Eudragit®-S100 Coated PLGA Nanoparticles for Colon Targeting of Etoricoxib: Optimization and Pharmacokinetic Assessments in Healthy Human Volunteers

Enas El-Maghawry, Mina I Tadros, Seham A Elkheshen, Ahmed Abd-Elbary

1Department of Pharmaceutics & Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt; 2Department of Pharmaceutics & Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Aim: Etoricoxib is a selective inhibitor of COX-2 enzyme. It is proposed as a potent anti-inflammatory drug intended for the control of irritable bowel syndrome. The current work aimed at developing etoricoxib-loaded nanoparticles for colon-targeting.

Materials and Methods: PLGA nanoparticles were developed via nano-spray drying technique. The D-optimal design was adopted for the investigation of the influence of i) DL-lactide-coglycolide (PLGA) concentration, ii) polyvinylpyrrolidone K30 (PVP K30) concentration and iii) lactide:glycolide ratio in the copolymer chain on the yield%, the encapsulation efficiency (EE%), particle size (PS) and percentage of drug release after 2h (P_{2h}), 4h (P_{4h}) and 12h (P_{12h}). To promote colon targeting of the systems, the best achieved system (M14) was either directly coated with poly(methacrylic acid-co-methyl methacrylate) [Eudragit®-S100] or loaded into hard gelatin capsules and the capsules were coated with poly(methacrylic acid-co-methyl methacrylate) (E-M14C). The pharmacokinetic parameters of etoricoxib following oral administration of E-M14C in healthy volunteers were assessed relative to commercial etoricoxib tablets.

Results: M14 system was prepared using PLGA (0.5% w/v) at a lactide:glycolide ratio of 100:0, in the presence of PVP K30 (2% w/v). M14 system was nano-spherical particles of 488 nm size possessing promising yield% (63.5%) and EE% (91.2%). The percentage drug released after 2, 4 and 12 hours were 43.41%, 47.34 and 64.96%, respectively. Following M14-loading into hard gelatin capsules and coating with poly(methacrylic acid-co-methyl methacrylate) [Eudragit-S100], the respective P_{2h}, P_{4h} and P_{12h} were 10.1%, 28.60% and 65.45%. Significant (p < 0.05) differences between the pharmacokinetic parameter of E-M14C in comparison with the commercial product were revealed with a delay in T_{max} (from 2.5h to 6h), a prolongation in MRT_{0-∞} (from 24.4h to 34.7h) and an increase in the relative oral bioavailability (4.23 folds).

Conclusion: E-M14C is a potential system for possible colon targeting of etoricoxib.

Keywords: colon targeting, etoricoxib, nano spray drying, PLGA, Eudragit-S100

Introduction

The colon is an organ that is vulnerable to major inflammatory bowel diseases (IBD). Two of these diseases are ulcerative colitis and Crohn’s disease, which are relapsing chronic inflammatory disorders of the intestinal mucosa.1 Ulcerative colitis affects the lining of the large intestine (colon) and rectum. Repeated inflammatory swelling leads to thickening of the intestinal wall and formation of scar
tissues in the rectum. Death of colon tissue or severe infection with sepsis formation may occur in severe cases.2

Colon-specific drug delivery systems have gained increasing attention for the treatment of such diseases. A targeted colonic carrier of drugs provides local delivery of the drug to the colon.3 Some of the advantages of targeting a drug to the colon include lowering incidences of adverse side effects, delivering the drug to the target site and lowering the conventional dose.4 Many non-steroidal anti-inflammatory drugs for the treatment of colon diseases have been formulated into colon-targeted delivery systems as diclofenac,5 piroxicam,6 ibuprofen,7 ketoprofen,8,9 flurbiprofen,10 lornoxicam,11 celecoxib12 and etoricoxib.13

Compared to other NSAIDs, etoricoxib (5-chloro-6-methyl-3-[4-methylsulfonyl) phenyl]-2,3-bipyridine) is considered as an effective drug for the treatment of IBD.14,15 It is characterized by being a selective inhibitor of cyclooxygenase-2 (COX-2) enzyme leading to the production of fewer prostaglandins; hence, pain and inflammation are lessened and the side effects produced by blocking the COX-1 enzyme are reduced.16 Few attempts investigated the design of colon-targeting systems for etoricoxib including the preparation of tablets coated with three layers of Eudragit® E100, HPMC and Eudragit® L100 as enteric coat13 and capsules coated with a three-layered film consisting of an acid-soluble polymer, a water-soluble polymer, and cellulose acetate phthalate as enteric polymer.17

The dimensions of the delivery system greatly influence the targeting of the inflamed colon where nanosized particles are more readily taken up by macrophages or dendritic cells at the site of active inflammation.18 Furthermore, they have a better ability to adhere to the mucus layers, thus delivering higher amounts of the entrapped drugs to the inflamed areas, which result in better therapeutic efficacy and reduced systemic adverse effects.3,19 The spray drying technique is one of the advanced technologies to develop nanoparticles possessing narrow particle size distributions and relatively high yields and drug entrapment efficiencies.

PLGA is a highly biocompatible, biodegradable,20 mechanically processable polymer that degrades yielding water-soluble and non-toxic products of normal metabolism.21–24 Degradation of PLGA occurs through hydrolysis of its ester linkages in the presence of water.25 Through this hydrolytic attack, random chain scission occurs, causing its degradation into lactic and glycolic acids.26 PLGA monomers; lactic acid and glycolic acid are non-toxic and can be removed from the body by normal metabolic pathways.27 It has been shown that the time required for the degradation of PLGA is related to the ratio of monomers used in its production, ie, controlling the degradation rate can be achieved by adjusting the ratio of DL-lactide to glycolide in the copolymer.28 The degradation rate of PLGA decreases with increasing either the lactide or the glycolide content of the copolymer.29 Copolymers of PLGA were approved by the FDA for numerous applications including extended-release pharmaceuticals.30,31 Practically, it is possible to tune the overall physical properties of the drug-polymer matrix by controlling certain parameters such as molecular weight of the polymer, lactide to glycolide ratio as well as the nature and concentration of the drug.32–34

The current work aimed at developing and optimizing a promising etoricoxib-loaded nanosystem for colon targeting applying the D-optimal design via nano-spray drying technique using poly (lactide-co-glycolide) (PLGA) copolymers. To promote the colon targeting potential of PLGA nanoparticles, the best achieved nanosystem was optimized via either coating the nanoparticles with poly (methacrylic acid-co-methyl methacrylate) [Eudragit® S100] during the nanospray drying process or through loading the nanoparticles into hard gelatin capsules and coating the capsules with poly (methacrylic acid-co-methyl methacrylate). The pharmacokinetic parameters of etoricoxib following oral administration of the most optimized system were assessed in healthy volunteers relative to the market product. To the best of our knowledge, the colon targeting potential of etoricoxib was never explored using our optimized nanospray-dried system nor the colon targeting of the drug was pharmacokinetically validated in healthy volunteers.

Materials and Methods

Materials

Etoricoxib was purchased from Provizer Pharma (Gujarat, India). PURASORB PDLG 5002 (DL-lactide/Glycolide 50:50 copolymer; with an inherent viscosity midpoint of 0.20 dL/g), PURASORB PDLG 7502 (DL-lactide /Glycolide 75:25 copolymer; with an inherent viscosity mid-point of 0.22 dL/g) and PURASORB PDL 02 (DL-lactide /Glycolide 100:0 copolymer; with an inherent viscosity mid-point of 0.20 dL/g) were kindly supplied by PURAC Biomaterials (Gorinchem, Netherlands). Dialysis tubing cellulose membrane (Molecular weight cutoff 12,000–14,000
Dovepress

Development of Etoricoxib-Loaded PLGA Nanoparticles
Etoricoxib-loaded PLGA nanoparticles were prepared via the spray drying technique. Accurate amounts of etoricoxib (2% w/v), PLGA (copolymer) and PVP K30 (carrier) were dissolved in acetonitrile. The solution was spray dried using B-90 Nano Spray Dryer (Büchi Labortechnik, Flawil, Switzerland). A mesh of 7 μm was used along with 60 Hz ultrasonic actuator frequency. The Nano Spray Dryer was operated in a closed-mode configuration. The instrument was connected to a cooling unit (the Inert Loop B-295) in order to offer the safe operation of solvents in a closed-mode configuration. An inert gas (Nitrogen) was used at 1.5 bars to prevent the explosion of gas mixture. An electric field was generated using CO₂ gas at 1.5 bars for separation of the particles. The O₂ concentration in the closed loop was kept below 4% v/v. The drying gas flow rate was 110 L/min, the inlet temperature was 85°C, the outlet temperature was 45°C, the pressure was 39 mbar and the spraying rate was 100%. The dried powders were collected from the particle collecting chamber using a scraper and were stored in a desiccator for further characterization.

Experimental Design
Design Expert software Ver. 7 (Stat-Ease Inc., Minneapolis, MN) was used for the design of experiments and assessment of results using the D-optimal design (DOD). The design maximizes the determinants obtained from the information matrix generated from all the possible combinations of the involved factors. It was involved in optimizing many nano-drug delivery systems and was proved to be better than the one-factor-at-a-time (OFAT) experiments.

Evaluation of the Developed Etoricoxib-Loaded PLGA Nanoparticles
Determination of Percentage Yield
The percentage yield of each system was calculated by dividing the weight of the obtained nanoparticles by the total initial weights of drug, polymer and carrier.

Determination of Drug Content and Encapsulation Efficiency %
A definite weight of the nanoparticles (which theoretically contains the equivalent of 10 mg of drug) was dissolved in 100 mL solution of 0.1 N HCl. After continuous shaking for 10 minutes, the solution was filtered through a 0.2-μm membrane filter. The solution was appropriately diluted and the drug concentration was measured spectrophotometrically (Shimadzu UV-1800 double beam spectrophotometer, Kyoto, Japan) at a predetermined λ_max of 234 nm. The actual drug content was determined from which the drug encapsulation efficiency % (EE%) was calculated using the following equation:

\[
\text{EE} = \left( \frac{\text{Drug Content in nanoparticles}}{\text{Drug Content in solution}} \right) \times 100
\]
The particle size and the polydispersity index (PDI) of the appropriately diluted water dispersions were measured using a Zetasizer (Nanoseries Instrument, Malvern-ZEN® 3600, Worcestershire, UK) via photon correlation spectroscopy. Measurements were conducted, in triplicates, at 25°C using an electric field strength of 23.2 V/cm.

In-vitro Drug Release Studies

The in-vitro drug release studies from the developed systems were conducted, in triplicates, in a USP dissolution tester apparatus (Vision G2 Elite 8™, Hanson, CA) at 37 ± 0.5°C. The paddles were adjusted to rotate at a speed of 100 rpm. A definite weight of the nanoparticles (containing the equivalent of 60 mg of drug) was dispersed in 0.1N HCl (1 mL, pH 1.2) and loaded into the dialysis bag. The latter was secured by two clamps at each end. The dialysis bag was suspended in a dissolution medium of 0.1N HCl (600 mL, pH 1.2) for the first 2 hours. At the end of the second hour, the pH was raised to 6.8. Furthermore, it was raised to 7.4 at the end of the fourth hour and was maintained until the end of the study. The adjustments of the pH (pH meter; Grifﬁn and George Ltd., London, England) were conducted using 0.235 M dibasic sodium phosphate solution. Perfect sink conditions prevailed during the studies. Five mL samples of the dissolution medium were withdrawn at predetermined time intervals, and immediately replaced with an equivalent volume of fresh medium to keep the volume constant. The drug concentration in the withdrawn samples was analysed spectrophotometrically at λ_max of 234 nm. The cumulative percent of drug released (mean ± S.D.) after 2 h (P_{2h}), 4 h (P_{4h}) and 12 h (P_{12h}) were determined.

Selection of the Best Achieved System

After confirming the polynomial equations relating the responses and the independent variables using the Design Table 1 The Variables and the Responses (Mean ± SD; n = 3) of the Investigated Etoricoxib-Loaded PLGA Systems; According to the D-Optimal Design

| Systems | Variables | Responses |
|---------|-----------|-----------|
| M1      | 2, 3.5    | 37.81 ± 4.90, 77.12 ± 10.20 |
| M2      | 1.25, 2   | 54.29 ± 4.90, 90.50 ± 8.50 |
| M3      | 2, 5      | 68.90 ± 8.60, 80.08 ± 5.90 |
| M4      | 2, 5      | 49.77 ± 8.50, 75.49 ± 4.70 |
| M5      | 2, 5      | 62.38 ± 6.00, 88.65 ± 8.40 |
| M6      | 0.5, 3.5  | 48.44 ± 4.80, 83.35 ± 6.30 |
| M7      | 2, 5      | 59.19 ± 5.00, 80.55 ± 9.10 |
| M8      | 2, 2      | 100.0 ± 4.50, 50.30 ± 6.30 |
| M9      | 1.25, 2   | 32.57 ± 4.50, 83.35 ± 6.30 |
| M10     | 1.25, 2.75| 37.81 ± 4.90, 48.50 ± 6.00 |
| M11     | 2, 2.5    | 61.4 ± 6.50, 84.60 ± 2.40 |
| M12     | 0.5, 2    | 63.50 ± 3.80, 91.20 ± 7.30 |
| M13     | 0.5, 2    | 64.0 ± 6.50, 91.20 ± 7.30 |
| M14     | 0.5, 2    | 61.4 ± 6.50, 84.60 ± 2.40 |
| M15     | 0.5, 2    | 59.19 ± 5.00, 80.55 ± 9.10 |
| M16     | 0.5, 2    | 32.57 ± 4.50, 83.35 ± 6.30 |
| M17     | 2, 2      | 75.25 ± 9.20, 75.80 ± 7.60 |
| M18     | 1.25, 3.5 | 59.33 ± 7.70, 90.50 ± 9.10 |
| M19     | 0.5, 2    | 67.50 ± 6.50, 91.20 ± 7.30 |
| M20     | 2, 5      | 59.88 ± 7.50, 82.60 ± 6.10 |
| M21     | 0.5, 2    | 59.33 ± 7.70, 90.50 ± 9.10 |
| M22     | 1.25, 2   | 46.50 ± 3.20, 97.50 ± 4.60 |

Abbreviations: *X1, DL-lactide-coglycolide copolymer concentration in system solution; X2, polyvinylpyrrolidone K30 concentration in system solution; X3, lactide:glycolide ratio in copolymer chain; EE, encapsulation efficiency %; PDI, polydispersity Index; P_{2h}, amount released in 2 hours; P_{4h}, amount released in 4 hours; P_{12h}, amount released in 12 hours; PLGA, DL-lactide-coglycolide copolymer.

\[
EE\% = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100
\]
Expert software, the desirability function was activated to select the best achieved system with respect to the following constraints: maximizing the yield%, the EE% and P_{12h} while minimizing the particle size.

**Morphologic Examination**

The topographic examination of the best achieved system (M14) was conducted using a scanning electron microscope fitted with an image analysis system (Quanta FEG250, Hillsboro, OR). The systems were fixed to brass grids and were sputter-coated (Edwards S-150A, Crawley, England) with a layer of gold (150 Å, 2 min) in a vacuum of argon gas (3 × 10⁻¹ atm). Finally, samples were examined at 20 kV.

**Differential Scanning Calorimetry (DSC)**

The thermotropic properties of etoricoxib, PURASORB PDL 02, PVP K30, their physical mixture and M14 were explored to assess the degree of drug crystallinity in the nanoparticle. Three to four milligrams of each sample were encapsulated in an aluminium pan, heated up to a temperature of 300°C with 10 °C/min rate. Dry nitrogen was used as a carrier gas with a flow rate of 25 mL/min. The DSC thermogram was plotted using differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan).

**Optimization of the Best Achieved System**

To promote the colon targeting potential of M14 system, two approaches were used for coating the selected system with the pH-dependent coating polymer; poly (methacrylic acid-co-methyl methacrylate) [Eudragit-S100]. The first one involved coating the nanoparticles (M14) with poly (methacrylic acid-co-methyl methacrylate) solution (2%, w/v in a 1:1 solvent mixture of acetone: isopropyl alcohol) using spray drying technique. Two poly (methacrylic acid-co-methyl methacrylate): copolymer ratios were investigated; 1:1 (E-M14A system) and 2:1 (E-M14B system). The second approach involved loading the nanoparticles (M14 system) into hard gelatin capsules followed by coating the capsules with the same Eudragit solution applying the dip-coating method (E-M14C system). Briefly, each capsule was dipped for 90 seconds in a beaker containing 20 mL of the previously mentioned coating solution. The process was repeated fifteen times and the coat was dried with a stream of air after each dip.

The particle size of E-M14A and E-M14B was compared to M14 while the weight gain % of E-M14C was evaluated relative to M14-loaded uncoated hard gelatin capsules. Each capsule was weighed before and after coating and the weight gain % was determined according to the following equation:

\[
\text{Weight gain} \% = \left( \frac{W_c - W_{un}}{W_{un}} \right) \times 100
\]

where \(W_c\) is the weight of the coated capsule and \(W_{un}\) is the weight of the uncoated capsule.

Furthermore, the in-vitro drug release studies of market product of etoricoxib, E-M14A, E-M14B and E-M14C were similarly conducted as previously noted. The percentages of drug released after 2 h (P_{2h}), 4 h (P_{4h}) and 12 h (P_{12h}) were determined from the dissolution profiles and compared to the corresponding values for formula M14.

**Pharmacokinetic Assessments of the Optimized System in Healthy Volunteers**

**Study Design**

The studies were carried out to compare the pharmacokinetics of etoricoxib from E-M14C (test treatment) and market tablets (reference treatment) following oral administration of single 60 mg doses in healthy volunteers; under fasting conditions. This study was conducted according to a two-treatment, two-period, randomized, crossover design with a two-week washout period between the two phases. The protocol of the studies was reviewed and approved by the Research Ethics Committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt [Serial no. PI 1902] and the Research Ethics Committee of Faculty of Pharmaceutical Sciences and Pharmaceutical Industries, Future University in Egypt, Cairo, Egypt [Serial no. REC-FPSPI-12/83]. The studies were carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

**Administration of the Treatments**

Six healthy male volunteers between 24 and 43 years old and weighing from 60 to 84 kg were invited to participate in the study after giving informed written consent. All subjects were judged to be healthy after conducting a general physical examination, reviewing their medical history and conducting the necessary biochemical and haematological laboratory assessments. The volunteers were instructed to avoid receiving any medication for one-week prior to-and during- the study period.

The volunteers were randomly divided into two equal groups. In Phase I, the first group of volunteers received the test treatment. The other group received the reference
treatment. Following the washout period, the reverse of randomization took place in Phase II. Both products were administered with 150 mL of water in the morning after a 12-h overnight fasting. Food and drinks were withheld for at least 2 h after dosing.

Blood Sample Collection and Preparation
Blood samples (4 mL) were collected into heparinized tubes for the measurement of etoricoxib at zero time and at 30 min., 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 24, 48 and 72 h post-dosing. The blood samples were centrifuged at 3000 rpm for 10 min, and the derived plasma samples were transferred to separate glass tubes and kept frozen at −80°C until analyses.

For the preparation of the plasma samples, each thawed sample (0.5 mL) was spiked with 100 µL of the internal standard solution (Loratadine; 200 ng/mL) and mixed using a vortex. For extraction of the drug, tertiary butyl methyl ether (4 mL) was added and mixed using a vortex. The organic layer was evaporated in a vacuum concentrator and finally, reconstituted in the mobile phase (0.5 mL).

Estimation of Etoricoxib Concentration in Plasma via LC-MS/MS
The determination of etoricoxib in the prepared plasma samples was conducted using a Shimadzu prominence HPLC system (Riverwood Drive, Columbia) consisted of an HPLC pump (LC-20AD), a degasser (DGU-14A), an autosampler (SIL-20AC). The system was connected to a Triple Quad 4500 mass spectrometer (AB SCIEX instruments, Darmstadt, Germany) equipped with a turbo ion spray [pneumatically assisted electron spray ionization (ESI)] source. The ESI interface was used in positive mode and the turbo ion spray source was heated to 399°C.

Chromatographic separation of extracted plasma samples was performed in the isocratic mode using an Agilent zorbax C18 column (50 mm × 5um diameter). The mobile phase consisted of a mixture of acetonitrile and 0.01% ammonium acetate (80:20). The system operated at a flow rate of 1 mL/min and the volume of injection was 10 µL.

The detector was operated in the multiple reaction monitoring mode with the precursor-to-product ion transitions were 358.96/279.90 Da for etoricoxib and 383.02/336.90 Da for Loratadine. A calibration curve was portrayed by plotting the peak area ratio of drug/IS against drug concentration in plasma over the concentration range of 10–5000 ng/mL. The calibration curve was found to be linear (y = 0.0017x + 0.0013). The estimated R² value was found to be 0.995.

The analyst software Ver.1.6.3 (Applied Biosystems; Waltham, Massachusetts) was used to acquire the output signals from the detector, to integrate the peak areas, to calculate the drug/internal standard peak area ratios and to estimate the drug concentration.

Pharmacokinetic and Statistical Analyses
The pharmacokinetic parameters; Cmax (ng/mL), Tmax (h), MRT(0–∞) (h), AUMC0–72 (ng.h.²/mL⁻¹), AUMC0–∞ (ng.h.²/mL⁻¹), AUC(0–72h) (ng.h/mL), AUC(0–∞) (ng.h/mL) of the test and the reference treatments were estimated for each volunteer. The data were analysed via non-compartmental analysis using WinNonlin software Ver. 4.1 (Scientific consulting Inc., Cary, NC). The relative oral bioavailability of etoricoxib was estimated from the AUC(0–∞) values of the test and the reference treatments.

Statistical analysis of the parametric data was performed, using SPSS software Ver. 17 (SPSS Inc., Chicago, IL), applying two-way analysis of variance (ANOVA) at P values of <0.05. The data were expressed as mean values ± SD. Statistical analysis of Tmax values was performed using Wilcoxon signed-rank test and the data were expressed as median (range).

Results and Discussion
Development of Etoricoxib-Loaded Nanoparticles
Etoricoxib-loaded nanoparticles were successfully developed via the nano-spray drying technique. Three concentrations of PLGA were explored (0.5%, 1.25%, 2%), along with three ratios of lactide:glycolide in the copolymer chain (50:50, 75:25, 100:0). Preliminary studies revealed partial loss of the spray-dried powders; possibly due to stickiness on the outlet drying chamber walls. To minimize these problems, PVP K 30 was incorporated as a carrier at three concentrations (2%, 3.5, 5%).

Experimental Design
Twenty-two systems were suggested by the Design Expert Software (Ver. 7) according to the D-optimal design. The normal plot of residuals and the graphs of residual vs predicted and predicted vs actual of the investigated responses confirmed that there was no need for data transformation and/or further analysis. The generated model equations were statistically significant and were best fit for the prediction of the desired response outcomes. The
goodness of fit of the models was checked via the determination of regression coefficient ($r^2$) and adjusted coefficients (adj. $r^2$). Relatively high values (>0.7) could indicate a high significance of fit of the model.$^{53,54}$

**Determination of Yield%**

Nanoparticles were weighed after the spray drying process. The yield percentages of the developed systems ranged from 32.57% (M8) to 70% (M12) (Table 1). The statistical analysis of data (ANOVA) confirmed that the linear model is valid with a significant $p$-value and insignificant lack of fit value; F value = 20.33 ($p<0.0001$) (Table 2). The most effective parameter on the yield (%) of nanoparticles was the Lactide:glycolide ratio in the copolymer chain with $F$-value of 19.87 ($p<0.0001$). This was followed by the influence of the copolymer concentration in the system solution with $F$-value of 19.65 ($p<0.0004$) and finally the contribution of PVP k30 concentration in the system solution with $F$-value of 12.51 ($p<0.002$). The equation showing the relation between the independent variables and yield (%) of nanoparticles is presented in Table 2 ($r^2 = 0.8271$). The predicted $r^2$ (0.702) was in reasonable agreement with the adjusted $r^2$ (0.7864). The adequate precision was 14.756; indicating an adequate signal. Thus, this linear model can be used to navigate the design space.

The response surface and the contour plots showing the effect of various factors on the yield (%) are shown in Figure 1A (A) and 1B (A), respectively. By observing the effect of one-factor plot on the percentage yield, and analysing the equation that describes the relation between the yield and parameters (Table 2), it was clear that the negative sign of the coefficient of the copolymer concentration in the system solution could indicate that the increase in the copolymer concentration would adversely affect the percentage yield. This could be ascribed to the increase in the viscosity of the solution and the resulting difficulties in spraying. This result agrees with Murakami et al,$^{55}$ who used the modified spontaneous emulsification solvent diffusion method and found that the increase in PLGA copolymer concentration had a negative influence on the yield of the nanoparticles. On the other hand, the positive sign of the coefficient of the carrier concentration in the system solution could point out the positive impact of the carrier concentration on the yield (%) of the nanoparticles. This could justify the ability of PVP to prevent the aggregation of the nanoparticles and/or minimize their adherence to the wall of the drying chamber, and hence, facilitating their removal and collection. These findings were in line with those reported by Shakeri et al$^{56}$ for the development of protein-loaded PLGA-PVP blend nanoparticles by the nanoprecipitation method. In a parallel line, the positive significant impact of lactide:glycolide ratio in the copolymer chain on the percentage yield was similarly revealed. The highest percentage yield was revealed with those systems prepared at a lactide:glycolide ratio of 100:0. Actually, the increase in the lactide:glycolide ratio in the copolymer chain is accompanied by an increase in the glass transition temperature ($T_g$) and in the yield percentage as well.$^{57-59}$

**Estimation of Drug Encapsulation Efficiency (EE%)**

The drug encapsulation efficiency (%) of the nanoparticles varied from 75.49% (M4) to 98.92% (M10) (Table 1). The quadratic equation was the best fitting model for EE% data with $F$ value of 14.64 ($p<0.0001$). The most effective parameter on the EE% of nanoparticles was the copolymer concentration; on squaring ($A^2$) had an $F$-value of 75.08 ($p<0.0001$). The equation ($r^2 = 0.9415$) that describes the relation between the independent variables and EE% of nanoparticles is presented in (Table 2). The predicted $r^2$ (0.8772) was in reasonable agreement with the adjusted $r^2$ (0.8301). The adequate precision was 12.703; indicating an adequate noise. It could be concluded that the quadratic equation could be used to navigate the design space.

**Figure 1A(B) and 1B(B)** represent the effect of the independent variables on the EE%; as represented by a quadratic model. It was clear that there is a direct correlation between the copolymer concentration and the drug EE%. This finding was previously demonstrated for rivastigmine-loaded PLGA nanoparticles, where the copolymer concentration was the major factor affecting the drug EE%.$^{55}$ It could be inferred that higher amounts of the copolymer are available to entrap the loaded drug. Similar findings were reported by Bhamere et al$^{60}$ for paclitaxel-loaded PLGA nanoparticles, by Kizilbey$^{61}$ for rutin-loaded PLGA nanoparticles, by Nahata et al$^{61}$ for aripiprazole-loaded PLGA microspheres and by Wagh et al$^{62}$ for cyclosporine A-loaded PLGA nanoparticles.

Parallel to the effect of the copolymer concentration, the PVP k30 concentration showed a significant positive impact on EE%. PVP has proven history for improving the solubility of poorly soluble drugs and preventing their crystallisation.$^{63}$ These results were in agreement with
Figure 1 (A) Response surface plots showing the effects of copolymer concentration and Polyvinylpyrrolidone K30 concentration, at a lactide:glycolide ratio in the copolymer chain of 100:0, on (A) yield %, (B) encapsulation efficiency %, (C) particle size and (D) cumulative % of drug released after 12 h. (B) Contour plots showing the effects of copolymer concentration and Polyvinylpyrrolidone K30 concentration, at a lactide:glycolide ratio in the copolymer chain of 100:0, on: (A) yield %, (B) encapsulation efficiency %, (C) particle size and (D) cumulative % of drug released after 12 h.

Abbreviations: PVP K30, polyvinylpyrrolidone K30; C:L:G, coded factor of lactide to glycolide ratio in copolymer chain; E.E., encapsulation efficiency %; PSD, particle size diameter; Rel. aft., release after; Conc., concentration.
those reported by Shakeri et al and Coombes et al. Unlike the positive contributions of the aforementioned variables on drug EE%, the lactide:glycolide ratio in the copolymer chain showed a significant negative influence on the drug EE%. This finding does not match what was reported by Chung et al who revealed a positive contribution of the lactide:glycolide ratio in the copolymer chain on the EE% of albumin-loaded microspheres. At lower lactide:glycolide ratios, PLGA copolymers possessing low glass transition temperatures could be achieved; allowing for fast shell formation, effective encapsulation and better protection for the loaded drug. On the other hand, at higher lactide:glycolide ratios, more hydrophobic PLGA copolymers would be revealed; allowing for the development of small particles with more drug opportunities to escape from nanoparticles.

**Measurements of Particle Size (PS)**

The particle size of the developed nanoparticles varied significantly from 250.52 nm (M10) to 2431.00 nm (M17), while the PDI values of the systems ranged from 0.350 to 1 (Table 1).

The linear model was statistically proved to be the best fitting model for particle size data with F value of 42.75 ($p<0.0001$). The most effective parameter on the size of the nanoparticle was the lactide:glycolide ratio in the copolymer chain with F-value of 54.27 ($p<0.0001$). The equation ($r^2 = 0.9415$) that described the relation between the independent variables and the particle size is presented in (Table 2). The predicted $r^2$ (0.888) was in reasonable agreement with the adjusted $r^2$ (0.841). The precision was 20.972 indicating an adequate noise; suggesting that the linear model could be used to navigate the design space.

*Figure 1A (C) and 1B (C) represent the effect of the independent variables on the particle size, as presented by a linear model. The polymer concentration had a significant positive effect on the particle size (Table 2). The increase in the particle size might be attributed to the increase in the viscosity of the sprayed solution which could retard the droplets subdividing. Similar findings were reported by Wagh et al for cyclosporine A-loaded PLGA nanoparticles and Joshi et al for rivastigmine-loaded PLGA nanoparticles. The subsequent slower nucleation rates would favour the generation of larger particles. On the other hand, the negative sign in the linear equation indicates that increasing the concentration of PVP K30 would lead to a decrease in the particle size. This might be attributed to the higher hydrophilicity associated with the use of higher amounts of this polymer. These findings are correlated to those of Quintanar-Guerrero et al who reported the production of smaller polymeric particles upon increasing PVP concentration. Furthermore, Zweers et al and Scholes et al reported that increasing the concentration of the carrier would decrease the size of the nanoparticles till an optimum carrier concentration. Beyond this level, it would positively influence the particle size.

The lactide:glycolide ratio had a significant effect on the particle size as indicated by its F-value of 54.27 ($p<0.0001$). The positive and negative signs in the linear equation indicate that it can affect the particle size positively or negatively depending on the investigated ratio. Observing the one-way plot curve, it can be seen that increasing the ratio from 50:50 to 75:25 would increase the particle size. Interestingly, the particle size decreased upon raising the ratio to 100:0. Contrary to the current finding, Mehrotra et al reported that lactide:glycolide ratio had a non-significant influence on the particle size during developing lomustine-loaded PLGA nanoparticles via the interfacial deposition method.

**The Cumulative Drug Released (%) After 12 h ($P_{12h}$)**

The cumulative drug released percentages from the developed nanoparticles after 12 h ($P_{12h}$) ranged from 22.78% (M3) to 69.38% (M2). The linear model was the best fitting model for $P_{12h}$ data ($p<0.0001$). The most effective parameter on the cumulative % release of the drug after 12 hours was the PVP K30 concentration with F-value of 77.60 ($p < 0.0001$). The relation between independent variables and $P_{12h}$ was presented in the equation ($r^2 = 0.841$) (Table 2). The predicted $r^2$ (0.746) was in reasonable agreement with the adjusted $r^2$ (0.803). The adequate precision was 12.509 indicating an adequate noise. It could be assumed that this linear model could be used to navigate the design space.

The drug release profiles (not shown) of the developed etoricoxib-loaded nanoparticles showed a biphasic pattern (Table 1). The initial fast release rate might be related to the presence of small particles, along with larger ones. The former would have smaller diffusion paths, so that the drug could rapidly access the solid/dissolution medium interface. The release rate in the second phase was assumed to be controlled by the drug diffusion rate across the polymer matrix. The negative sign indicates the inverse relationship between the copolymer concentration...
and \( P_{12h} \). This could be attributed to the formation of thicker coats of the copolymer around the drug at higher concentrations of the polymer thus, hindering the drug release percentages.

Figure 1A(D) and 1B(D) represent the effect of the independent variables on \( P_{12h} \), as represented by a linear model. As shown in the equation (Table 2), increasing the concentration of PVP K30 had a negative influence on \( P_{12h} \). These findings relatively match with the finding of Coombes et al.\(^{63} \) who used PVP as a stabilizer for the development of ovalbumin-loaded microparticles and proved that its presence contributed to a reduction in the burst effect of the drug release as well as a prolongation of the drug release pattern.

An inverse correlation could be established between the lactide:glycolide ratio and \( P_{12h} \). PLGA copolymers possessing a lactide:glycolide of 50:50 usually degrade more rapidly than PLA alone.\(^70 \) PLGA copolymers possessing a lactide:glycolide ratio of 50:50 are more hydrophilic than

### Table 2: Equations of Models that Best Fit the Investigated Responses

| Response     | Type of Equation Fitting the Model | Final Equation | Lactide:Glycolide Ratio in Copolymer | Equation |
|--------------|-----------------------------------|----------------|-------------------------------------|----------|
| Yield(%)     | Linear                            | In terms of coded factors | Yield = 55.43 − 5.68 * A + 4.32 * B + 5.94 * C[1] + 3.86 * C[2] |
|              | In terms of actual factors         | 50:50           | Yield = 60.75972 − 7.56888 * X1 + 2.87709 * X2 |
|              |                                    | 75:25           | Yield = 58.68318 − 7.56888 * X1 + 2.87709 * X2 |
|              |                                    | 100:0           | Yield = 45.01779 − 7.56888 * X1 + 2.87709 * X2 |
| EE(%)        | Quadratic                         | In terms of coded factors | E.E. = 91.01 − 4.39 * A + 2.30 * B − 0.12 * C[1] − 1.68 * C[2] − 0.16 * AB − 0.45 * AC[1] − 0.38 * AC[2] + 2.07 * BC[1] + 0.67 * BC[2] − 11.97 * A^2 + 5.52 * B^2 |
|              | In terms of actual factors         | 50:50           | E.E. = 84.97 + 47.22 * X1 − 14.09 * X2 − 0.139 * X1 * X2 − 21.276 * X1^2 + 2.455 * X2 |
|              |                                    | 75:25           | E.E. = 86.56544 + 47.31810 * X1 − 15.02631 * X2 − 0.13949 * X1 * X2 − 21.27609 * X1^2 + 2.45455 * X2^2 |
|              |                                    | 100:0           | E.E. = 95.98090 + 48.93050 * X1 − 17.29774 * X2 − 0.13949 * X1 * X2 − 21.27609 * X1^2 + 2.455 * X2^2 |
| Particle size(nm) | Linear                           | In terms of coded factors | PS = 920.58 + 307.43 * A - 243.49 * B −176.04 * C[1] + 584.18 * C[2] |
|              | In terms of actual factors         | 50:50           | PS = 800.30 + 409.91 * X1 − 162.33 * X2 |
|              |                                    | 75:25           | PS = 1560.52 + 409.91 * X1 − 162.33 * X2 |
|              |                                    | 100:0           | PS = 568.19 + 409.91 * X1 − 162.33 * X2 |
| \( P_{12h} \) | Linear                            | In terms of coded factors | \( P_{12h} = 48.61 − 3.66 * A - 12.64 * B - 0.79 * C[1] + 2.22 * C[2] \) |
|              | In terms of actual factors         | 50:50           | \( P_{12h} = 83.42 − 4.88 * X1 - 8.43 * X2 \) |
|              |                                    | 75:25           | \( P_{12h} = 86.42 − 4.88 * X1 - 8.43 * X2 \) |
|              |                                    | 100:0           | \( P_{12h} = 82.78 − 4.88 * X1 - 8.43 * X2 \) |

**Abbreviations:** X1, DL-lactide-coglycolide copolymer concentration in system solution; X2, polyvinylpyrrolidone K30 concentration in system solution; E.E., encapsulation efficiency %; PS, particle size; \( P_{12h} \), amount of drug released after 12 hours; A, coded factor of DL-lactide-coglycolide copolymer; B, coded factor of Polyvinylpyrrolidone K30; C, coded factor of lactide:glycolide ratio in the copolymer chain.
those possessing a ratio of 75:25.71 The latter copolymers possess a greater number of the more hydrophobic lactic acid monomers which would degrade in a longer period of time. In other words, the absolute value of the degradation rate would be positively correlated to the lactide:glycolide ratio.72

Selection of the Best Achieved System
The desirability function was explored to allow proper selection of the best achieved system which possesses a compromise between the targeted constraints; maximizing the yield%, the EE% and P12h, while minimizing the PS. The best achieved system, as suggested by the designed expert software, was M14. It had a promising yield (%) of 63.5%, EE% of 91.2%, P12h of 64.96% and PS of 488 nm.

Morphologic Characteristics
Figure 2 illustrates the scanning electron micrographs of the drug crystals (A) and the best achieved system; M14 (B). Spherical particles with smooth surfaces were revealed for the latter. The lack of the surface-adsorbed drug crystals could indicate the efficient entrapment of etoricoxib within the matrix of the nanoparticles.

Differential Scanning Calorimetry (DSC)
Figure 3 shows the DSC thermograms of pure drug, PURASORB PDL 02 copolymer, PVP K30, their physical mixture and M14 system. Etoricoxib had a sharp endothermic peak at 139°C; corresponding to its melting point.73 The DSC thermograms of the copolymer (PURASORB PDL 02) and the carrier (PVP K30) showed endothermic peaks at 125°C.9,74 The permanence of the characteristic endothermic peak of the drug in the physical mixture and its disappearance in M14 system could indicate that the drug has changed from the crystalline to the more soluble amorphous state in the latter.

Optimization of the Best Achieved System
A successful colon targeted drug delivery should allow the release of minimum drug percentages during its transit in the stomach and in the upper intestine to ensure maximum drug delivery in the colon75. The respective P2h and P4h of M14 were relatively high; 43.41–47.34% (Table 1). To minimize the drug released percentages at the stomach (P2h) and the small intestine (P4h) and to promote the colon targeting potential of M14, two approaches were adopted for coating the system with poly (methacrylic acid-co-methyl methacrylate) [Eudragit-S100]. The first involved the incorporation of poly (methacrylic acid-co-methyl methacrylate) in a coating solution, at two poly (methacrylic acid-co-methyl methacrylate): copolymer ratios of 1:1 (E-M14A) and 2:1 (E-M14B) followed by coating of nanoparticles with spray drying. The second approach explored the dip coating of M14-loaded hard gelatin capsules with poly (methacrylic acid-co-methyl methacrylate) solution (2%, w/v) (E-M14C). The first approach led to an increase in the mean PS from 488 nm (M14) to 1250 nm (E-M14A) and 2699 nm
The larger PS of the E-M14B system could be related to the use of a higher poly (methacrylic acid-co-methyl methacrylate) concentration in the sprayed coating solution. On the other hand, the weight gain % of E-M14C system, relative to M14-loaded uncoated hard gelatin capsules, was \( \approx 25\% \).

The in-vitro drug release profiles of market tablets of etoricoxib, M14, E-M14A, E-M14B and E-M14C systems are depicted in Figure 4. It was clear that the drug showed almost 100% release within 4 h from the commercial tablets. On the other hand, the investigated systems showed more retarded drug release profiles. The \( P_{2h} \) of M14, E-M14A, E-M14B and E-M14C were 43.41%, 33.67%, 24.3% and 9.85%, respectively. According to the pharmacopeial specifications for enteric-coated systems, the \( P_{2h} \) should not exceed 10%. It was clear that the implementation of the first approach (E-M14A, E-M14B) did not allow the development of coherent coating barriers which were unable to retard the rate of drug release into the acidic milieu; even with the higher poly (methacrylic acid-co-methyl methacrylate) concentration (E-M14B). On the other hand, a significantly lowered \( P_{2h} \) was achieved following the adoption of the dip coating approach on the surface of the hard gelatin capsules filled with the nanoparticles (E-M14C).

The \( P_{4h} \) of M14, E-M14A, E-M14B and E-M14C were 47.34%, 40.5%, 32.6 and 28.6%, respectively. The superiority of the dip coating approach to minimize the drug released percentages in the simulating small intestine medium could be inferred. Interestingly, no significant differences (\( p > 0.05 \)) were observed between the \( P_{12h} \) values for M14 (64.96%), E-M14A (64.82%), E-M14B (64.56%) and E-M14C systems (65.45%). It is worth to note that the respective cumulative percentages of drug released after 24 h were 81.8 %, 81.2%, 80.52% and 81.5%. The maximization of the percentages of drug release in the colon could be expected with E-M14C system. Furthermore, this latter system will preserve the nano-size of the PLGA nanoparticles which will allow the uptake by macrophages or dendritic cells at the site of active inflammation in the colon after the dissolution of the enteric-cote shield so it was selected for the In Vivo assessment.

**Pharmacokinetic Assessments of the Optimized System in Healthy Volunteers**

The mean (± S.D.) etoricoxib plasma concentration – time curves following oral administration of single 60 mg oral doses of market tablets of etoricoxib and E-M14C system in healthy volunteers, under fasting conditions, are portrayed in Figure 5.

The \( C_{max} \) values of the reference and the test treatments were 619.20 ± 212.46 ng/mL and 487.68 ± 134.63 ng/mL, respectively. The statistical analysis of data revealed the lack of significant differences (\( p = 0.205 \)), Table 3.

On contrary, the statistical analysis of the non-parametric data (\( T_{max} \)) via Wilcoxon’s signed-rank test as well as the parametric data (MRT(0-\( \infty \)), AUMC(0-0.72h) (ng.h\(^2\)mL\(^{-1} \)), AUMC(0-\( \infty \)) (ng.h\(^2\)mL\(^{-1} \)), AUC(0-72h) and AUC(0-\( \infty \)) via two-way ANOVA revealed the superiority of E-M14C system (Table 3). The respective median \( T_{max} \) values for the market tablets of etoricoxib and E-M14C system were 2.5 and 6 h. These results are in correlation...
Figure 4 The in-vitro drug release profiles of Arcoxia® tablets, M14 (uncoated nanoparticles), E-M14A (nanoparticles with Eudragit®-S100 coat: PURASORB PDL02 ratio of 1:1), E-M14B (nanoparticles with Eudragit®-S100 coat: PURABORB PDL02 ratio of 2:1), E-M14C systems (M14 nanoparticles filled in Eudragit®-S100-coated hard gelatin capsules) (mean ± S.D., n = 3).

Abbreviation: PDL02, DL-lactide coglycolide copolymer of Lactide:glycolide ratio of 100:0 and inherent viscosity 0.2 dL/g.

Figure 5 Plasma concentration–time curves of etoricoxib following oral administration of single oral doses (60 mg) of Arcoxia® tablets and E-M14C system (M14 nanoparticles filled in Eudragit®-S100-coated hard gelatin capsules) in healthy volunteers, under fasting conditions (mean ± S.D., n = 6).
with the dissolution pattern of the drug from the two products in gradient pH medium simulating the GIT fluid. Both results confirm the ability of the latter system to minimize the percentages of drug released in the stomach and the small intestine, allowing for the delivery of most of its payload at the colon.

In a parallel line, the prolongation in \( MRT_{(0-\infty)} \) from 24.41 h to 34.70 h could point out the sustained drug release capabilities of E-M14C system. With respect to \( AUC_{(0-\infty)} \) values of both treatments, the relative oral bioavailability would be 177.35%. Taking into consideration \( AUC_{(0-72)} \) values of both treatments, the relative oral bioavailability would be raised to 423.32%.

**Conclusions**

Etoricoxib-loaded nanoparticles were successfully developed, according to the D-optimal design, via the nanospray drying technique. M14 system was prepared using PLGA (0.5% w/v) at a lactide:glycolide ratio of 100:0, in presence of PVP K30 (2% w/v). Further coating of M14-loaded hard gelatin capsules with poly (methacrylic acid-co-methyl methacrylate) (E-M14C) was necessary to minimize the percentages of drug released in the stomach and small intestine. The pharmacokinetic assessments of E-M14C in healthy human volunteers revealed its colon-targeting potential and proved its ability to increase the oral bioavailability of etoricoxib, relative to the marketed tablets. Further clinical studies are required to confirm the efficacy of this system in the management of irritable bowel syndrome.

**Disclosure**

The authors declare that there are no conflicts of interest in this work.

**References**

1. Carter MJ, Lobo AJ, Travis SP. Guidelines for the management of inflammatory bowel disease in adults. *Gut*. 2004;53(suppl 5):v1–v16. doi:10.1136/gut.2004.034372
2. Badhana S, Garud N, Garud A. Colon specific drug delivery of mesalazine using Eudragit S100-coated chitosan microspheres for the treatment of ulcerative colitis. *Int Current Pharm J*. 2013;2(3):42–48. doi:10.3329/icpj.v2i3.13577
3. Coco R, Plapied L, Pourcelle V, et al. Drug delivery to inflamed colon by nanoparticles: comparison of different strategies. *Int J Pharm*. 2013;440(1–2):3–12. doi:10.1016/j.ijpharm.2012.07.017
4. Rubinstein A. Colonic drug delivery. *Drug Discov Today Technol*. 2005;2(1):33–37. doi:10.1016/j.ddtc.2005.05.021
5. Oriente I, Cerchiera T, Luppi B, Bigucci F, Zucari G, Zecchi V. Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. *Int J Pharm*. 2002;238(1–2):51–59. doi:10.1016/S0378-5173(02)00060-1
6. Lamoudi L, Chaumeil JC, Daoud K. PLGA nanoparticles loaded with the non-steroid anti-inflammatory factor influence study and optimization using factorial design. *Int J Chem Eng Appl*. 2013;4(3):369. doi:10.7763/IJCEA.2013.V4.327
7. Salunke KS, Kulkarni MV. Formulation and in-vitro evaluation of dextran matrix tablet of ibuprofen for colon specific drug delivery. *Pak J Pharm Sci*. 2008;21(1):17–20.
8. de Alencar RG, de Oliveira AC, Lima EM, da Cunha-filho MSS, Taveira SF, Marreto RN. Compacted multiparticulate systems for colon-specific delivery of ketoprofen. *AAPS Pharm Sci Tech*. 2017;18(6):2260–2268. doi:10.1208/s12249-016-0700-2
9. Abbas HK, Wais FMH, Abood AN. Preparation and evaluation of ketoprofen nanosuspension using solvent evaporation technique. *Iraqi J Pharm Sci*. 2017;41–55.
10. Orlu M, Cevher E, Araman A. Design and evaluation of colon specific drug delivery system containing flurbiprofen microspheres. *Int J Pharm*. 2006;318(1–2):103–117. doi:10.1016/j.ijpharm.2006.03.025
11. Hadi MA, Rao NR, Rao AS, Mahtab T, Tabassum S. A review on various formulation methods in preparing colon targeted mini-tablets for chronotherapy. *J Applied Pharm Sci*. 2018;8(03):158–164.
12. Krishnaiah Y, Satyanarayana V, Dinesh Kumar B, Karthikeyan R. Studies on the development of colon-targeted delivery systems for celecoxib in the prevention of colorectal cancer. *J Drug Target*. 2002;10(3):247–254. doi:10.1080/10611860290022697

---

**Table 3** Pharmacokinetic Parameters for Etoricoxib Following Oral Administration of Single Oral Doses (60 mg) of Arcoxia® Tablets and E-M14C System (M14 Nanoparticles Filled in Eudragit®-S100-Coated Hard Gelatin Capsules) in Healthy Volunteers, Under Fasting Conditions. Results are Presented as Mean ± S.D., n = 6

| Pharmacokinetic Parameter | Arcoxia® Tablets | E-M14C System | P-values | Significance |
|---------------------------|------------------|---------------|----------|-------------|
| \( C_{\text{max}} \) (ng/mL) | 619.20 ± 212.46 | 487.68 ± 134.63 | 0.205 | Non-significant |
| \( T_{\text{max}} \) (h) | 2.5 (2.3–3.5) | 6 (0) | 3.48 × 10^{-9} | Significant |
| MRT (h) | 24.41 ± 2.06 | 34.70 ± 0.99 | 5.37 × 10^{-7} | Significant |
| \( AUMC_{0-72h} \) (ng.h.mmL^{-1}) | 309.738.60 ± 83.091.01 | 781.033.90 ± 245.799.10 | 0.001 | Significant |
| \( AUMC_{0-\infty} \) (ng.h.mmL^{-1}) | 654.634.80 ± 244.498.70 | 11.361.278 ± 3.221.904 | 8 × 10^{-5} | Significant |
| \( AUC_{0-72h} \) (ng.h.mmL^{-1}) | 12.687.92 ± 3016.89 | 22.502.60 ± 692.48 | 0.01 | Significant |
| \( AUC_{0-\infty} \) (ng.h.mmL^{-1}) | 15.692.65 ± 4242.39 | 66.430.56 ± 16.401.58 | 6 × 10^{-5} | Significant |

Note: Median (range). Abbreviations: \( C_{\text{max}} \), maximum concentration of drug in blood; \( T_{\text{max}} \), time needed to reach the maximum concentration of drug; MRT, mean residence time; AUMC, area under moment curve from time 0 to \( \infty \); \( AUC_{0-72h} \), area under curve from time 0 to 72 hours; \( AUC_{0-\infty} \), area under curve from time 0 to \( \infty \).
13. Trivedi HD, Puranik PK. Formulation and development of colon specific etoricoxib CODESTM tablet: statistical optimization and in vivo roentgenography. *Int J Pharm Res Clin Res*. 2016;7(1):185–215.

14. El Miedany Y, Youssef S, Ahmed I, El Gaafary M. The gastrointestinal safety and effect on disease activity of etoricoxib, a selective cox-2 inhibitor in inflammatory bowel diseases. *Am J Gastroenterol*. 2006;101(2):311. doi:10.1111/j.1572-0241.2006.00384.x

15. Miao XP, Li JS, Ouyang Q, Hu RW, Zhang Y, Li HY. Tolerability of selective cyclooxygenase 2 inhibitors used for the treatment of rheumatological manifestations of inflammatory bowel disease. *Cochrane Database Systematic Rev*. 2014;10.

16. Rodrigues AD, Halpin RA, Geer LA, et al. Absorption, metabolism, and excretion of etoricoxib, a potent and selective cyclooxygenase-2 inhibitor, in healthy male volunteers. *Drug Metab Disposition*. 2003;31(2):224–232. doi:10.1124/dmd.31.2.224

17. Kiran KGB, Naresh YS, Mohammed GA, Abdul Nasir K. Design and evaluation of a new capsule-type dosage form for colon-targeted delivery of etoricoxib. *Int J Pharm Sci Clin Res*. 2013;3(1):147–151.

18. Lamprecht A, Schäfer U, Lehr C-M. Size-dependent bioadsorption of micro-and nanoparticulate carriers to the inflamed colonic mucosa. *Pharm Res*. 2001;18(6):788–793. doi:10.1023/A:1011032380604

19. Chaudhary A, Das S. Recent advancement of chitosan-based nanoparticles for oral controlled delivery of insulin and other therapeutic agents. *AAPS Pharm Sci Tech*. 2011;12(1):10–20. doi:10.1208/s12249-010-9561-2

20. Sharma S, Parmar A, Kori S, Sandhir R. PLGA-based nanoparticles: a new paradigm in biomedical applications. *TrAC Trends Anal Chem*. 2016;80:30–40. doi:10.1016/j.trac.2015.06.014

21. Stevanović M, Savić J, Jordović B, Uskoković D. Fabrication, in vitro degradation and the release behaviours of poly (D,L-lactide-co-glycolide) nanospheres containing ascorbic acid. *Colloids Surf B Biointerfaces*. 2007;59(2):215–223. doi:10.1016/j.colsurfb.2007.05.011

22. Taylor D, Thomas R, Penfold J. Polymer/surfactant interactions at the air/water interface. *Adv Colloid Interface Sci*. 2007;132(2):69–110. doi:10.1016/j.cis.2007.01.002

23. Budhan A, Siegel SJ, Winey KJ. Controlling the in vitro release profiles for a system of haloperidol-loaded PLGA nanoparticles. *Int J Pharm*. 2008;346(1):151–159. doi:10.1016/j.ijpharm.2007.06.011

24. Fambri L, Migliarese C, Kesenci K, Piskin E. Biodegradable polymers. Editor; Ronaldo Barbucci: *Integrated Biomaterials Sci*. Springer; 2002:119–187.

25. Dunne M, Corrigan O, Ramtoola Z. Influence of particle size and dissolution conditions on the degradation properties of poly(lactide-co-glycolide) microspheres. *Biomaterials*. 2000;21(16):1659–1668. doi:10.1016/S0142-9612(00)00405-0

26. Raghuvanshi RS, Singh M, Talwar G. Biodegradable delivery system for single step immunization with tetanus toxoid. *Int J Pharm*. 1993;93(3–4):R1–R5. doi:10.1016/S0378-5173(93)90188-L

27. Stevanović M, Jordović B, Uskoković D. Stereological analysis of micro- and nanometric drug particles with specific drug targeting and drug release properties. *J Pharm Sci*. 2008;97(1):71–87. doi:10.1016/j.jspis.2010.02.001

28. Mundargi RC, Babu VR, Mangwami V, Patel P, Aminabhavi TM. Nano/micro technologies for delivering macromolecular therapeutics using poly (D, L-lactide-co-glycolide) and its derivatives. *J Controlled Release*. 2008;125(3):193–209. doi:10.1016/j.jconrel.2007.09.013

29. Li X, Anikó N, Arpagaus C, Bellelay F, Vandamme TF. Nanoparticles by spray drying using innovative new technology: the büchi nano spray dryer B-90. *J Controlled Release*. 2010;147(2):304–310. doi:10.1016/j.jconrel.2010.07.113

30. Gautier S, Arpagaus C, Schafroth N, Meuri M, Deschamps A, Maquet V. Fine chitosan microparticles with narrow & controlled size distribution using spray-drying technologies. *Drug Delivery Technol*. 2010;10(8):30–37.

31. Triefenbach F Design of experiments: the D-optimal approach and its implementation as a computer algorithm. *Bachelor's Thesis in Information and Communication Technology*. 2008.

32. Arai H, Suzuki T, Kaseda C, Takayama K. Effect of an experimental design for evaluating the nonlinear optimal formulation of theophylline tablets using a bootstrap resampling technique. *Chem Pharm Bull*. 2009;57(6):572–579. doi:10.1248/cpb.57.572

33. Dixit R, Nagarshenker M. Self-nanomulsifying granules of ezetimibe: design, optimization and evaluation. *Eur J Pharm Sci*. 2008;35(3):183–192. doi:10.1016/j.ejps.2008.06.013

34. Elmaggar YS, El-Massik MA, Abdallah YO. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: design and optimization. *Int J Pharm*. 2009;380(1–2):133–141. doi:10.1016/j.ijpharm.2009.07.015

35. Nahata T, Saini TR. D-optimal designing and optimization of long acting microsphere-based injectable formulation of aripiprazole. *Drug Dev Ind Pharm*. 2008;34(7):668–675. doi:10.1080/03639040701836545

36. Czitrom V. One-factor-at-a-time versus designed experiments. *Am Stat*. 1999;53(2):126–131.

37. Abdel-Hafez SM, Hathout RM, Sammour OA. Towards better modeling of chitosan nanoparticles production: screening different factors and comparing two experimental designs. *Int J Biol Macromol*. 2014;64:334–340. doi:10.1016/j.ijbiomac.2013.11.041

38. Garud N, Garud A. Preparation and in-vitro evaluation of metformin microspheres using non-aqueous solvent evaporation technique. *Trop J Pharm Res*. 2012;11(4):577–583. doi:10.4134/tjpr.v11i4.1148

39. Singh S, Mishra A, Verma A, Ghosh AK, Mishra AK. A simple Ultraviolet spectrophotometric method for the determination of etoricoxib in dosage formulations. *Adv Pharm Technol Res*. 2012;3(4):237. doi:10.4103/2231-4040.104715

40. Elbary AA, Tadros MI, Alaa-ElDin AA. Sucrose stearate-enriched lipid matrix tablets of etodolac: modulation of drug release, diffusional modeling and structure elucidation studies. *AAPS Pharm Sci Tech*. 2013;14(2):656–668. doi:10.1016/j.aaps.2013.09.051-3

41. Yogesh P, Madhuri M, Vaishnavi M, Atul C, Pankaj G. Preparation, characterization, and evaluation of anti-inflammatory activity of etoricoxib loaded soluplus® nano composites. *Int J Appl Pharma*. 2018;10:6.

42. El-Bary AA, Aboelwafa AA, Al Sharabi IM. Some formulation variables on the optimization of pH-dependent, colon-targeted, sustained-release mesalamine microspheres. *AAPS Pharm Sci Tech*. 2012;13(1):75–84. doi:10.1208/s12249-011-9721-z

43. Mehta R, Chawla A, Sharma P, Pawar P. Formulation and in vitro evaluation of Eudragit S-100 coated naproxen matrix tablets for colon-targeted drug delivery system. *J Adv Pharm Technol Res*. 2013;4(1):31. doi:10.4103/2231-4040.107498
50. Qureshi J, Ali J, Baboota S, Ahuja A, Mallikarjun C. Development and evaluation of chronotherapeutic drug delivery system for the management of nocturnal asthma. *Trop J Pharm Res*. 2012;11(5):703–712.

51. Alisharkas L, Kumar PV, Wei YS. Preparation and in vitro evaluation of enteric coated oral vancomycin hydrochloride sustained release formulation with mucoadhesive properties. *Am J Adv Drug Delivery*. 2018;06. doi:10.21767/2321-547X.1000029

52. Elbury AA, Tadros MF, Alaa-Eldin AA. Development and in vitro/in vivo evaluation of etodacol controlled porous osmotic pump tablets. *AAPS Pharm Sci Tech*. 2011;12(2):485–495. doi:10.1088/122249-011-9968-x

53. Joshi SA, Chavhan SS, Sawant KK. Rivastigmine-loaded PLGA and PBCA nanoparticles: preparation, optimization, characterization, in vitro and pharmacodynamic studies. *Eur J Pharm Biopharm*. 2010;76(2):189–199. doi:10.1016/j.ejpb.2010.07.007

54. Hinkelmann K, Kępnowe O. Design and Analysis of Experiments. Vol. 1. Wiley Online Library; 1994.

55. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y. Preparation of poly (DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *Int J Pharm*. 1999;187(2):143–152. doi:10.1016/S0378-5173(99)00187-8

56. Shakeri S, Roghanian R, Emtiaziz G, Errico C, Chiellini E, Chiellini F. Preparation of protein-loaded PLGA-PVP blend nanoparticles by nanoprecipitation method: entrapment, initial burst and drug release kinetic studies. *Nanomed J*. 2015;2(3):175–186.

57. Muzaffar K, Wani SA, Dinkarao BV, Kumar P. Determination of production efficiency, color, glass transition, and sticky point temperature of spray-dried pomegranate juice powder. *Cogent Food Agriculture*. 2016;2(1):114444. doi:10.1080/23319192.2016.114444

58. Fang Z, Bhandari B. Comparing the efficiency of protein and maltodextrin on spray drying of bayberry juice. *Food Res Int*. 2012;48(2):478–483. doi:10.1016/j.foodres.2012.05.025

59. Lee LY, Wang CH, Smith KA. Micro-porous paclitaxel-loaded PLGA foams—a new material for controlled release of chemotherapeutic agents. 2007.

60. Bhamibe D, Shivravstava B, Sharma P, Gide P. Effect of polymer and formulation variables on properties of self-assembled polymeric micellar nanoparticles. *J Nanomed Biotechnol*. 2014;4(129):2. doi:10.4172/2155-983X.1000129

61. Kuribey K. Optimization of rutin-loaded PLGA nanoparticles synthesized by single-emulsion solvent evaporation method. *ACS Omega*. 2019;4(1):555–562. doi:10.1021/acsomega.8b02767

62. Wagh YD, Apar DU. Cyclosporine a loaded PLGA nanoparticles for dry drug delivery: in vitro characterization studies. *J Nanotechnol*. 2014;2014.

63. Coombes A, Yeh M-K, Lavelle E, Davis S. The control of protein release from poly (DL-lactide-co-glycolide) microparticles by variation of the external aqueous phase surfactant in the water-in oil-in water method. *J Controlled Release*. 1998;52(3):311–320. doi:10.1016/S0168-3659(98)00006-6

64. Chung TW, Tsai YL, Hsieh JH, Tsai WJ. Different ratios of lactide and glycolide in PLGA affect the surface property and protein delivery characteristics of the PLGA microspheres with hydrophobic additives. *J Microencapsulation*. 2006;23(1):15–27.

65. Baysan U, Elmas F, Koç M. The effect of spray drying conditions on physicochemical properties of encapsulated propolis powder. *J Food Process Eng*. c13024.

66. Quintanar-Guerrero D, Fessi H, Allèmann E, Doelker E. Influence of stabilizing agents and preparative variables on the formation of poly (D, L-lactic acid) nanoparticles by an emulsification-diffusion technique. *Int J Pharm*. 1996;143(2):133–141. doi:10.1016/S0378-5173(96)04697-2

67. Scholes P, Coombes A, Illum L, Daviz S, Vert M, Davies M. The preparation of sub-200 nm poly (lactide-co-glycolide) microspheres for site-specific drug delivery. *J Controlled Release*. 1993;25(1–2):145–153. doi:10.1016/0168-3659(93)00103-C

68. Mehrotra A, Pandit JK. Preparation and characterization and biodistribution studies of lomustine loaded PLGA nanoparticles by interfacial deposition method. *J Nanomed Biotechnol*. 2015;5(138):2. doi:10.4172/2155-983X.1000138

69. Corrigan OI, Li X. Quantifying drug release from PLGA nanoparticulates. *Eur J Pharm Biopharm*. 2009;73(3–4):477–485. doi:10.1016/j.ejpb.2009.04.004

70. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. *Biomaterials Science: An Introduction to Materials in Medicine*. Elsevier; 2004.

71. Rajapakse TE, Lo DD. Microencapsulation of vaccine antigens and adjuvants for mucosal targeting. *Curr Immunol Rev*. 2010;6(1):29–37. doi:10.2174/157339510790231798

72. Park TG. Degradation of poly (lactide-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials*. 1995;16(15):1123–1130. doi:10.1016/0142-9612(95)93575-X

73. Chauhan B, Shimpi S, Paradkar A. Preparation and characterization of etoricoxib solid dispersions using lipid carriers by spray drying technique. *AAPS Pharm Sci Tech*. 2005;6(3):E405–E409. doi:10.1208/pst060350

74. Erbetta CDAC, Alves RJ, Resende JM, de Souza Freitas RF, de Sousa RG. Synthesis and characterization of poly (D, L-lactic-co-glycolide) copolymer. *J Biomater Nanobiotechnol*. 2012;3(02):208. doi:10.4236/jbnn.2012.32027

75. Chandran S, Sanjay KS, Ali Asghar LF. Microspheres with pH modulated release: design and characterization of formulation variables for colonic delivery. *J Microencapsulation*. 2009;26(5):420–431. doi:10.1080/0265204080242021

---

**International Journal of Nanomedicine**

**Publish your work in this journal**

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®, Clinical Medicine, Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.