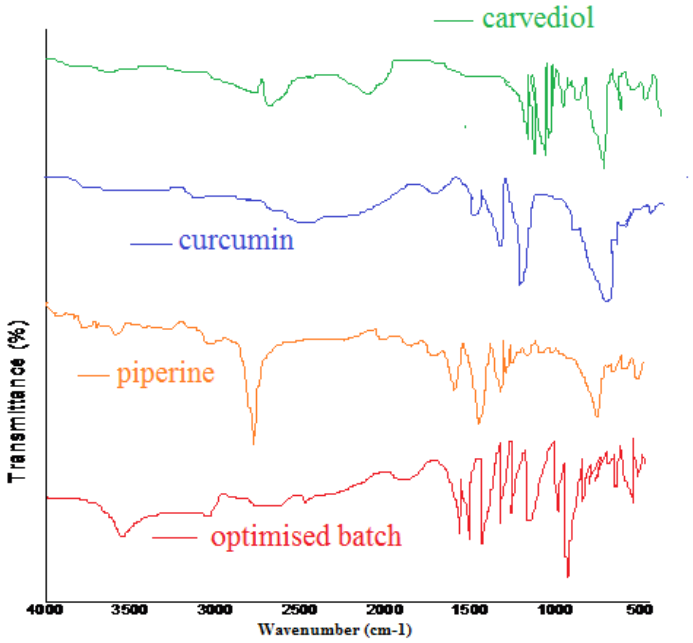


Figure 1. Spectrum of Carvedilol, Piperine, Curcumin and Optimized Batch A3

Differential Scanning Calorimetry

DSC thermograms of carvedilol showed sharp endothermic peaks at 115.21ºC. A sharp endothermic peak at 186ºC was observed in the thermogram of Curcumin. While the formulation of carvedilol did not showed any melting point at 115.21ºC, which reveals drug is in amorphous state. The absence of thermal event of drug indicates possible hydrogen binding resulting in interaction of excipients and carvedilol (Figure 2).

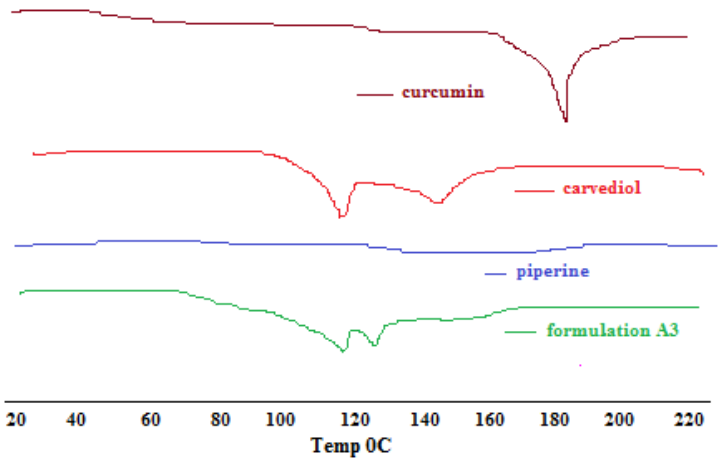


Figure 2. DSC pattern of Pure Carvedilol and Formulation A3

X- ray Diffraction study

X- ray diffraction spectra of pure carvedilol and optimized batch of formulation was given in Figure 3. Pure Carvedilol was in crystalline state as it showed sharp distinct peaks notably at 2θ diffraction angles of 15.54°, 17.23°, 21.38°, 23.76°and 26.05°. While the optimized batch of the drug carvedilol do not show any dominant peak which indicates the amorphous nature of the formulation. It was thus concluded that the crystallinity of drug was reduced due to interaction between the drugs and the excipients resulting in development of amorphous nature of the drug.

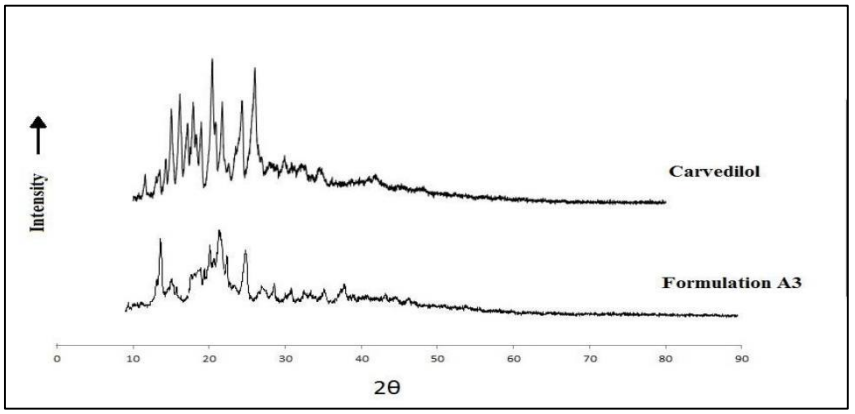


Figure 3. X-ray Diffraction Study of Carvedilol and Formulation A3

In- vitro Dissolution studies

The In-vitro drug release of pure piperine, curcumin and marketed formulation were about 79.83 %, 76.20 % and 77.25 respectively, at the of 60 minutes duration. The % drug release of formulations A1, A2 and A3 were 85.41 %, 86.79 % and 89.95 % respectively at the end of 60 mins (**Figure 4**).

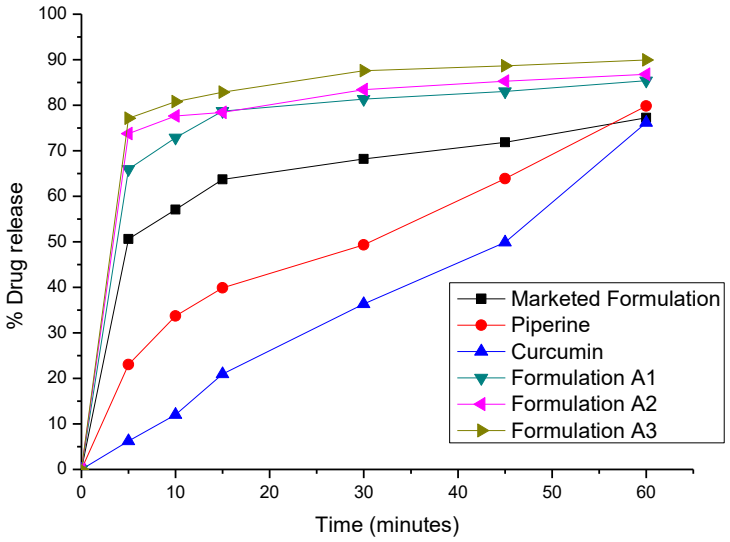


Figure 4. In-vitro Drug Release

Drug Permeation studies

In-vitro permeation studies of optimized formulation batch A3 are given in figure 5. The % drug permeation of the composites of optimized formulation batch A3 was about 64.27% at the end of 60 mins.

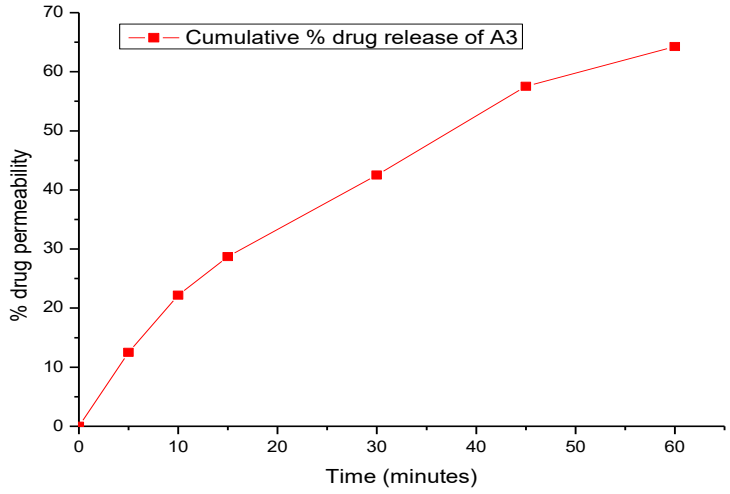


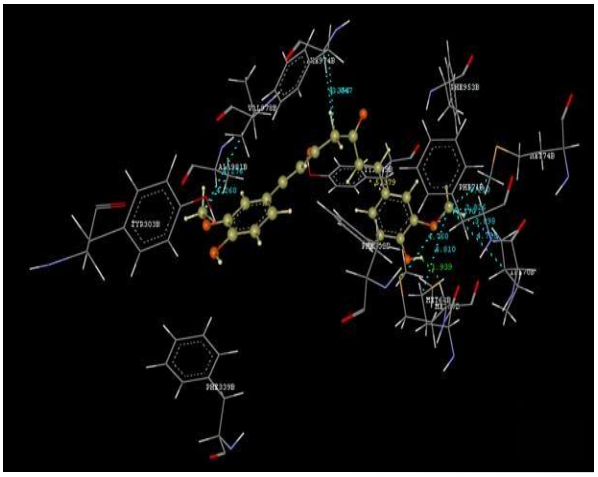
Figure 5. Plot of Cumulative % Permeation of Optimized Composites

Docking studies

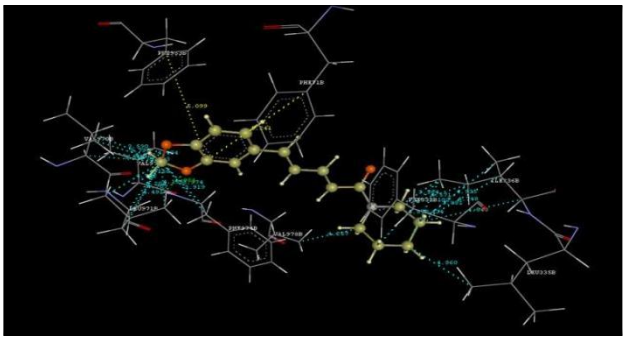
Molecular Docking Study of Carvedilol

Docking study was carried with an objective to study the interactions of P-gp amino acid residues to P-gp inhibitors so Docking study was initiated for Curcumin and Piperine molecule with Nucleotide Binding Domain (NBD) of P-gp protein. The docking score was calculated based on the interaction studies and distance calculated with binding strategies and expressed as in form of wanderwalls interaction, hydrogen bond and hydrophobic interactions. Thus docking score of curcumin

and piperine was found to be -45.73 and -40.08 respectively. The crystal structure of P-gp protein was taken from RSCB-protein data bank and formed as 2HYD (PDB id).



(A)



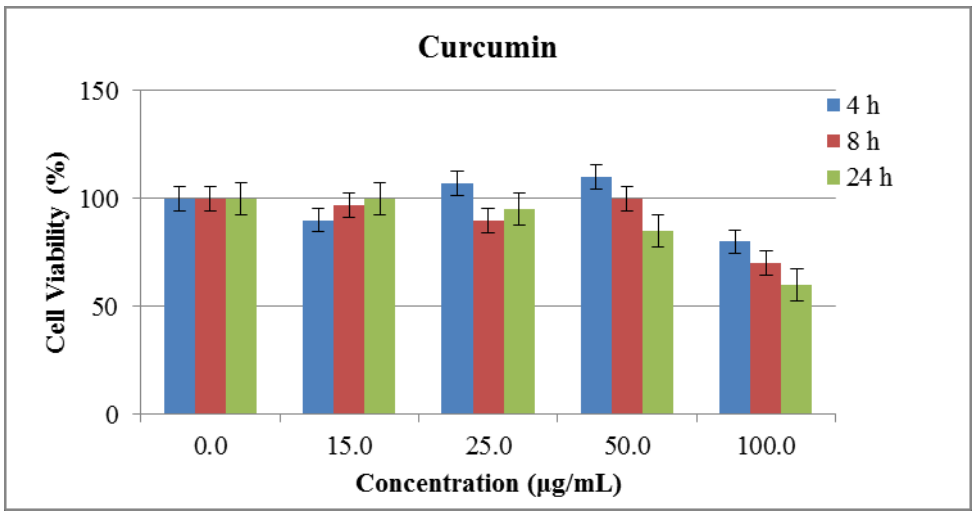
(B)

Figure 6. Docking study of (a) Curcumin (b) Piperine with P-gp

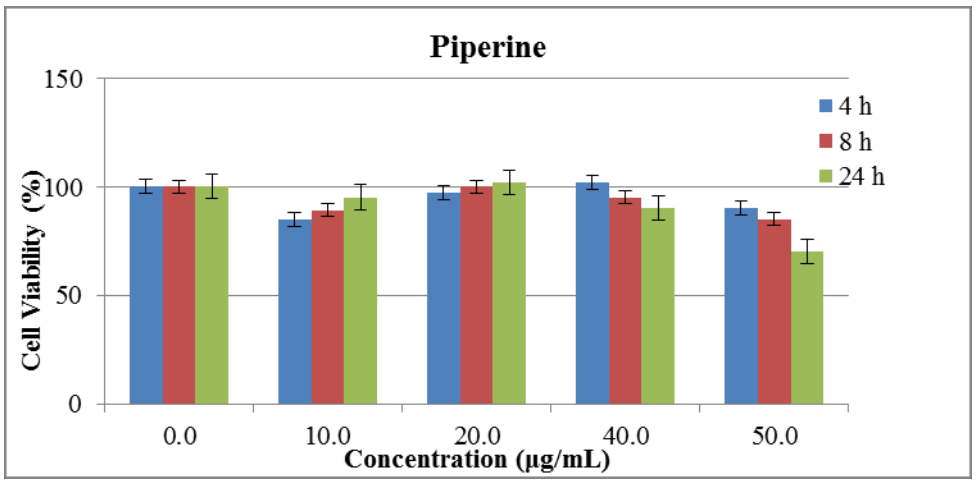
Pharmacokinetic Evaluation using In-vitro Models

Cell Viability (Modified MTT assay)

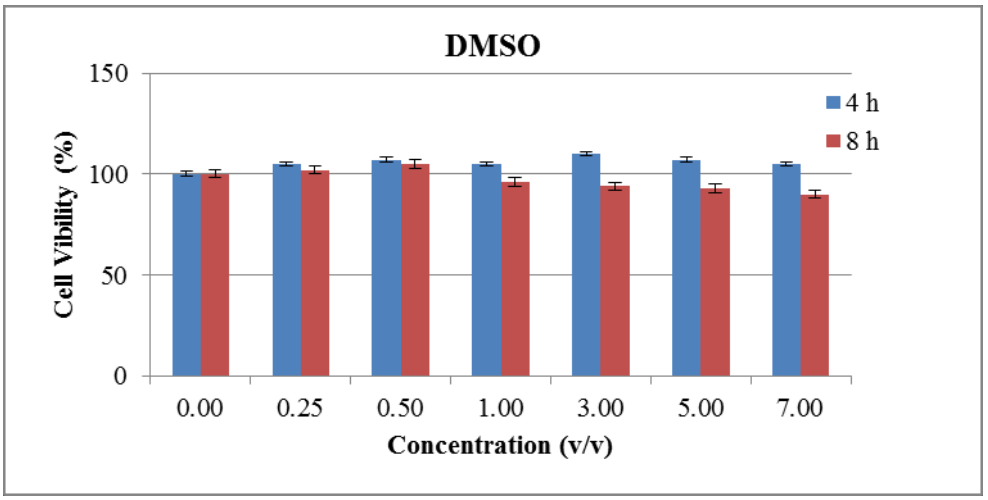
The cell viability study was carried with an objective to optimize the concentration of P- gp inhibitors required to remain the cells viable for the further study. The modified MTT assay was done for cell viability study. The modified MTT assay showed the concentration of curcumin, piperine and DMSO required to remain the cell viable by the amount of reduced form of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to be present inside the cell in color form. Results are expressed as percentages of 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide absorbance with respect to the untreated vehicle control wells (Figure 7).



(A)



(B)



(C)

Figure 7. Effect of a) Curcumin, b) Piperine and c) Dimethyl sulfoxide (DMSO) on cell viabilities of Caco-2 cells

After 4, 8, or 24 hours of treatment with increasing doses of the compounds, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test was used to assess the viability of the cells. The percentages of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide absorbance in relation to the vehicle control wells that were not treated are used to express the results.

Conclusion

The present work attempt to improve pharmacokinetic pattern of low bioavailable drug candidate by inhibiting P-glycoprotein (P-gp) with the help of P-gp inhibitors curcumin and piperine. Chemical compatibility of the three components together has been confirmed by carrying out DSC and FTIR studies. The formulation containing the three components was characterized to estimate any incompatibility present in it by carrying out FTIR, DSC, and PXRD studies based upon which it was confirmed that the made formulation was chemically sound product. The PXRD graph of formulation when compared to pure drug showed peaks of low intensity which confirmed the stability of the formulation thus enhancing its solubility in the systemic circulation. Docking studies have confirmed the interaction of the two P-gp inhibitors with the targeted protein P-gp and have shown various Hydrophobic interactions, Vander-wall interactions and Hydrogen bond interactions which supports the strong binding of piperine and curcumin with the P-gp protein. In-vitro testing studies such as activity of Carvedilol composites over Caco-2 cell line culture, have confirmed the enhancement of bioavailability of Carvedilol by 28% to that of 10% in Carvedilol alone, based on the concentration of drug uptake by the cells. Through this approach, this work would thus provide a viable means for a once daily formulation of Carvedilol possibly with reduced dosing.

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