DATA DRIVEN MODELLING OF THE CHLOROQUINE PHOSPHATE PHARMACOKINETICS OF DEGRADABLE PLGA BASED NANOPARTICLES

Sandeep Kumar Shukla, Chandni Pachouri, Sandhya Pathak, Bharat Patel, Satyendra Kumar Tripathi, Archna Pandey

Abstract

Chloroquine phosphate loaded PLGA nanoparticles were prepared using the modified nanoprecipitation method. PLGA is a synthetic copolymer that can be easily functionalized to achieve desired outcomes. PLGA nanoparticles are biodegradable, biocompatible, non-toxic and easily prepared.The primary focus of the study was concerned with the preparation of Chloroquine phosphate-loaded PLGA nanoparticles in order to improve drug tolerance, reduce the frequency of doses and less toxic. Formulation of nanoparticles were characterized for encapsulation efficiency using UV-vis. spectroscopy; particle size distribution, polydispersity index, and zeta potential using photon correlation spectroscopy; and in vitro drug release profile using UV-vis. spectroscopy. The sizes of nanoparticles formulation were 152.3 nm, polydispersity index is 0.272, encapsulation efficiency was 75 % and drug loading efficiency was 60 % respectively. Ready nanoparticles were spherical shape, smooth surface and free-flowing nature.In vitro drug release studies showed that prepared nanoparticles-maintained drug release over 24 hours. Infra-red (IR) spectra showed the stability of Chloroquine phosphate loaded PLGA nanoparticles and the absence of drug-polymer interactions.

Keywords: Biodegradable, entrapment efficiency, nanoprecipitation, PLGA, nanoparticles.

Department of Chemistry, Dr. Harisingh Gour Vishwavidyalaya (Central University), 470 003 Sagar, (M.P.), India

[*]Corresponding author, s.shukla3634@gmail.com, prof.archnapandey@gmail.com

Eur. Chem. Bull. **2023**,*12(Special Issue 1), 283-291*

283

Introduction

Malaria is often confronted with the problem of instability and poor biological distribution, which causes an alteration of pharmacokinetics. [1]. Considerable efforts have been directed towards malaria eradication in endemic areas. Chemotherapy has been a major factor in the fight against the disease. Without an effective vaccine, chemotherapy is the only easily accessible option for malaria management.Much of this morbidity and mortality could be prevented if the medicines available to patients were effective, of high quality and used correctly[2,3]. Chloroquine phosphate is the main antimalarial agent. Chloroquine phosphate has been the antimalarial drug of choice for many decades because of its safety, high efficiency and low cost [4].However, given the widespread prevalence of chloroquine-resistant (CQR) parasitic strains, CQ was replaced as front-line antimalarial chemotherapy in the late 1990s [5].

Nanomedicine has been praised for its success in the distribution of medicines and its improved effectiveness [6-8]. The aim of the study was to assess the antiplasmodial efficacy. Chemotherapy involves the development of multiple drug resistance and non-specific targeting of intracellular parasites, leading to high dose requirements and subsequent intolerable toxicity. Nanocarriers have been given special attention with the aim of minimizing the side effects of drug therapy, such as poor bioavailability and drug selectivity [9-11].New anti-malarial drugs must meet the requirements of fast efficacy, minimum toxicity and low cost.So nanotechnology is a vital part of that.Ideally, the nanoparticle delivery system would selectively accumulate in the required organ or tissue and, at the same time, penetrate the target cells to release the bioactive agent [12-14]. These nanocarriers are known to enhance the effectiveness of currently available anti-malarial drugs and contribute to the formulation and administration of new chemical entities. Targeting drugs specifically on their site of action has a huge benefit in malaria since malaria parasites often develop drug resistance through the administration of low levels of drugs in the presence of high numbers of parasites. Moreover, nanomedicine has the potential to restore the use of old and toxic medicines by altering their biological distribution, improving bioavailability and reducing toxicity. [15,16]. PLGA use as a nano-carrier in the present study because it is a biodegradable polymer and it co-polymer including poly (esters), including poly (lactic acid), poly (glycolic acid) is nature biocompatibility and biodegradability provide a versatile range of polymer in pharmaceutical industry [17,18].

The aim of the work is prepared chloroquine phosphate were loaded in poly (d,l-lactic-co-glycolic acid) (PLGA) using modified nanoprecipitation technique. The nanoparticle formed was characterized for size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The in vitro release of the drug was also determined by dialysis beg method. Nanoparticles have been widely applied as promising antimalarial drug nanocarriers thanks to their biocompatibility, high loading capacity, chemical stability and straightforward synthesis/ surface functionalization.

Experimental
Materials

Chloroquine phosphate was gifted from Lupin Pharma Laboratories Ltd., Bhopal, Madhya Pradesh, India. Polyvinyl alcohol (PVA, Mw: 160000, 86.5-89.0% hydrolyzed) are purchased from HiMedia Laboratories Pct. Ltd., Nashik, India. Poly (D-acid, L-lactic-co-glycol) (PLGA, 50:50, Mw: 30000-60000) was obtained from Sigma Aldrich, Bangalore, India. Acetone were purchased from the Central Drug House (CDH), Delhi, India. Phosphate buffer, spam 80 were purchased from HiMedia Laboratory Pvt. Ltd., Mumbai, India. All solvents and chemicals were analytical grade.

Preparation of Chloroquine phosphate loaded PLGA nanoparticles

The optimized formulation (CQP-PLGA-NPs), were prepared by using modified nanoprecipitation technique as follows: 10 mg drug dissolved in distilled water and 50 mg PLGA dissolved in 5 ml acetone both are mixed. The organic phase added dropwise into aqueous phase containing 1% PVA solution (pH 7.4 phosphate buffer saline) under

Eur. Chem. Bull. **2023,***12(Special Issue 1), 283-291*

284

magnetic stirring at 1200 rpm. The solution was kept for 5 hours to permit acetone to evaporate. Subsequent to this, the suspended nanoparticles were freeze-dried [19].

Particle size and polydispersity index

The newly developed CQP-PLGA-NPs were released into deionized water.Suspension has been characterised for particle size and polydispersity index (PDI) by using the dynamic light scattering technique (DLS) (Malvern Instruments Ltd. and Nanoplus Particulated System).

Determination of drug entrapment efficiency and drug loading

The determination of drug entrapment efficiency (%EE) and drug loading (%DL) of prepared nanoparticles by using UV spectrophotometer(LABINDIA analytical, UV 3092) at 220 nm.The newly prepared suspension was centrifuged at 18000 rpm for 15 minutes to obtain a clear supernatant.The encapsulation efficiency (EE) and drug loading (DL) were calculated as following equations [20]:

$$\%EE = \frac{\text{Total amount of drug} - \text{Free amount of drug in supernatant}}{\text{Total amount of drug}} \times 100 \qquad (1)$$

$$\%DL = \frac{\text{Weight entrapped drug}}{\text{Weight of nanoparticles recoverd}} \times 100 \qquad (2)$$

Morphological analysis

The morphologies of the optimizedCQP-PLGA-NPs were studied by transmission electron microscope (TEM, TECNA), scanning electron microscope (SEM, NOVA NANO FESEM 450) and atomic force microscopy (AFM, INNOVA, ICON analytical equipment, Bruker). In the TEM analysis, lyophilized nanoparticles were then diluted with 2 mL ethanol and mixed uniformly by sonication for 5 min. The samples were prepared by placing a drop from the nanoparticle suspension on the Formvar-coated, air-dried copper grid.For SEM analysis, lyophilized nanoparticles were mounted on double-sided adhesive carbon strains, and the particles were examined under low vacuum and high potential. The 3D organization and surface morphology of the

nanoparticles were investigated by AFM microscopy in threaded mode with 100 mm long tips and cantilevered beams. The small quantity of nanoparticle suspension was attached to the magnetic study with the glass lid holder and dried at 50°C in the oven.

Fourier transform infrared spectroscopy analysis

The FTIR spectra of PLGA, chloroquine phosphate, and CQP-PLGA-NPs were recovered by using the Bruker-FTIR Spectrophotometer. The spectra were recovered in a wavelength range between 4000 and 400 cm^{-1} and interpreted with the help of FT-IR software.
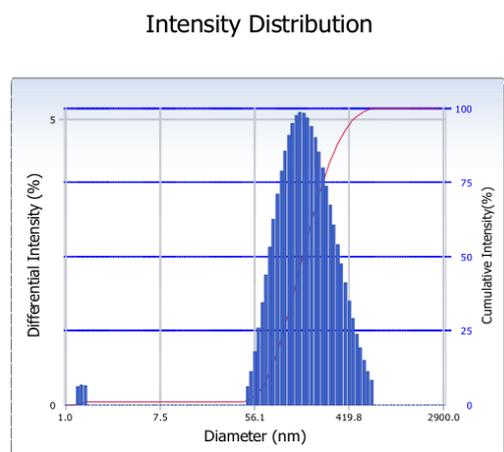
In vitro drug release study and data analysis

An *in vitro* drug release study was conducted to determine the drug release profile by using CQP-PLGA-NPs. The *in vitro* release study was carried out using the dialysis bag method with the help of a dialysis membrane (Mw: 12000-14000 Da). CQP-PLGA-NPs containing nanoparticles was suspended in 10 mL of phosphate buffer saline (PBS, pH = 7.4) in the dialysis bag at a rotation speed of 100 rpm and temperature of 37 °C. Periodically, 5 mL of the sample was collected and the same volume of PBS (pH = 7.4) was added. The quantity of drug released was determined by UV-VIS spectrometry at a wavelength of 220 nm at various moments. The cumulative % of the drug released was analysed through various kinetic models such as Zero order kinetic model, First-order kinetic model, Higuchi model and Korsmeyer-Peppas model.

Results and discussion
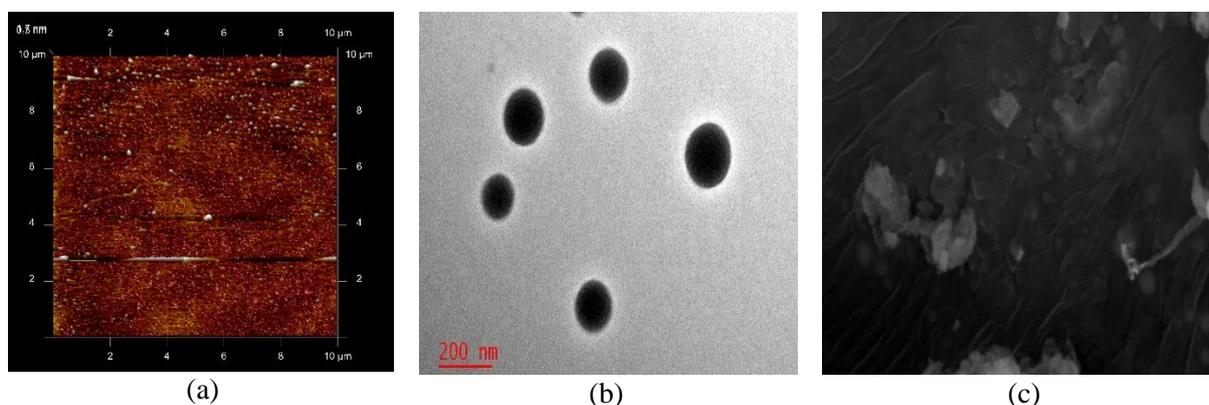## Particle size and polydispersity index

The size of the optimized formulation CQP-PLGA-NPs was about 152.3nm (Figure 1), which was within the nano range. The PDI values of the nanoparticles were in the range of 0.272. The PDI value ≤ 1 showed that stability of the prepared nanoparticles.

Eur. Chem. Bull. **2023**,*12(Special Issue 1), 283-291*

285

Intensity Distribution

(a)

Figure 1. (a) Particle size distribution of nanoparticles
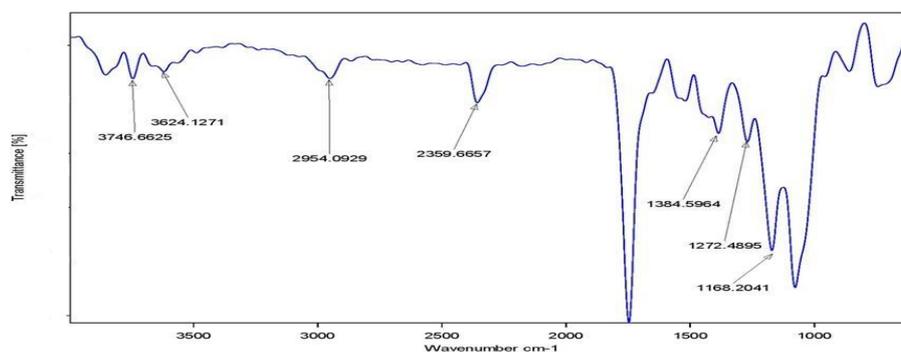
Morphological analysis

The shape and surface morphology of the nanoparticles were identified by using AFM, TEM, and SEM techniques. The AFM image of nanoparticles formulation is shown in Figure 2a. However, TEM and SEM images of the formulation are shown in Figures 2b and c, respectively. The SEM and TEM images of CQP-PLGA-NPs confirmed that the particle is spherical in shape, homogeneous size distribution and smooth. The analysis also indicates that the size of the nanoparticles is in the range of nano size and the diameter of the particle is 152.3 nm.

(a) (b) (c)

Figure 2. (a) 3D view of AFM of the formulation, (b) TEM image, and (b) SEM image of the nanoparticles.

Determination of drug entrapment efficiency and drug loading

The entrapment efficiency of CQP-PLGA-NPswas calculatedusing the spectrophotometric method. The %EE and %DL of the CQP-PLGA-NPswere measured as 75%and 60%, respectively.

Fourier transform infrared spectroscopy (FT-IR) analysis

The FT-IR spectrum of chloroquine phosphate, PLGA and CQP-PLGA-NPs is presented in Figure 3.The absorption bands in the spectra were recorded for the drug and its charged PLGA nanoparticles in the 400-4000 cm^{-1} area.

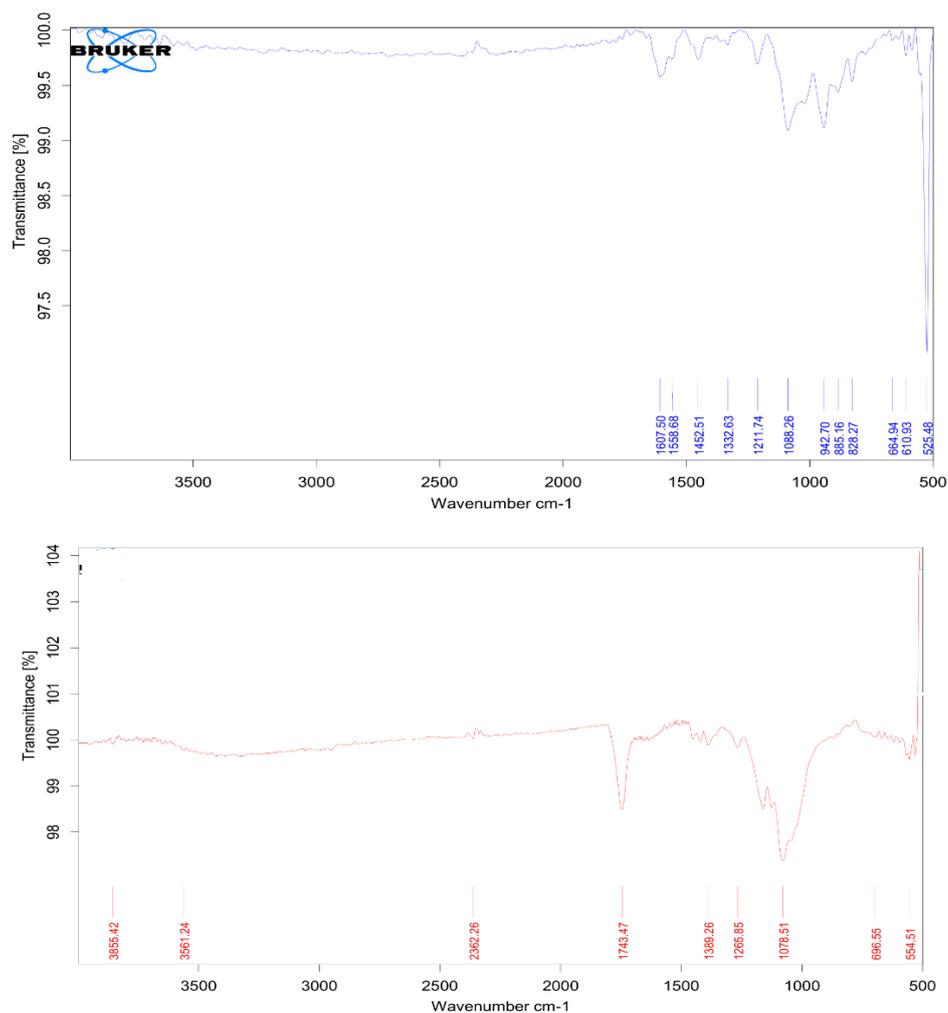*Eur. Chem. Bull. 2023,12(Special Issue 1), 283-291*

286

Figure 3. FTIR spectra of PLGA (a), chloroquine phosphate (b), and CQP-PLGA-NPs (c).

FT-IR spectra of chloroquine phosphate showed a characteristic peak of NH_2 bending at 1743.47 cm^{-1}, aromatic C=C stretching at 1389.26 cm^{-1}, C-N stretching at 1256.85, 1078.51 cm^{-1}. The spectra for PLGA polymer showed peaks at 3746-3624 cm^{-1} which is its characteristic peak of O-H stretching. The C-H stretching peak was found at 2954 cm^{-1}, and the C-O stretching peaks at 1168 and 1272 cm^{-1} were also observed in the spectra. For CQP-PLGA-NPs, the peaks of chloroquine phosphate are much less intense due to the low concentration of the drug in the nanoparticles (Figure 3c). There is also no significant change in functional peaks between the spectrums of drugs, polymers and prepared nanoparticles. Spectral analysis indicated that the specific functional groups of the polymer material in the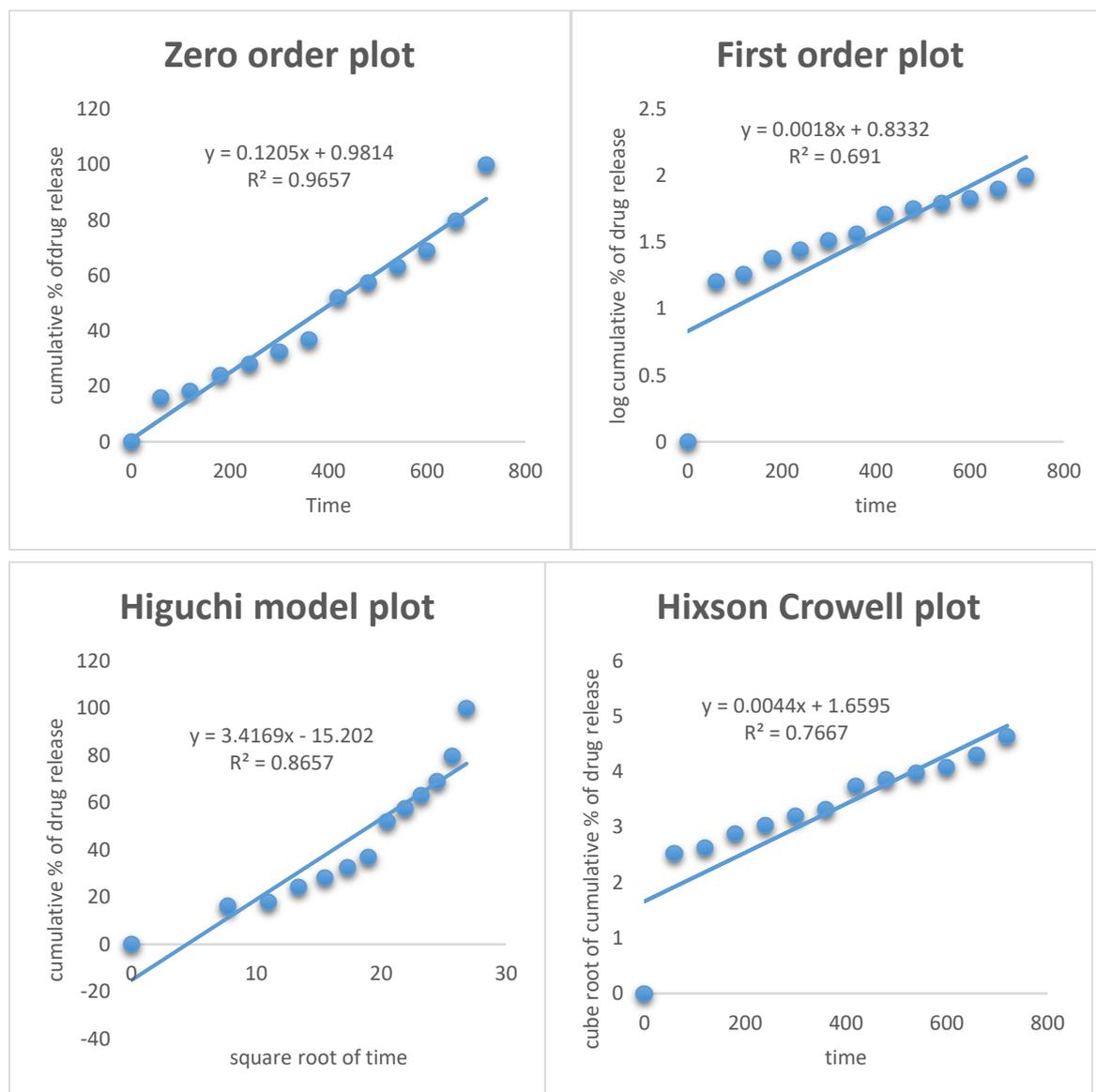 nanoparticle surface have nearly the same chemical characteristics between the pure polymer and the drug-trapped polymer.

In-vitro **drug release studies**

In vitro drug release studies were conducted in phosphate buffer pH 7.4 for the optimized formulation CQP-PLGA-NP.Release patterns indicate that CQP-PLGA-NPs showed sustained release of the drug from the polymer matrix.Therefore, it proves that the formulated nanoparticles show sustained release of the drug within 24 hours, the formulation has shown high entrapment efficiency as shown in Figure 4. The release of the drug with different time data were plotted by different kinetic models such as Zero order kinetic model, First-order kinetic model, Higuchi model and Korsmeyer-Peppas model.

Eur. Chem. Bull. **2023**,*12(Special Issue 1), 283-291*

287

Table 1. R^2 values and rate constants of different models for *in vitro* drug release studies.

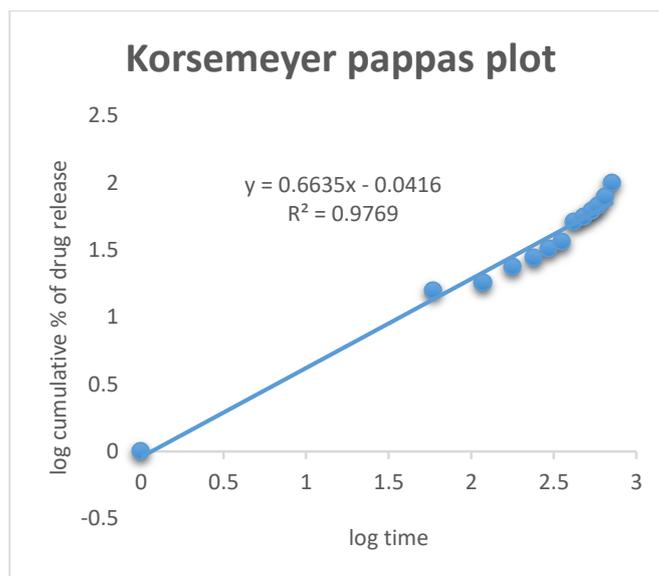| No	Kinetic model	R^2	k	N
1	Zero-order	0.9657	-	-
2	First-order	0.691	-	-
3	Higuchi	0.8657	-	-
4	Hixson-Crowell	0.7667	-	-
5	Koersemeyer-Peppas	0.9769	-	-

Figure 4. Drug release kinetics plots: (a) zero order plot, (b) first order plot, (c) Higuchi plot,(d) Hixon-Crowell model, and (e) Korsmeyer-Peppas plot.

The drug release correlation coefficients (R) obtained from different kinetic models are shown in Table 1. According to the best-fitted model, the maximum regression value (R^2) is taken for consideration. It is concluded that the formulation follows the zero-order model, the correlation coefficient value of the zero-order model is 0.9657.

Conclusions

In this study, chloroquine phosphate was successfully encapsulated in PLGA nanoparticles through the modified nanoprecipitation technique.This work suggests that the aqueous phase (pH 7.4 phosphate buffer), the stabilizer (PVA) and optimization based on the polymer drug report PLGA (1:5) and stabilizer concentration (1%) play a key role in achieving higher entrapment efficiency (75%), optimal partical size (152.3 nm) and narrow polydispersity index (0.272). The prepared CQP-PLGA-NPs have lots of potential, increased bioavailability, better patient compliance, decreased frequency of dose and reduce toxicity. In vitro drug release studies showed that prepared nanoparticles maintained drug release over 24 hours.The drug-polymer interaction was assured by the FTIR spectra.

References

1. Oyetunde Oyeyemi, Olajumoke Morenkeji, Funmilayo Afolayan, Kabiru Dauda, Zulaikha Busari, Jairam Meena and Amulya Panda, Curcumin-Artesunate Based Polymeric Nanoparticle; Antiplasmodial and Toxicological Evaluation in Murine Model, frontiers in pharmacology,2018.

Eur. Chem. Bull. **2023**,*12(Special Issue 1), 283-291*

289

2. World Health Organization. *World Malaria Report 2019*; World Health Organization: Genève, Switzerland, 2019.

3. Roli Jain, Sandeep Sukla and Archna Panday, Release Behavior of PLGA-loaded Nanoparticle of Anti - Malarial Drug using Dialysis Membrane, Nanomedicine and Nanotechnology Journal. 2019; 2(1):123.

4. Nayyar GM, Breman JG, Newton PN, Herrington J. Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. Lancet Infect Dis. 2012; 12:488–96.

5. Tábata Loíse Cunha Lima, Renata de Carvalho Feitosa, Emanuell dos Santos-Silva, Alaine Maria dos Santos-Silva, Emerson Michell da Silva Siqueira, Paula Renata Lima Machado, Alianda Maira Cornélio, Eryvaldo Sócrates Tabosa do Egito, Matheus de Freitas Fernandes-Pedrosa, Kleber Juvenal Silva Farias, and Arnóbio Antônio da Silva-Júnior, Improving Encapsulation of Hydrophilic Chloroquine Diphosphate into Biodegradable Nanoparticles: A Promising Approach against Herpes Virus Simplex-1 Infection,Pharmaceutics 2018, 10, 255; doi:10.3390/pharmaceutics10040255

6. S.K.Tripathi, B.Patel, S Pathak, C.Pachouri ,S.Shukla,A.Pandey, Donepezil loaded PLGA nanoparticles, from modified nano-precipitation, an advanced drug delivery system to treat Alzheimer disease, Journal of Physics : conference series, 1849(2021)012001.

7. B. Patel, S. K. Tripathi, C. Pachouri, S. Pathak, S. Shukla, A. Pandey, Primaquine phosphate-loaded chitosan Nanoparticles: formulation optimisation to In vitro drug release kinetics study, Oxidation Communications, 44, No 3, 603–614 (2021).

8. S. Pathak, S. Tripathi, B. Patel, C. Pachouri, S. Shukla, A. Pandey, Development and characterisation of Raloxifene-loaded soy lecithin – chitosan Nanoparticles for osteoporosis treatment, Oxidation Communications, 44, No 3, 615–624 (2021).

9. Bohrey, S.; Chourasiya, V.; Pandey, A. Polymeric Nanoparticles Containing Diazepam: Preparation, Optimization, Characterization, in-Vitro Drug Release and Release Kinetic Study. *Nano Converg.***2016**, *3* (1). https://doi.org/10.1186/s40580-016-0061-2.

10. Tiwari, R.; Aharwal, R. P.; Shukla, S.; Pandey, A. Controlled Drug Release for Poorly Water Soluble Drugs- a Role of Polymeric Nanoparticles. *Int. J. Pharm. Sci. Res.***2014**, *5* (5), 1661–1670.

11. Bohrey, S.; Chourasiya, V.; Pandey, A. Preparation, Optimization by 2^3 Factorial Design, Characterization and in Vitro Release Kinetics of Lorazepam Loaded PLGA Nanoparticles. *Polym. Sci. Ser. A***2016**, *58* (6), 975–986.

12. Sarvesh Bohrey, Vibha Chourasiya and Archna Pandey, Polymeric nanoparticles containing diazepam: preparation, optimization, characterization, in-vitro drug release and release kinetic study,*Nano Convergence (2016) 3:3* DOI 10.1186/s40580-016-0061-2.

13. Farhana Akbar Mangrio,Pankaj Dwivedi,Shuya Han, Gang Zhao, Dayong Gao,Ting Si,and Ronald X. Xu, Characteristics of Artemether-Loaded Poly(lactic-co-glycolic) Acid Microparticles Fabricated by Coaxial Electrospray: Validation of Enhanced Encapsulation Efficiency and Bioavailability, Mol. Pharmaceutics 2017, 14, 4725−4733, DOI: 10.1021/acs.molpharmaceut.7b00862.

14. Xiaoxia Xie, Qing Tao, Yina Zou, Fengyi Zhang, Miao Guo, Ying Wang, Hui Wang,‡ Qian Zhou, and Shuqin Yu, PLGA Nanoparticles Improve the Oral Bioavailability of Curcumin in Rats: Characterizations and Mechanisms,J. Agric. Food Chem. 2011, 59, 9280–9289, dx.doi.org/10.1021/jf202135j.

15. Sebastian Piepera, Klaus Langera, Doxorubicin-loaded PLGA nanoparticles - a systematic evaluation of preparation techniques and parameters, Materials Today: Proceedings 4 (2017) S188–S192.

16. G. Mittal a, D.K. Sahana a, V. Bhardwaj a, M.N.V. Ravi Kumar, Estradiol loaded

*Eur. Chem. Bull. **2023**,12(Special Issue 1), 283-291*

290

PLGA nanoparticles for oral administration: Effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo, Journal of Controlled Release 119 (2007) 77–85.

17. Fabienne Danhier, Eduardo Ansorena, Joana M. Silva, Régis Coco, Aude Le Breton, Véronique Préat, PLGA-based nanoparticles: An overview of biomedical applications, Journal of Controlled Release 161 (2012) 505–522.

18. Yen Cu, Carmen J. Booth, W. Mark Saltzman, In vivo distribution of surface-modified PLGA nanoparticles following intravaginal delivery, Journal of Controlled Release 156 (2011) 258–264.

19. Leena Peltonen,Johanna Aitta,Samuli Hyvönen,Milja Karjalainen,and Jouni Hirvonen, Improved entrapment efficiency of hydrophilic drug substance during nanoprecipitation of poly(l)lactide

nanoparticles, AAPS Pharm SciTech 2004;5 (1).

20. Vibha Chourasiya, Sarvesh Bohrey, Archna Pandey, Formulation, optimization, characterization and in-vitro drug release kinetics of atenolol loaded PLGA nanoparticles using 33 factorialdesign for oral delivery, Materials Discovery 5 (2016) 1–13.

21. S. K. SHUKLA, S. GOHELB, A. PANDEY: Nanonization of CNS Drug by Co-precipitation of Carbamazepine with PEG 400/ CTAB Surfactant. J Appl Res Social Sci, **5** (23), (2018).

22. V. CHOURASIYA, S. BOHREY, A. PANDEY: Hydrochlorothiazide Containing PLGA Nanoparticles:Desing, Characterization, *in-vitro* Drug Release and Release Kinetics Study. Polym Sci Ser B, **57**, 645 (2015).

23. R. JAIN, S. K. SHUKLA, N. NEMA, A. PANDEY: Drug Nano-particle: a Release Kinetics. Nanome Nanotechnol, **6** (5), 1000317 (2015).

Eur. Chem. Bull. 2023,12(Special Issue 1), 283-291

291