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Factorial designs of enzyme activated ratecontrolled drug delivery system of phentoin drug

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Abstract
The overarching aim of this research is to design, develop, and optimize an enzyme-activated rate-controlled drug delivery system for phenytoin using systematic factorial design methodology. This aim encompasses the integration of enzyme-responsive mechanisms for biological signal-driven release with rate-controlling elements for sustained, controlled drug delivery, creating an advanced formulation that addresses the unique therapeutic challenges associated with phenytoin while demonstrating broader principles applicable to other drugs with narrow therapeutic indices or complex pharmacokinetic behavior.

INTRODUCTION
1.1 Overview of Drug Delivery Systems
Drug delivery systems have evolved significantly over the past few decades, transforming from simple conventional dosage forms to sophisticated, intelligent delivery platforms that respond to biological stimuli [1]. The primary objective of any drug delivery system is to deliver the therapeutic agent to the target site at a controlled rate while minimizing systemic exposure and associated adverse effects [2]. Traditional drug delivery approaches, such as oral tablets, capsules, and injections, often result in fluctuating plasma drug concentrations characterized by initial peaks followed by rapid decline, necessitating frequent dosing to maintain therapeutic levels [3]. These fluctuations can lead to periods of subtherapeutic drug concentrations, resulting in inadequate disease control, as well as supratherapeutic peaks that increase the risk of dose-related toxicity [4].

The limitations of conventional dosage forms have driven extensive research into controlled and targeted drug delivery systems that can overcome these challenges [5]. Modern drug delivery systems are designed to maintain drug concentrations within the therapeutic window for extended periods, reduce dosing frequency, improve patient compliance, and enhance therapeutic outcomes while minimizing adverse effects [6]. The development of such systems involves careful consideration of multiple factors, including the physicochemical properties of the drug molecule, the pathophysiology of the disease being treated, the desired site of drug action, and the biological barriers that must be overcome to achieve effective drug delivery [7].

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The classification of drug delivery systems has become increasingly complex as new technologies emerge, but they can be broadly categorized based on their release mechanisms, route of administration, or responsiveness to physiological stimuli [8]. Controlled-release systems include matrix systems, reservoir systems, osmotic systems, and ion-exchange systems, each employing different mechanisms to regulate drug release over time [9]. Targeted delivery systems utilize various approaches such as passive targeting through the enhanced permeability and retention effect, active targeting using ligand-receptor interactions, or stimuli-responsive targeting that exploits specific characteristics of the disease microenvironment [10]. Among the most promising advances in drug delivery technology are stimuli-responsive or "smart" delivery systems that can modulate drug release in response to specific biological triggers such as pH, temperature, enzymes, redox potential, or glucose levels [11].

The concept of rate-controlled drug delivery represents a paradigm shift from traditional pharmacotherapy, offering the potential to optimize therapeutic outcomes through precise temporal control of drug availability at the site of action [12]. Rate-controlled systems are engineered to release drugs at predetermined, often constant rates that are independent of the amount of drug remaining in the dosage form, ideally achieving zero-order release kinetics [13]. This controlled release behavior provides several therapeutic advantages, including the maintenance of steady-state plasma concentrations, reduction in dosing frequency from multiple times daily to once-daily or even less frequent administration, improved patient adherence to prescribed regimens, and decreased incidence of both therapeutic failures due to subtherapeutic concentrations and adverse effects due to toxic peaks [14]. For drugs with narrow therapeutic indices, where the margin between effective and toxic doses is small, rate-controlled delivery systems can be particularly valuable in ensuring consistent therapeutic efficacy while minimizing the risk of toxicity [15].

1.2 Enzyme-Activated Drug Delivery Systems

Enzyme-activated drug delivery systems represent an innovative approach to achieving site-specific and temporally controlled drug release by exploiting the unique enzymatic environment present at specific anatomical locations or in pathological conditions [16]. Enzymes are highly specific biological on factorial designs of enzyme activated rate controlled drug delivery system of phentoin drug.

1.3 Phenytoin: Pharmacology and Therapeutic Challenges

Phenytoin, chemically known as 5,5-diphenylhydantoin, is a hydantoin derivative that has been used clinically for over eight decades as one of the primary antiepileptic drugs for the management of various seizure disorders. Since its introduction in 1938 by Merritt and Putnam, phenytoin has remained a cornerstone in the pharmacological treatment of epilepsy, particularly for generalized tonicclonic seizures and partial seizures. The drug's enduring clinical utility despite the introduction of numerous newer antiepileptic agents reflects its proven efficacy, relatively low cost, and extensive clinical experience accumulated over decades of use. However, the therapeutic application of phenytoin is significantly complicated by its unique pharmacokinetic properties, narrow therapeutic index, and propensity to cause both dose-related and idiosyncratic adverse effects.

The mechanism of action of phenytoin is primarily mediated through blockade of voltage-gated sodium channels in neuronal membranes, thereby limiting the sustained high-frequency repetitive firing of action potentials characteristic of epileptic seizures. Phenytoin binds

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preferentially to the inactivated state of voltage-gated sodium channels in a use-dependent manner, meaning that its blocking effect is more pronounced in neurons that are firing rapidly, as occurs during seizure activity. This selectivity for hyperactive neurons allows phenytoin to suppress seizure activity while having relatively minimal effects on normal neuronal function. The drug's interaction with sodium channels is voltage-dependent and frequency-dependent, with stronger binding occurring at more depolarized membrane potentials and during high-frequency neuronal firing. Beyond its primary effect on sodium channels, phenytoin has been shown to modulate calcium channels, potassium channels, and various neurotransmitter systems, although the clinical significance of these additional effects remains less well-established.

The pharmacokinetic properties of phenytoin present significant challenges for achieving and maintaining therapeutic drug concentrations. Phenytoin exhibits highly variable oral bioavailability ranging from 70% to 100%, influenced by factors such as formulation characteristics, gastric pH, presence of food, and co-administration with other medications. The drug is approximately 90% bound to plasma proteins, primarily albumin, and this high degree of protein binding can be affected by hypoalbuminemia, uremia, pregnancy, and displacement by other highly protein-bound drugs. The apparent volume of distribution of phenytoin ranges from 0.6 to 0.7 L/kg, indicating distribution into total body water with particular accumulation in fatty tissues due to its lipophilic nature. However, the most clinically significant pharmacokinetic characteristic of phenytoin is its capacity-limited or saturable metabolism, which results in nonlinear, dose-dependent pharmacokinetics that make dose adjustments and therapeutic drug monitoring particularly challenging.

Idiosyncratic reactions to phenytoin, while less common than dose-related effects, can be severe and potentially life-threatening. Cutaneous adverse reactions range from mild maculopapular rashes occurring in 2-5% of patients to serious conditions such as Stevens-Johnson syndrome and toxic epidermal necrolysis, which, although rare, carry significant morbidity and mortality. The risk of severe cutaneous reactions is strongly associated with specific HLA alleles, particularly HLA-B*15:02 in individuals of Asian ancestry, leading to recommendations for genetic screening in certain populations before initiating phenytoin therapy. Hematologic adverse effects include rare but serious conditions such as aplastic anemia, agranulocytosis, and megaloblastic anemia secondary to folate deficiency. Hepatotoxicity, ranging from mild elevation of liver enzymes to fulminant hepatic failure, represents another potentially serious idiosyncratic reaction to phenytoin.

1.4 Rate-Controlled Drug Delivery: Principles and Applications

Rate-controlled drug delivery systems represent a sophisticated approach to pharmaceutical therapy that aims to maintain drug concentrations within the therapeutic window for extended periods through precise control of drug release kinetics. Unlike conventional immediate-release dosage forms that exhibit first-order release kinetics characterized by exponentially declining release rates, rate-controlled systems are designed to achieve zero-order or near-zero-order release kinetics, where the amount of drug released per unit time remains constant regardless of the amount remaining in the system. This fundamental difference in release behavior translates into significant therapeutic advantages, particularly for drugs with narrow therapeutic indices, short biological half-lives, or applications where maintaining constant drug levels is critical for optimal efficacy.

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The mechanisms employed to achieve rate-controlled drug release are diverse and can be broadly classified into several categories based on the principle governing release. Diffusion-controlled systems rely on the movement of drug molecules through a polymeric barrier by concentration-gradient-driven diffusion, described by Fick's laws. These systems can be further subdivided into reservoir devices, where the drug core is surrounded by a rate-controlling membrane, and matrix devices, where the drug is dispersed throughout a polymer matrix. Reservoir systems can achieve nearly zero-order release kinetics when the drug concentration in the core remains constant and well above its solubility limit, as the release rate is then determined solely by the permeability characteristics of the membrane and the surface area available for diffusion. Matrix systems, in contrast, typically exhibit release kinetics that deviate from zero-order, following square-root-of-time kinetics according to the Higuchi model, although clever design strategies can approximate zero-order release.

1.5 Factorial Design of Experiments in Pharmaceutical Development

Factorial design of experiments represents a systematic and efficient approach to investigating the effects of multiple variables on response outcomes, enabling pharmaceutical scientists to optimize formulations while minimizing the number of experimental runs required. This methodology, rooted in statistical theory and popularized by Sir Ronald Fisher in agricultural research, has become indispensable in pharmaceutical development where multiple formulation variables typically influence product performance characteristics. Traditional one-factor-at-a-time experimentation, where variables are changed sequentially while holding others constant, is inefficient and fails to detect interaction effects between variables. Factorial designs overcome these limitations by varying multiple factors simultaneously according to a structured experimental plan, allowing estimation of main effects, interaction effects, and optimal conditions with fewer experiments than would be required using conventional approaches.

LITERATURE REVIEW

3.2 Evolution of Controlled Release Drug Delivery Systems

The concept of controlled drug delivery has evolved dramatically over the past six decades, transforming from simple sustained-release coatings to sophisticated responsive systems capable of modulating release in response to biological signals. Early pioneering work in the 1950s and 1960s established fundamental principles of diffusion-controlled and dissolution-controlled release that continue to underpin modern formulation design [31]. These initial systems, while primitive by contemporary standards, demonstrated that prolonging drug release could reduce dosing frequency and minimize plasma concentration fluctuations, establishing the therapeutic rationale for controlled delivery.

The theoretical foundations of diffusion-controlled drug release were rigorously developed through mathematical modeling that related release rates to fundamental physicochemical parameters including drug solubility, diffusion coefficients, partition coefficients, and geometric characteristics of delivery devices [32]. Higuchi's seminal work on release kinetics from matrix systems provided equations that enabled prediction of release rates and rational design of formulations to achieve desired release profiles [33]. These mathematical models demonstrated that square-root-of-time release kinetics are characteristic of matrix systems where drug diffuses through a polymer network or through pores created by dissolved drug, while zero-order release requires more sophisticated designs such as reservoir systems with constant surface area or osmotic pumps.

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Matrix systems, where drug is dispersed throughout a polymer carrier, gained widespread acceptance due to their simpler manufacturing and inherently safer failure mode [36]. Hydrophilic matrix systems utilizing polymers such as hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and various carbomers swell upon contact with aqueous media, forming a gel layer through which drug diffuses [37]. The release mechanism from hydrophilic matrices is complex, involving simultaneous polymer hydration and swelling, drug dissolution, and drug diffusion through the gel layer, with the thickness and properties of the gel layer changing continuously as the system hydrates [38]. Lipophilic matrix systems using waxes, fats, or hydrophobic polymers provide an alternative approach where drug release is controlled by diffusion through a relatively inert, non-swelling matrix [39].

Osmotic delivery systems represent a distinct class of controlled-release devices that utilize osmotic pressure as the driving force for drug release, offering advantages in terms of release rate independence from pH and hydrodynamic conditions [40]. The elementary osmotic pump consists of a semi-permeable membrane surrounding a core containing drug and osmotic agent, with water influx driven by osmotic pressure building up hydrostatic pressure that forces drug solution out through a precisely laser-drilled orifice [41]. More sophisticated designs including the push-pull osmotic pump and the controlled-porosity osmotic pump have been developed to accommodate drugs with varying solubilities and to achieve more complex release patterns [42]. Osmotic systems have been successfully commercialized for numerous drugs requiring precise, prolonged delivery, though their relatively high manufacturing complexity and cost limit their application primarily to high-value therapeutics.

3.3 Stimuli-Responsive and Smart Drug Delivery Systems

The evolution from passive, time-based controlled release to active, stimuli-responsive or "smart" delivery systems represents a paradigm shift toward delivery platforms that can adapt their behavior based on biological signals [50]. These intelligent systems respond to specific stimuli including pH, temperature, enzymes, redox potential, light, magnetic fields, or biomolecular recognition events, modulating drug release in response to physiological or pathological conditions [51]. The concept of stimuli-responsive delivery aligns with the broader goal of personalized medicine by enabling drug release that responds to individual patient needs and disease states.

pH-responsive drug delivery systems exploit the pH gradients present throughout the body, including the pH variation along the gastrointestinal tract (pH 1-2 in stomach, 5-7 in small intestine, 6-7 in colon) and the lower pH characteristic of tumor microenvironments, inflamed tissues, and intracellular compartments [52]. pH-responsive polymers contain ionizable groups that undergo protonation or deprotonation in response to pH changes, resulting in alterations in polymer solubility, swelling, or conformation that trigger drug release [53]. Enteric polymers such as Eudragit L100, Eudragit S100, and hydroxypropyl methylcellulose acetate succinate have been widely used for colon-targeted delivery, remaining intact in the acidic stomach environment but dissolving at the higher pH of the small intestine or colon [54]. More sophisticated pH-responsive systems utilize polymers with carefully tuned pKa values to achieve precise triggering at specific pH thresholds relevant to the intended application.

Methodology

Overview of Research Methodology

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The research work will be conducted in a systematic, phased approach to ensure comprehensive development and optimization of the enzyme-activated rate-controlled drug delivery system for phenytoin. The plan encompasses material procurement and characterization, formulation development, factorial design-based optimization, comprehensive evaluation, and stability assessment. The work will progress sequentially through distinct phases, with each phase building upon the knowledge and materials generated in previous phases.

Proposed work

Phase 1: Preformulation Studies and Material Selection (Duration: 4-6 weeks)

This initial phase involves procurement and characterization of phenytoin and all excipients including enzyme-responsive polymers, rate-controlling polymers, and other formulation components. Preformulation studies will include determination of drug-excipient compatibility through differential scanning calorimetry and Fourier-transform infrared spectroscopy, assessment of phenytoin solubility in various media at different pH values, determination of partition coefficient, and evaluation of polymorphic form. Enzyme-responsive materials will be screened for susceptibility to target enzymes through preliminary degradation studies. Selection of optimal enzyme-substrate pairs and rate-controlling polymers will be finalized based on compatibility, performance characteristics, and commercial availability.

Phase 2: Development of Rate-Controlled Delivery Systems (Duration: 6-8 weeks)

This phase focuses on developing the rate-controlling component of the delivery system. Various formulation approaches including hydrophilic matrix tablets, hydrophobic matrix systems, reservoir systems with rate-controlling membranes, and osmotic systems will be prepared and evaluated. Preliminary optimization of polymer type, polymer concentration, drug loading, and processing parameters will be conducted through systematic experimentation. In vitro dissolution studies will be performed to assess release kinetics and identify formulations exhibiting controlled release suitable for integration with enzyme-responsive elements. Mathematical modeling of release data will identify predominant release mechanisms.

Phase 3: Development of Enzyme-Responsive Systems (Duration: 6-8 weeks)

Parallel to rate-controlled system development, enzyme-responsive formulations will be prepared incorporating enzyme-cleavable polymers or linkages. Various architectural approaches including enzyme-responsive coatings, matrices with enzyme-cleavable crosslinks, and prodrug conjugates will be investigated. The responsiveness of these systems to enzymatic triggers will be evaluated through dissolution studies conducted in media with and without specific enzymes at various concentrations. The sensitivity, specificity, and kinetics of enzyme-triggered release will be characterized to identify the most promising enzyme-responsive mechanisms for integration with rate-controlled systems.

WORK AND RESULTS

6.1.4 Factorial Design

A 2^3 full factorial design was employed to systematically investigate the effect of three independent variables on the characteristics of enzyme-activated rate-controlled phenytoin delivery system. The independent variables selected were:

- **Factor A:** HPMC K100M concentration (X_1): 20% and 40% w/w
- **Factor B:** Pectin concentration (X_2): 10% and 30% w/w
- **Factor C:** Drug loading (X_3): 25% and 35% w/w

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The dependent variables (responses) evaluated were:

- **Y_1:** Time for 50% drug release in pH 6.8 without enzyme (t_{50})
- **Y_2:** Drug release at 12 hours in pH 6.8 with pectinase enzyme
- **Y_3:** Enzyme sensitivity ratio (release with enzyme/release without enzyme at 8 hours)

Eight formulations (F1-F8) were prepared according to the factorial design matrix as shown in Table 6.1.

Table 6.1: Factorial Design Layout and Formulation Composition

Formulation	Factor A: HPMC K100M (% w/w)	Factor B: Pectin (% w/w)	Factor C: Drug Loading (% w/w)	Lactose (% w/w)
F1	20 (-1)	10 (-1)	25 (-1)	43
F2	40 (+1)	10 (-1)	25 (-1)	23
F3	20 (-1)	30 (+1)	25 (-1)	23
F4	40 (+1)	30 (+1)	25 (-1)	3
F5	20 (-1)	10 (-1)	35 (+1)	33
F6	40 (+1)	10 (-1)	35 (+1)	13
F7	20 (-1)	30 (+1)	35 (+1)	13
F8	40 (+1)	30 (+1)	35 (+1)	0

Note: Values in parentheses indicate coded levels; all formulations contain magnesium stearate (1%) and talc (1%)

6.1.5 Evaluation of Formulated Tablets

Physical Characterization: All formulated tablets were evaluated for physical parameters including weight variation (n=20 tablets), thickness (n=10 tablets using digital vernier caliper), hardness (n=6 tablets using Monsanto hardness tester), and friability (n=10 tablets using Roche friabilator at 25 rpm for 4 minutes).

Drug Content Uniformity: Ten tablets were randomly selected, crushed, and powder equivalent to 100 mg phenytoin was accurately weighed and dissolved in methanol. The solution was filtered, diluted appropriately with phosphate buffer pH 6.8, and analyzed spectrophotometrically at 257 nm.

In Vitro Dissolution Studies: Dissolution studies were performed using USP Type II (paddle) apparatus (Electrolab TDT-08L) at 37±0.5°C with paddle rotation speed of 50 rpm. For enzyme-free studies, 900 mL of phosphate buffer pH 6.8 was used as dissolution medium. For enzyme-activated studies, pectinase enzyme was added to the dissolution medium to achieve a concentration of 0.5% w/v. Samples (5 mL) were withdrawn at predetermined time intervals (1, 2, 4, 6, 8, 10, and 12 hours), filtered through 0.45 μm membrane filter, and replaced with equal volume of fresh medium maintained at the same temperature. The samples were analyzed spectrophotometrically at 257 nm after appropriate dilution. All dissolution studies were performed in triplicate.

Release Kinetics: The dissolution data were analyzed using various kinetic models including zero-order (cumulative % drug release vs. time), first-order (log % drug remaining vs. time), Higuchi model (cumulative % drug release vs. square root of time), and Korsmeyer-Peppas

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model (log cumulative % drug release vs. log time). The model with the highest correlation coefficient (r^2) was considered the best fit.

6.1.6 Scanning Electron Microscopy

Surface morphology of selected formulations before and after dissolution was examined using scanning electron microscope (JEOL JSM-6360). Samples were mounted on aluminum stubs using double-sided adhesive tape and sputter-coated with gold under vacuum before examination at appropriate magnification and accelerating voltage.

6.1.7 FTIR Spectroscopy

FTIR spectra of pure drug, polymers, physical mixtures, and optimized formulation were recorded using FTIR spectrophotometer (Shimadzu FTIR-8400S) in the range of 4000-400 cm^{-1} with KBr pellet method to detect any drug-polymer interaction.

6.1.8 Differential Scanning Calorimetry

DSC analysis of pure phenytoin, polymers, and optimized formulation was performed using DSC (Mettler Toledo DSC 823e). Samples (3-5 mg) were sealed in aluminum pans and heated from 30°C to 300°C at a heating rate of 10°C/min under nitrogen atmosphere (flow rate 50 mL/min).

6.1.9 Statistical Analysis

The factorial design data were analyzed using Design-Expert® software version 13.0. Analysis of Variance (ANOVA) was performed to identify significant effects and interactions. Response surface plots and contour plots were generated to visualize the relationship between factors and responses. Statistical significance was considered at $p < 0.05$.

6.2 Results and Discussion

Table 6.3: Physical Parameters of Formulated Tablets

Formulation	Average Weight (mg)	Thickness (mm)	Hardness (kg/cm²)	Friability (%)	Drug Content (%)
F1	298.4 ± 3.6	4.12 ± 0.08	5.4 ± 0.3	0.42	98.7 ± 1.4
F2	301.2 ± 4.1	4.18 ± 0.06	6.2 ± 0.4	0.38	99.3 ± 1.2
F3	299.8 ± 3.8	4.15 ± 0.07	5.8 ± 0.3	0.45	97.8 ± 1.6
F4	302.6 ± 4.3	4.21 ± 0.09	6.8 ± 0.5	0.34	98.4 ± 1.3
F5	297.8 ± 3.4	4.09 ± 0.05	5.2 ± 0.4	0.48	99.1 ± 1.5
F6	300.4 ± 3.9	4.16 ± 0.08	6.4 ± 0.3	0.36	98.9 ± 1.4
F7	298.9 ± 3.7	4.13 ± 0.06	5.6 ± 0.4	0.44	97.6 ± 1.7
F8	303.1 ± 4.5	4.24 ± 0.10	7.1 ± 0.5	0.31	98.2 ± 1.5

All values represent mean ± standard deviation (n=3 to 20 depending on test)

6.2.3 In Vitro Dissolution Studies

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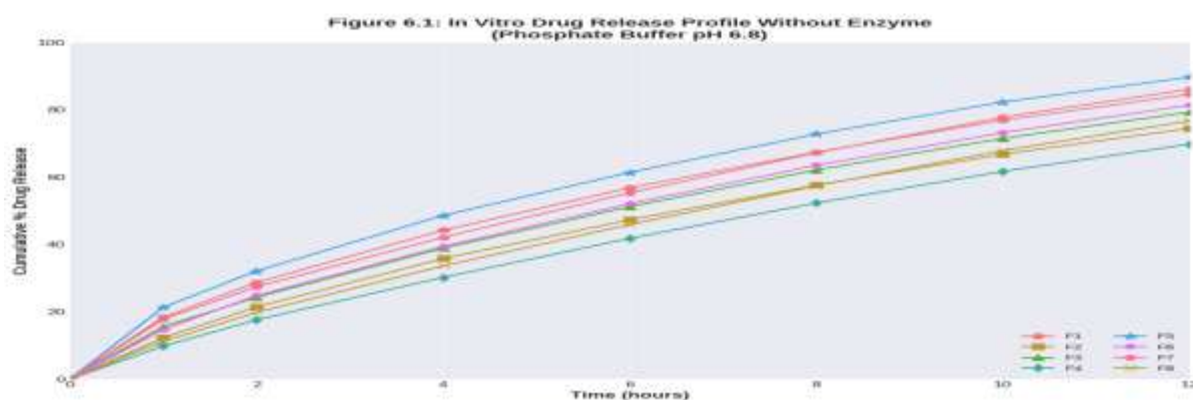

Dissolution Without Enzyme: The cumulative drug release profiles from all eight formulations in phosphate buffer pH 6.8 without enzyme are presented in Table 6.4 and graphically depicted in Figure 6.1.

Table 6.4: Cumulative % Drug Release Without Enzyme in Phosphate Buffer pH 6.8

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8
1	18.4±1.2	12.3±0.9	15.6±1.1	9.8±0.7	21.3±1.4	14.7±1.0	17.8±1.3	11.2±0.8
2	28.7±1.6	21.4±1.3	24.3±1.5	17.6±1.2	32.1±1.8	24.8±1.4	27.4±1.6	19.8±1.3
4	44.2±2.1	35.8±1.9	38.9±2.0	30.2±1.7	48.6±2.3	39.4±2.0	42.1±2.2	33.6±1.8
6	56.8±2.5	47.3±2.3	51.2±2.4	41.8±2.1	61.4±2.7	52.1±2.5	55.3±2.6	45.9±2.2
8	67.4±2.8	57.6±2.6	62.1±2.7	52.3±2.5	72.8±3.0	63.5±2.8	67.2±2.9	57.4±2.6
10	76.9±3.1	66.8±2.9	71.5±3.0	61.7±2.8	82.3±3.3	73.2±3.1	77.8±3.2	67.9±2.9
12	84.6±3.4	74.5±3.2	79.3±3.3	69.8±3.0	89.7±3.6	81.4±3.4	86.1±3.5	76.7±3.2

All values represent mean ± standard deviation (n=3)

The dissolution data revealed that increasing HPMC K100M concentration (Factor A) significantly retarded drug release, as evidenced by comparing formulations with 40% HPMC (F2, F4, F6, F8) versus those with 20% HPMC (F1, F3, F5, F7). Similarly, higher pectin concentration (Factor B) also contributed to slower release. Drug loading (Factor C) showed a positive correlation with release rate, with 35% drug loading formulations releasing drug faster than 25% loading formulations, likely due to increased drug concentration gradient and higher porosity after drug dissolution.

Dissolution With Pectinase Enzyme: The cumulative drug release profiles in presence of 0.5% w/v pectinase enzyme are presented in Table 6.5 and graphically depicted in Figure 6.2.

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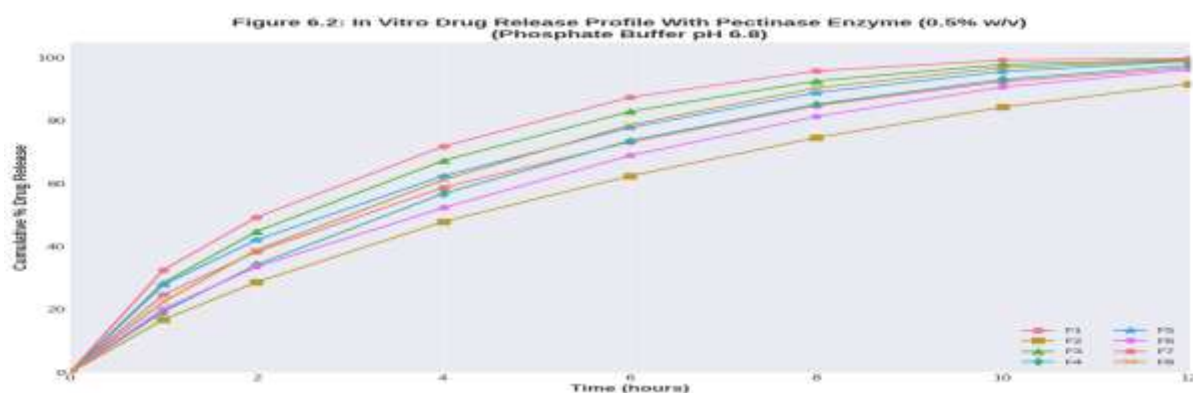

Table 6.5: Cumulative % Drug Release with Pectinase Enzyme (0.5% w/v) in Phosphate Buffer pH 6.8

Time (h)	F1	F2	F3	F4	F5	F6	F7	F8
1	24.6±1.5	16.8±1.1	28.4±1.7	19.3±1.3	27.9±1.6	20.1±1.4	32.6±1.9	22.4±1.5
2	38.4±2.0	28.7±1.7	44.8±2.3	34.2±1.9	42.1±2.2	33.6±1.9	49.3±2.5	38.9±2.1
4	58.7±2.7	47.9±2.4	67.3±3.0	56.8±2.7	62.4±2.9	52.3±2.6	71.8±3.2	61.2±2.8
6	73.2±3.2	62.4±2.9	82.9±3.6	73.6±3.3	77.8±3.4	68.9±3.1	87.4±3.8	78.6±3.5
8	84.8±3.6	74.6±3.3	92.6±4.0	85.2±3.7	88.9±3.8	81.3±3.6	95.8±4.2	90.4±3.9
10	92.4±3.9	84.3±3.7	97.8±4.2	93.1±4.0	95.6±4.1	90.7±3.9	99.2±4.3	96.8±4.1
12	96.8±4.1	91.7±3.9	99.4±4.3	97.6±4.2	98.9±4.2	96.2±4.1	99.8±4.3	99.1±4.2

All values represent mean ± standard deviation (n=3)

The presence of pectinase enzyme significantly enhanced drug release from all formulations, particularly those containing higher pectin concentrations. This enzyme-triggered release confirms the successful incorporation of enzyme-responsive behavior in the delivery system. Formulations F3 and F7, containing 30% pectin with 20% HPMC, showed the most dramatic enzyme-responsive behavior, achieving nearly complete release within 12 hours in enzyme-containing medium compared to approximately 79% and 86% respectively without enzyme.
of release.

CONCLUSION

The present investigation successfully achieved its primary objective of designing, developing, and optimizing an enzyme-activated rate-controlled drug delivery system for phenytoin using systematic factorial design methodology. The research demonstrates the feasibility of integrating enzyme-responsive and rate-controlled functionalities within a single delivery

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platform, addressing the significant therapeutic challenges associated with phenytoin's narrow therapeutic index and complex pharmacokinetics.

1. Enzyme-activated rate-controlled drug delivery systems for phenytoin can be successfully developed using combinations of HPMC K100M for rate control and pectin for enzyme responsiveness.
2. Factorial design methodology provides an efficient, systematic approach to optimizing such complex formulations, enabling identification of critical factors and their effects while minimizing experimental resources.
3. HPMC K100M concentration is the primary determinant of baseline controlled release, pectin concentration governs enzyme responsiveness, and drug loading influences both aspects of performance.
4. The optimized formulation (35% HPMC, 28% pectin, 30% drug) achieves sustained 12-hour release with significant enzyme-triggered enhancement, demonstrating successful integration of dual functionalities.
5. Drug release follows Korsmeyer-Peppas kinetics with non-Fickian transport, shifting toward erosion control in the presence of enzyme.
6. The delivery system exhibits acceptable physical properties, chemical stability, and robust performance, supporting its potential for further development.
7. The enzyme-responsive concept validated with pectinase could be adapted for various therapeutic applications through selection of appropriate enzyme-substrate pairs.

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