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Establishing In Vitro-In Vivo Correlation (IVIVC) for Formulations of Vitamin C and Iron in Daily Plus Tablets

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ABSTRACT

Introduction: The aim of this study was to establish an in vitro–in vivo correlation (IVIVC) model for ferrous fumarate (iron) and ascorbic acid (vitamin C). **Methods:** In vitro dissolution data from 13 samples of a multinutrient botanical formulation containing iron and vitamin C (Daily Plus extended-release tablets) were analyzed using mathematical models to estimate the absorption kinetics of vitamin C and iron in vivo following oral administration. Plasma concentrations were estimated for both iron and vitamin C from the generated models. **Results:** Our analysis revealed a quadratic and a linear relationship between in vivo absorption and in vitro dissolution rates for both vitamin C and iron, respectively, indicating that the dissolution rate of these compounds in vitro is a critical factor affecting their absorption kinetics in vivo. Furthermore, we found that the IVIVC model correlation is specific to the formulation, as different formulations may have different absorption kinetics and IVIVC models. By leveraging this quadratic relationship, we were able to make precise predictions of the entire pharmacokinetic profile based solely on in vitro dissolution data for the formulation. **Conclusion:** A robust IVIVC (level A) was established for vitamin C and iron release from Daily Plus tablets. This correlation can serve as a surrogate bioequivalence studies.

KEYWORDS: dissolution, in vitro–in vivo correlation (IVIVC), vitamin C, iron

INTRODUCTION

The U.S. Food and Drug Administration (FDA) describes in vitro–in vivo correlation (IVIVC) as a predictive mathematical model that establishes a relationship between an in vitro characteristic of a dosage form, such as its dissolution or release profile, and its corresponding in vivo behavior. In 1997, the FDA issued regulatory guidance outlining the development, assessment, and applications of IVIVC for extended-release (ER) oral dosage forms (1–3). This guidance identified deconvolution and convolution-based approaches as standard techniques for establishing an IVIVC, emphasizing that these methods represent an in vitro–in vivo relationship more than a direct correlation (*4–6*).

IVIVC is classified into five distinct levels (level A, B, C, D, and multiple C) based on the nature of data utilized in the analysis. A level A correlation is typically represented as a linear relationship between the in vitro and in vivo dissolution of an ingredient from its dosage form. A level A IVIVC can be achieved with direct superimposition of the in vitro and in vivo dissolution curves or with the use of a scaling factor for superimposition. A level B IVIVC involves applying statistical moment analysis principles to the in vitro and in vivo dissolution data. Therefore, the predictive power of a level B IVIVC is limited, and a more precise model is needed to accurately predict the in vivo behavior of the active ingredient. A level C IVIVC establishes a relationship between a single dissolution parameter and a pharmacokinetic (PK) parameter;

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however, this correlation does not fully capture the entire shape of the plasma concentration–time curve, which is a critical factor in evaluating the performance of ER products (*7, 8*). By extension, the multiple level C correlation relates one or several PK parameters to the dissolution of the drug at multiple timepoints. IVIVC levels B and C are less powerful than level A, as they do not incorporate all the information pertaining to both the in vitro and in vivo behavior of the formulation.

The current study aims to establish a level A IVIVC for iron and vitamin C release from a multinutrient botanical formulation of Daily Plus tablets (*9*). The ingredients in Daily Plus are gotu kola (*Centella asciatica*); vitamins A, E, D, K, B2, B5, B9, B3, B7, B12, B1, and C; as well as copper, manganese, iodine, molybdenum, zinc, selenium, iron, chromium, calcium, magnesium, phosphorus, acerola cherry, purple carrot, and elderberry. Ferrous fumarate does not dissolve in water but is soluble in dilute hydrochloric acid (HCl); it is bioavailable, inexpensive, and thus, an attractive iron source. Ascorbic acid is recognized to be the most effective enhancer of iron absorption and counteracts iron absorption inhibitors such as phytic acid and polyphenols (*10–16*). This is an important formulation for iron supplementation because iron deficiency is a major health problem in developing countries. Utilizing deconvolution techniques and mathematical modelling, predicted (in vitro) and published (in vivo) PK values for these ER formulations were used to validate how well in vitro dissolution testing can predict their behavior after oral administration (*6, 7, 17–23*).

METHODS

Thirteen samples of Daily Plus ER tablets (Amway India Enterprise, India) were used for this study. HCl, oxalic acid, and metaphosphoric acid were obtained from Merck.

HCl, methanol, acetic acid, sodium acetate, sodium hydroxide pellets, and sodium phosphate monobasic crystals were supplied by J.T. Baker (Mexico). AMD reference substance was provided by Sigma-Aldrich (USA).

In Vitro Dissolution Studies

The release characteristics of iron and vitamin C from 13 samples of Daily Plus ER tablets were studied using a United States Pharmacopeia (USP) paddle dissolution apparatus operating at 75 rpm with dissolution media maintained at 37 °C.

For dissolution of vitamin C, the dissolution media contained 0.5% oxalic acid and 3% metaphosphoric acid at pH 2.8 (total of 900 mL for each vessel) to provide a stable environment that preserves integrity of vitamin C, enhances solubility, and ensures accurate analysis. Metaphosphoric acid complexes with other metal ions and prevents the oxidative degradation of vitamin C, while oxalic acid creates an acidic environment that improves vitamin C solubility and mimics the physiological conditions under which ascorbic acid is absorbed in human body. A 10-mL sample was collected from each of six vessels and combined to create a pooled sample for ascorbic acid analysis using reverse phase–high-performance liquid chromatography (RP-HPLC). Sampling time points were 1, 3, and 6 h.

An aliquot of the sample was introduced into the HPLC system and analyzed against an external reference standard for vitamin C quantification. The analysis utilized an Agilent Zorbax SB-AQ column (4.6 × 150 mm, 5 µm) or equivalent. The run time was set to 10 minutes, with a flow rate of 0.6 mL/min and a detection wavelength of 245 nm.

For dissolution of iron, 900 mL of 0.4-N HCL buffer was used as dissolution media. A 10-mL sample was collected from each of six vessels and mixed to prepare a pooled sample. Iron was analyzed using optical emission spectroscopy/mass spectrometry.

In Vivo Studies

A literature survey was performed to examine in vivo absorption kinetics for dosages of 60-mg vitamin C and 14-mg iron, which are closely aligned with the formulation's dosages of 65-mg vitamin C and 19-mg iron. The published in vivo profiles for vitamin C and iron were taken from studies by Piotrovskij et al. and Husmann et al., respectively (*20, 24*).

Pharmacokinetic Model Building

An ordinary differential equation (ODE) was utilized to develop an IVIVC for determining the in-vivo absorption profile. The ODE-based PK model was calibrated using observed plasma concentrations of vitamin C and iron at doses of 60 mg and 14 mg, respectively (*20, 24*).

Among the three PK models reported by Piotrovskij et al., Levine et al., and Padayatty et al., the model proposed by Piotrovskij et al. was selected for the in vivo studies of vitamin C (*20, 25, 26*). This selection was made because the model's parameters were calibrated using a 60-mg dose, which closely approximates the 65-mg vitamin C content of the product under investigation. A comprehensive list of model parameters used in the current study can be found in Table 1.

Table 1. Optimized Parameter Values for Models of Vitamin C and Iron Release

Parameter	Value	Unit	Description
Vitamin C			
α	932.2	µmol h^{-1}	Maximum rate of ascorbic acid (AA) intestinal absorption
β	1824.4	µmol	Rate at which AA intestinal absorption is equal to one-half of α
K_effxtiss	0.163	h^{-1}	Rate of efflux from tissue
CLren	23.18	L h^{-1}	Renal clearance by filtration from plasma into renal tubule
Vc	3.48	L	Plasma volume
V_maxtiss	425.85	µmol L^{-1} h^{-1}	Maximum rate of AA tissue uptake
KMtiss	5.88	µmol L^{-1}	Rate at which AA tissue uptake is equal to one-half of Vmaxtiss
Kmet	0.0003	h^{-1}	Rate of conversion to metabolites
Kurn	109.17	h^{-1}	Rate of urine loss
Kgut	0.018	h^{-1}	Fecal loss
gmax	5.25	µmol L^{-1} h^{-1}	Transport maximum rate at which AA tubular can be reabsorbed
Iron			
K1	h^{-1}	0.0061	Rate of absorption
Q_{pt}	mL/h	0.0161	Rate of transport
V_p	mL	18.3626	Volume of tissue
V_t	mL	13.1320	Volume of tissue
K2	mL/h	0.0955	Rate of removal from plasma
K3	mL/h	6.7647	Rate of removal from tissue

To ensure model accuracy, the model was validated against available data for doses of 65-mg vitamin C and 19-mg iron in the Daily Plus formulation. Using the validated model, plasma concentrations over time were simulated to determine the percentage of active ingredient absorbed. This involved employing a deconvolution technique based on the Wagner-Nelson Method, which enables the calculation of absorption profiles for a one-compartment model. Specifically, we applied the Loo-Riegelman absorption method to calculate the fraction of vitamin or iron absorbed at different time points, assuming complete elimination of the absorbed ingredient from the system at time t = ∞ (*17*). The fraction (*F*) of active ingredient absorbed in the system at time was defined as $F_t = V_p \times C_p + V_t \times C_t + K_{el} \times V_p \times (AUC_0^t)$. At time, $t = \infty$, $F_\infty = K_{el} \times V_p \times (AUC_0^\infty)$, where V_p is the plasma or serum volume, C_p is plasma or serum concentration, Vt is tissue volume, C_t is tissue concentration, and K_{el} denotes the elimination rate. The percentage of vitamin C or iron absorbed into the system is calculated as $(F_t / F^\infty) \times 100$.

Statistical Analysis

Data analysis was performed using MatLab R2022a (MathWorks, Inc). The relationship between the percentage of active ingredient absorbed in vivo and dissolved in vitro was evaluated using linear regression and multiple regression. The performance of the model was evaluated based on the coefficient of determination (R^2) and the root mean square error (RMSE). Model selection was performed by maximizing R^2 and minimizing RMSE, as a lower RMSE reflects improved model performance by reducing prediction errors. The proportion of variance in in vivo and in vitro data were quantified based on R^2. Confidence intervals for regression coefficients were computed for precision. All analyses were conducted according to established statistical protocols.

RESULTS AND DISCUSSION

The in vitro dissolution tests demonstrated that most ingredients were absorbed within the first 10 hours following administration, with complete absorption occurring within 15 hours for iron and 20 hours for vitamin C. The in vitro dissolution rates were strongly correlated with the published in vivo absorption data for both active ingredients (*20, 24*).

Vitamin C

To develop the IVIVC for vitamin C release, a three-compartment mathematical model was used, as shown in Figure 1A. The model parameters were optimized for this study (Table 1), and the output was the predicted PK values for the 65-mg dose of vitamin C. The predicted values were validated with in vivo plasma concentrations for a 60-mg dose of vitamin C (*20*). The model provided an excellent representation of the predicted time course of ascorbic acid plasma levels. As shown in Figure 1B, the concentration of vitamin C reaches its maximum (86 µM) at approximately 3 hours after administration and gradually decreases to 62 µM within 20 hours (for a 65-mg dose of vitamin C).

The results indicate that nearly 85% of vitamin C was assimilated within 5–10 hours after oral administration, after which the absorption rate became stable, as demonstrated in Figure 2A. Figure 2B shows that over 50% of the ingredient dissolved in less than the designated time, and 100% of the ingredient is dissolved in less than 6 hours. Following this, a comparative analysis was conducted between the in vivo absorption data and in vitro dissolution data. The in vivo absorption data were plotted against the in vitro dissolution data for time points ranging from 0 to 6 hours for the formulation. A quadratic

fit (Fig. 2C) and cubic fit (not shown) analysis of the data produced a reasonable agreement. The results show that the optimized model parameters yield a good fit for the in vivo plasma concentration-time curve corresponding to a 65-mg dose of vitamin C.

The PK evaluation of vitamin C in the Daily Plus ER tablet formulation revealed several parameters essential for establishing a strong IVIVC (*11*, *27–29*). Initially, the model highlighted a rapid absorption rate from the intestine, characterized by a maximum rate of intestinal absorption (α = 932.2 µmol h^{-1}). This parameter signifies a close alignment between the in vitro dissolution rate and the rate of absorption observed in vivo, ensuring accurate prediction of plasma concentrations following oral administration.

Moreover, the model incorporated parameters indicative of a sustained release profile in vitro, including a low rate of efflux from tissue (K_effxtiss = 0.163 h^{-1}) and a high maximum rate of tissue uptake (V_maxtiss = 425.85 µmol L^{-1} h^{-1}). These findings indicate that vitamin C is progressively utilized by tissues over time, aligning with the temporal relationship observed between the in vitro dissolution profile (> 50% dissolution within the specified time and complete dissolution within 6 hours) and in vivo absorption kinetics (peak plasma concentration around 3 hours and saturation at 20 hours). This temporal alignment substantiates a robust IVIVC for vitamin C at a dose of 65 mg (*18–20*).

Iron

To develop the IVIVC for iron release, a two-compartment model was used, with one designated as the central compartment, representing plasma/serum, and the other as tissue, as shown in Figure 3A. The concentrations of iron from the in vivo dissolution experiments and derived from the model using published in vivo data are shown in Figure 3B (*24*, *30*). The concentration of iron reached its maximum level 3 hours after oral administration, and

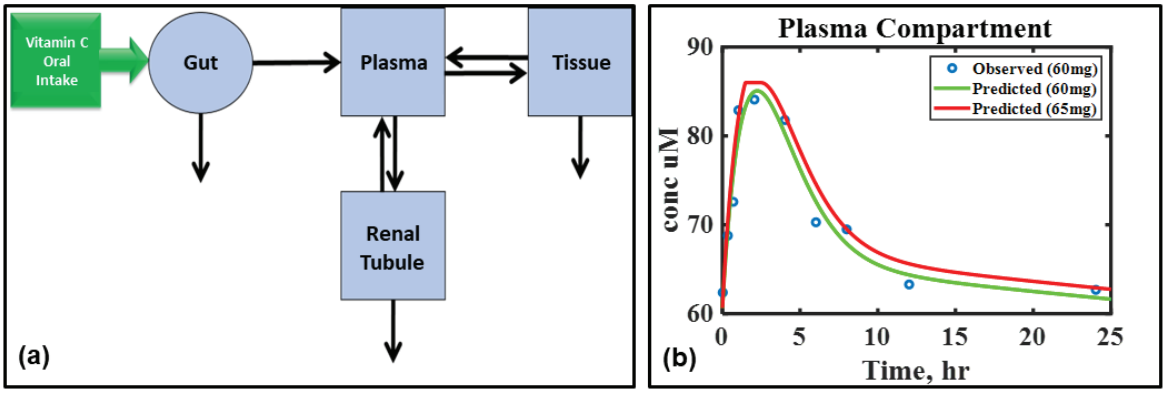

*Figure 1. Schematic representation of model to simulate in vivo concentration of vitamin C parameters. (**a**) Three-compartment model used to study vitamin C, encompassing the central plasma compartment, the tissue compartment, and the renal tubule compartment. (**b**) Predicted values for a 60- and 65-mg dose for vitamin C (green and red line, respectively) and published in vivo data for a 60-mg dose (blue circles) (20). Maximum concentration for the 65-mg dose was 86 µM at 3 hours.*

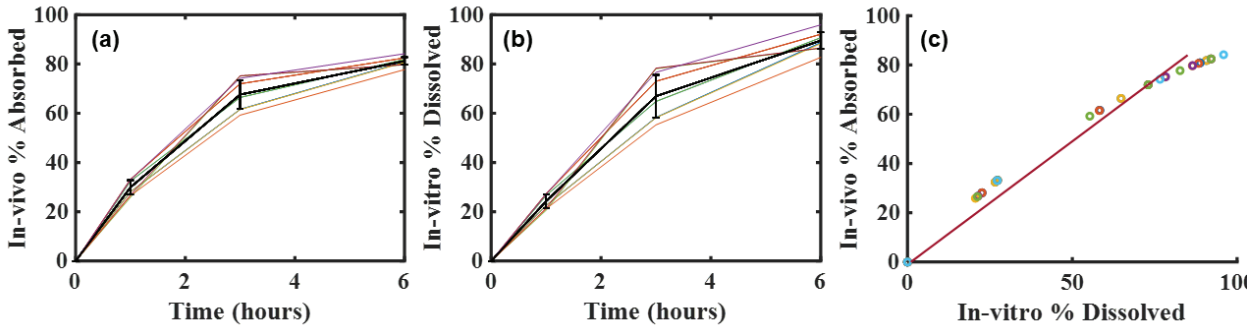

*Figure 2. Plots depicting the release behavior of vitamin C in vivo absorption and in vitro dissolution. (**a**) In vivo absorption profile of 60-mg vitamin C as determined by deconvolution analysis, showing approximately 85% release within 5–6 hours. (**b**) In vitro dissolution profile of 65-mg vitamin C, showing complete release occurring in under 6 hours (n = 13). (**c**) Linear regression plots of the in vitro-in vivo correlation (IVIVC) for percent dissolved versus percent absorbed of vitamin C in the formulation, showing a strong correlation (R^2 = 0.97).*

then gradually decreased to zero within 20 hours (for a 19-mg dose of iron).

The percentage of iron absorbed was determined by deconvoluting the in vivo iron concentration within the plasma/serum compartment of the model (*31–33*). Approximately 98% of iron was absorbed within 6 hours after oral administration in vivo (Fig. 4A) and 100% was released in vitro within the same time (Fig. 4B). Fitting the in-vitro dissolution data against the absorption percentage for each hour from 0 to 6 h obtained a good correlation (Fig. 4C).

The PK evaluation of iron in the Daily Plus ER tablet formulation exhibited steady absorption kinetics (K1 = 0.0061 hr^{-1}) and efficient transport from central to peripheral compartments (Q_{pt} = 0.0161 mL/hr). These parameters show that the in vitro dissolution profile accurately predicts the absorption kinetics observed in vivo, where nearly 98% of the iron dose (19 mg) is absorbed within 10 hours after oral administration.

The model further integrated parameters such as plasma volume (V_p = 18.3626 mL) and tissue volume (V_t = 13.1320 mL), which are crucial for correlating in vitro dissolution data with in vivo PK values. Rapid removal rates from plasma/serum (K2 = 0.0955 mL/hr) and tissue (K3 = 6.7647 mL/hr) underscored efficient utilization and recycling of iron, consistent with the complete absorption observed within 20 hours. This comprehensive PK evaluation of iron supports a robust IVIVC, corroborated by linear fit analysis demonstrating good agreement between in vitro dissolution data and in vivo absorption data (*34–37*).

IVIVC

Quadratic fit analysis of the in vitro and in vivo data validated the capability of dissolution tests to predict PK values for vitamin C and iron (Fig. 5). This statistical

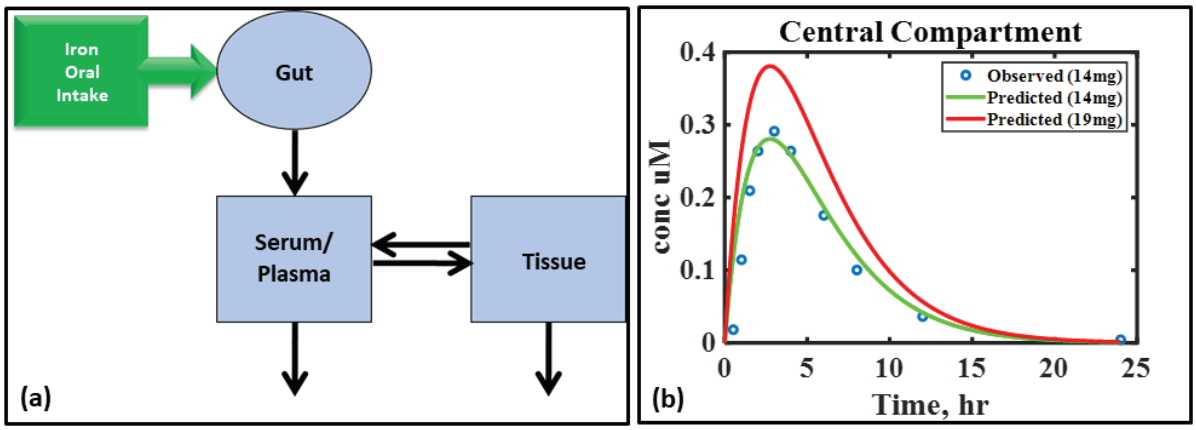

*Figure 3. Schematic representation of model to simulate in vivo concentration of ferrous fumarate parameters. (**a**) Two-compartment model to study iron release, encompassing a central compartment for serum/plasma and a compartment for tissue. (**b**) Predicted values for a 14- and 19-mg dose of iron (green and red lines, respectively) and published in-vivo data for a 14-mg dose (blue circles) (24). Maximum concentration for the 14-mg dose was 0.38 µM at 3 hours.*

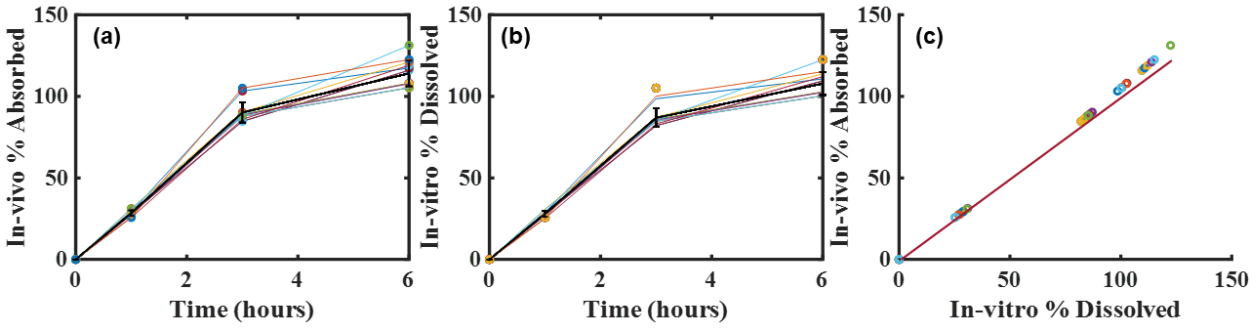

*Figure 4. Plots depicting the release behavior of ferrous fumarate (iron). (**a**) In vivo absorption profile of 14-mg iron as determined by deconvolution analysis in the plasma/serum compartment, showing approximately 90% absorption within 6 hours. (**b**) In vitro dissolution profile of 19-mg iron, showing complete dissolution within 6 hours (n = 13). (**c**) Linear regression plots of the in vitro-in vivo correlation (IVIVC) for percent dissolved versus percent absorbed of iron at each hour from 0 to 6 in the formulation, showing a strong correlation (R^2 = 0.97).*

approach confirmed a reasonable agreement between the two datasets, which is pivotal for regulatory purposes, potentially reducing the need for extensive in vivo bioavailability studies during formulation development (*38*, *39*). The models predicted that approximately 98% of iron and 85% of vitamin C are absorbed within 6 hours, with complete absorption achieved by 15 and 20 hours, respectively. These predictions mirror the in vitro dissolution profile and confirm a robust IVIVC for both nutrients in the Daily Plus ER tablet formulation.

Limitations

Although the developed IVIVC model demonstrates strong predictive capability, several limitations must be acknowledged. The model relies on the assumption that in vitro conditions adequately replicate the in vivo environment. However, physiological variability in vivo, such as pH fluctuations, gastrointestinal (GI) transit time, enzymatic activity, and bile salt concentrations may affect drug dissolution, absorption, and ultimately the predictability of the model. Variability in gastric and intestinal pH between individuals, particularly in special populations (e.g., pediatric, geriatric, or those with GI disorders) can alter drug solubility and absorption rates. Additionally, differences in GI transit time due to factors such as diet, motility disorders, or co-administered medications may introduce variability not captured by the in vitro experimental setup (*40–42*). Although the IVIVC model provides a simplified framework for predicting in vivo drug behaviour, its application should consider these sources of variability. Further studies incorporating biorelevant dissolution conditions and dynamic physiological models (e.g., physiologically based pharmacokinetic modelling) could enhance the robustness and generalizability of the IVIVC model (*43*).

CONCLUSION

The study successfully demonstrated a robust IVIVC for the Daily Plus formulation containing vitamin C and ferrous fumarate. The integration of mathematical modelling with comprehensive pharmacokinetic data provided clear insights into the absorption, distribution, metabolism,

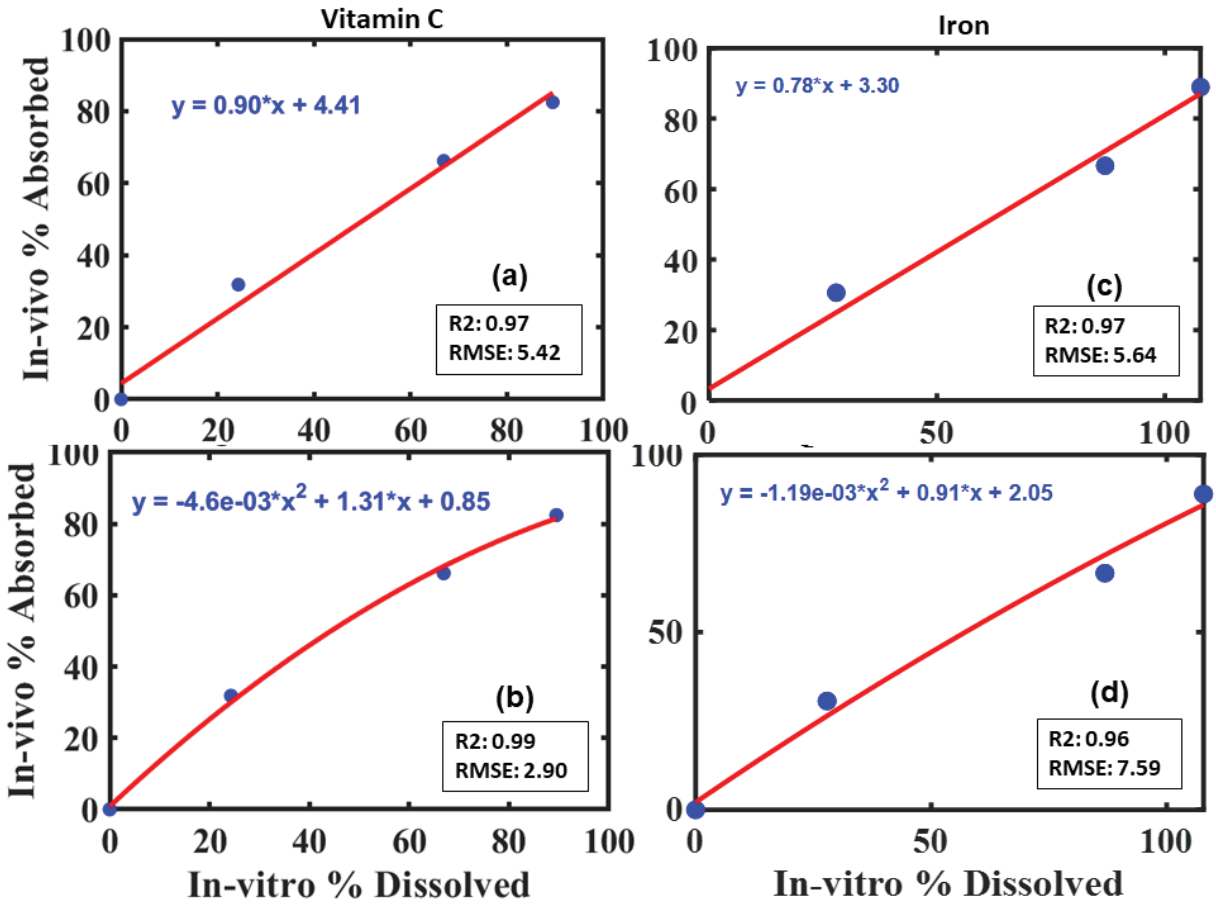

*Figure 5. Quadratic fit analysis of the predicted in vivo absorption and in vitro dissolution profiles for vitamin c (***a** *and* **b***) and iron (***c** *and* **d***). Blue dots represent the data. Red line represents the fit. R^2 and root mean square error (RMSE) values show that the dissolution data fit is quadratic for vitamin C (left) and linear for iron (right).*

and excretion profiles of both nutrients. These insights enhance understanding of their pharmacokinetics and facilitate the development of optimized formulations meeting therapeutic requirements effectively.

The strong correlation between in vitro dissolution rates and in vivo absorption kinetics supports the validity and utility of IVIVC in predicting the performance of formulations in clinical settings. Future research could explore additional parameters to further refine models and enhance the predictive accuracy of dissolution testing for diverse formulations and active ingredients. This advancement holds promise for streamlining formulation development and regulatory processes, ultimately benefiting patient care and therapeutic outcomes in the pharmaceutical industry.

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DISCLOSURES

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SUPPLEMENTAL MATERIAL

Supplemental material is available for this article and may be requested by contacting the corresponding author.

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