Alqalam Journal of Medical and Applied Sciences. 2025;8(3):1539-1543
https://doi.org/10.54361/ajmas.258339

Original article

The Effect of PEG-grafted-PLA Nanoparticles Loaded with Famotidine on Apical Efflux Using CaCo-2 Cell Monolayer

Mohamed Mokhtar[1]*, Fadia Mussa[2]

[1]*Pharmaceutical Department Faculty of Health Sciences, Sirte University, Sirte, Libya*
[2]*Pharmaceutical Department, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya*
Corresponding Email. *mohamed.alhouni@su.edu.ly*

Abstract
Bioavailability of some orally administered drugs may be limited by an intestinal secretion process that is mediated by P-glycoprotein. Such an effect could be seen on Famotidine as it has a relatively low bioavailability. Encapsulation of Famotidine with pegylated nanoparticles (NPs) might enhance its bioavailability by inhibiting its secretion from the basolateral to apical side due to the effect of P-gp. Grafted PLA-PEGs were prepared with two different grafting ratios of 1 and 5 % of different molecular weights of poly (ethyleneglycol) methyl ether, 750 and 2000Da. Nanoparticles loaded with Famotidine and pure Famotidine were tested in vitro on the CaCo-2 monolayer. Apical-to-Basolateral and Basolateral-to-Apical for permeability assessment. Results showed that Famotidine formulated with NPs at 5 % ratio of PEG at different molecular weights and 1 % with 2000 Da decreased the secretion of Famotidine from Basolateral to Apical across CaCo-2 monolayer cells compared to 1% ratio of PEG at 760Da, free Famotidine, and physical mixtures.
Keywords. PEG, Nanoparticles, Famotidine, Caco-2 Monolayer.

Introduction

The gastrointestinal tract acts as a barrier for orally administered drugs. The tight junctions of the epithelium act as a physical barrier, while the metabolic enzymes act as a biochemical barrier. In order to be absorbed, drug molecules have to be dissolved into gastrointestinal fluids, be chemically stable, resistant to enzymatic degradation, and not be bound to food [1]. Moreover, drug molecules need to diffuse across the gastrointestinal membrane by entering epithelial cells from the apical (luminal) side and exiting from the basolateral or passing through the intercellular space (paracellular) to reach systemic circulation [2].

Amongst those transport proteins, P-gp is a plasma membrane glycoprotein of about 170 kDa, also called an apical efflux transporter. P-gp is expressed in various normal tissues, including the liver, the kidney, the adrenal glands, the brain, the testis, and the intestinal brush border membranes[3]. P-gp is also expressed in tumor cells and is responsible for the efflux of chemotherapeutic agents from multidrug-resistant (MDR) cancer cells. P-gp acts as a drug efflux pump and lowers intracellular drug concentration in acting as an absorption barrier by secreting drugs from the intestinal cells' cytosol to the intestinal lumen, reducing permeability and bioavailability, and having a profound impact on several drug pharmacokinetics [3]. Therefore, oral bioavailability of drugs that are P-gp substrates could be improved by inhibiting P-gp efflux function within the intestinal membrane [3].

Some pharmaceutical excipients could inhibit the secretion function of P-gp in the intestinal membrane and therefore improve the oral bioavailability of drugs that are P-gp substrates[4]. Polyethylene glycol (PEG) is uncharged, hydrophilic, and soluble in many organic solvents. PEG is commonly used as an excipient in pharmaceutical formulations, and it is available in standard molecular weights (Mw) ranging from 200 to 8000 Da[5]. Moreover, PEG has been shown to enhance the bioavailability of some drugs by inhibiting P-glycoprotein efflux[6]. Indeed, PEG with a range of 300 to 4000 Da molecular weights has been used to improve oral drug permeability of P-gp substrates [7].

Nanosizing formulation has been proven to be an efficient technique to enhance drug absorption and bioavailability of several drugs[8]. NPs drug carriers have a size varying from 10 to 1000 nm and can be either matrices or nanospheres in which the drug is physically or molecularly and uniformly dispersed, while nanocapsules are a vesicular system in which the drug is confined to a cavity surrounded by a unique polymer membrane[9]. Recently, biodegradable NPs have been increasingly tested in pharmaceutical research as a promising drug delivery system [10]. Nanoencapsulation may provide several benefits to the drug adsorption and It could prevent premature degradation of the drug in a hostile GIT environment, contribute to the target drug to a specific part of the GIT, and allow the delivery of poorly soluble drugs[11]. Indeed, improvement of biological barriers crossed by nanocarriers in oral delivery has been reported for several encapsulated drugs[7].

Pegylated drug carriers have been developed initially to prevent rapid elimination by the mononuclear phagocytosis system (MPS) and increase circulation time in systemic circulation to optimize drug release[12]. However, pegylated drug carriers could also benefit in oral delivery, by improving their penetration of the mucus barrier covering the epithelium and favoring interaction with the epithelium cell membrane, decreasing interaction with food, and eventually, as discussed earlier, by decreasing P-gp activity[13]. Famotidine, an H2-receptor antagonist is inhibiting gastric acid secretion and heals gastric & duodenal

Received*: 24-05-2025 -* ***Accepted****: 20-07-2025 -* ***Published****: 28-07-2025*

ulcers. Famotidine has been chosen as a drug model because of its relatively low and variable oral bioavailability, apparently due to its relatively low aqueous solubility and efflux of transporter P-glycoprotein inhibition [14]. Therefore, encapsulation of this drug in a pegylated nanocarrier might be a promising approach. In spite of some limitations, a CaCo-2 cell monolayer has been used as a tool to evaluate and test drug absorption permeability for orally administered drugs across the gastrointestinal tract[3, 15]. PEG could be used to enhance drug bioavailability as it has been proven to inhibit P-glycoprotein efflux[16-19]. In this study, Famotidine, an H2 antagonist, has been chosen as a drug model because P-glycoprotein is a transporter efflux of Famotidine. To overcome P-gp efflux of Famotidine, dispersion within pegylated (PEG5%-g-PLA) nanoparticles (NPs) can be used to protect, stabilize, and block P-gp pump activity. CaCo-2 as in vitro model was used to evaluate NPs' ability to inhibit P-gp efflux. The aim of the study was to develop PEG-grafted PLA (PEG-g-PLA) nanoparticles to encapsulate Famotidine and evaluate their effect on P-gp-mediated efflux and permeability using CaCo-2 cell monolayers.

Methods

PLA was synthesized by ring ring-opening method of D, Lactide and allyl glycidyl ether. Copolymers were prepared with two ratios of pendant groups, with 1 and 5% mol/mol. Grafting of methoxy PEG was done by esterification after the oxidation of allyl into a carboxylic group. The copolymer was grafted with two different molecular weights of poly (ethylene glycol) methyl ether (762 and 2000 Da) that are intended to act as a bioavailability enhancer [20].

The copolymers were characterized by GPC, DSC, and NMR. Nanoparticles (NPs) loaded with Famotidine were prepared by the single emulsion evaporation method. Four types of NPs were prepared: PEG $_{2000\ Da\ 5\%}$ -g-PLA, PEG $_{2000\ Da\ 1\%}$ -g-PLA, PEG $_{762\ Da\ 1\%}$-g-PLA, and PEG $_{762\ Da\ 5\%}$-g-PLA, as well as controls. NPs were characterized by encapsulation efficiency, morphology (AFM), XRPD, DSC, surface charge (Zeta potential), particle size, and size distribution. NPs were also evaluated for their cytotoxicity toward CaCo-2 cells using the MTT test [21].

Permeability study across monolayer CaCo-2 cells line using polycarbonate membrane (Transwells™12 mm i.d., 3µ mm pore size, Costar, Corning, NY, USA) has been carried out in different directions. Apical-to-Basolateral and Basolateral-to-Apical. Free Famotidine, loaded nanoparticles, and physical mixtures of control NPs and Famotidine were evaluated. Cell monolayers were preincubated for 30 min. at 37°C in transport Hank's Buffer Supplement solution (HBSS). Basolateral-to-Apical and Apical-to-Basolateral transport experiments were performed by adding 1.5 and 0.4mL of solutions containing concentrations of 40, 80, and 160 ug/mL for pure Famotidine or corresponding quantities of drug/ control NPs physical mixtures and drug-loaded NPs in the Basolateral (BL) and Apical (AP) compartments, respectively.

High performance liquid chromatography (SHIMADZU with SPD-20A UV/vis detector) and a Hyperclone 5 microns packing 130 A° pore size BDS C18 column were used to quantify Famotidine in Basolateral and Apical compartments as a function of time. The mobile phase flow rate was set at 1 mL/min, and Famotidine was detected at 267 nm with 7.6 min. retention time.

Results

Grafted copolymers with molecular weights ranging from 6000 to 16000 Da were obtained. Spherical nanoparticles with smooth surfaces were produced. The average drug loading was determined to be 1.5 %. Nanoparticles had a mean diameter of approximately 150 nm. Control and loaded with Famotidine NPs were found to be non-toxic within the evaluated concentrations over 24 hours. DSC results have shown that free Famotidine and NPs formulated from 5% 165C°, while NPs formulated from 1% grafted PEG have shown a change in the Famotidine peak position that may be due to interaction between the drug and ungrafted part of the copolymer. Famotidine crystalline characteristic peaks were detected in NPs formulated from 1% grafted PEG, while 5% grafted PEG NPs led to amorphous material (Figure 1)

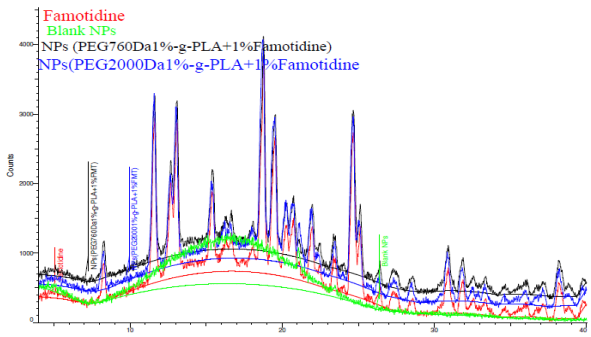

Figure 1. Famotidine crystalline Peaks from different formulations.

Results from permeability study have shown that secretion of Famotidine from basal to apical significantly decreased from NPs formulations 5 % grafted copolymer at both 750 and 2000 Da compared to 1%

Received*: 24-05-2025 -* ***Accepted****: 20-07-2025 -* ***Published****: 28-07-2025*

formulations as well as free Famotidine and physical mixtures as it can been seen in Figures 2, 3 and 4 at 40, 80 and 160 ug/mL, respectively[6, 22-25].

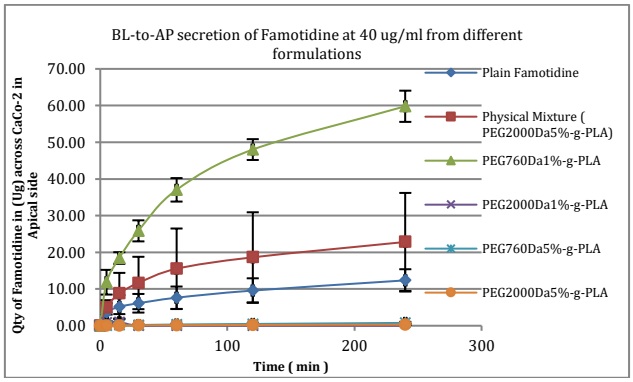

Figure 2. BL-to-AP secretion of Famotidine at 40 ug/ml from different formulations

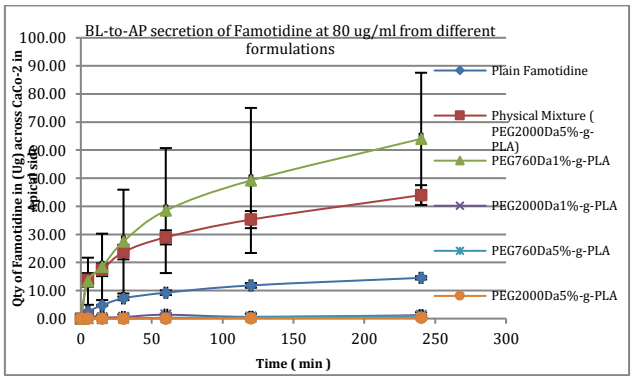

Figure 3. BL-to-AP secretion of Famotidine at 80 ug/ml from different formulations

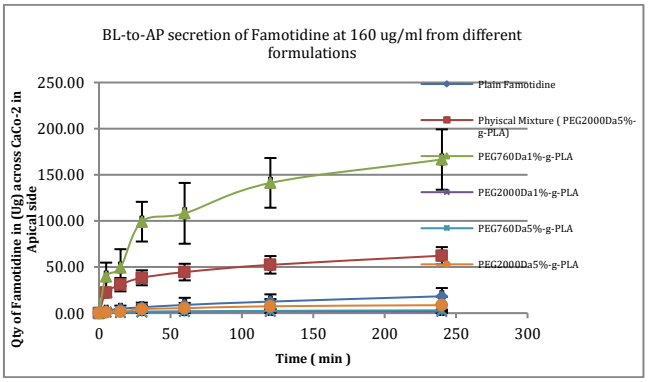

Figure 4. BL-to-AP secretion of Famotidine at 160 ug/ml from different formulations

Discussion

The NPs characterization results demonstrate that NPs formulations were prepared with a mean particle size ranging from 156 to 211nm, AFM images confirm the shape and the size obtained by DLS. X-ray diffraction analysis confirmed the crystalline nature of pure Famotidine through sharp, well-defined peaks. The XRPD scans of loaded NPs showed that those two types of scans varied more with grafting ratios rather than with PEG molecular weight. For NPs prepared from PEG1%, the Famotidine peak was detected (Figure 1), while for PEG5%) a change in Famotidine from crystal to an amorphous state, or that it exists in nanocrystals, but XRPD could not detect all Famotidine peaks. Such absence of Famotidine peaks in loaded NPs with 5% grafting ratio and detecting peaks with low intensity indicates whether PEG exists in high density at NPs surface or Famotidine converted from crystalline to amorphous that Famotidine cannot be detected by XRPD.

The transport experiments were conducted to evaluate the permeability of Famotidine and to examine the potential effect of PEG on P-gp efflux of encapsulated Famotidine across the CaCo-2 cell *in vitro* model. Different loaded NPs formulations of PEG-g-PLA polymer, PM of blank NPs formulations (corresponding to loaded formulations) with Famotidine, and PM of PEG2000Da and PEG750Da were used. Pure Famotidine and loaded PLA NPs (no PEG) formulation were used as a control to evaluate the effect of PEG on P-gp efflux of Famotidine from different formulations. Transport experiments were carried out in triplicate for all formulations, and average results were reported. The permeability study showed a significant decrease in

Received: *24-05-2025* - ***Accepted***: *20-07-2025* - ***Published***: *28-07-2025*

the BL to AP secretion of Famotidine when formulated with 5% PEG-g-PLA at both 750 and 2000 Da compared to 1% formulations, free Famotidine, and physical mixtures. This effect was consistently observed across different concentrations of Famotidine (40, 80, and 160 ug/ml), as shown in Figures 2, 3, and 4. Famotidine has low permeability, and its oral absorption is primarily limited by its intestinal permeability. The observed reduction in BL-to-AP transport suggests that the 5 % PEG-g-PLA NPs are effectively inhibiting the efflux of Famotidine, likely mediated by P-gp.

Polyethylene glycol (PEG), a part of the PEG-g-PLA polymer, is known as P-gp inhibitor. Therefore, encapsulation of Famotidine within PEG-g-PLA NPs appears to enhance Famotidine permeability. Our results show a concentration-dependent effect of PEG-g-PLA polymer (5% vs 1%), further, the potential of these NPs formulations to overcome the permeability limitation of Famotidine. To summarize transport results, it was observed that P-gp efflux inhibition of Famotidine is attributed to PEG content. It can be noticed from the results that the best results were obtained from formulations and combinations containing PEG 5%, irrespective to molecular weights, which is consistent with other findings[6, 26, 27]. These results confirmed our hypothesis, i.e. that pure PEG in blank or loaded NPs can inhibit P-gp Famotidine efflux.

Loaded NPs prepared from polymer grafted at 5 % PEG, irrespective to molecular weights, showed a decrease of P-gp efflux of Famotidine and they have a good absorptive transport profile. It has been reported by some authors that NPs transported via transcellular transport, in which NPs enter the cell by an endocytic process, takes place at cell apical membrane, intracellular trafficking toward exocytic release at the basolateral compartment[7]. In this study the presence of hydrophilic polymers (PEG) on the surface of NPs grafted at 5 % PEG, irrespective to molecular weights, provides an effective way to control the interface between NPs and the biological systems (P-gp in this case) as they are designed to interact with.

Conclusion

Permeability results have shown that Famotidine secretion from BL-to-AP across CaCo-2 cell monolayer was decreased in NPs formulated from polymer grafted with 5 % PEG of both molecular weights and at 1 % PEG with 2000 Da compared to NPs with 1% PEG grafted 750Da, physical mixtures, and free Famotidine. Those results might be explained by the impact of the PEG grafted ratio and molecular weight on P-glycoprotein inhibition.

Acknowledgments

In this section, you can acknowledge any support given that is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Aulton ME. Aulton's pharmaceutics: the design and manufacture of medicines. 3rd ed. Edinburgh; New York: Churchill Livingstone; 2007. x, 717 p.
2. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins - Introduction. Pflugers Arch. 2004;447(5):465-8.
3. Akhtar N, Ahad A, Khar RK, Jaggi M. The emerging role of P-glycoprotein inhibitors in drug delivery: a patent review. Expert Opin Ther Pat. 2011;21(4):561-76.
4. Ashiru-Oredope DA, Patel N, Forbes B, Craig DQ. The effect of polyoxyethylene polymers on the transport of ranitidine in Caco-2 cell monolayers. Int J Pharm. 2011;409(1-2):164-8.
5. Rowe RC, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients. London; Chicago, Washington, DC: Pharmaceutical Press; American Pharmacists Association; 2009. xxvii, 888 p.
6. Hugger ED, Novak BL, Burton PS, Audus KL, Borchardt RT. A comparison of commonly used polyethoxylated pharmaceutical excipients on their ability to inhibit P-glycoprotein activity in vitro. J Pharm Sci. 2002;91(9):1991-2002.
7. Rabanel JM, Aoun V, Elkin I, Mokhtar M, Hildgen P. Drug-loaded nanocarriers: passive targeting and crossing of biological barriers. Curr Med Chem. 2012;19(19):3070-102.
8. Sepassi S, Yadollahi R, Asgarzadeh A, Braun DE, Ober CA, Goodwin DJ. Effect of polymer molecular weight on the production of drug nanoparticles. J Pharm Sci. 2007;96(10):2655-66.
9. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci. 2003;18(2):113-20.
10. Li SD, Huang L. Pharmacokinetics and biodistribution of nanoparticles. Mol Pharm. 2008;5(4):496-504.
11. Bhardwaj V, Kumar MN. Pharmaceutical aspects of polymeric nanoparticles for oral drug delivery. J Biomed Nanotechnol. 2005;1(3):235-58.
12. Owens DE 3rd, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm. 2006;307(1):93-102.
13. Mert O, Lai SK, Ensign L, Yang M, Wang YY, Wood J, et al. A poly(ethylene glycol)-based surfactant for formulation of drug-loaded mucus penetrating particles. J Control Release. 2012;157(3):455-60.

Received*: 24-05-2025 -* ***Accepted****: 20-07-2025 -* ***Published****: 28-07-2025*

14. Thakker DR, Burton PS, Borchardt RT. Saturable absorptive transport of the hydrophilic organic cation ranitidine in Caco-2 cells: role of pH-dependent organic cation uptake system and P-glycoprotein. Pharm Res. 2006;23(6):1165-77.
15. Anderle P, Niederer E, Rubas W, Hilgendorf C, Spahn-Langguth H, Wunderli-Allenspach H, et al. P-glycoprotein (P-gp) mediated efflux in Caco-2 cell monolayers: the influence of culturing conditions and drug exposure on P-gp expression levels. J Pharm Sci. 1998;87(6):757-62.
16. Gref R, Lück M, Quellec P, Marchand M, Dellacherie E, Harnisch S, et al. Stealth corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf B Biointerfaces. 2000;18(3-4):301-13.
17. Zhang K, Tang X, Zhang J, Lu W, Lin X, Zhang Y, et al. PEG-PLGA copolymers: their structure and structure-influenced drug delivery applications. J Control Release. 2014;183:77-86.
18. Bazile D, Prud'homme C, Bassoullet MT, Marlard M, Spenlehauer G, Veillard M. Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. J Pharm Sci. 1995;84(4):493-8.
19. Shah P, Jogani V, Bagchi T, Misra A. Role of Caco-2 cell monolayers in prediction of intestinal drug absorption. Biotechnol Prog. 2006;22(1):186-98.
20. Nadeau V, Leclair G, Hildgen P. Synthesis of new versatile functionalized polyesters for biomedical applications. Polymer. 2005;46(25):11263-72.
21. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63.
22. Donovan MD, Flynn GL, Amidon GL. Absorption of polyethylene glycols 600 through 2000: the molecular weight dependence of gastrointestinal and nasal absorption. Pharm Res. 1990;7(8):863-8.
23. Rabanel JM, Hildgen P, Banquy X. Assessment of PEG on polymeric particles surface, a key step in drug carrier translation. J Control Release. 2014;185:71-87.
24. Jung T, Kamm W, Breitenbach A, Kaiserling E, Xiao JX, Kissel T. Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? Eur J Pharm Biopharm. 2000;50(1):147-60.
25. Iqbal J, Hombach J, Matuszczak B, Bernkop-Schnürch A. Design and in vitro evaluation of a novel polymeric P-glycoprotein (P-gp) inhibitor. J Control Release. 2010;147(1):62-9.
26. Hugger ED, Audus KL, Borchardt RT. Effects of poly(ethylene glycol) on efflux transporter activity in Caco-2 cell monolayers. J Pharm Sci. 2002;91(9):1980-90.
27. Hugger ED, Burton PS, Borchardt RT. Automated analysis of polyethylene glycol-induced inhibition of P-glycoprotein activity in vitro. J Pharm Sci. 2003;92(1):21-6.

Received*: 24-05-2025 -* ***Accepted****: 20-07-2025 -* ***Published****: 28-07-2025*