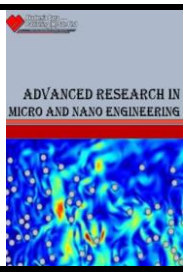
Penerbit Akademia Baru

Journal of Advanced Research in Micro and Nano Engineering

Journal homepage:
https://www.akademiabaru.com/submit/index.php/armne/index
ISSN: 2756-8210



Kinetic and Mechanism of Zerumbone Release from Cross-linked Gelatin-Zeolite Y Hybrid for Oral Anticancer Drug Delivery

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ARTICLE INFO

Article history:
Received 16 January 2024
Received in revised form 20 February 2024
Accepted 23 March 2024
Available online 30 April 2024

Keywords:
Gelatin; zeolite Y; zerumbone; natural chemotherapy; oral controlled release

ABSTRACT

A hybrid of zeolite Y-gelatin film as an oral dosage form for the natural anticancer drug was achieved by homogenously incorporating the drug-loaded zeolite Y into the gelatin solution. Drug ability was analyzed using computational and experimental approaches, drug encapsulation efficiency via the BET method, and possible interactions by FTIR analyses. Zerumbone released was done in both pH 1.2 and pH 7.4 mimicking the human gastrointestinal tract conditions for 24 hrs and subjected to kinetics study via suitable mathematical models to determine what governs the drug release with the results indicating that a sustained delivery of once-daily oral dosage form could be achieved.

1. Introduction

Cervical cancer is one of the most common cancers among Malaysian women and despite being potentially preventable, this disease is known as a 'silent killer' as most women do not realize they have the disease earlier as the symptoms mostly tend to appear in later stages. Moreover, the death rate is more than two times higher than in other Asian and Western countries even with the practice of screening programs and immunizations, triggers worry among health and medical practitioners [1-3]. Treatments for cervical cancer is highly known for the use of powerful synthetic cytotoxic chemotherapy drugs to kill fast-growing cancerous cell [4] is an aggressive form of chemical drug therapy had to cause endless serious side effects that can severely impact the quality of life [5]. Therefore, notable opportunities for new cancer drug discovery had undergone a significant change over the last decade [6] leading to the potential of natural products [7] as anticancer drugs with the first anticancer drug recognized in the 1950s by U.S National Cancer Institute.

Most chemo drugs can be released intravenously (IV) or orally, except for cervical cancer treatment that only necessitates IVs, which is quite challenging and burdensome for cancer patients as it can only be done at cancer clinics or appointed hospitals. This method required patients to sit for hours to finish the treatment is unacceptable, hence modern-day chemo drug delivery should

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https://doi.org/10.37934/armne.18.1.3243

utilize the controlled release technology via the oral route for drug administration [8,9]. It greatly offers advantages including prolonged drug delivery within the therapeutic range, single dosage daily, self-administer scheduled dose at home, undisturbed patient's daily routines as well as reducing the number of resources needed for the care services relating to chemotherapy, therefore much more convenient, economical and manageable [1].

Nature is the best source of drugs in terms of overcoming cell toxicity and adverse reaction as well as improving chemotherapy treatment efficiency [7]. Medicinal plants and herbs were practiced since ancient times to help humans pursue better health and treat ailments. Plant-derived anticancer agents have dramatically changed conventional chemotherapy treatment as there are much simpler, safer, eco-friendly, and low cost of production with faster and less toxic sides effect [10]. Zerumbone (ZER) is a bioactive compound isolated from the rhizome of ginger from the Zingiberaceae family and can be found abundantly in rhizomes of *Zingiber Zerumbet (L) Smith* and known as *lempoyang* in Malaysia [11]. Anticancer properties of zerumbone have been reported in various studies at different concentrations and doses, both in vitro and in vivo. Studies also show that zerumbone exhibits antiproliferative properties, which can retard the spread of several malignant cans into surrounding tissues with minimal effect on normal cells [12].

Drug carriers must be inert and compatible when exposed to the living tissue and/or bodily fluids and formulations designed for prolonged delivery required high initial drug loading. Among diverse groups of materials, biodegradable polymers are the most successful drug carriers due to their biocompatibility and biodegradability (avoiding toxic effects on the biological system), flexibility in design, and low cost [13]. Among all biopolymers, gelatin can be used for sustained drug delivery. Due to its biocompatibility and biodegradability, it can easily be excreted through the usual metabolic process by the body. To accomplish prolonged (extended) release, the formulation requires enormous initial drug loading. Hence, the total dose per delivery also increased significantly and resulted in an unlikely larger size of dosage form that is impossible to swallow. Formulation with higher drug content also shows higher release rate constant, as they mostly ended up with initial burst release and exponentially increased the drug concentration in blood plasma several times than normal dose (induced toxicity), limiting the amount of drug that can be practically incorporated into such system. As a single component, gelatin can exhibit undesirable mechanical properties and does not have the advantages of drug encapsulation [14], and limit its potential as a drug carrier for prolonged drug release [15]. Therefore, zeolite Y a porous aluminosilicate with the advantages of small size and large surface area per unit volume attained the capability of the highest drug loading and drug entrapment but permits faster drug release [16] and is paired together with gelatin to improve their performance. Hence, a proper formulation was developed in this study involving organic-inorganic hybrid (OIH) materials [14, 17,18] namely gelatin (GEL) and porous zeolite Y for oral controlled release of natural anticancer drug zerumbone (ZER) for cervical cancer treatments. In this study, we discussed the drug-ability of ZER and its' release mechanism from the OIH formulation via selected mathematical models.

2. Methodology

ZER crystals obtained from the rhizomes of Zingiber Zerumbet (L) Smith were isolated and purified according to methods in earlier studies [19] and were first loaded into porous zeolite Y with continuous stirring and dried at room temperature. Then, 10 grams of GEL powder was soaked in 50 ml of deionized water and heated to 70 ºC with the addition of glycerin (5 grams). The drug-loaded zeolite Y prepared earlier was mixed into a GEL solution and homogenously stirred before casting into a thin film. The hybrid solution was left to cool in the refrigerator and cut into the desired

dimension before cross-linked with glutaraldehyde (GTA) at 0.2 v/v% into previously cooled sunflower oil (10 ºC for 24 hrs). The cross-linked sample was freed from oil through repeated washing with isopropyl alcohol, and this experiment is repeated for 5,10, and 15 v/v% of ZER concentrations. All samples were kept in a desiccator until further use [20].

Sample characterization is a method of classifying the physicochemical properties of the sample. Samples were subjected to various testing methods such as drug-ability analysis via computational and experimental methods. Computational analysis was achieved by using MARVIN BEAN software Porosity analysis was done via BET analyzer model Quantachrome Autosorb Automated Gas Sorption, where the result of the analysis will give information on surface area and pore characterization before and after drug loading. FTIR analysis was carried out on NICOLET 6700 model to determine the presence of specific chemical groups as well as to determine any interactions that might occur between the components of the hybrid film with the FTIR spectrum presented as plots of intensity versus wavenumber (cm^{-1}).

The drug release analysis was measured by using a UV-VIS spectrophotometer. Approximately 0.5 grams of sample was placed in 250 ml of phosphate buffer saline (PBS) at pH 1.2 and pH 7.4, using an ultrasonic bath sonicator at a speed of 100 rpm and maintained temperature of 37 ºC to mimic the human GIT conditions. 5 ml of aliquots were removed at regular time intervals for 24 hrs and the dissolution media was refilled with fresh buffer after each removal. The aliquots were filtered to ensure no solid was in suspension and the filtered aliquots were analyzed for ZER released at λ= 212 nm at pH 1.2 and λ= 217 nm at pH 7.4 using a UV-Vis spectrophotometer model PERKIN ELMER UV/VIS Lambda 20. The experiments were conducted in triplicate and the absorption values, *A*, were averaged.

Kinetics study was done by using several mathematical models representing dissolution drug profile as a function of time-related to the amount of drug dissolved from the dosage form, which are zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas kinetics model. ZER release data obtained from UV-Vis were plotted into the above-mentioned mathematical models with the correlation coefficient, r^2 value calculated from each kinetic model were used as an indicator of the best fit model, and the drug release kinetic parameter namely diffusion coefficient, *n* calculated from the slope of Korsmeyer -Peppas models describes the mechanism of drug released.

3. Results

3.1 Physicochemical Properties of ZER

The physical properties of the drug are very crucial to determine its bioavailability of the drug. The software analysis described ZER as an unsaturated cyclic aliphatic compound with a chemical formula of $C_{15}H_{22}O$, 218.16 Da of molecular weight with an estimated ring size of 11Å, possess logP= 5.34 and logD= 4.72 (logP≠logD) and having oxygen as a polar atom with PSA value of 17 Å has categorized ZER as a small molecular weight drug (MW<500 Da) with ability to diffuse through the lipid-based cell membrane and can be transported in aqueous media like blood and intracellular fluid and suitable for the oral route as main delivery path according to Biopharmaceutics Classification System (BCS).

3.2 Incorporation of ZER into Zeolite Y

The adsorption-desorption isotherms of zeolite Y (Figure 2) shows to exhibits Type I isotherms, which is characteristic of microporous materials where nitrogen uptake increased quickly at low relative pressure (P/P_o) by adsorption in micropores and external surface. After the monolayer

adsorption at low P/P_o, the nitrogen uptake was almost constant, and a small hysteresis loop at high relative pressure ($P/P_o > 0.45$) was formed in the adsorption-desorption curves suggested some narrow mesopores in zeolite Y, indicating that zeolite Y exhibits both Types I and Type IV isotherms which are micro and mesoporous size-structured material.

BET pore analysis complemented the aforementioned analysis as zeolite Y exhibits pore size distributions at 14.55 Å, 38.8 Å, and 156.6 Å. According to the IUPAC classification of porosity, the pore opening of zeolite Y was classified into micro-porous material with a pore size less than 20 Å, and mesoporous material with a pore size distribution between 20-500 Å. The pore volume of zeolite Y before and after ZER loading was tabulated in Table 1, and the mass of ZER incorporated into zeolite Y was determined respectively by Eq. (1) and (2).

$$\mathrm{m} = \rho \mathrm{v} \tag{1}$$

m = Mass of ZER after incorporated into zeolite Y
ρ = Density of ZER (0.888 g/cm^3)
V = Volume of zeolite Y pores that have been incorporated by ZER

$$\text{Encapsulation efficiency} = \frac{\mathrm{M2}}{\mathrm{M1}} \text{ x } 100 \tag{2}$$

M_1= Initial mass of ZER
M_2= Mass of ZER loaded in zeolite Y pores

Table 1
Pore size distribution and encapsulation efficiency of ZER

Conc	M_1	Pore distribution	Vb	Va	V_{ZER}	M_2	EE
µM	G	Å	cm^3	cm^3	cm^3	g	%
	0.0109	14.55	0.141	0.147	0.006	0.006	51.4
100		38.80	0.111	0.114	0.004	0.003	28.4
		156.60	0.061	0.063	0.003	0.002	20.2
					Total encapsulation		100

From the calculation obtained in Table 1, 100 % of ZER was found to incorporate into porous zeolite Y, with a slight change of the hysteresis loop that could be observed at $P/P_o > 0.45$ after drug loading. Zeolite Y with a higher silica ratio (Si/Al ratio = 5.1) is prone to the adsorption of less polar molecules accountable towards high ZER loading. Moreover, the above-mentioned analyses have classified ZER as a small molecular weight drug that could easily enter the pore opening of zeolite Y and scattered within the pores, with more than 50 % of ZER found residing near the pore opening as compared to mesopore pore size distribution. The size of zeolite as nanoparticles has a great impact on its drug loading i.e., small nanoparticles have a large surface area [21]. Hence, drug loading into the porous system can be maximized by understanding the nature of host-guest chemistry, and factors such as polarity, surface area, and pore diameter that influence the loading of drugs into the system.

3.3 Fourier Transform Infra-Red Analysis

The absorption bands of pure GEL in the infrared spectra were situated in the amide band regions. GEL in nature resembled a broad peak at 3261cm^{-1} signifies Amide A (-OH and -NH groups), a sharp peak of C=O stretching (Amide I) at 1630 cm^{-1}, N-H bending (Amide II) at 1540 cm^{-1}, with several peaks arise from 1460 -1200 cm^{-1} related to C-H deformation (Amide III) (Figure 1a). Formation of GEL film (Figure 1b) resulted in shifted amides peaks to a higher wavenumber, Amide A peaks shifted to ~3300cm^{-1}, Amide I to 1642 cm^{-1} and Amide II to 1555 cm^{-1}, with a strong intense peak found at 1038 cm^{-1} presumably related to the possible interactions arising between glycerin and GEL during film forming process [22]. The inorganic component, zeolite Y (Figure 1c) provides information with a strong peak at 1059 cm^{-1} that corresponds to Si-O-Al and Si-O-Si bonds with ZER-zeolite Y (Figure 1e) showing a prominent peak ~1640 cm^{-1} assigned to the carbonyl group (C=O) of ZER. The shift of the C=O band from 1659 cm^{-1} to 1640 cm^{-1} upon ZER loading into zeolite Y is observed presumably due to the effect of hydrogen bonding with zeolite Y.

Upon incorporation of zeolite Y with GEL (Figure 2b), zeolite Y-related peaks at 1059 cm^{-1} reduced its peak intensity and shifted to 1068 cm^{-1}, generally due to hydrogen bonding with charged ions NH^{3+} and COO^{-} belonged to GEL, and similar outcome observed in the hydroxyl group (-OH) stretching region of zeolite Y indicated homogenous blend without any phase separation between the two components [23]. Hydrogen bonding between ZER-GEL (Figure 2d) was observed at 1640 cm^{-1} with overlapped Amide I and shifted bending (Amide II) to 1559 cm^{-}1. GTA (Figure 2e) presented a broad peak at 3380 cm^{-1} due to the stretching vibration of water, CH_2 vibration of aldehyde close to 2755 cm^{-1}, C=O at 1640 cm^{-1}, and CH_2 bending vibrations occur at 1442 cm^{-1} and 1334 cm^{-1}. The group of peaks between 1200 cm^{-1} - 900 cm^{-1} is due to C-O vibrations illustrated by the hydration of GTA [24]. It was observed that the intensity of the hybrid film increased after cross-linked at 1639 cm^{-1} (Figure 4g) mainly contributed by the formation of the imine linkages (C=N) from GEL-GTA cross-linking chain overlapped with the C=O stretching from the GEL film. The intensity of the absorption band at 1558 cm^{-1} was also increased due to ethylenic (C=C) groups overlapping with the N-H bend groups from GEL, which is similar to the hydroxyl groups. This is evidenced by the presence of intra or inters hydrogen overlapped with –NH and –OH groups of GEL [25].

3.4 ZER Release from Zeolite Y

The release profiles at 5, 10, and 15 v/v% of ZER loadings were shown in Figure 3 in both acidic (pH 1.2) and basic (pH 7.4) respectively. A nearly complete release of ZER is observed after~ 90 minutes, with 15 v/v% showing the fastest release followed by 10 and 5 v/v% with more than 50% of the ZER being released within the first hour. The release data were fitted using a first-order kinetic model for a porous matrix that showed a release rate constant of 0.118, 0.146, and 0.152 min^{-1} as increasing the ZER loading. Faster drug release is presumably because of ZER molecules present close to the pore opening of zeolite Y. In particular, ZER possessed weak bonding with zeolite Y as well as high solvation towards the release solutions also contributes to faster release.

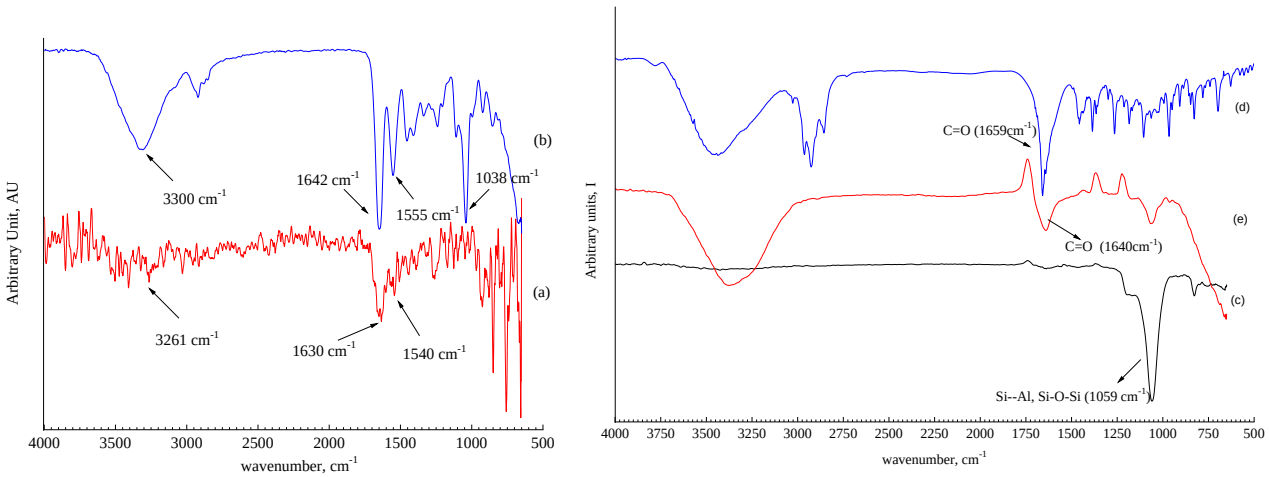


Fig. 1. FTIR spectra of (a) GEL and (b) GEL film, (c) zeolite Y, (d) ZER crystal, and (e) ZER-zeolite Y

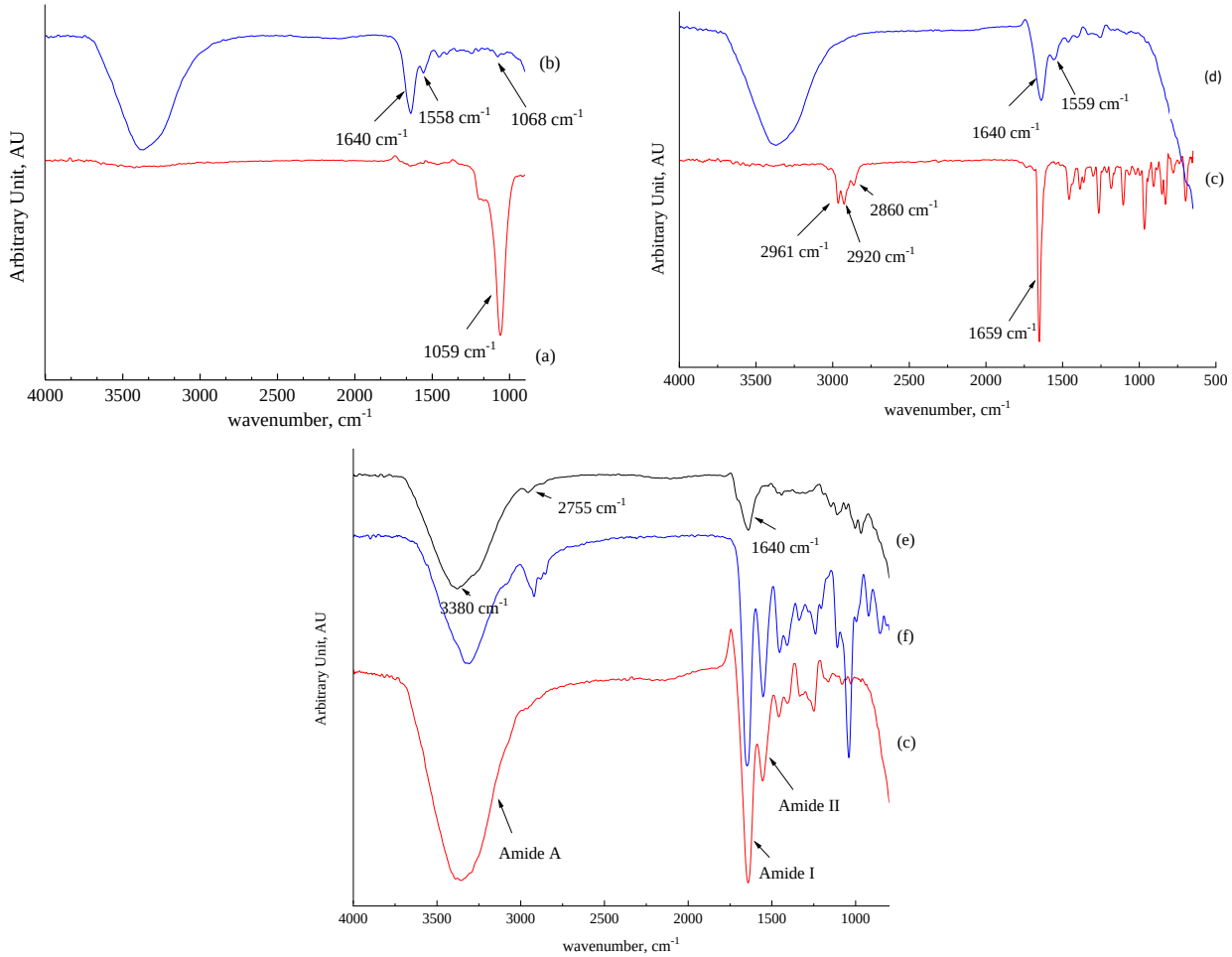


Fig. 2. FTIR spectra of (a) zeolite Y, (b) zeolite Y-GEL, (c) ZER crystal, and (d) ZER-GEL, (e) GTA, (f) ZER-ZEO Y/GEL, and (g) cross-linked ZER-ZEO Y/GEL film

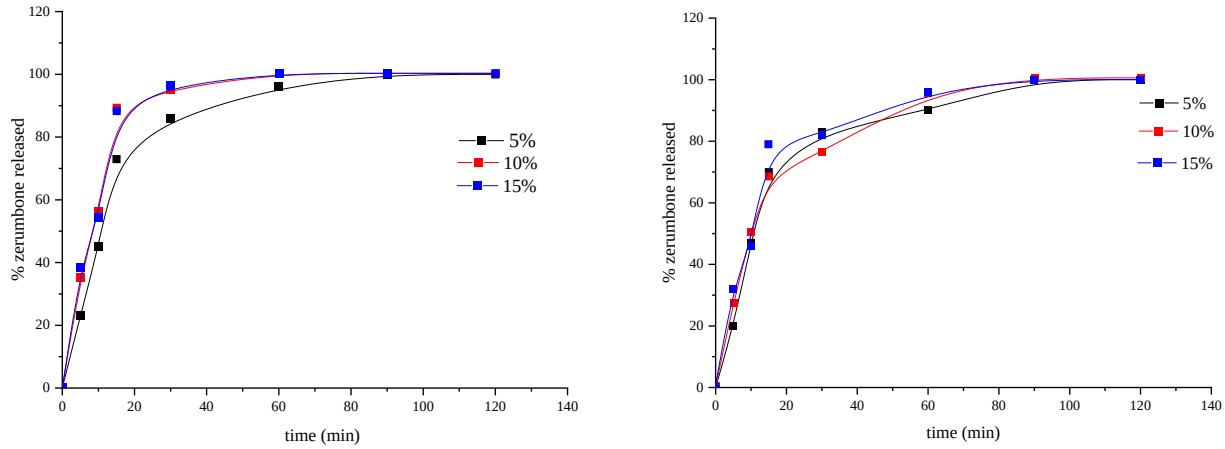


Fig. 3. ZER release from zeolite Y at (a) pH 1.2 and (b) pH (7.4) at ZER loadings 5-15 v/v%

3.5 ZER Release from OIH Film

The released characteristics of ZER from cross-linked zeolite Y-GEL hybrid films were investigated in both acidic (pH 1.2) and basic (pH 7.4) conditions that mimic the human gastrointestinal tract for 24 hours at a maintained temperature of 37 ºC. The measured percentage of drug release in both dissolution media presented in Table 2 indicates a significant increase in ZER release with increasing drug concentrations, interestingly, more than 90% of ZER is released from the composites at pH 1.2 within 24 hours as compared to ZER released at pH 7.4. This suggests that the ionic interaction of the hybrid films easily broken at acidic pH, leading to more ZER being released rapidly at the upper GIT (stomach area) as compared to the small intestines (i.e., the higher affinity of GEL towards acidic pH as compared to alkaline).

However, a similar release pattern of ZER with the first initial burst release followed by slower release within 8 to 10 hours and fast release as reaching 24 hours of drug delivery, subjected to both pH conditions (Figure 4). An initial high release or burst release is mainly due to the position of drug molecules that are hat held just beneath the matrix surface contributes to the burst effect, which is a common effect for a matrix-type system (i.e., homogenous drug dispersion in the matrix) as there was no protective barrier to decelerate drug diffusion [26]. Slower ZER released was mainly due to the slower diffusion of the drug entrapped at the inner part of the cross-linked gel network providing a sturdier gelatinous layer, allowing a longer pathway for drug diffusion into the surrounding media, and faster release was observed after that as polymer chains disentangle with time.

3.6 Kinetics and Mechanism of ZER Release

To investigate the drug release kinetics, release data were plotted into various kinetics models as shown in Figure 5, where models that obtained the highest correlation coefficient (r^2) value indicate the best fit model. Several kinetics models relating to the drug release from the matrix system are described as follows:

Qt versus t	Zero order [27]
log Qt versus t	First order [28]
Qt versus square root of t	Higuchi diffusion model [28]
Qt versus cube root of t	Hixson-Crowell (erosion model) [29]
log % Qt versus log %t	Korsmeyer-Peppas [30]

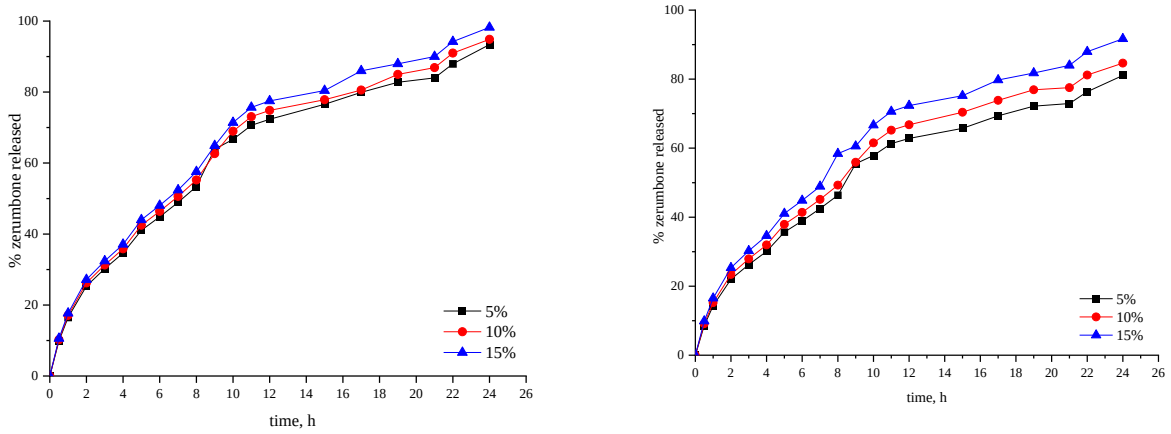


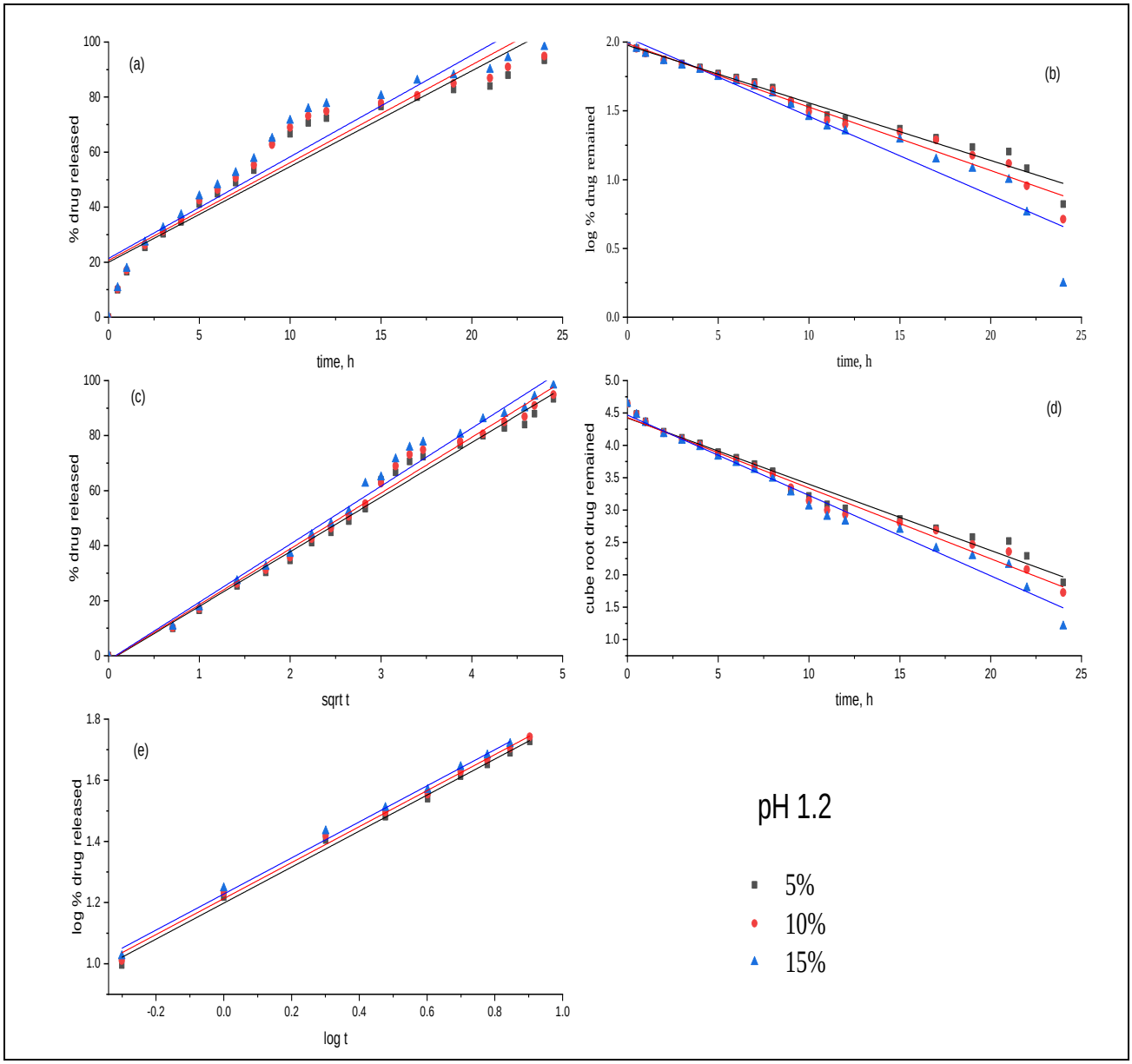
Fig. 4. Cumulative percentage of drug release at 5-15% ZER loadings at (a) pH 1.2 and (b) pH 7.4 from cross-linked zeolite Y-GEL composite

where Qt is the amount of ZER released at the time, t.

ZER release of a zero-order kinetics model defines the process of constant drug release with calculated r^2 = 0.891-.0.892 for pH 1.2 and r^2= 0.8860-0.8929 for pH 7.4. The first order model (i.e., the rate is directly proportional to the concentration of the drug undergoing reaction i.e., the greater the concentration faster the reaction) exhibits r^2=0.9291-0.9739 and r^2=0.9755-0.9836, ZER release from Higuchi models involves both dissolution and diffusion shows r^2= 0.9836-0.9849 and r^2=0.9836-0.9853, Hixson-Crowell cube root law describes ZER drug release from systems with a change in surface area and diameter with r^2=0.9724-0.9784 and r^2=0.9565-0.9722, while Korsmeyer-Peppas model presents r^2= 0.995-0.996 and r^2= 0.993-0.995 at pH 1.2 and pH 7.4 respectively. Most kinetic models (except the zero-order model) present r^2 values closely approaching the trendline, but ZER release is best fitted into the Korsmeyer-Peppas model which described the drug diffusion from a polymeric system. To determine which type of diffusion it follows, the *n* value obtained from the slope of the graph is used to characterize different release mechanisms as tabulated in Table 2, where the release exponent is 0.5 < n < 1, which indicates an anomalous diffusion.

Table 2
The calculated value of 'r^2' and 'n' from selected kinetics models

A	pH	%drug released, t = 24 hrs	Zero Order	First Order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas	*n*
			r^2					
5		93	0.879	0.976	0.982	0.971	0.996	0.5889
10	1.2	94	0.881	0.980	0.984	0.979	0.996	0.5889
15		100	0.881	0.943	0.984	0.981	0.995	0.590
5		81	0.879	0.973	0.982	0.951	0.993	0.608
10	7.4	84	0.880	0.981	0.983	0.960	0.995	0.595
15		91	0.874	0.986	0.982	0.971	0.993	0.608


(a)
% drug released
time, h
(b)
log % drug remained
time, h
(c)
% drug released
sqrt t
(d)
cube root drug remained
time, h
(e)
log % drug released
log t
pH 1.2
5%
10%
15%

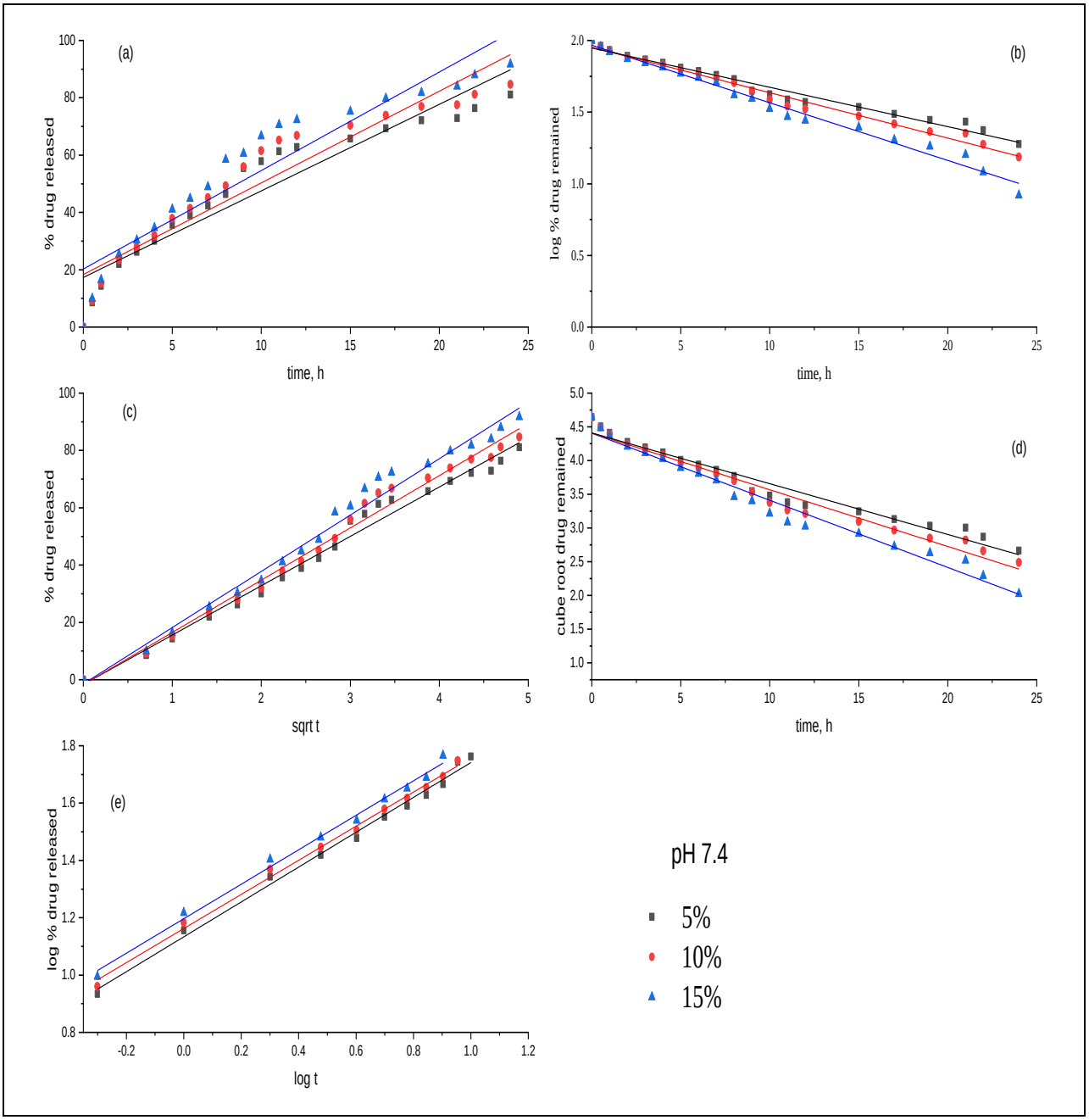


Fig. 5. ZER release from (a) zero order, (b) first order, (c) Higuchi, (d) Hixson-Crowell, and (e) Korsmeyer Peppas kinetic models from both pH 1.2 and pH 7.4 at ZER concentration 5-15v/v%

Due to the hydrophilicity of GEL film, ZER diffusion is due to film swelling followed by erosion of the matrix. On exposure to aqueous fluid, the GEL matrix took up water and started hydrating to form a gel layer, changed from a glassy to a rubbery state associated with the swelling process. A sharp distinction between glassy and rubbery regions is observed as the matrix increases in volume because of swelling in which this phenomenon activates the ZER released. With time, the aqueous media continuously infiltrated into the matrix until a fully hydrated system formed, then proceeded to erosion action [8]. Hence, this indicates that the release of ZER from the zeolite Y-GEL matrix is greatly influenced by the type of dosage form (matrix or reservoir system), pH, nature of the drug, and course of the drug carrier itself. The weak interactions between the inorganic carrier, polymer, and drug components do not help in slowing drug release. Amine as the predominant group in GEL highly interacts with drugs, especially with high molecular weight opposite charged drugs via the

formation of poly-ion complexes (ionic interactions or electrostatic interactions), where these characteristics are not owned by ZER (i.e., ZER is a positively charged drug with low molecular weight). Therefore, we could emphasize that the impact of cross-linking indirectly renders excessive water uptake of the hydrophilic matrix giving rise to a prolonged or sustained ZER delivery of up to 24 hours.

4. Conclusions

This study compares the possible advantages and disadvantages of natural chemotherapy drugs for treating cervical cancer to those of traditional chemotherapy agents (and their side effects). We deduced from the findings that the zeolite Y-GEL hybrid system accomplished sustained oral delivery of a natural anticancer drug (ZER) for 24 hours. Given that GEL is highly susceptible to the pH of the GIT, cross-linking was induced using GTA at a concentration of 0.2 v/v% for 24 hours, representing the ideal circumstance for attaining 24 hours of drug delivery. The drug was diffused from the hybrid system by a reinforced polymeric layer under controlled swelling, and the subsequent erosion process will control the remaining drug distribution. Due to its precise manufacturing, availability of materials calculated kinetics, and mechanism determining drug release, this system is relatively easy to produce on a large scale. With the advancements in the field of drug delivery based on hybrid materials, this work illustrates the feasibility of oral natural chemotherapeutic drug delivery for cervical cancer therapies. We have also worked to develop a hybrid drug delivery system, as finding the perfect drug delivery system remains.

Acknowledgment

The author would like to thank to The Institute of Graduate Studies (IPSis) for financial support, and to the faculty of Applied Sciences, UiTM Shah Alam for the facilities and guidance.

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