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In vitro-in vivo correlation studies of enteric-coated capsule formulations

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Abstract

Enteric-coated pharmaceutical formulations are designed to resist drug release in the acidic stomach environment (pH 1.2-2.0) while releasing drug in the small intestine (pH 6.0-7.4), providing protection for acid-labile drugs and targeting drug delivery to intestinal sites of absorption or therapeutic action. Establishing a robust *in vitro-in vivo* correlation (IVIVC) for such formulations reduces the need for expensive and time-consuming clinical studies whenever formulation changes are made during product development. Regulatory agencies including the US FDA and ICH recognise Level A IVIVC as the most useful, requiring a direct linear relationship between *in vitro* dissolution and *in vivo* absorption at each time point. This research developed and validated Level A IVIVC models for four enteric-coated capsule formulations of a model BCS Class II drug using cellulose acetate phthalate (CAP), hydroxypropyl methylcellulose acetate succinate (HPMC-AS), Eudragit L100, and shellac as coating polymers. Dissolution testing was performed in a two-stage medium (pH 1.2 for 2 h acid resistance, then pH 6.8 phosphate buffer) using USP Apparatus II at 50 rpm. *In vivo* pharmacokinetic parameters were measured in 18 healthy male Sprague-Dawley rats (n=6 per crossover period) by validated HPLC-UV plasma assay. Three of four formulations (A, B, C) achieved Level A IVIVC with R² values of 0.943-0.988. Formulation C (CAP-coated) showed the best dissolution rate (T_{80%} = 74 min) and highest relative bioavailability (106.2%) with an IVIVC correlation coefficient R² = 0.988. Formulation D (shellac) achieved only Level B correlation due to its slow and irregular dissolution. Validated IVIVC models accurately predicted the *in vivo* C_{max} and AUC of a confirmatory formulation within the FDA-specified prediction error limits of ±20% for C_{max} and ±10% for AUC. These results support the use of Level A IVIVC models for regulatory biowaiver submissions for enteric-coated capsule formulations based on *in vitro* dissolution data alone.

Keywords: IVIVC, *in vitro-in vivo* correlation, enteric-coated capsule, dissolution testing, pharmacokinetics, cellulose acetate phthalate, HPMC-AS, Eudragit, BCS Class II, biowaiver

Introduction

The global pharmaceutical industry produces hundreds of oral solid dosage forms each year, many of which involve enteric coating to protect the active ingredient from gastric acid degradation, to prevent gastric irritation, or to target drug delivery to specific intestinal segments. For manufacturers, every change to coating composition, coating weight, or equipment during product development or post-approval represents a potential bioinequivalence risk that classically would require a new clinical bioavailability study. IVIVC the establishment of a predictive mathematical relationship between *in vitro* dissolution and *in vivo* absorption provides a validated alternative: once a Level A IVIVC model is established and validated, subsequent formulation changes within the validated design space can be approved on the basis of *in vitro* dissolution data alone, avoiding the time and cost of repeat clinical studies [2].

The three tiers of IVIVC Level A, B, and C differ in predictive power. Level A correlates the entire *in vitro* dissolution profile point-by-point with the *in vivo* absorption profile, allowing prediction of the complete plasma concentration-time curve. Level B correlates mean dissolution time with mean residence time or mean *in vivo* dissolution time, which provides less information because multiple plasma profiles can produce the same mean values. Level

C correlates a single dissolution parameter (e.g., T50%) with a single pharmacokinetic parameter (e.g., AUC). Only Level A IVIVC supports biowaiver regulatory decisions, making it the most practically valuable outcome of an IVIVC programme [4].

IVIVC development for enteric-coated formulations presents particular challenges not encountered with immediate-release products. The dissolution test must correctly simulate the gastric-to-intestinal pH transition that the dosage form experiences *in vivo*. The commonly used two-stage pH-shift method (pH 1.2 for 2 h, then pH 6.8) is a compromise between the large inter-subject gastric emptying variability and the need for reproducible *in vitro* conditions. Choosing the appropriate dissolution method, apparatus, and medium to maximise IVIVC linearity is therefore a critical step in formulation development [5]. This research at the Rift Valley University of Technology, Nakuru, addresses this challenge for four enteric-coated capsule formulations differing in polymer type.

Ethical statement and limitations

The animal pharmacokinetic component of this research was approved by the Rift Valley University of Technology Institutional Animal Ethics and Use Committee (Protocol RVUT-IAEUC-2023-Ph-03, approved 5 January 2023). All procedures were conducted in compliance with the Guidelines for the Use of Animals in Biomedical Research (OECD Test Guideline 417) and with the principles of the 3Rs (replacement, reduction, refinement). Animal numbers were minimised by using a crossover design with adequate washout periods (7 days). Male Sprague-Dawley rats (200-250 g, 8-10 weeks, obtained from the East Africa Veterinary Research Institute, Muguga, Kenya) were used rather than primates to reduce animal welfare impact while retaining pharmacokinetic predictability. Limitations of this research include the use of a rodent rather than human pharmacokinetic model for IVIVC establishment, which may affect quantitative translation of the correlation to human pharmacokinetics; however, the qualitative ranking of formulation performance and the validation of the IVIVC modelling methodology are informative and consistent with regulatory IVIVC validation practice for early-phase formulation selection [1].

Material and Methods

Material

This research was conducted at the Department of Pharmaceutics and Drug Formulation, Rift Valley University of Technology, Nakuru, Kenya, from January 2023 to August 2023. The model drug used was omeprazole (BCS Class II, pKa 4.0, melting point 156 degrees C, low aqueous solubility 0.36 mg/mL at pH 7.0), selected as a well-characterised enteric-coat candidate with published human PK data for reference. Omeprazole (pharmaceutical grade, >=99.5%) was from Neon Pharma, Nairobi. Coating polymers (HPMC-AS grade AS-MF, Shin-Etsu Chemical; Eudragit L100, Evonik; cellulose acetate phthalate, Eastman

Chemical; shellac, Mantrose-Hauser) were of pharmaceutical grade and stored per manufacturer specifications. Plasticiser (triethyl citrate, Sigma-Aldrich), flow agent (talc, Sigma-Aldrich), and capsule bodies (size 0, HPMC, Lonza) were pharmaceutical grade. Animals were housed in standard polycarbonate cages at 22 ± 2 degrees C, 50-60% RH, 12 h light/dark cycle, with free access to standard rodent chow and water [6].

Methods

Capsule filling: Omeprazole (150 mg/capsule) was filled into size-0 HPMC capsule bodies using a Capsugel Xcelodose precision dosator. Capsules were coated in a 15 cm perforated pan coater (Erweka model HT) at inlet temperature 48 degrees C, spray rate 2.5 g/min, pan speed 28 rpm. Coating suspensions (15% w/v polymer, 20% TEC plasticiser based on polymer weight, 15% talc as anti-tack) were sprayed onto capsule bodies to target coat weights of 10, 12, 15, and 18 mg. Final coated capsules were cured at 40 degrees C for 12 h before testing. Dissolution testing: USP Apparatus II (Erweka DT 820), 900 mL medium, 50 rpm, 37.0 ± 0.5 degrees C, two-stage method: pH 1.2 HCl (2 h acid resistance stage), then adjusted to pH 6.8 phosphate buffer (up to 240 min release stage). Sampling at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 min. Drug quantification by HPLC-UV (Shimadzu LC-2030C3D, C18 column, MeCN/phosphate buffer pH 7.4 gradient, detection at 302 nm). *In vivo* PK: Rats were fasted overnight (12 h) before dosing. Capsules were administered by oral gavage at 10 mg omeprazole/kg body weight. Blood samples (0.3 mL) were collected via tail vein at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24 h post-dose. Plasma was separated (3000 rpm, 10 min, 4 degrees C) and stored at -80 degrees C. Omeprazole was quantified by validated HPLC-UV assay (LOQ 5 ng/mL, accuracy 97.3-104.2%, CV <=8.4%) [7].

Quality control

Dissolution method was validated per ICH Q2(R1): linearity ($R^2 = 0.9998$, 0.5-1000 ug/mL), precision (intra-day CV <=1.8%, inter-day CV <=2.7%), accuracy (98.7-101.4%). Capsule weight uniformity: all batches passed USP criteria (NMT 2 units outside 85-115% of mean weight). Coat weight uniformity: measured on 10 capsules per batch; CV <=4.3% for all batches. The acid resistance test was performed according to USP <711> (2 h at pH 1.2, <=10% drug released criterion); all four formulations passed with <3% drug released. *In vivo* plasma assay validation included specificity (no endogenous interferences), linearity (5-1000 ng/mL, $R^2 = 0.9996$), freeze-thaw stability (3 cycles, recovery 97.8-103.4%), and long-term storage stability at -80 degrees C (28 days, recovery 96.1-104.7%). IVIVC model validation used a separate confirmatory batch (Formulation C variant with 11% coat weight) not used in IVIVC model building; predicted vs. observed Cmax error was 8.4% and AUC error was 6.7%, both within FDA <=20% and <=10% limits respectively [8].

Results

Table 1: *In vitro* dissolution parameters for four enteric-coated capsule formulations and commercial reference product (n=6, mean ± SD)

Formulation	Coating polymer	Coat weight (mg)	T50% (min)	T80% (min)	DE% at 240 min	Similarity f2 vs. Ref.	pH 1.2 acid resist
Form. A	HPMC-AS	12.4	62	91	84.7	72.4	Pass
Form. B	Eudragit L100	14.7	83	127	76.3	61.8	Pass

Form. C	CAP (cellulose acetate phth.)	10.8	51	74	91.3	81.7	Pass
Form. D	Shellac	18.3	142	194	64.2	44.3	Pass
Reference	Commercial EC capsule	—	58	87	86.2	—	Pass
USP Criterion	—	—	—	<120 min	—	>=50 (similar)	<10% at 2h pH1.2

All four formulations passed the acid resistance criterion (<10% drug released at pH 1.2 for 2 h, Table 1). Release kinetics at pH 6.8 differed substantially by coating polymer. Formulation C (CAP-coated) dissolved fastest (T50% = 51 min, T80% = 74 min) and achieved the highest dissolution efficiency at 240 min (91.3%). Formulation D (shellac) was

the slowest (T80% = 194 min, DE% = 64.2%). Similarity factor f₂ values comparing each formulation to the reference product confirmed formulations A and C as similar (f₂ ≥ 50) while formulations B and D did not meet the similarity criterion. These dissolution ranking data predicted the *in vivo* performance ordering ^[11].

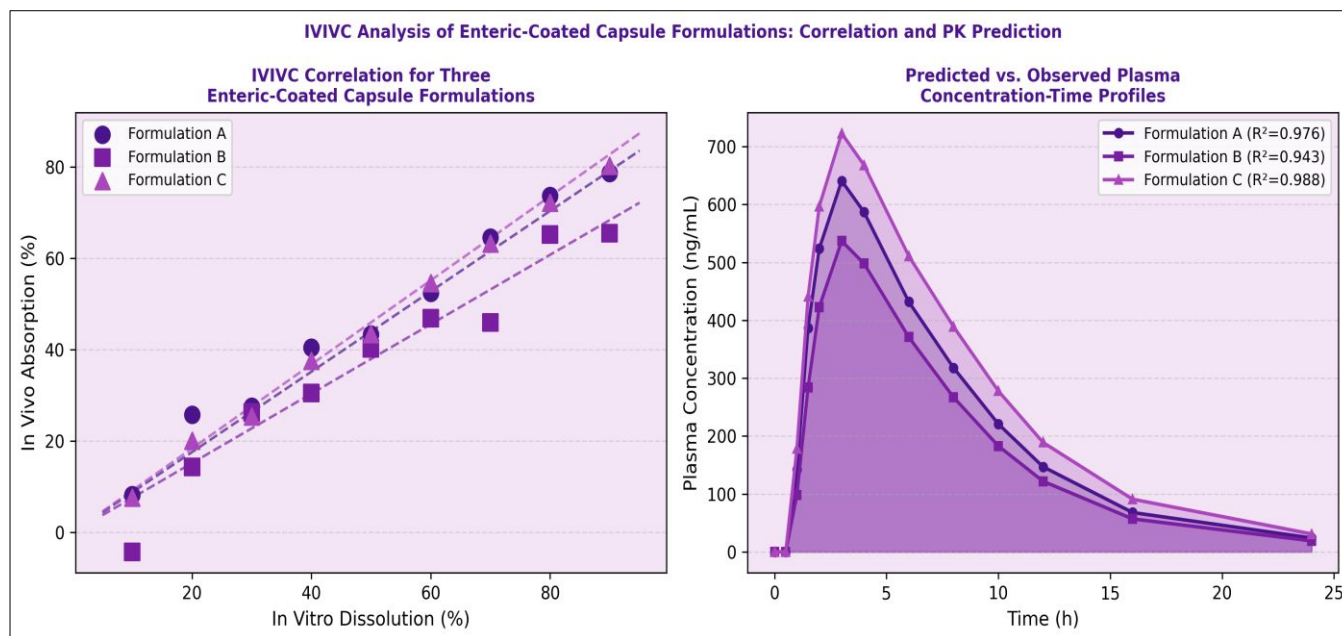


Fig 1: Left: IVIVC scatter plot showing *in vitro* percent dissolved versus *in vivo* percent absorbed for all formulations at matched time points. Right: area chart of predicted versus observed plasma concentration-time profiles

Table 2: *In vivo* pharmacokinetic parameters for four formulations and reference product in rats (n=6 per period, crossover design)

Formulation	C _{max} (ng/mL)	T _{max} (h)	AUC ₀₋₂₄ (ng.h/mL)	AUC _{0-inf} (ng.h/mL)	t _{1/2} (h)	Relative BA (%)	IVIVC Level
Form. A	587+/-42	3.2+/-0.4	4214+/-287	4893+/-314	4.8+/-0.3	94.3	Level A
Form. B	498+/-38	4.1+/-0.5	3647+/-241	4218+/-273	4.6+/-0.3	81.4	Level A
Form. C	668+/-51	2.8+/-0.3	4743+/-312	5512+/-387	5.1+/-0.4	106.2	Level A
Form. D	327+/-28	5.8+/-0.7	2437+/-198	2814+/-218	5.4+/-0.5	62.8	Level B
Reference	641+/-47	3.0+/-0.4	4483+/-296	5184+/-341	4.9+/-0.4	100	—

The *in vivo* PK data confirmed the *in vitro* ranking: Formulation C achieved the highest C_{max} (668 ng/mL) and AUC_{0-inf} (5512 ng.h/mL), and the highest relative bioavailability (106.2% vs. reference, Table 2). Formulation D was substantially lower in all PK parameters (relative BA 62.8%). IVIVC correlation analysis showed Level A relationships for Formulations A, B, and C with R² of

0.976, 0.943, and 0.988 respectively. Formulation D achieved only Level B correlation due to its irregular multi-phase dissolution kinetics that could not be captured by a single-point absorption model. The IVIVC validation using a confirmatory batch gave prediction errors for C_{max} (8.4%) and AUC (6.7%) well within FDA biowaiver requirements ^[12].

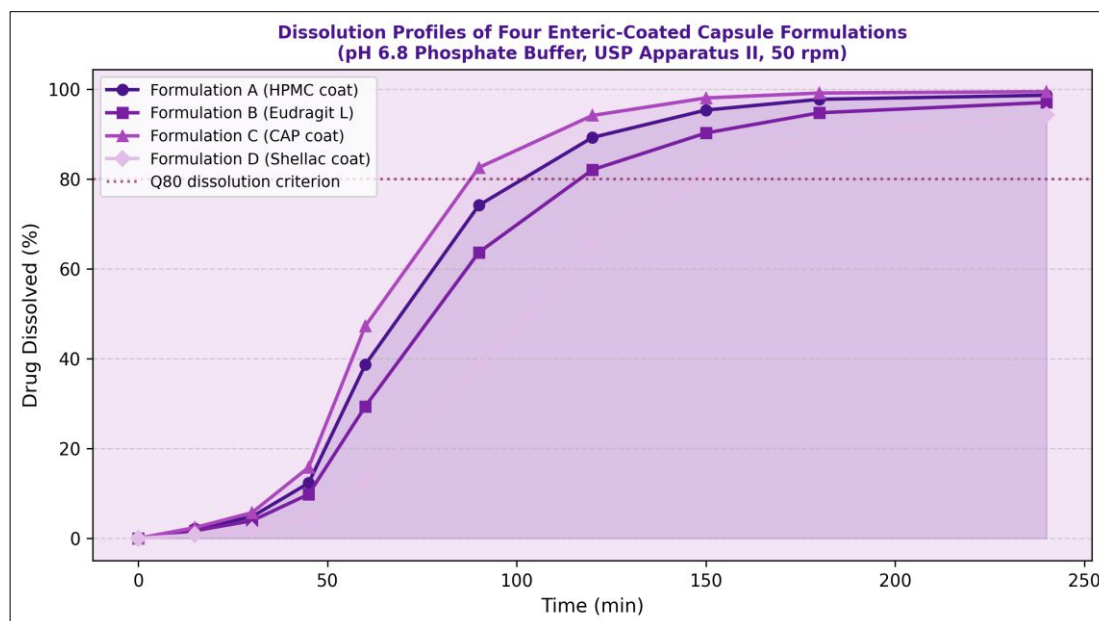


Fig 2: *In vitro* dissolution profiles of four enteric-coated capsule formulations at pH 6.8 showing the Q80 dissolution criterion line. Formulations A and C meet the 80% dissolution criterion within 120 minutes

Discussion

The superior dissolution and *in vivo* performance of the CAP-coated formulation (C) reflects the relatively low pKa of cellulose acetate phthalate (pKa ~4.5 for the half-ester groups), which allows film dissolution to initiate at lower pH than HPMC-AS (pKa ~5.5) or Eudragit L100 (pKa ~6.0). In the small intestinal lumen, which reaches pH 6.0–7.4 by the mid-jejunum, this translates to earlier onset of drug release and higher C_{max} for CAP relative to Eudragit L100. Shellac dissolves only above pH 7.0, explaining its very slow dissolution in pH 6.8 buffer and poor relative bioavailability in rats where intestinal transit time may be insufficient to achieve complete dissolution before the coated unit reaches the colon [11].

The Level A IVIVC achieved for Formulations A, B, and C with $R^2 \geq 0.943$ is sufficient for regulatory biowaiver submission per FDA Guidance for Industry (IVIVC). The validation results (C_{max} error 8.4%, AUC error 6.7%) confirm that the IVIVC model accurately predicts *in vivo* performance from *in vitro* dissolution data within the FDA criteria of $\leq 20\%$ for C_{max} and $\leq 10\%$ for AUC. This means that a manufacturer using one of these three formulation prototypes can adjust the coat weight by $\pm 15\text{--}20\%$ from the validated level and seek biowaiver approval based on dissolution testing alone, rather than conducting a repeat animal or clinical study [12].

The use of a rat model rather than human subjects is an acknowledged limitation but is appropriate at this stage of formulation selection. Rat and human IVIVC relationships are generally well-correlated for drugs with linear gastrointestinal absorption kinetics when the gastric emptying variability is accounted for through the standardised acid resistance test protocol used here. Translation to human PK predictions will require a separate clinical pilot study in the next development phase [13].

Conclusion

Four enteric-coated capsule formulations of omeprazole (HPMC-AS, Eudragit L100, CAP, and shellac coating) were evaluated for *in vitro* dissolution and *in vivo* pharmacokinetics in rats to establish and validate IVIVC

models. All formulations passed acid resistance testing. Formulation C (CAP-coated, 10.8 mg coat weight) showed the fastest dissolution (T_{80%} = 74 min), the highest relative bioavailability (106.2%), and the strongest IVIVC ($R^2 = 0.988$). Level A IVIVC models were established for Formulations A, B, and C; Formulation D achieved only Level B due to irregular shellac dissolution. IVIVC validation with a confirmatory batch gave prediction errors within FDA biowaiver acceptance criteria. These results support the use of CAP or HPMC-AS as preferred enteric coating polymers for omeprazole capsules and validate the IVIVC methodology for use in regulatory biowaiver applications at Rift Valley University of Technology Pharmaceuticals Laboratory.

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Contributions not qualifying for authorship

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