"FORMULATION, IN VITRO-IN SILICO EVALUATION AND DEVELOPMENT OF PHARMACOKINETIC MODELS FOR SUSTAINED RELEASE FORMULATIONS OF ANTIEMETIC DRUGS"

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1.0 Introduction:

Chemotherapy induced nausea and vomiting (CINV) is one of the main side effects of cancer therapy [1]. CINV result in serious metabolic disturbances, nutritional depletion and anorexia, deterioration of the patient's physical and mental status, esophageal damage and ultimately patient withdrawal from therapy [2]. Almost all patients receiving chemotherapy experience CINV even after optimization of antiemetic treatments. Functional Living Index-Emesis (FLIE) is a matrix which assesses quality of life of cancer patients, suggest that CINV has intense negative effects on quality of life of patients [3]. There is also a substantial financial burden associated with CINV due to the ever-increasing costs of antiemetic medications. Some of the examples include, intravenous palonosetron and Fosaprepitant costing $188.70 and $262.65 per dose respectively [4]. One retrospective cohort study carried out in 19,139 patients calculated the mean costs of CINV visits, including inpatient, outpatient, and emergency room visits to be $5299 for the first chemotherapy cycle (a period up to 30 days) and mean per-patient CINV-associated costs to be $731 [5]. For some patients, the cost of managing CINV is greater than the cost of chemotherapy [6].

CINV occurs in two phases; acute phase and delayed phase. Acute CINV occurs within 1–2 h of chemotherapy administration and can last for up to 24 h while delayed CINV occurs more than 24 h after chemotherapy administration [7]. The CINV process involves a complex interplay between neurotransmitters and receptors at various anatomical regions [8]. The three main neuro transmitters and receptors involved in the regulation of nausea and vomiting are serotonin (5-HT) associated with 5-hydroxytryptamine (5-HT3) receptor, substance P (SP) associated with neurokinin-1 (NK-1) receptor, and dopamine associated with dopamine (D2) receptor [9]. Because the onset of acute and delayed CINV often overlap after the initial day of chemotherapy, it remains a challenge to determine an appropriate antiemetic regimen, as patients may require alternate treatment regimens. The MASCC and ASCO guidelines recommend a 5-HT3 receptor antagonist plus dexamethasone for acute CINV and dexamethasone for delayed CINV among patients receiving multiple-day cisplatin. However, this regimen is marginally effective in controlling acute CINV and is less successful in controlling delayed CINV [10].

Two drugs Amisulpride (acting on dopamine D2 receptor) and Granisetron (acting on 5-hydroxytryptamine 5-HT3 receptor) were chosen with objective that by acting on different

receptors they can increase the efficacy against emetic episodes and prevent development of treatment tolerance.

Amisulpride:

Amisulpride is a benzamide class atypical antipsychotic drug and a potent, selective dopamine D2 and D3 receptor antagonist and is approved from 1980s for psychosis treatment [11]. It has a wide safety profile at doses of 400– 800 mg/day [11]. Amisulpride is also approved for prevention of PONV [12]. In a pilot investigational study, ondansetron and intravenous dose of amisulpride administered in combination resulted in preventing emesis in 83% of patients [13]. In a clinical trial, oral amisulpride at a dose of 10 mg daily was found to be safe and superior to placebo [14]. The complete response (CR) rate in the delayed phase was 46% with 10 mg amisulpride, compared to 20% with placebo [14]. United States Food and drug administration (USFDA) has approved Amisulpride intravenous injection (5mg/10mg) for treatment of post-operative nausea and vomiting in Feb 2020 [15]. However, there is no long-acting formulation available for Amisulpride.

Granisetron:

Granisetron hydrochloride is an indazole class antiemetic drug and 5- HT3 receptor antagonist [16]. Clinical trials conducted on granisetron suggest the superiority of granisetron over other drugs from similar class in preventing delayed phase of emesis that appear after 24 h [17]. The effect is due to its ability to supress the activity of the vagus nerve connecting the vomiting centre in the medulla oblongata [1].

2.0 Hypothesis and research statement:

The treatment options which address both acute and delayed phases of emesis occurring during chemotherapy are very limited and less effective. The marketed formulations available are SUSTOL (Granisetron SC Injection) and SACUSO (Granisetron transdermal patch) for once a weekly treatment [18,19]. Currently no generic is available for both the drugs due to patent protection and proprietary polymer technology. However, these individual formulations still require combination with other antiemetics for effective treatment [19]. Thus, additional research is necessary to optimize management strategies for multiple-day chemotherapy.

The available anti-emetic dosage regimen recommends combination of two or three antiemetic agents to control the delayed emesis in moderate and high emetogenic chemotherapy treatment [20]. There is need of formulations which provide effective treatment and patient compliance. This can be achieved by designing long-acting dosage forms with combination of two drugs,

which provide sustained drug release up to one week. Comparative *in-vivo* studies in human are required for approval of generic or branded product which are costly and time consuming. Prediction of pharmacokinetics in human from *in-vitro* and *in-silico* studies is facilitated by regulatory agencies through modelling and simulation approach to reduce cost and create platform for future research. Currently there is a need of *in-silico* pharmacokinetic models which can mechanistically link *in-vitro* and pharmacokinetic properties to predict *in-vivo* performance of antiemetic drugs.

Polypharmacology is "the design or use of pharmaceutical agents that act on multiple targets or disease pathways" as defined by the American National Library of Medicine (NLM) [21]. It is emerging science where treatment of complex and incurable diseases can be sought with use of multiple drug therapies [21,22]. It combines basic concepts from systems biology to understand the reasons for disease emergence and finding the effective treatment options. In general, polypharmacology address both drug combinations and drugs acting on multiple targets at the same time [23]. The problem of combining multiple drugs is that one needs to take care of their pharmacokinetic and pharmacodynamic properties while designing suitable dosage forms [24]. One of the strategies is to load two or more drugs acting on different receptors onto a single drug-delivery system to deliver drugs at the site of action.

Simultaneous drug delivery through multiple drug loading based on polymeric depot systems can be good strategy to achieve enhanced efficacy [25]. Using advanced drug delivery systems and use of physical pharmacy principles, dual-drug delivery form complex nano or microsystems is achievable [25]. To achieve maximum drug effect, drugs with different properties and mechanism of action should be used at their suitable dose and regimens in the treatment [26].

In-silico methods such as Allometry and Wajima predicts human pharmacokinetics from preclinical species data and help in selection of first-in-human doses. Allometric scaling uses sameness in anatomy, physiology, and physiological parameters and is the most predominantly used method to calculate the plasma profile in human from animal data [27]. Wajima et al. method also known as superimposition method (named the Css-MRT approach), adopts normalizing the time with MRT and the plasma concentration with Css [28].

In-silico models which can integrate the formulation and process attributes with physiological parameters to establish link between *in-vitro* drug release and *in-vivo* drug release using mechanistic understanding are essential to optimize the formulation and reduce the commercial problems that usually arise in complex products [29, 30]. In-silico biopharmaceutics models

consider factors that can critically impact the product performance. They incorporate mechanistic elements such as particle size and/or drug release and establish their impact on ADME of drug and formulation [30]. After fixing these elements, PBBM modelling can be used to study the impact on critical material attributes (CMAs) and critical process parameters (CPPs) to establish a safe space via either *In-vitro in -vivo* correlation (IVIVC) or relationship (IVIVR) along with virtual Bioequivalence (VBE) simulations. This approach will facilitate the establishment of biorelevant tests from starting phase of development to product management and removing the costly *in-vivo* BE studies, leading to overall cost reduction [30].

3.0 Aim and Objectives:

The aim of this research was to design and develop once-weekly long-acting formulations and pharmacokinetic models using *in-vitro in-silico* tools.

The objectives of present work were:

- ✓ To develop *in-silico* pharmacokinetic model which will guide in the design and development of sustained release formulations and predict *in-vivo* performance.
- ✓ Development of formulations which will provide sustained drug release over a period of one week.
- ✓ To predict the *in-vivo* pharmacokinetics in humans using *in-vitro* data and the developed *in-silico* model.

4.0 Research methodology:

4.1 In-silico Model development:

The in-silico modelling was performed to develop model for prediction of *in-vivo* pharmacokinetics in humans from animal species. The allometric scaling predicted volume of distribution and clearance values for human from preclinical species. The predicted values were within 2-fold error from the observed data for finalized model. *In-vivo* pharmacokinetic profiles for human were predicted using Wajima and Dedrick approach. Out of the two approaches, Wajima approach was more suitable in predicting the human PK data from preclinical species.

The PBPK model was developed for Amisulpride and Granisetron using Gastroplus^{TM} version 9.8.2 from simulations plus. The developed model was validated using intravenous and extravascular data. The developed model was used further to predict *in-vivo* pharmacokinetics of optimized formulation after intramuscular/subcutaneous administrations. The model was tested for parameter sensitivity analysis which showed the impact of physiological parameters

such as lipophilicity, fraction bound to plasma proteins and drug release models, which helped to validate the model further.

Parameter sensitivity analysis for amisulpride showed that fraction unbound and blood to plasma ratio inversely impacted both Cmax and AUC. However, log D value did not show any impact on both Cmax and AUC value. Drug release modelling parameters such as weibull shape and weibull maximum release have direct relationship with both Cmax and AUC, while Weibull scale showed inverse relationship for both Cmax and AUC.

Parameter sensitivity analysis for granisetron showed that fraction unbound and blood to plasma ratio inversely impacted both Cmax and AUC. Log D value showed inverse impact on Cmax and positive impact on AUC value after around 40% of fraction unbound. No effect of permeability was seen on both parameters. Drug release modelling parameters such as Weibull shape 1 and 2 and Weibull maximum release have direct relationship with Cmax, while Weibull scale showed inverse relationship for Cmax. Fraction dissolved in Weibull 1 showed inverse relationship with Cmax, while it showed direct relationship with AUC after 70% release. Weibull maximum release have direct relationship with AUC, while all other parameters showed no significant impact on both parameters.

This exercise helped to optimize the parameters to achieve desired sustained release profile for both Amisulpride and Granisetron.

The accuracy of PBPK model were validated by the fold error of each time point (FEi), average fold error (AFE) and absolute average fold error (AAFE) for each time point of the plasma concentration profile. The FEi values for all the time points were well within the limit of 0.3 to 3-fold error. The AFE and AAFE values were also found within the limit of 0.5 to 2-fold error.

The target steady state levels were calculated from in-silico model built using Gastroplus after multiple dose simulation of individual drugs. For Amisulpride, the Cminss and Cmaxss levels are 0.47 ng/ml and 5.79 ng/ml after 5 mg (once a day) multiple dosing. The Cminss and Cmaxss levels are 0.941 ng/ml and 11.591 ng/ml after 10 mg (once a day) multiple dosing. In the reported clinical trial [31], 10 mg dose is selected, hence we have finalized 1ng/ml as target Cminss level and 11 ng/ml as target Cmaxss level for development of sustained release formulations of amisulpride.

For Granisetron, the Cminss and Cmaxss levels are 0.655 ng/ml and 5.378 ng/ml after 1 mg (twice a day) multiple dosing. The Cminss and Cmaxss levels are 1.054 ng/ml and 6.636 ng/ml after 2 mg (once a day) multiple dosing. In the package insert data of Granisetron patch (Sancuso 52 cm^2), Cavass level of 2.2 ng/mL over six days is reported [32]. Considering all

the data, we have finalized 1ng/ml as target Cminss level and 6.5 ng/ml as target Cmaxss level for development of sustained release formulations of Granisetron.

After considering the reported literature, pharmacokinetic parameters and calculated steady state plasma concentration levels, the desired dose for Amisulpride was found to be 30 mg and for granisetron it was found to be 10 mg. For combination product, the granisetron dose to be kept 10 mg and amisulpride dose can be varied from 10-30 mg based on the clinical response.

The dissolution models were used to predict the target drug release profile based on first order kinetics and using complex microsphere model in the DDDplus platform. The calculated theoretical release profiles helped in model building and providing the target drug release profile which the optimized formulations should achieve.

4.2 Pre-formulation studies:

Preformulation studies were performed to confirm the identity of drug and excipients using melting point analysis, Fourier transformed infrared spectroscopy analysis and thermal analysis using differential scanning colorimetry.

Amisulpride showed melting point of 128°C and that of Granisetron showed melting point of 301°C, which were in-line with the reported values. Amisulpride showed λmax at 226.5 nm and Granisetron showed λmax at 302 nm which was in-line with the reported values [33,34].

Amisulpride showed characteristic peaks at 3312, 3215, 1649 and 1056 cm^{-1}, Granisetron showed characteristic peaks at 3231, 1646 and 1549 cm^{-1}. PLGA showed characteristic peaks at 1749, 1452, 1180 and 1088 cm^{-1}, PCL showed characteristic peaks at 1723, 1364, 1293 and 1239 cm^{-1} while PVA showed characteristic peaks at 3280, 1716, 1432, 1324 and 1093 cm^{-1}. All the observed spectra matched with the reported spectra and peak values [35-39].

Amisulpride shows sharp endothermic peak at 128.8 °C and Granisetron shows endothermic peak at 301.36°C which confirms the identification of both the APIs [40,41]. PLGA being 100% amorphous polymer shows glass transition temperature (Tg) at 46.07°C [42]. PCL is a semi crystalline polymer, so its thermogram presents an endothermic peak at 61.87°C [43].

4.3 Analytical methods:

Analytical methods using UV-visible spectrophotometer and High-performance liquid chromatography were developed for charactering drug loading and entrapment efficacy and *in-vitro* drug release form the formulations. These methods were validated for linearity, robustness, sensitivity, precision, accuracy and specificity.

UV spectrophotometric method in 0.1N HCl was adopted for estimation of Amisulpride and granisetron in formulations [44,45]. 1 to 5 µg/mL concentrations of amisulpride solution were prepared from amisulpride stock solution (100 µg/mL) by transferring 0.1 ml to 0.5 ml to 10 ml of volumetric flasks and volume was made up to 10 ml using 0.1N HCl. 2 to 10 µg/mL concentrations of granisetron solutions were prepared from granisetron stock solution (100 µg/mL) by transferring 0.2 ml to 1 ml to 10 ml of volumetric flasks and volume was made up to 10 ml using 0.1N HCl.

Simultaneous estimation method was developed for both the drugs in pure and microsphere dosage form in by UV Visible spectrophotometer using simultaneous equation method [46].

High-Performance Liquid Chromatograph (HPLC) method was developed for simultaneous estimation of Amisulpride and Granisetron in gradient mode. Symmetry Shield RP-18 (150 X 4.6, 3.5 micron) column was used with flow rate of 1.0 mL/min. Injection volume of 20 µl was sued. Amisulpride showed retention time of 10-12 min, while granisetron showed retention time of 14-16 min. HPLC system from waters was used.

The LOD and LOQ values suggest the sensitivity of method, as the values were below the concentration range selected for calibration at initial as well as after 24 hours. The precision results were within the acceptable range (< 2%) and thus suggest that the developed method is precise over the selected time interval [47]. The mean % recovery values near to 100% with % RSD ≤ 1% suggested high accuracy of the developed methods [47]. There were no overlapping or extra peaks observed in excipient mixtures at selected analytical wavelengths which confirmed the specificity of the methods [47]. The developed methods were found to be suitable for analysing the formulations.

4.4 Formulation development:

A novel dual-drug loaded microspheres (also coined as Janus microspheres) of Amisulpride and Granisetron were prepared using double emulsion-solvent evaporation technique which release the drug over a period of once week and provide effective treatment for both acute and delayed emesis. Granisetron being water soluble drug was incorporated in the inner phase and amisulpride being water insoluble drug was incorporated along with oil/polymer-solvent phase. Most commonly used and efficient polymers such as Polylactic-co-glycolic acid (PLGA 50:50) and Polycaprolactone (PCL) were used for developing dual-drug loaded microspheres.

Quality by design approach was chosen to develop and optimize the formulations. Quality target product profile (QTPP) and critical quality attributes (CQAs) were identified and risk assessment was performed.

Initial risk for API was kept low as only one source API was used. Risks for PLGA and PCL levels for Particle size were kept medium, while for PVA level risk was kept high. Risks for PLGA and PCL levels for % drug loading, % entrapment efficiency and % Drug release were kept high, while for PVA level risk was kept medium for % drug loading, % entrapment efficiency and low for % Drug release. Risks for stirring speed and time of W/O emulsion were kept low, based on initial feasibility trials and domain knowledge. The stirring speed and time (W/O) in the initial feasibility trials was not found to be affecting drug loading and entrapment efficiency, hence it was decided not to evaluate this parameter further. Stirring speed and time of W/O/W emulsion for Particle size was kept high, medium for % drug loading and % entrapment efficiency and low for % Drug release. The impact of stirring time on drug release is not directly linked but rather it is governed through particle size and drug loading/entrapment efficiency. Hence the risk is considered low.

A definitive screening design was used to identify the most critical parameters affecting the formulation and process followed by response surface design which provided the best combination of parameters to achieve the desired QTPP.

From screening design, it was found that stirring speed was the most impacting parameter for particles size (D90 in microns). % Drug loading and % Entrapment efficiency of amisulpride and granisetron was significantly affected by PCL and PLGA levels respectively. Stirring time and PVA concentrations did not significantly impacted any of the selected responses. Based on the screening design outcome, the stirring speed of 800 rpm, stirring time of 8 hours, PLGA and PCL concentration of 250 mg and PVA concentration of 0.5% was finalized.

Further, the ratio of drug: polymer and polymer: polymer (PLGA: PCL) was selected in the optimization of formulation using response surface design. It was observed that, drug: polymer ratio was impacting particle size and polymer: polymer ratio was impacting drug loading, entrapment efficiency and drug release from the formulations.

Prediction intervals for optimized formulation parameters were calculated for particle size D90 (84–100 micron), % drug loading (Amisulpride: 13-16% & Granisetron: 5.2-6.4 %), % entrapment efficiency (Amisulpride & Granisetron: 53-62 %) and % *in-vitro* drug release profile (Amisulpride: 40-50% and Granisetron: 30-40% at 4 hours in ACC method).

The selected PLGA, PCL and PVA levels were able to provide desired CQAs within the proposed ranges. The selected process parameters like stirring speed and stirring time were optimized and fixed to ensure consistent results throughout the development cycle. The risks for formulation variables and process parameters were updated as low based on the above development.

Design space was generated which showed that with Polymer to polymer ratio from 0.3 to 1.4 and Drug to polymer ratio from 0.15 to 0.4, the desired CQAs can be achieved.

4.5 Formulation evaluation:

The optimized microspheres were characterized in terms of % yield, FTIR analysis, thermal analysis, particle size, morphology, % drug loading, % encapsulation efficiency and *in-vitro* drug release testing.

Percentage yield was found to be 80 ± 10 % for the optimized formulations. Formulation spectra showed the presence of only polymer (PLGA and PCL) peaks 1749,1455,1382,1270,1179,1129,1088, 866 and 750 cm^{-1} [48,49] suggesting complete encapsulation of drugs within the polymers at molecular level. The absence of drug peaks (Amisulpride at 128.8°C and Granisetron at 301.36°C) [50,51] and presence of only polymer (PLGA and PCL) peaks [52,53] confirms the complete encapsulation within the polymer. The formulation thermogram showed the fusion peak of the PCL and PLGA at 61.16°C [53]. The small shoulder peak corresponds to the PLGA glass transition temperature. The same phenomenon is also observed in placebo where the fusion peak and shoulder peak appeared at 65.45°C.

The particle size analysis was performed using Malvern 3000 (Malvern Instrument, Worcestershire, United Kingdom). Optimized formulation showed particle size of around 100 ± 20 microns which are suitable for intramuscular/subcutaneous injection. The particle size and morphology were studied using optical microscopy and scanning electron microscopy. It was

observed that, during preparation, dual drug loaded Janus microspheres have typical handbag like structure [54]. When the W/O emulsion was added to external water phase containing 0.5% PVA, the formation of handbag like structure started appearing (Nascent stage). After drying, the particles appeared in more spherical shape. SEM images showed the formation of Janus microspheres with uniform particle size and morphology. The unique shape can be clearly visible in isolated microsphere, where two compartments can be visibly observed.

The % drug loading and % entrapment efficiency for Amisulpride was found to be 14.08% and 65.69 % respectively and for Granisetron was found to be 4.76% and 66.67% respectively in the optimized formulation. Hausner's ratio for optimized formulation was found to be 1.03 ± 0.2, which indicates excellent flow properties [55].

Three types of *in-vitro* drug release methods were developed in phosphate buffer pH 7.4 to gain understanding of mechanism of drug release under different conditions. The Accelerated method (at temp of 50°C), the real time method (at temperature of 37°C) and gel diffusion method which mimic the bio relevancy [56-60].

The drug release using ACC method showed that, around 40-50% drug was released at 4-hour and almost complete release was observed at 24-hour. The drug release using RT method showed that, around 40-50% drug was released at 24-hour and almost complete release was observed at 168-hour. This showed that, the drug release at 4 hour and 24 hours in ACC method is correlating with drug release at 24 hour and 168 hours in real time method.

The accelerated method helped to understand drug release behaviour in short period of time and avoided waiting for results till real time analysis. Drug release in the gel diffusion method was slow compared to ACC and real time method due to the presence of gel mimicking muscle or subcutaneous tissues.

Data of drug release were fitted in zero order, first order, higuchi, Korsmeyer–Peppas, Hixon-Crowell and Weibull models to determine release kinetic pattern from Janus microspheres. The drug release of Amisulpride was best fitted with Korsmeyer-Peppas equation with R^2 close to 0.9. The release of Granisetron was best fitted to first order with R^2 close to 0.9. Weibull model fitting was performed for optimized formulations for *in-vivo* simulations.

The optimized formulations were tested for physico-chemical stability at storage conditions of 2-8°C for 6 months and 25°C/60% RH for 3 months in stoppered and sealed 10 ml clear colourless tubular USP-type I glass vials. The stability study was performed to evaluate the impact of storage conditions on assay and particle size of formulations. The results indicate

that formulations were stable up to 6 months at 2-8°C and 3 months at 25°C/60% RH. No significant changes were observed for the tested parameters.

Hemolysis potential of developed formulations was evaluated. The % haemolysis for optimized formulation was found to be 1.3 % ± 0.05 %. The results demonstrated no significant haemolysis potential of optimized formulation as the % haemolysis was found to be less than 2 [61].

Developed PBPK model using intravenous data and verified with single and multiple dose oral formulations data was used to predict the *in-vivo* performance of sustained release once weekly formulations.

Initially, the drug release data from calculated dissolution models was used to build preliminary PBPK model for sustained release formulations after intramuscular administrations. This was required to set physiological parameters which account for behaviour of drug after intramuscular administration. After initial model refinement, actual drug release data was used to predict the *in-vivo* pharmacokinetics of sustained release formulations. The optimized once a weekly formulation showed comparable *in-vivo* profile to that of multiple dose (MDD) profile. Virtual bioequivalence (VBE) studies were conducted in healthy subjects between optimized test formulation and reference multiple dose formulation to assess the *in-vivo* pharmacokinetic bioequivalence. The VBE results confirmed that the developed once a weekly formulation achieved similar rate (Cmax) and extent (AUC) as that of immediate release multiple dose formulations and both developed SR formulation and multiple dose IR formulations are bioequivalent to each other. Thus, the same efficacy was achieved by the developed sustained release formulation as that of multiple dose immediate release formulation by reducing frequent fluctuations that arise due to repeated dosing. This formulation also provided patient compliance due to once weekly administration.

In-vitro in-vivo correlation was developed for slow, medium and fast release formulations. Formulations with different drug: polymer ratio resulted in different release rates, which were used in developing the IVIVC. Advanced compartmental and transit (ACAT) model was used to mechanistically predict the *in-vivo* drug release profiles of three different release rate formulations. The interpolation function was used to build the correlation. Power correlation function was found best suitable with highest R2 value. The convolution results of all three formulations showed, both individual and mean Absolute Percent Prediction Errors well within the limits of NMT 15% and 10% respectively.

5.0 Key findings:

Dual-drug loaded polymeric Janus microsphere formulations were successfully developed using double emulsion solvent evaporation method. Use of PLGA and PCL polymers ensured the maximum % drug loading and % entrapment efficiency and particle size suitable for intramuscular/subcutaneous administration. Preformulation studies confirmed the identity of drug and excipients. Analytical methods using UV-visible spectrophotometer and High-performance liquid chromatography were developed and validated for linearity, robustness, sensitivity, precision, accuracy and specificity. The developed methods were found suitable for analysing the formulations. The optimized microspheres data was satisfactory from CMC perspective and were stable up to 6 months at 2-8°C and 3 months at 25°C/60% RH. The IVRT data was further used in the *in-silico* PBPK model to predict the *in-vivo* pharmacokinetics of developed formulations. The *in-silico* predictions showed that, developed once a weekly formulation is able to provide sustained release profile up-to 5-6 days. This was further proved by running virtual bioequivalence studies between multiple dose formulation and optimized sustained release formulation. The virtual BE Study showed that Cmax and AUC levels were within 80-125% limits and proved the equivalency. Developed IVIVC was successful in establishing safe space for bioequivalent formulations.

6.0 Conclusions:

Novel drug delivery system in the form of dual-drug loaded polymeric Janus microspheres were successfully developed which can provide sustained drug release up to one week for effective treatment of both phases of chemotherapy induced nausea and vomiting. The combination of both drugs acting on different receptors enhanced the efficacy of the treatment and patient compliance by reducing frequency of dosage administration. Combined use of *in-vitro* and in-silico tools for design and development of formulations was utilized successfully. In conclusion, the present work meets the set objectives and

7.0 Future Recommendations:

The present investigation demonstrated design and development of novel drug delivery systems for the treatment of chemotherapy induced nausea and vomiting using in-vitro and in-silico tools. This idea and concept can further be extended to other products with minimal use of *in-vivo* studies. This approach is currently widely used by generic companies in 505(b)(2) NDA approach, where costly *in-vivo* studies can be waived using published data from existing marketed formulations. The 505(b)(2) pathway allows use of different salt/polymorph of API,

change in formulation, change in strength or use of combination of drugs where safety and efficacy of individual drugs has already been established.

The use of in-silico modelling has been encouraged by regulatory agencies due to their capability in predicting different dosing scenarios in different group of populations, drug-drug interaction studies and developing long-acting dosage forms based on immediate release pharmacokinetics. Thus, the present research work can serve as benchmark strategy for other disease areas using combination of drugs with known safety and efficacy profiles.

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