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Research Article

Enhancing the Dissolution Performance of PVP-Based Boluses by Graphene Oxide Incorporation for Veterinary Applications.

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Abstract

Drug delivery systems for the food-processing animals in the veterinary field is particularly limited to the disease prevention and growth promotion. Therefore, optimizing dissolution performance of a targeted formulation may be achieved using a performance enhancer together with a commercial binder. In this study, poly(vinyl pyrrolidone) (PVP) was modified with graphene oxide (GO) to form a more stable binder matrix. The dissolution profiles of the formulations with and without the addition of GO into the bolus matrix were examined using two different methods: the continuous dissolution rig based on an artificial saliva and an in vitro dissolution test by the means of Daisy-II incubator. The kinetic models of First Order, Higuchi, Hixson-Crowell and Korsemeyer-Peppas were compared for a best fit of the experimental results obtained using the continuous dissolution rig and Daisy II. The results showed that PVP-containing tablets dissolved more rapidly, whereas PVP-GO combination provided a more controlled and prolonged release profile. Daisy II results in higher rate of dissolution for both formulations compared to the ones in the continuous dissolution rig.

Keywords: Sustained-release, Release kinetics, Ruminants, Graphene oxide, Artificial saliva.

Veterinerlik Uygulamalarında PVP Bazlı Bolusların Grafen Oksit Eklenerek Salım Performansının Artırılması.

Öz

Veterinerlik alanında gıda kaynağı olarak yetiştirilen hayvanlar için ilaç salım sistemleri özellikle hastalık önleme ve büyümeyi teşvik etme ile sınırlıdır. Bu nedenle, hedeflenen bir formülasyonun salım performansının optimize edilmesi, ticari bir bağlayıcı ile birlikte bir performans arttırıcı kullanılarak gerçekleştirilebilir. Bu çalışmada, poli(vinil pirolidon) (PVP), daha kararlı bir bağlayıcı yapısı oluşturmak için formülasyon grafen oksit (GO) ile modifiye edilmiştir. Bolus yapısına GO eklenmiş ve eklenmemiş formülasyonların salım profilleri iki farklı yöntem kullanılarak incelenmiştir: yapay tükürük bazlı sürekli salım sistemi ve Daisy-II inkübatörü aracılığıyla bir in vitro çözünme testidir. Sürekli salım sistemi ve Daisy II kullanılarak elde edilen deneysel sonuçların en iyi uyumu için Birinci Derece, Higuchi, Hixson-Crowell ve Korsemeyer-Peppas'ın kinetik modelleri karşılaştırılmıştır. Sonuçlar, PVP içeren tabletlerin daha hızlı çözündüğünü, PVP-GO kombinasyonunun ise daha kontrollü ve uzun süreli bir salım profili sağladığını göstermiştir. Daisy II, sürekli salım sisteminde gerçekleştirilen formülasyonlara kıyasla, her iki formülasyon için de daha yüksek salım oranına neden olmuştur.

Anahtar Kelimeler: Kontrollü salım, Salım Kinetiği, Ruminantlar, Grafen oksit, Yapay tükürük.

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1. Introduction

There are numerous studies regarding the sustained-release drug delivery systems usually with a less than 1 g tablet weights in human applications. However, the application of similar delivery systems for the food-processing animals in the veterinary field is particularly limited to the disease prevention and growth promotion [1-3]. And, these formulations mostly rely on much heavier tablet sizes (bolus >> 10 g). Therefore, the understanding of release kinetic of such heavy bolus forms by taking also the complexity of a stomach of a ruminant into consideration requires more attention from scientists. Designing and optimizing performance of boluses for ruminants may require performance enhancers that could be used together with excipients/binders. There are recent studies showing that these materials can interact and change dissolution, absorption and bioavailability characteristics of tablet formulations [4].

Binders are preferred in pharmaceutical formulations to enhance drug bioavailability, improve solubility, and optimize dispersibility and release properties [5, 6]. Natural, semi-synthetic, or synthetic binders that are classified depending on their chemical nature are used in bolus production through either dry or wet granulation techniques. Using additives or so-called performance enhancers together along with binders could have a significant effect on release profiles of boluses. These systems aim to enhance the therapeutic efficacy of drugs while minimizing side effects [7]. Graphene oxide (GO) exhibits superior biological properties due to its hydrophilic functional groups, such as hydroxyl, epoxide, and carboxylic groups. These groups not only facilitate better dispersion of GO in water and physiological environments [8, 9] but also enable easy binding with antibodies, enzymes, and other biological molecules [10]. Therefore, GO is considered to have a broader potential for use, particularly in drug carrier systems. The unique physicochemical properties of GO, combined with the stabilizing and dispersing abilities of PVP, offer a powerful combination to enhance the efficacy of drug delivery systems. GO has been shown to act as a nanocarrier platform in such systems due to its high surface area and stability in biological environments [11, 12]. Moreover, modifying the surface of graphene with biocompatible polymers further enhances its efficiency in biological systems by increasing its loading capacity [11,13-14]. PVP is a common industrial binder often used in the wet granulation process among many others [15]. PVP, an amphiphilic polymer with hydrophilic and lipophilic groups [16, 17], stands out as an ideal stabilizing and binding agent for drug delivery systems. The low toxicity, excellent solubility, and complexation ability of PVP with various materials make it a unique component in drug delivery systems [11]. Additionally, the ability of PVP to successfully disperse single-layer graphene in both organic solvents and water enhances the efficacy of graphene- and GO-based systems in biomedical applications [16, 17]. GO with PVP (PVP-GO) has been shown to enable the efficient loading and controlled release of pharmaceutical agents such as quercetin and gefitinib, while also maintaining system stability and enhancing bioavailability [18]. The binding properties of PVP support the adhesion of drug particles, thereby optimizing homogeneous distribution.

There are three main parameters, rumen fluid, contraction and microflora that affects dissolution and biodegradation of materials in a rumen [19, 20]. The dissolution studies of tablets can be carried out either using an artificial saliva for the effect of rumen fluid or Daisy

II incubator in which artificial rumen saliva and microflora of a rumen are taken into consideration. In this study, the dissolution profiles of two bolus formulations of PVP and PVP-GO were studied using a continuous system (the continuous dissolution rig) based on a fluid flow of artificial saliva [21] and in vitro dissolution test carried out by the means of the Daisy-II incubator [22]. The release kinetics of both formulations in these two systems were calculated according to the models of Zero order, Higuchi, Hixson-Crowell and Korsemeyer-Peppas.

2. Material and Method

2.1. Material

Graphite powder (60 mesh, 99.9%, Sigma-Aldrich, America), Polyvinylpyrrolidone (PVP) (Kollidon 30, Std Huzhou Sunflower Pharma, China), Calcium Formate (Perstorp, Sweden), Magnesium Oxide (MKM Maltas, Türkiye), Vitamin D3 and Vitamin E and Niasinamid (Adisseo, Fransa), Sorbitol (Sunsorb, Türkiye), Microcrystalline cellulose (MCC) (Vivapur 101, JRS Rettenmaier, Germany), Saccharomyces cerevisiae (Phileo by Lesaffre, France), Magnesium Stearate (Zorlu Kimya, Türkiye) were used as received without any further purification.

2.2. Method

2.2.1. Synthesis of graphene oxide (GO)

GO was synthesized using a modified Hummer's method [23]. Initially, 20 g of graphite, 10 g of P_2O_5, 10 g of $K_2S_2O_8$, and 50 mL of concentrated sulfuric acid were mixed and subjected to the oxidation at a temperature of 80 °C for ca. 6 hours. Upon completion, 200 mL of ultrapure water (UPW) was added to the reaction mixture. The oxidized graphite was then subsequently filtered and washed with UPW until that a neutral pH was achieved, and then dried at a temperature of 70 °C at the oven (Mikrotest MIN-120, Türkiye). Subsequently, a mixture of 1 g of oxidized graphite, 2 g of sodium nitrate, and 50 mL of sulfuric acid were stirred in an ice bath for 30 mins. Following this, 5 g of potassium permanganate was gradually and slowly introduced, and then the reaction proceeded in a water bath at a temperature of 35 °C for about 180 mins.

Following the reaction, 50 mL of UPW was slowly added, and the mixture was then stirred in an ice bath for an additional 30 mins. 8 mL of 30% hydrogen peroxide (H_2O_2) v/v was added dropwise, resulting a yellowish suspension. The mixture was then subjected to a centrifugation at 3000 rpm for 5 minutes and the obtained solid was washed with 10% v/v HCl. Finally, the was rinsed multiple times with UPW and then dried under vacuum at 70 °C. The resulting GO was subsequently ground into a fine powder [24].

2.2.2. Preparation of Boluses with PVP and PVP-GO

Powder mix as given in Table 1 were granulated using the wet granulation technique [25]. The binder solutions containing (12.5% w/v PVP) with and without GO were slowly sprayed onto the powder during mixing.

Table 1 Composition and weight percentage of components in the formulated boluses

Function	Component	% w/w	
		PVP	PVP + GO
Mineral and Vitamin Supplements	Calcium Formate	74,15	74,00
	Magnesium Oxide	7,00	7,00
	Vitamin D3	0,30	0,30
	Vitamin E	0,80	0,80
Energy source	Sugar Alcohol	3,00	3,00
Filler and dispersant	Cellulose derivative	10,00	10,00
Microflora regulator	*Saccharomyces cerevisiae*	0,75	0,75
Binder	PVP	2,00	2,00
	GO	0,00	0,15
Lubricant	Magnesium Stearate	2,00	2,00

Preparation of PVP binder solution: for the preparation of the PVP solution, 3.90 g PVP was dissolved in water to achieve a concentration of 12.5% w/v and stirred until a complete dissolution was occurred.

Preparation of PVP-GO binder solution: First, 0.25 g of GO was added into 50 mL water and sonicated in an ultrasonic water bath for ca. 2.5 hours. Following this, 3.90 g of PVP (equivalent to 2% w/w PVP in dry bolus formulation) was added, and the mixture was stirred until a homogeneous solid blend was obtained. The powder components used in Table 1 were individually sieved, and then mixed until a homogeneous blend was achieved. Solutions of PVP prepared with and without GO were slowly sprayed onto the powder mix. Both formulations were dried until reaching around 1.5% w/w water content. Drying was performed in an oven at 50 ºC for 90 mins to ensure uniform moisture removal without thermal decomposition of components. A general drying profile of both formulations are shown in Fig. 1. The prepared granules were weighed at 2.5 g and tableted using a manual hydraulic press under a pressure of 16 MPa.

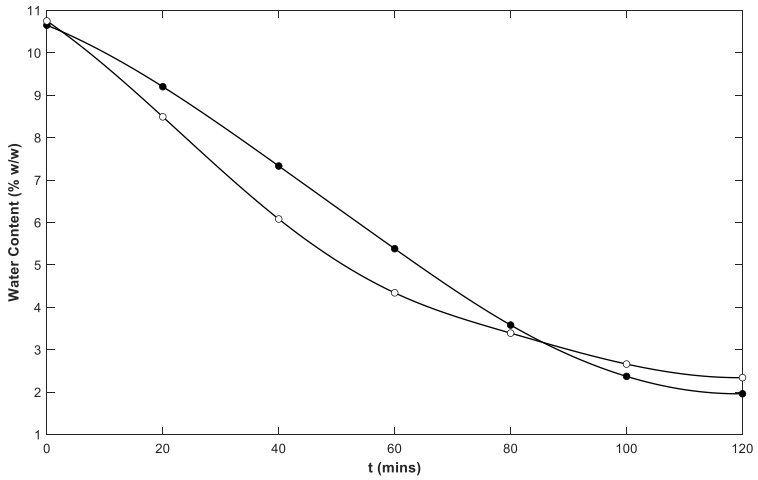


Figure 1 Drying processes of two granules obtained with PVP (●) and PVP-GO (○) solutions.

2.2.3. Sustained-Release Studies of Boluses

2.2.3.1. The Continuous Dissolution Rig Studies

The release studies of the boluses were conducted using a continuous-feed controlled-release experimental system, as illustrated in Fig. 2 [26]. The bolus was placed inside the BF (Bag Filter) and subsequently positioned within the JF (Jacketed Flask). During the experiment, the continuous stirring was maintained using an MS (Magnetic Stirrer) to ensure a homogeneous solution in the JF. A volume of 600 mL for the JF and a flow rate of 5 mL/min of artificial saliva were utilized to imitate physical conditions of a rumen of a grown cattle. Artificial saliva was prepared in the laboratory according to the composition reported by McDougall[27]. The rumen fluid was continuously supplied to the experimental system using a peristaltic pump (Shenchen, BT100N).

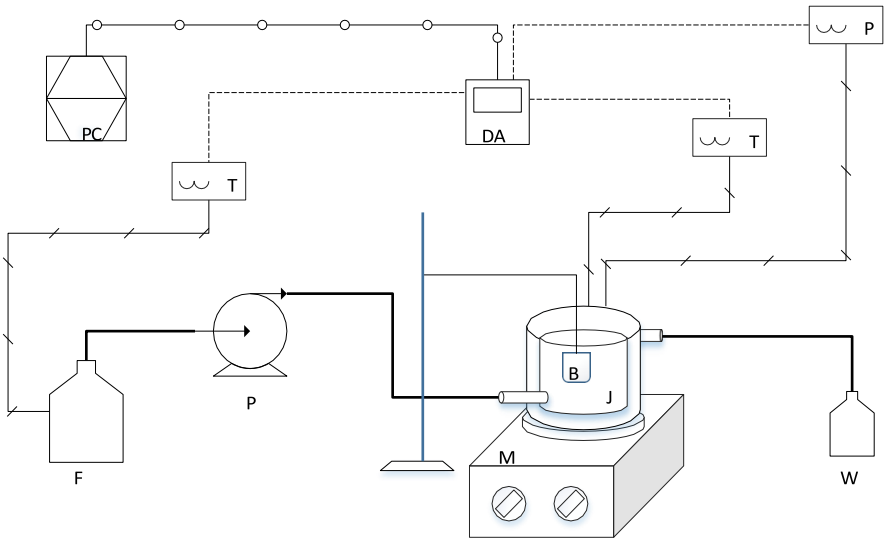


Figure 2 The process flow diagram of continuous dissolution rig. **PC**; Computer, **DAQ**; NI DAQ, **T1**, **T2**; K-type thermocouples, **BF**; Bag Filter, **JF**; Jacketed Flask, **MS**; Magnetic Stirrer, **P**; Peristaltic Pump, **FT**; Feed Tank, **WC**; Waste Container.

The temperature (Omega, K-type thermocouples), pH and conductivity (WTW PH/Cond 3320 SET 2) were monitored and recorded online for each experiment. For waste fluid management, the system's outlet was connected to a waste container to collect output flow. The temperature, pH and conductivity of the release medium mostly remains constant within the given fluctuations as shown in Fig. 3.

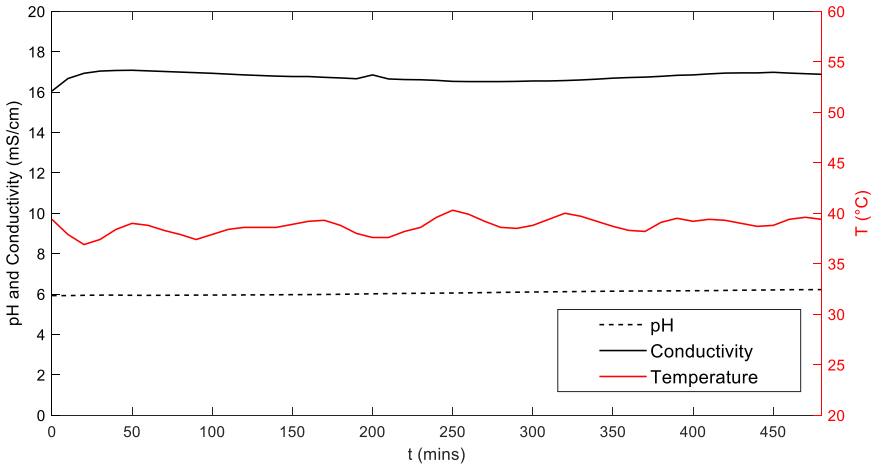


Figure 3 The variation of T, pH and conductivity of release medium throughout an experiment in the continuous dissolution rig.

The boluses were removed at predefined time intervals from the JF and subjected to freeze-drying under vacuum at a pressure of 10-3 mbar for 180 mins using a lyophilizer (Teknosem Toros, TRS-2/2V). The weight of the boluses was then recorded.

2.2.3.2. In vitro Dissolution Studies, Daisy II

To investigate the release kinetics of boluses, woven polyester filter bags with a pore size of 25 µm and rotating jars of the Daisy-II incubator (ANKOM Technology, Fairport, New York, USA) were used. Buffer solutions as proposed by Marten and Barnes (1980), was used in the Daisy-II incubator which was set to a temperature of 39 ºC. Subsequently, 1600 ml of buffer solution and 400 ml of rumen fluid were added to each jar under a CO2 gas atmosphere as described elsewhere [28]. Each jar is divided into two separate compartments via internal mixing chambers, with each compartment representing a one-hour time interval in the experiment. Thus, a total release study for about 8 hours incubation in four jars was conducted. In each compartment, three boluses were placed on one side for three replicates, while another three boluses were placed on the opposite side for the remaining three replicates. Throughout the study, at the end of each hour, three bolus samples from the respective compartment of the jar were collected, freeze-dried and weighed. The dissolution data as an average of three replicates were calculated for each time point.

2.2.4. Characterization of granule morphology and binder interactions

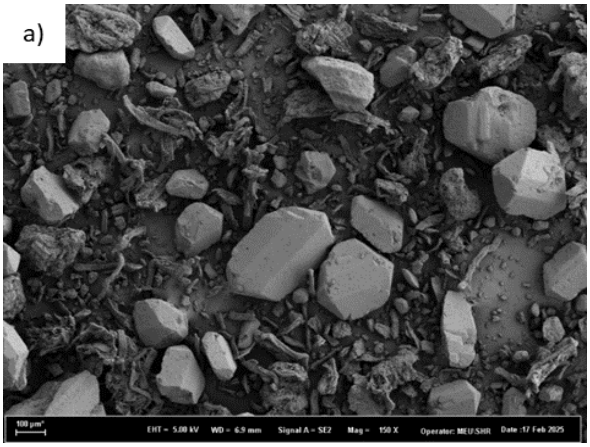
The morphology of powder mix and granules prepared with and without GO were examined using FEI Quanta 650 Field Emission Scanning Electron Microscopy (FESEM). To understand the chemical interaction between PVP and GO were analyzed by Fourier Transform Infrared

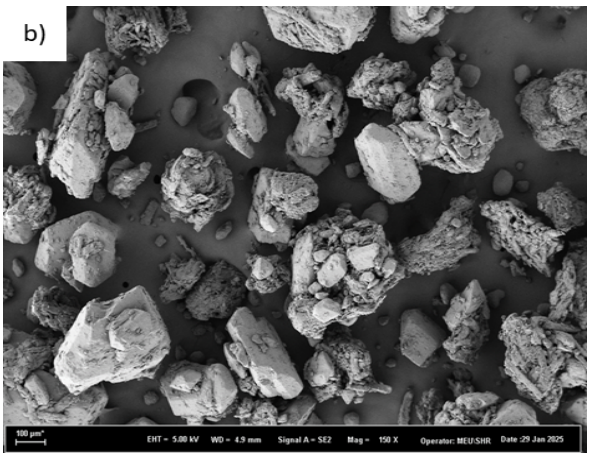
Spectroscopy (FTIR, BRUKER VERTEX70) using the Attenuated Total Reflectance (ATR) method. The mechanical strength of the boluses was measured using a TQC LD0551 Shore Durometer Type D.

3. Results and discussion

3.1. Granulation and tablet morphology

The release kinetic of a bolus may greatly be affected by volume, interaction and morphology. Fig. 4 shows that granule SEM images of the plain and the granulated powders. The SEM analyses were performed on three different samples: (Fig. 4-a) pure powder mixture, (Fig. 4-b) granules formed with PVP, and (Fig. 4-c) granules formed with PVP-GO. It was observed that the pure powder mixture contained distinct and dispersed particles as seen in Fig. 4-a.





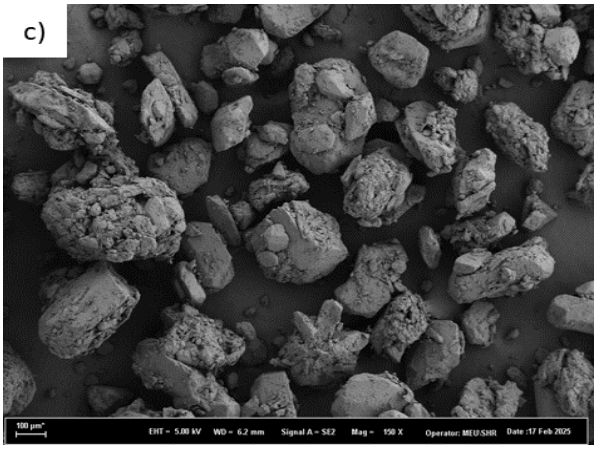


Figure 4 SEM images of (a) powder (no granulation), (b) granules with PVP and granules with PVP-GO.

The granules with PVP-GO were observed to be smaller and more compact compared to the one with PVP. The high surface area and layered structure of GO morphology may create surface coating effect that could result in better particle size distribution. In contrast, when only PVP was used, granulation was achieved, but the bonding between particles remained more pronounced.

Fig. 5 shows the spectra of PVP, GO and the one resulting from the chemical interaction between GO and PVP. The shift observed at 1650 cm-¹ that may suggests the possibility of hydrogen bonding formation, in additon to vibrations seen at the wavenumber of 1426 cm-¹. [29, 30].

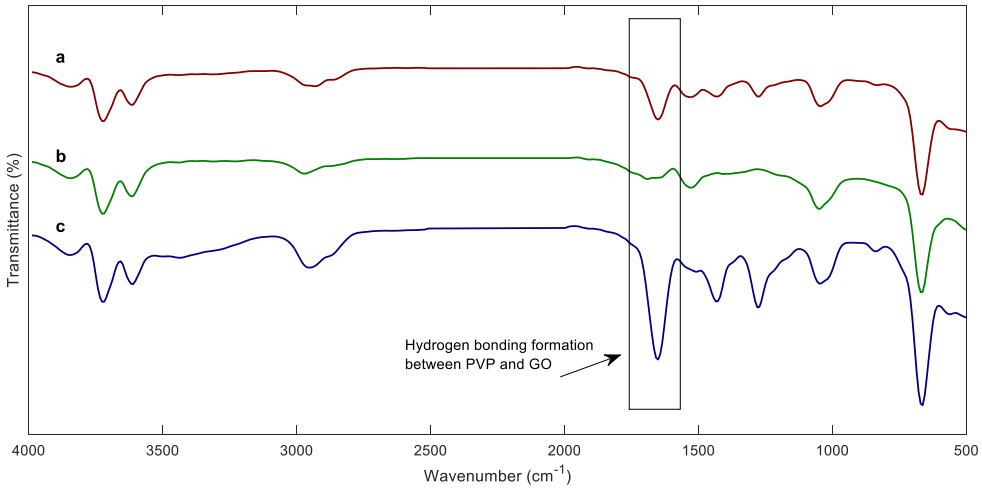


Figure 5 FTIR-ATR Spectra of PVP (a), GO (b), and PVP-GO (c).

The hardness of a bolus is a crucial quality parameter in pharmaceutical formulations, as it ensures the physical durability of a bolus and its ability to resist breakage or cracking during transportation and storage. The hardness of PVP-GO boluses was measured at 54 N, while tablets containing only PVP had a hardness of 46 N. The chemical interactions within the

granule structure may lead to a more compact and uniform tablet morphology and therefore more resistant to fractures.

3.2. Dissolution Profiles of the Formulations with and without GO

The dissolution profiles of both formulations in two different systems, the continuous dissolution rig and the in vitro (Daisy-II) systems were investigated. As seen in Fig. 6, the formulation with PVP exhibits a faster release, while the one with PVP-GO demonstrates a slower, a more controlled release profile. This difference may be attributed to the incorporation of GO into the PVP matrix, which can reduce the dissolution rate and results in a more stable release profile.

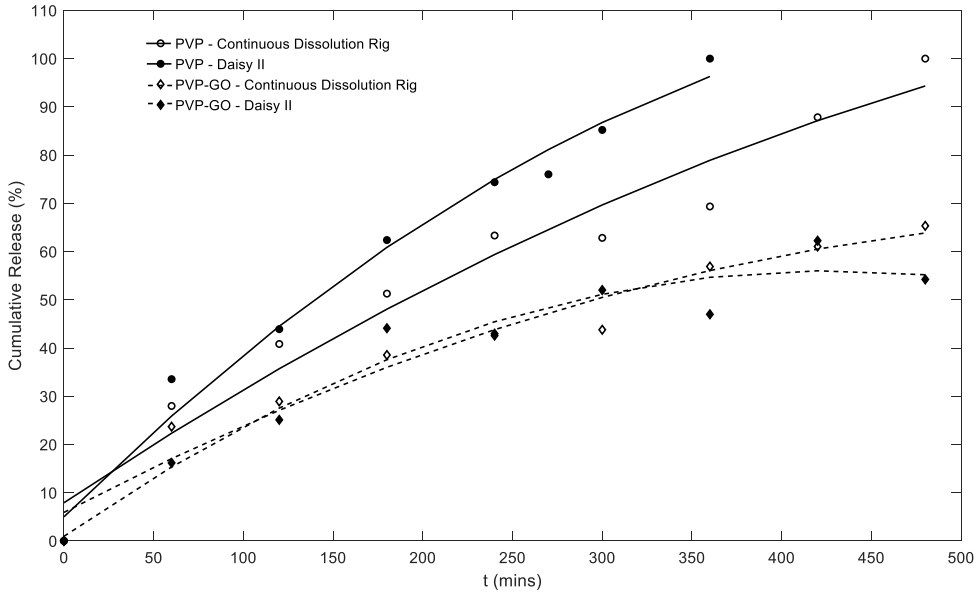


Figure 6 The release profile of formulations with and without GO in both the continuous dissolution rig and Daisy-II.

The addition of GO into the PVP solution exhibited a stronger binding effect, leading to a slower dissolution rate. The high surface area of GO, its ability to balance hydrophilic and hydrophobic regions, and its capacity to form hydrogen bonds may contribute such a behavior. The oxygen-containing functional groups of GO (hydroxyl, carboxyl) that can interact with PVP may lead to the formation of a more stable and compressible granule structure. FTIR spectrum in Fig. 5 confirms the presence of these chemical interactions which may result in a reduced dissolution rate through the enhanced binder properties.

PVP is recognized as an effective binder, and its water-soluble nature facilitates rapid disintegration upon contact with the dissolution medium, promoting faster drug release. However, the interaction between PVP and GO could create a stronger binding matrix, which further reduced the dissolution rate and therefore resulting in a more controlled release profile. As seen in Fig. 6, both formulations show a relatively slower release kinetic in the continuous dissolution rig compared to the Daisy II. To sum up, it is expected to reach slower release kinetics in the continuous dissolution rig than in vitro environment obtained in Daisy II.

3.3. Release kinetics of boluses

Various mathematical models are employed to model drug release behavior under different environmental conditions [31]. The release kinetics of the formulations with and without GO were evaluated using four common kinetic models under artificial rumen saliva and in vitro conditions [32]. The kinetic curves obtained using these models for the continuous dissolution rig and Daisy II are given in Fig. 7.

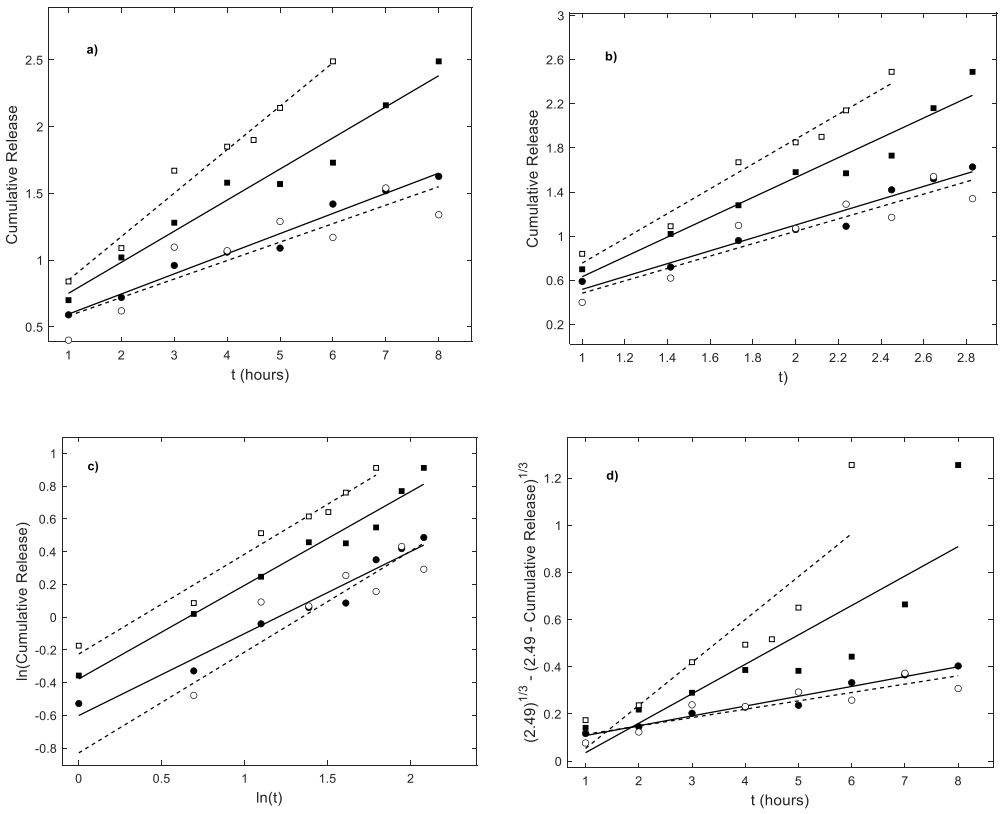


Figure 7 Zero order (a), Higuchi (b), Korsmeyer-Peppas (c) and Hixson-Crowell (d) kinetic models of both formulations in two systems. PVP by the continuous dissolution rig (□), PVP by Daisy II (■), PVP-GO by the continuous dissolution rig (●) and PVP-GO by Daisy II (○).

The coefficient of determination (R^2) and the Akaike Information Criterion (AIC) were employed to evaluate the regression analysis and model fits for both systems. While R^2 indicates the model's agreement with experimental data, AIC assesses model accuracy and complexity to determine the most suitable model [33]. As shown in Table 2, when evaluating the performance of kinetic models of the formulation without GO, the highest R^2 value (0.9728) in the continuous dissolution rig was obtained for the Korsmeyer-Peppas Model. Additionally, the lowest AIC value (-37.94) was also calculated for this model, demonstrating its optimal balance between accuracy and simplicity.

Table 2. Comparison of Release Kinetic Models in Rumen Saliva and In Vitro Conditions: Regression and Model Fitting Analysis with PVP Incorporation.

Model	Equation	Continuous Dissolution Rig		Daisy II	
		R^2	AIC	R^2	AIC
Zero order	F=K.t	0.9646	-30.18	0.9775	-29.01
Higuchi	$F=K.t^{1/2}$	0.9461	-26.83	0.9709	-27.21
Hixson-Crowell	$2.49^{1/2}-(2.49-F)^{1/2}=-K.t$	0.7469	-22.40	0.7938	-20.17
Korsemeyer-Peppas	Ln(F)=ln(K)+n.ln(t)	0.9728	-37.94	0.9730	-33.33

Although the highest coefficient of determination (R^2 = 0.9775) in Daisy II conditions was obtained for the Zero-order kinetic model, the lowest AIC value (-33.33), which reflects the balance between model accuracy and complexity, was calculated for the Korsmeyer-Peppas model. This suggests that the Korsmeyer-Peppas model provides a better overall fit due to its optimal trade-off between goodness of fit and model simplicity. For PVP-GO formulation, the highest R^2 value (0.9769) was obtained for the Zero-Order Kinetics Model, suggesting a strong agreement with experimental data for the continuous dissolution rig as presented in Table 3. However, the lowest AIC value (-59.96) was recorded for the Hixson-Crowell Model. It is suggested that PVP-GO systems may be primarily influenced by surface area and particle size distribution of the bolus formulations. Under Daisy II conditions, the highest R^2 value (0.9121) was observed for the Korsmeyer-Peppas Model. Nevertheless, the lowest AIC value (-45.36) was again found for the Hixson-Crowell Model, reinforcing its suitability for describing drug release behavior in PVP-GO systems.

Table 3. Comparison of Kinetic Models in the Continuous Dissolution Rig and Daisy II: Regression and Model Fitting Analysis for the Formulation of PVP-GO.

Model	Equation	Continuous Dissolution Rig		Daisy II	
		R^2	AIC	R^2	AIC
Zero order	F=K.t	0.9769	-40.66	0.8002	-23.19
Higuchi	$F=K.t^{1/2}$	0.9615	-36.57	0.6873	-14.86
Hixson-Crowell	$2.49^{1/2}-(2.49-F)^{1/2}=-K.t$	0.9730	-59.96	0.8083	-45.36

Korsemeyer-Peppas	Ln(F)=ln(K)+n.ln(t)	0.9628	-37.49	0.9121	-26.86

The lowest AIC value (-59.96) for the continuous dissolution rig for PVP-GO formulation was observed for the Hixson-Crowell Model. A similar trend was observed under Daisy II conditions as the lowest AIC value (-45.36) was calculated for the Hixson-Crowell Model for the PVP-GO formulation and the lowest AIC value (-33.33) was found the Korsmeyer-Peppas Model in the PVP formulation. In addition, for the continuous dissolution rig, the PVP formulation also showed the lowest AIC value (-37.94) with the Korsmeyer-Peppas Model. While the Korsmeyer-Peppas Model provides the best fit for PVP-containing systems, the Hixson-Crowell Model appears more suitable for PVP-GO systems.

4. Conclusion

The release studies of tablets and boluses for veterinary applications should involve in vitro and in vivo studies to obtain a more comprehensive understanding about the dissolution profiles. Therefore, using a representative approach based on artificial rumen saliva may be of a great importance to reduce experimental work in testing various formulations. In this study, a comparison of the continuous dissolution rig with a more common in vitro approach, Daisy II incubator provides valuable aspects to the literature in the veterinary pharmacy. The effect of a performance enhancer, GO, with a commercial binder, PVP, on the release profile was investigated using these two methods. It has been demonstrated that the interaction of GO with PVP could have a significant effect on dissolution rates. As expected, Daisy II results in higher rate of dissolution for both formulations compared to the ones in the continuous dissolution rig. Incorporation of GO as an enhancer to the binder matrix resulted in a change in kinetic mechanism. This work underlines that more research should be carried out regarding the comparison of the continuous dissolution rig and current in vitro studies present in the literature.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

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References

[1] Cardinal, J. R. (1985). Controlled drug delivery: Veterinary applications. Journal of Controlled Release, 2(1), 393-403.

[2] Martinez, M. N., Lindquist, D., & Modric, S. (2010). Terminology challenges: Defining modified release dosage forms in veterinary medicine. Food and Drug Administration, Center for Veterinary Medicine, Office of New Animal Drug Evaluation. Rockville, MD.

[3] Ruan, X., Gao, X., Gao, Y., Peng, L., Ji, H., Guo, D., & Jiang, S. (2018). Preparation and in vitro release kinetics of ivermectin sustained-release bolus optimized by response surface methodology. PeerJ, 6, e5418. https://doi.org/10.7717/peerj.5418

[4] Kalász, H., & Antal, I. (2006). Drug excipients. Current Medicinal Chemistry, 13, 2535-2563.

[5] Khairnar, R. G., Darade, A. R., & Tasgaonkar, R. R. (2024). A review on tablet binders as a pharmaceutical excipient. World Journal of Biology, Pharmacy and Health Sciences, 17(3), 295–302.

[6] Amol, M., & Bhairav, B. A., & Saudager, R. B. (2017). Co-processed excipients for tabletting: Review article. Research Journal of Pharmacy and Technology, 10(7), 2427–2432.

[7] Shen, S. I., Jasti, B. R., & Li, X. (2003). Design of controlled-release drug delivery systems. In M. Kutz (Ed.), Standard handbook of biomedical engineering and design (Chap. 22, pp. 22.1-22). McGraw-Hill.

[8] Li, D., Muller, M. B., Gilje, S., Kaner, R. B., & Wallace, G. G. (2008). Processable aqueous dispersions of graphene nanosheets. Nature Nanotechnology, 3, 101–105.

[9] Yang, X., Wang, Y., Huang, X., Ma, Y., Huang, Y., Yang, R., et al. (2010). Multi-functionalized graphene oxide-based anticancer drug-carrier with dual-targeting function and pH-sensitivity. Journal of Materials Chemistry, 20, 3448–3454.

[10] Turgut, F. N. A., Konakcı, C. O., Arıkan, B., Comak, G., & Yıldıztuğay, E. (2024). Graphene oxide-based aerogel stimulates growth, mercury accumulation, photosynthesis-related gene expression, antioxidant efficiency and redox status in wheat under mercury exposure. Environmental Pollution, 342, 123117.

[11] Eshaghi, M. M., Pourmadadi, M., Rahdar, A., & Díez-Pascual, A. M. (2022). Novel carboxymethyl cellulose-based hydrogel with core–shell Fe_3O_4@SiO_2 nanoparticles for quercetin delivery. Materials, 15(24), 8711.

[12] Yaghoubi, F., Motlagh, N. S. H., Naghib, S. M., Haghiralsadat, F., Jaliani, H. Z., & Moradi, A. (2022). A functionalized graphene oxide with improved cytocompatibility for stimuli-

responsive co-delivery of curcumin and doxorubicin in cancer treatment. Scientific Reports, 12(1), 1–18.

[13] Mostafavi, F. S., & Imani, R. (2021). Synthesis and characterization of gelatin-functionalized reduced graphene oxide for drug delivery application. Pathobiology Research, 23(5), 75–85.

[14] Pourmadadi, M., Yazdian, F., Hojjati, S., & Khosravi-Darani, K. (2021). Detection of microorganisms using graphene-based nanobiosensors. Food Technology and Biotechnology, 59(4), 496–506.

[15] Dinda, S. C., & Mukharjee, B. (2009). Gum cordia – A new tablet binder and emulsifier. Acta Pharmaceutica Sciencia, 51, 189–198.

[16] Wajid, A. S., Das, S., Irin, F., Ahmed, H. S. T., Shelburne, J. L., Parviz, D., Fullerton, R. J., Jankowski, A. F., Hedden, R. C., & Green, M. J. (2012). Polymer-stabilized graphene dispersions at high concentrations in organic solvents for composite production. Carbon, 50(2), 526–534.

[17] Zhang, J., Shen, G., Wang, W., Zhou, X., & Guo, S. (2010). Individual nanocomposite sheets of chemically reduced graphene oxide and poly(N-vinyl pyrrolidone): Preparation and humidity sensing characteristics. Journal of Materials Chemistry, 20, 10824–10828.

[18] Tiwari, H., Karki, N., Pal, M., Basak, S., Verma, R. K., Bal, R., Kandpal, N. D., Bisht, G., & Sahoo, N. G. (2019). Functionalized graphene oxide as a nanocarrier for dual drug delivery applications: The synergistic effect of quercetin and gefitinib against ovarian cancer cells. Colloids and Surfaces B: Biointerfaces, 178, 452–459.

[19] Soltis, M. P., Moorey, S. E., Egert-McLean, A. M., Voy, B. H., Shepherd, E. A., & Myer, P. R. (2023). Rumen biogeographical regions and microbiome variation. Microorganisms, 11(3), 747.

[20] Comtet-Marre, S., Parisot, N., Lepercq, P., Chaucheyras-Durand, F., Mosoni, P., Peyretaillade, E., & Forano, E. (2023). Lignocellulose degradation by rumen bacterial communities: New insights from metagenome analyses. Environmental Research, 226, 115785.

[21] McDougall, E. (1948). Studies on ruminant saliva: The composition and output of sheep's saliva. Biochemical Journal, 43, 99–109.

[22] Tassone, S., Fortina, R., & Peiretti, P. G. (2020). In vitro techniques using the Daisy II incubator for the assessment of digestibility: A review. Animals, 10(5), 775. https://doi.org/10.3390/ani10050775

[23] Hummers, W. S., & Offeman, R. E. (1958). Preparation of graphitic oxide. Journal of the American Chemical Society, 80(6), 1339.

[24] Bilik, K., Çomak, G., & Sönmez, O. (2024). Graphene oxide-catalyzed microwave-assisted esterification of oleic acid to biodiesel. Biofuels, 953–960.

[25] Vadaga, A. K., Gudla, S. S., Nareboina, G. S. K., Gubbala, H., & Golla, B. (2024). Comprehensive review on modern techniques of granulation in pharmaceutical solid dosage forms. Intelligent Pharmacy, 2, 609–629.

[26] Comak, G., Durmuş, M., & Erez, İ. (2024). In vitro release kinetics and in vivo field trial performance of a long-term sustained-release bolus for Saanen goats. Turkish Journal of Engineering, 8(4), 712–719.

[27] McDougall, E.I. (1948). Studies on Ruminant Saliva. The Composition and Output of Sheep's Saliva. Biochemical Journal, 43, 99–109.

[28] Marten, G. C., & Barnes, R. F. (1980). Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. In Standardization of Analytical Methodology for Feeds: Proceedings of a Workshop Held in Ottawa, Canada, 12–14 March 1979. International Development Research Centre.

[29] Yin, B., Wang, J., Jia, H., He, J., Zhang, X., & Xu, Z. (2016). Enhanced mechanical properties and thermal conductivity of styrene–butadiene rubber reinforced with polyvinylpyrrolidone-modified graphene oxide. Journal of Materials Science, 51, 5724–5737.

[30] Chang, X., Wang, Z., Quan, S., Xu, Y., Jiang, Z., & Shao, L. (2014). Exploring the synergetic effects of graphene oxide (GO) and polyvinylpyrrolidone (PVP) on poly(vinylidene fluoride) (PVDF) ultrafiltration membrane performance. Applied Surface Science, 316, 537–548.

[31] Huang, V., Ye, H., & Yam, K. L. (2013). Release mathematical model of active agent from packaging material into food. Mathematical Problems in Engineering, 10, 1–10.

[32] Paaeakh, M. P., Jose, P. A., Setty, C., & Christoper, G. V. P. (2018). Release kinetics–concepts and applications. International Journal of Pharmacy Research & Technology, 8, 1.

[33] Jahormi, L. P., Ghazali, M., Ashrafi, H., & Azadi, A. (2020). A comparison of models for the analysis of the kinetics of drug release from PLGA-based nanoparticles. Heliyon, 6(2), e03451.