Available online on www.ijddt.com
International Journal of Drug Delivery Technology 2016; 6(4); 116-122

ISSN: 0975 4415

Research Article

Controlled *in Vitro* Release of Levodopa from Sodium Alginate Membranes

Margarida Franco, Sara Biscaia, Tânia Viana, Nuno Alves, Pedro Morouço*

Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal.

Available Online: 1st December, 2016

ABSTRACT

Levodopa (LD) plays a central role in Parkinson's Disease therapeutics. In this study, we aimed to encapsulate LD in sodium alginate (SA) membranes, and to study its dissolution profiles. Two types of SA membranes, loaded with two different amounts of LD were prepared and compared (M_1: 85 mg per 50 ml SA/LD; M_2: 127.5 mg per 75 ml SA/LD); membranes production followed a solvent-casting methodology. Calcium chloride was used as a crosslinking agent. LD solubility tests were performed to predict sink conditions required for complete drug dissolution. LD dissolution assays were carried out and UV spectrophotometry was used for cumulative release percentage determination. The obtained data were mathematically evaluated and fitted into mathematical dependent models; the difference factor (f_1), the similarity factor (f_2) and other parameters like dissolution efficiency (DE) were also used. No differences in the dissolution profiles of both membranes were noticed. Thus, increasing the amount of LD, but keeping the same concentration, led to a similar controlled release. The membranes presented in this work are expected to be a promising contribution in the development of a new controlled drug delivery system for LD administration.

Keywords: Parkinson's disease, controlled delivery, dissolution assays, drug release kinetics, mathematical model fitting.

INTRODUCTION

Parkinson's Disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease[1]. Commonly it has an onset peak age of 65 years although in younger individuals of less than 40 years and rare juvenile forms may occur[2]. Its trigger factors may include: (i) toxic metals which are involved in oxidative stress chemical reactions leading to the production of oxygen reactive species and cellular apoptosis[3]; (ii) enteric bacteria toxins; (iii) lack of complex B vitamins or diets rich in meat, fat and simple sugars and (iv) genetic and occupational reasons[4,5]. This disorder appears to be related with a loss of dopamine production and storage, due to a degeneration of the nigrostriatal dopaminergic neurons[6]. Symptoms include the classic phenotype of tremor, rigidity and bradykinesia as well as a wide number of non-motor symptoms, mainly in cardiovascular and gastric systems[7,8]. Since last century, in the late 50's, that Levodopa (LD) - a dopamine's precursor - plays a central role in the pharmacological treatment of PD, via its decarboxylation into dopamine. The main reason is because it has the ability to cross the brain blood barrier while dopamine itself does not[7-10]. The enzymes responsible for this transformation are the aromatic aminoacid descarboxylase enzyme complex. They are also present at the Gastro-Intestinal-Tract and even in the peripheral circulation, so that oral LD bioavailability is affected and its half life time dramatically reduced. Thus, the induced on-off effects that result from the peaks and valleys on the therapeutic levels are a huge constraint in the patient's quality of life[11]. However, the transdermal route of administration may be very useful for drugs that undergo extensive first-pass metabolism such as LD[1]. Even though it does not solve the peripheral degradation problem, it can help with the enteric one. Strategies should be developed to enhance or better control transdermal delivery which include replacing passive diffusion by active penetration, among others[12]. Accordingly, a transdermal delivery would provide a progressive and sustained supply of LD to the systemic circulation avoiding under-therapeutic doses. The Lipinski criteria are widely used by medical chemists to predict, not only the absorption of compounds, but overall drug-likeness. Lipinski rule of five includes properties like: (i) molecular weight; (ii) compound's lipophilicity expressed as logP; (iii) number of groups in the molecule that can donate hydrogen atoms to hydrogen bonds; and (iv) number of groups that can accept hydrogen atoms to form hydrogen bonds[13]; being LD a drug that follows all the mentioned criteria. On the other hand, sodium alginate (SA) is known to be a whole family of linear copolymers containing blocks of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues. This biomaterial is obtained from brown seaweed or bacteria and has found numerous applications in biomedical science and engineering, due to its favorable properties, including biocompatibility, low toxicity, low cost and ease of gelation by addition of divalent cations such as Ca^{2+}. For that reason, SA gels have been investigated for the delivery of a variety of low molecular weight drugs[14]. The central purpose of this work consisted on the production of two

Author for Correspondence:

membranes of SA with distinct LD mass amount, aiming to evaluate and compare their dissolution profiles. The methodology chosen was the dissolution assays recommended and adapted from the different pharmacopoeias, followed by LD dosage via UV determination. Membranes dissolution profile's data underwent studies based on independent methods using a difference factor (f_1) as well as a similarity factor (f_2)[15,16]. Other parameters like dissolution efficiency (DE)[17] and some dependent methods using a fitting process into different mathematical models[18-20] were also used. Actually, a transdermal administration mediated by a controlled mechanism of delivery would provide a higher LD bioavailability and a steady state plasma level. This is supposed to avoid motor complications and consequently improve patient's compliance and comfort[21].

MATERIALS AND METHODS

Materials

Very-low viscosity sodium alginate was obtained from Alfa Aesar GmbH & Co. Kg (Germany); L-3,4-dihydroxyphenylalanine, ±98% from Sigma Aldrich (China); calcium chloride dihydrated, ACS reag., from Sigma Aldrich; methanol and hydrochloric acid, 37% ACS reag., came from Sigma (Germany); acetic acid glacial, analytical grade, ACS reag., from Scharlab.S.L. (Spain); sodium acetate trihydrate, reagent grade, ACS reag., from Scharlab.S.L. (Spain); distilled water. All the aforementioned materials were used without any change or modification relatively to original supplier.

Membranes preparation

A 3% (w/v) SA solution was prepared with $1x10^{-4}$ M hydrochloric acid (HCl) under magnetic stirring. A value of 4pH was selected and fixed (pH metter Scansci 901, Scansci, Portugal) due to the negligible oxidation in acidic conditions of the LD[22]. Two membranes were prepared with different LD amounts: 85 mg in 50 ml SA/LD for membrane 1 (M_1); and 127.5 mg in 75 ml for membrane 2 (M_2). Both membranes had the same drug concentration (0.17% w/v). A solvent casting methodology was employed to produce the membranes. The solution was poured into a Petri dish and let too dry at room temperature. To prepare SA/LD crosslinked membranes (SA/LD/$CaCl_2$), the dry SA/LD membranes were taken out from the Petri dish and submerged in 20% $CaCl_2$ solution for 5 minutes. The same technique was used to produce SA membranes, without LD, crosslinked with $CaCl_2$[23-25]. The membranes were rinsed with methanol to remove the residual $CaCl_2$, and dried at room temperature[26-28].

Solubility tests

Previously to the preparation of pharmaceutical dosage forms, it is of utmost significance to test the referred drugs solubility in the appropriate solvent for the dissolution assays[29]. It is imperative to avoid an oversaturated situation, as it may lead to misleading results. Apparent solubility (S_{app}) refers to the dynamic solubility of LD in the selected solvent and was determined by stirring an excess amount of the drug in 10 ml of acetate buffer (pH 4.5). A multistirrer thermostated (KS 4000 i control, IKA, Germany) was used at 20 ± 0.5°C of temperature, for 5-h so that the prepared saturated solutions could achieve the equilibrium, which means that no more LD molecules could be dissolved. Some filters (1 FC 20 M 0 90, from Filtres RS) were used for the samples filtration. Samples were then diluted (1:2, 1:10 and 1:20) and the absorbance measured at 280 nm using a UV-Vis spectrophotometer (spectrascan from Scansci, Portugal). The amount of dissolved drug was calculated through a LD calibration curve created in the same conditions. Three samples were used for each assay[26-30].

Dissolution assays

Dissolution is a process which occurs when a substance enters into a solvent to yield a solution. This occurrence is controlled by the affinity between the substance and the solvent. Dissolution procedure is a relevant instrument for monitoring batch-to-batch quality control and to accomplish drug release studies. The right *in vitro* method and choosing the proper apparatus provides a way to design effective drug products. Nowadays, dissolution testing is harmonized between United States Pharmacopoeia (USP), European Pharmacopoeia (EP), Japanese Pharmacopoeia (JP) and Portuguese Pharmacopoeia (PP)[31-34]. According to the guidelines imposed by those authorities, both membranes were submerged in 250 ml of the chosen solvent, which was an acetate buffer, pH 4.5 to respect the sink conditions. Sink conditions usually take place in a volume of dissolution medium that is three times the saturation volume. The undersaturated situation would provide enough volume of dissolution medium to dissolve the expected amount of drug released from the membrane. The analyses were performed at 32 ± 0.5ºC with 100 rpm of stirring speed. At predetermined sampling time points a 3 ml aliquot was removed and the same amount of fresh buffer was replaced in order to maintain the sink conditions[34,35]. The samples were then dosed at 280 nm and concentrations estimated using the calibration curve. Three samples were used for each assay[26-28].

Dissolution profile studies

The pharmaceutical industry and the registration authorities do focus, nowadays, on drug dissolution studies. To fulfill this goal, cumulative profiles of dissolved drug are more commonly used rather than their differential profiles[17]. Several methods have been proposed for the quantitative study of the values obtained in dissolution/release tests. They are related with mathematical model's independent and dependent methods (curve fitting) and some were applied in the present study. They included two pair-wise independent methods based on similarity and difference factors, another independent method based on the mathematical determination of the efficiency of dissolution (DE) and four methods which depend on mathematical models[15-17, 36]. Consequently, the values of the amount of drug dissolved up to 480 minutes from both membranes were studied in terms of the comparison of their release profiles. A simple independent method approach using a difference factor (f_1) and a similarity factor (f_2) to compare dissolution profiles was applied. The difference factor (f_1) calculates the difference between the two curves at each time point

$$D.E.(\%) = \frac{SA}{R} \times 100$$

SA – Shaded area

R – Rectangle area (y_{100} x t)

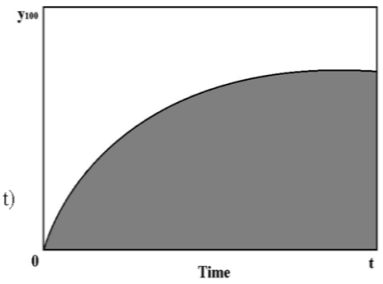

Figure 1: Dissolution of a drug from a pharmaceutical dosage form.

and is a measurement of the relative error between the two curves. It is represented by Equation 1:

$$f_1 = \{[\sum_{t=1}^{n}|R_t\text{-}T_t|]/[\sum_{t=1}^{n} R_t]\}\times 100 \quad (1)$$

The similarity factor (f_2) is a logarithmic reciprocal function of the difference in percentage dissolved at each time point between two profiles belonging to two formulations and is determined following Equation 2:

$$f_2 = 50 \cdot log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0,5} \times 100\right\} \quad (2)$$

For both, n is the number of time points, R_t and T_t are the percent dissolved of the reference and test products, respectively, at each time point t. For curves to be considered similar, f_1 values should be close to 0, and f_2 values should be close to 100. Generally, f_1 values up to 15 (0-15) and f_2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves[16]. Another parameter evaluated was the dissolution efficiency (DE). DE of a pharmaceutical dosage form is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It is represented in Figure 1 and can be calculated by Equation 3, where y is the cumulative release percentage and t is the elapsed time test[17].

$$D.E. = \frac{\int_0^t y \times dt}{y_{100} \times t} \times 100 \quad (3)$$

Finally, model dependent methods based on different mathematical functions were employed. Once a suitable function has been selected, the dissolution profiles can be evaluated depending on the derived model parameters. The model dependent approaches include Zero order, First order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Baker-Lonsdale, Weibull, Hopfenberg, Gompertz among others. These kinetic models, describe the overall release of the drug from dosage forms[20]. This work dissolution assay's results were fitted into Zero order (Equation 4), First order (Equation 5), and Higuchi (Equation 6) kinetic models as follows:

$$Q_t = Q_0 + K_0 t \quad (4)$$

where Q_t is the amount of drug dissolved in time t, Q_0 is initial amount of drug in solution, and K_0 is zero order release constant.

$$\ln\left(\frac{Q_t}{Q_0}\right) = K_1 t \quad (5)$$

where Q_t is the amount of drug dissolved in time t, Q_0 is initial amount of drug, and K_1 is first order release constant.

$$Q = K_H t^{1/2} \quad (6)$$

where Q is the amount of drug released in time t and K_H is Higuchi dissolution constant. Additionally, to characterize drug release mechanisms from the polymeric system studied, the Korsmeyer-Peppas model was also applied (Equation 7):

$$\frac{M_t}{M_\infty} = Kt^n \quad (7)$$

where, M_t/M_∞ is a fraction of drug release at time t, K is the release rate constant and n is the release exponent, which depends on the release mechanism and the shape of the matrix tested (Table 1)[17,37-39]. Korsmeyer-Peppas equation is not merely a fitting expression but a consequence of a detailed mechanistic process that involves a pure diffusional (Fickian) mechanism with an additional relaxational or convection mechanism. In fact, this equation rises from a molecular and mechanistic analysis, while other models have a more fitting approach[19].

RESULTS AND DISCUSSION

Solubility tests

The S_{app} value for LD, using the calibration curve previously prepared (Figure 2), was 1.69 ± 0.03 mg/ml. This value was obtained with a 1:10 dilution because: (i) 1:2 dilution revealed absorbance values too high for the calibration curve; and (ii) with 1:20 dilution the associated error would be higher than with 1:10 dilution.

Dissolution assays

Although some authors consider that the significance and mechanism of a rapid initial release have not been fully clarified[40], the burst effect of LD release (at 5 min: 25.97 ± 2.09% in M_1 and 18.79 ± 0.88% in M_2) suggests that it may be associated with the surface adsorbed drug. High availability of drug on surface will show higher burst release and only after that, the entrapped drug in the matrix during fabrication will be released over a period of time that depends on the polymer, on drug properties and on the pharmaceutical technology applied[41]. SA is a hydrophilic polymer that is more suitable for water-soluble drugs as compared to water-insoluble drugs. In both type of membranes, SA encapsulation efficiency of the drug loaded was increased because of the high intermolecular forces of the formulation at this pH, which delays the drug diffusion across the polymer membrane. In fact, in both cases, after the mentioned initial release, a portion of the drug have shown a more controlled release determined by the water absorption process and by the polymeric chains loosening, which promotes drug diffusion[41]. There is still another drug fraction that did not exhibit any release at all, and that in further studies should be considered. This could be due to its strong entrapment inside the hydrogel net because, as above-mentioned, at the pH of the experiment, SA is actually in its acidic form with a higher viscosity, as demonstrated in Figure 3.

Data analysis

As previously referred, the independent methods pair-wise procedures include the difference factor (f_1) and the similarity factor (f_2). In the present work, f_1 values obtained from the dissolution curves up to 480 minutes were 8.07 (considering M_1 as the reference) and 8.49 (when M_2 was considered as the reference). Small bias should not be

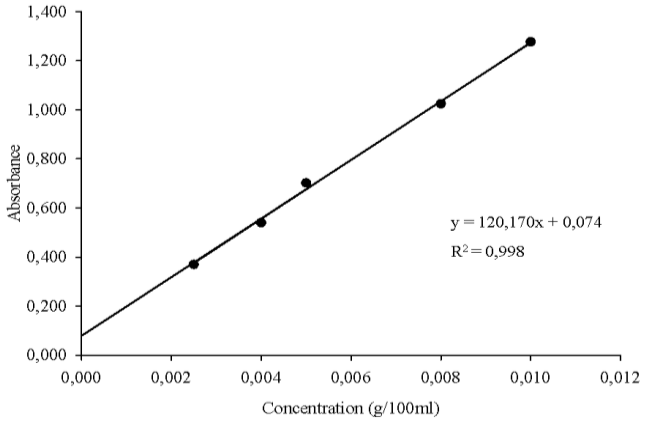

Figure 2: Levodopa calibration curve at 280 nm.

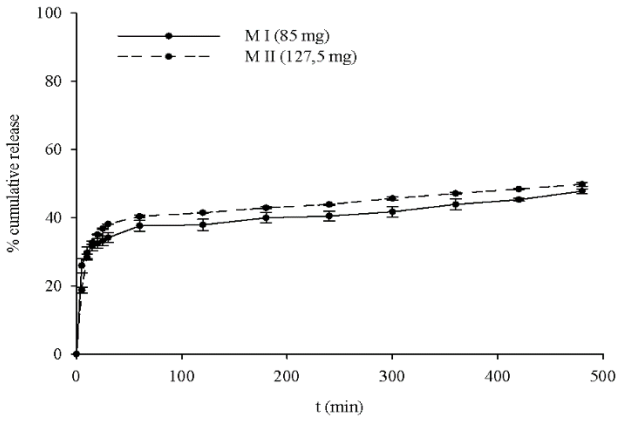

Figure 3: Cumulative release percentage of M_1 and M_2 membranes for the 8-h period.

Table 1: Release mechanisms of polymeric controlled delivery systems.

Release exponent (*n*)			Drug release mechanism
Thin film	*Cylinder*	*Sphere*	
0.5	0.45	0.43	Fickian diffusion
0.5<*n*<1.0	0.45<*n*<0.89	0.43<*n*<0.85	Anomalous transport
1.0	0.89	0.85	Case-II transport

Table 2: Mechanisms and kinetics of drug release from M_1 and M_2 membranes (R^2: determination coefficients; *n*: release exponents).

Membrane	Zero order		First order		Higuchi		Korsmeyer-Peppas		
	R^2	K	R^2	K	R^2	K	R^2	K	*n*
M_1	0.482	0.047	0.811	0.001	0.639	1.241	0.968	22.87	0.112
M_2	0.478	0.054	0.528	0.001	0.654	1.435	0.818	20.04	0.151

valorized as values of f_1 up to 15 conduct to similar dissolution profile's[16]. Additionally, the value of f_2 obtained was 72.12. This value also supports the similarity between the two release profiles. Furthermore, this similarity factor (f_2) has been adopted by the Center for Drug Evaluation and Research (FDA) and by Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicinal Products (EMEA), as a criterion for the assessment of the similarity between two *in vitro* dissolution profiles[17]. Likewise, the dissolution efficiency was similar between membranes (40.5% vs. 43.5%). Although this parameter contributes with low information to elucidate the release mechanism, it may be used in association with other variables[17]. In the present study, the *in vitro* release data was fitted in four different mathematical models, with the purpose of predicting mechanism and kinetics release of LD from SA membranes (cf. Figure 4); and as shown in Table 2, the investigated release profiles were better explained by Korsmeyer-Peppas equation (M_1 R^2 = 0.968; M_2 R^2 = 0.818). The studied drug delivery systems have film geometry, thus, once the values of release exponent (*n)* were lower than 0.5 in the two cases, indicated that the drug release followed a Fickian mechanism, i.e., the release depending only on a diffusion m echanism[37,42,43]. On the other hand, the value of the release exponent (*n*) obtained was below the limits imposed by Korsmeyer model (Table 1). Nevertheless, power law can only give limited insight into the exact release mechanism of the drug[44]. Consequently, the obtained values of the exponent *n* provided an indication for the diffusion controlled drug release mechanism, but may not be automatically valid for the LD/SA membranes studied.

CONCLUSIONS

In the present work, SA and SA/LD membranes with different amount of LD, both crosslinked with $CaCl_2$, were successfully produced. UV dosage results suggest that a fraction of the drug is strongly linked to the polymeric complex or more effectively encapsulated inside it, while another portion is adsorbed on the membranes surface and consequently more suitable to be released. This fact is one of the features contributing for the initial burst release. Under the experimental conditions, SA becomes alginic acid which is a gel with increased intermolecular forces, therefore with better ability to hold a molecule. This event can possibly explain that a part of the loaded LD is released in a more sustained way. The complex formed between the polymeric matrix and LD proved to control and retard the release of this drug. This occurrence may possibly have a role in the modulation of its bioavailability. In terms of the comparison of the two kinds of membranes studied, both proved to have similar dissolution profiles from the point of view of their release kinetics and mechanism, following Korsmeyer-Peppas model. Moreover, they showed quite identical DE. According to f_1 and f_2 values they can also be considered bioequivalents. Further studies should investigate mechanisms: (i) to increase the availability of

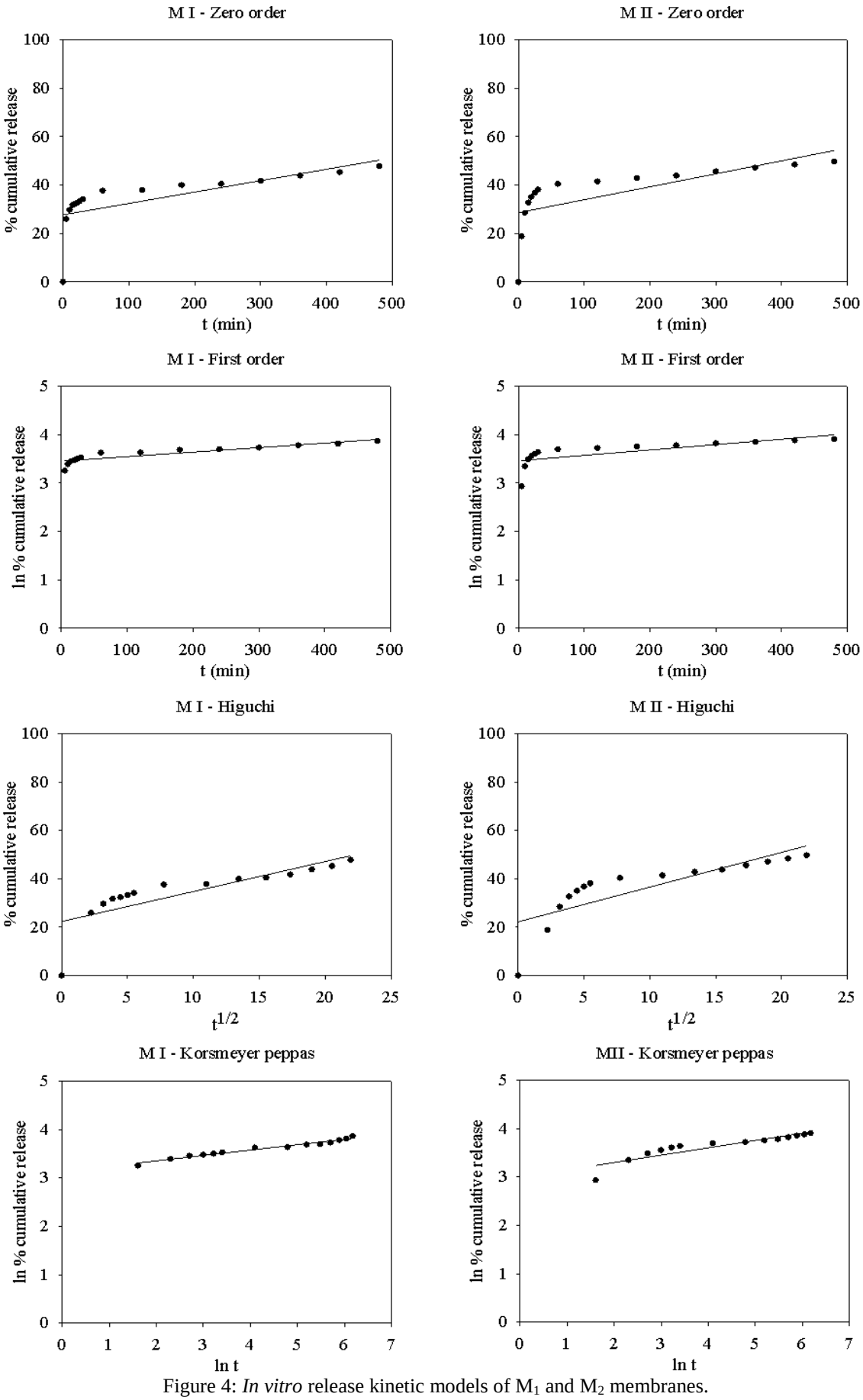

Figure 4: *In vitro* release kinetic models of M_1 and M_2 membranes.

SA membranes to support higher amount of LD; and (ii) to reduce the drug fraction that did not exhibit any release at all.

ACKNOWLEDGEMENTS

This research was funded by the Portuguese Government – Foundation for Science and Technology (UID/Multi/04044/2013).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

REFERENCES

1. Goole J, Amighi K. Levodopa delivery systems for the treatment of Parkinson's disease: an overview. Int J Pharm. 2009; 380:1-15.
2. Dick F D. Parkinson's disease and pesticide exposures. Br Med Bull. 2006;79-80:219-231.
3. Rodrigues M, Campos LC. Estratégia para o tratamento com levodopa na doença de Parkinson. Revista Analytica. 2006; 23:44-51.
4. Parkinson's Disease. 1995-2012 Life Extension®. www.lef.org. Assessed 23 Mar 2016.
5. Dick S, Semple S, Dick F, Seaton A. Occupational titles as risk factors for Parkinson's disease. Occ Med. 2007; 57:50-56.
6. Lindgren HS, Cenci MA, Lane EL. Dyskinesia – Advances in the Understanding of Pathophysiology and Possible Treatment Options. Eur Neurological Rev. 2010;5(2):34–40.
7. Gallagher DA, Schrag A. Impact of Newer Pharmacological Treatments on Quality of Life in Patients with Parkinson's Disease. CNS Drugs. 2008;22(7):563-586.
8. Rashed ER, El-Rehim HA, El-Ghazaly MA. Potential efficacy of dopamine loaded-PVP/PAA nanogel in experimental models of Parkinsonism: Possible disease modifying activity. J Biomed Mater Res Part A. 2015;103.5:1713-1720.
9. Perez-Lloret S, Rascol O. Dopamine Receptor Agonists for the Treatment of Early or Advanced Parkinson's Disease. CNS Drugs. 2010;24(11):941-968.
10. Chen Y, Liu L. Modern methods for delivery of drugs across the blood–brain barrier. Adv Drug Deliv Rev. 2012;64(7):640–665.
11. Jankovic J. Parkinson's disease therapy: treatment of early and late disease. Chin Med J. 2001;114(3):227-234.
12. Martini LG, Crowley PJ, Ibrahim S. Controlling Release from Dosage Forms: The Future. American Pharmaceutical Review 2012.
13. Leeson P. Drug discovery: Chemical beauty contest. Nature. 2012; 481:455–456.
14. Lee WL, Shi WX, Low ZY, Li S, Loo SCJ. Modeling of drug release from biodegradable triple-layered microparticles. J Biomed Mater Res Part A. 2012;100A:3353-3362.
15. Moore JW, Flanner HH. Mathematical Comparison of Dissolution Profiles. Pharma Tech. 1996;20(6):64-74.
16. Guidance for Industry: Dissolution testing of immediate release solid oral dosage forms. U. S. Department of Health and Human Services, Food and Drug Administration. Center for Drug Evaluation and Research (CDER). August 1997.
17. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001; 13:123–133.
18. Shoaib MH, Tazeen J, Merchant HA, Yousuf RI. Evaluation of drug release kinetics from ibuprofen matrix tablets using HPMC. Pak J Pharm Sci. 2006;19(2):119-124.
19. Peppas NA, Narasimhan B. Mathematical models in drug delivery: How modeling has shaped the way we design new drug delivery systems. J Control Release. 2014; 190:75-81.
20. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems. Acta Pol Pharm. 2010;67(3):217-223.
21. Jenner P, McCreary AC, Scheller DK. Continuous drug delivery in early- and late-stage Parkinson's disease as a strategy for avoiding dyskinesia induction and expression. J Neural Transm. 2011;118(12):1691–1702.
22. Kankkunen T, Huupponen I, Lahtinen K, Sundell M, Ekman K, Kontturi K, Hirvonen J. Improved stability and release control of levodopa and metaraminol using ion-exchange fibers and transdermal iontophoresis. Eur J Pharm Sci. 2002; 16:273-280.
23. Mi FL, Liang HF, Wu YC, Lin YS, Yang TF, Sung HW. pH-sensitive behavior of two-component hydrogels composed of N, O-carboxymethyl chitosan and alginate. J Biomater Sci Polym Ed. 2005;16(11):1333-1345.
24. Saeedi M, Akbari J, Enayatifard R, Morteza-Semnani K, Tahernia M, Valizadeh H. In Situ Cross-Linking of Polyanionic Polymers to Sustain the Drug Release from Theophylline Tablets. Iran J Pharm Res. 2009;8(4):241-249.
25. Nokhodchi A, Tailor A. In situ cross-linking of sodium alginate with calcium and aluminum ions to sustain the release of theophylline from polymeric matrices. Farmaco. 2004;59(12):999-1004.
26. Franco MR, Biscaia S, Viana T, Bártolo PJ. Levodopa controlled release from alginate membranes. In: Bártolo PJ et al. editors. High Value Manufacturing – Advanced Research in Virtual and Rapid Prototyping. London: CRC Press; 2014. p 189-194. ISBN: 978-1-138-00137-4.
27. Franco MR, Viana TF, Biscaia SI, Bártolo PJ. Levodopa incorporation in alginate membranes for drug delivery studies. Adv Mat Res. 2013; 749:423-428.
28. Franco M, Viana T, Biscaia S, Bártolo PJ. Levodopa controlled release from alginate membranes. Rev Saude Publica. 2014;48:161.

29. Salústio P. Aumento da solubilidade de fármacos pouco solúveis em complexos com a β-ciclodextrina. PhD thesis, Faculty of Pharmacy, University of Lisbon 2009.
30. Petralito S, Zanardi I, Memoli A, Annesini MC, Millucci V, Travagli V. Apparent Solubility and Dissolution Profile at Non-Sink Conditions as Quality Improvement Tools. In: Basnet P. editor. Promising Pharmaceuticals. InTech; 2012;5. p 83-101. ISBN: 978-953-51-0631-9, DOI: 10.5772/37656.
31. Kadajji VG, Betageri GV, Venkatesan N. Approaches for Dissolution Testing of Novel Drug Delivery Systems. American Pharmaceutical Review 2011.
32. Hawley M. Stage Appropriate Dissolution Methods in Formulation Development. American Pharmaceutical Review 2013.
33. USP General Chapter on Dissolution (711). United States Pharmacopeia and National Formulary. United States Pharmacopeial Convention, Inc.: Rockville, MD 2008:267-274.
34. Portuguese Pharmacopeia 9, (2.9.4.): Ensaio de dissolução dos adesivos transdérmicos. INFARMED Mds, I.P. Lisboa 1 2010:304-306.
35. Qureshi S. Sink Condition: Solely an in vitro (analytical chemistry) and not the in vivo or physiological requirement. www.drug-dissolution-testing.com.
36. Carvalho-Silva B, Moreira-Campos LM, Nunan EA, Vianna-Soares CD, Araujo-Alves BL, Cesar IC, Pianetti GA. Optimization and statistical evaluation of dissolution tests for indinavir sulfate capsules. Farmac.o 2004;59(11):921–927.
37. Li L, Wang L, Shao Y, Ni R, Zhang T, Mao S. Drug release characteristics from chitosan-alginate matrix tablets based on the theory of self-assembled film. Int J Pharm. 2013; 450:197-207.
38. Rajesh N, Siddaramaiah. Feasibility of xantham gum-sodium alginate as a transdermal drug delivery system for domperidone. J Mater Sci Mater Med. 2009;20(10):2085-2089.
39. Kamboj S, Saini V, Bala S. Formulation and characterization of drug loaded noionic surfactant vesicles (Niosomes) for oral bioavalability enhancement. Scientific World Journal. 2014; 2014:1-8.
40. Costa PJC. Avaliação *in vitro* da lioequivalência de formulações farmacêuticas. Braz J Pharm Sci. 2002;38(2):141-153.
41. Nagarwal RC, Kumar R, Pandit JK. Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: In vitro characterization and in vivo study in rabbit eye. Eur J Pharm Sci. 2012; 47:678-685.
42. Schliecker G, Schmidt C, Fuchs S, Ehinger A, Sandow J, Kissel T. In vitro and in vivo correlation of buserelin release from biodegradable implants using statistical moment analysis. J Control Release. 2004; 94:25-37.
43. Veronovskim A, Knez Z, Novak Z. Comparison of ionic and non-ionic drug release from multi-membrane spherical aerogels. Int J Pharm. 2013; 454:58-66.
44. Siepmann J, Peppas NA. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev. 2001; 48:139-157.