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Biosynthesized poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) as biocompatible microcapsules with extended release for busulfan and montelukast

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ABSTRACT

An extended release dosage form based on encapsulating the challenging drug busulfan within microspheres of the biodegradable, biocompatible and biosynthesized poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) polyester was achieved. The used (PHBV) polymer was biosynthesized by the halophilic archaeon *Haloferax mediterranei* from date waste biomass as feed-stock. PHBV microspheres of 1.2–2.1 μm diameter were successfully fabricated and loaded with busulfan with an encapsulation efficiency of 29.2 ± 0.2%. In addition, PHBV microspheres of 1.5–3.5 μm diameter and loaded with montelukast sodium (MK) drug were also fabricated with an encapsulation efficiency of 16.0 ± 0.4%. The double-emulsion solvent evaporation method was used to fabricate the drug-loaded microspheres. The drug-loaded microspheres have been characterized by XRD, FTIR and SEM, and confirmed to be successfully fabricated. The drugs *in vitro* release profiles have shown extended release for up to 3 days in case of busulfan and 8 h in case of montelukast sodium. The *in vitro* release profiles for busulfan and montelukast suggest that these drug-loaded microcapsules can be efficiently used as new dosage forms to solve the current issues of busulfan administration protocols, and to introduce a new dosage form for montelukast with extended release performance.

1. Introduction

Busulfan (1,4-butanediol dimethanesulfonate) drug (Fig. 1) is an alkylating agent that has been used in chemotherapy for decades. It is mainly used to treat a specific type of cancer of white blood cells called chronic myelogenous leukemia (CML) [1]. The first introduced dosage form was as tablets for oral administration [2,3]. Currently, busulfan is mostly used in a dosage form for intravenous administration under the tradename Busulfex® and as tablets under trade name Myleran®. Busulfex is a very critical medication in lymphoma, leukemia, myeloproliferative disorders and most importantly in pediatrics for inborn errors and in immunodeficiency [4,5], and it is used for myeloablation to destroy the bone marrow and cancer cells in preparation for a bone marrow transplant as preconditioning before hematopoietic stem cell transplantation (HSCT) [6]. Busulfex® has many issues and limitations that include: (a) severe side effects have been reported, of which hepatic veno-occlusive disease is the most frequent and severe [7]. Liver toxicity was explained by high systemic exposure to the drug [8,9], (b) large inter-patient variability and hence variations in bioavailability [10], (c) low solubility in aqueous solution which imposed the use of the toxic organic solvent *N,N*-dimethylacetamide (DMA) [11], (d) the hydrolysis and inactivation of the drug in aqueous solutions [12,13], (e) the administration protocol is considered harsh and lengthy, especially for children; it is administered intravenously for a total of 16 doses during four consecutive days, each dose is administered through a catheter as a two-hour infusion every 6 h.

All these aforementioned concerns on Busulfex® have raised the need for a sustained release dosage form. To address this need,

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nanocarriers of porous Metal-Organic Framework (MOF) nanoparticles based on iron (III) trimesate (MIL-100) nanocarrier encapsulating considerable amounts of busulfan (32%) were administered to rats and studied in comparison with the commercial Busilvex™ [14]. These MOF materials have the disadvantage of the increasing risk of heavy metals accumulation and long term side effects. Also, when compared to organic polymers, it is well-known that MOF materials are more difficult to fabricate, process, scale up and do fine tuning on their structures. In another similar study, MOF nanoparticles, based on crystalline porous iron (III) carboxylates, have shown a high busulfan loading of up to 25 wt% [15]. To utilize the advantages of organic polymeric materials, another attempt to encapsulate busulfan was reported [16], the researchers used poly(ethylene glycol) (PEG)-coated nanospheres using polyester-PEG diblock copolymers, but the results showed very poor drug loading percentage down to 1%, and also relatively low encapsulation efficiency of 4–11% in addition to the impractical very fast release; only 30 min were more than sufficient to get 100% of busulfan released completely. In another study, busulfan entrapment by nanoprecipitation into poly(isobutyl cyanoacrylate) and poly(ethyl cyanoacrylate) was reported with maximum loading ratios of 5.9% (*w*/w) [17], and the clear disadvantage here was the very low and impractical loading percentage in addition to the well-known cytotoxicity associated with these short alkyl chains cyanoacrylate polymers [18].

The aforementioned issues and limitations whether in the current busulfan commercial dosage form, or in the published research attempts, all highlight the need to develop a new dosage form with ideally the following features: (a) to be based on an organic biodegradable and biocompatible polymeric material, (b) can achieve high loading and encapsulation efficiencies, (c) exhibits extended and slow release to reduce side effects, control systemic exposure to the drug in addition to enhancing patient compliance and receptivity to the drug, (d) avoids the use of heavy metals and toxic materials and hence lowers risks of cytotoxicity, (e) protects the drug from degradation before reaching the target and (f) reduce the differences in bioavailability. To the best of our knowledge, and till the time of conducting this study, busulfan has not been commercially introduced or registered as a sustained release dosage form.

Montelukast sodium (MK) (Fig. 1) is prescribed to prevent and treat asthma. It can decrease the number of acute asthma attacks and the symptoms as well. This drug is also used to treat symptoms of allergic rhinitis (hay fever) and to prevent exercise-induced bronchoconstriction (EIB). MK hasn't been available as sustained release dosage form so far.

PHBV, poly-3-hydroxybutyrate (PHB) and many of polyhydroxyalkanoates (PHAs) polymers have been intensively studied in the biomedical and drug delivery arenas [19,20]. Biodegradability and biocompatibility are the two main features that dragged the attention for PHBV utilization as a drug delivery system. In addition, some other properties have also helped, for example its structure is more amorphous compared to the PHB, and this is considered more efficient and practical for drug release as drugs can diffuse more readily through the

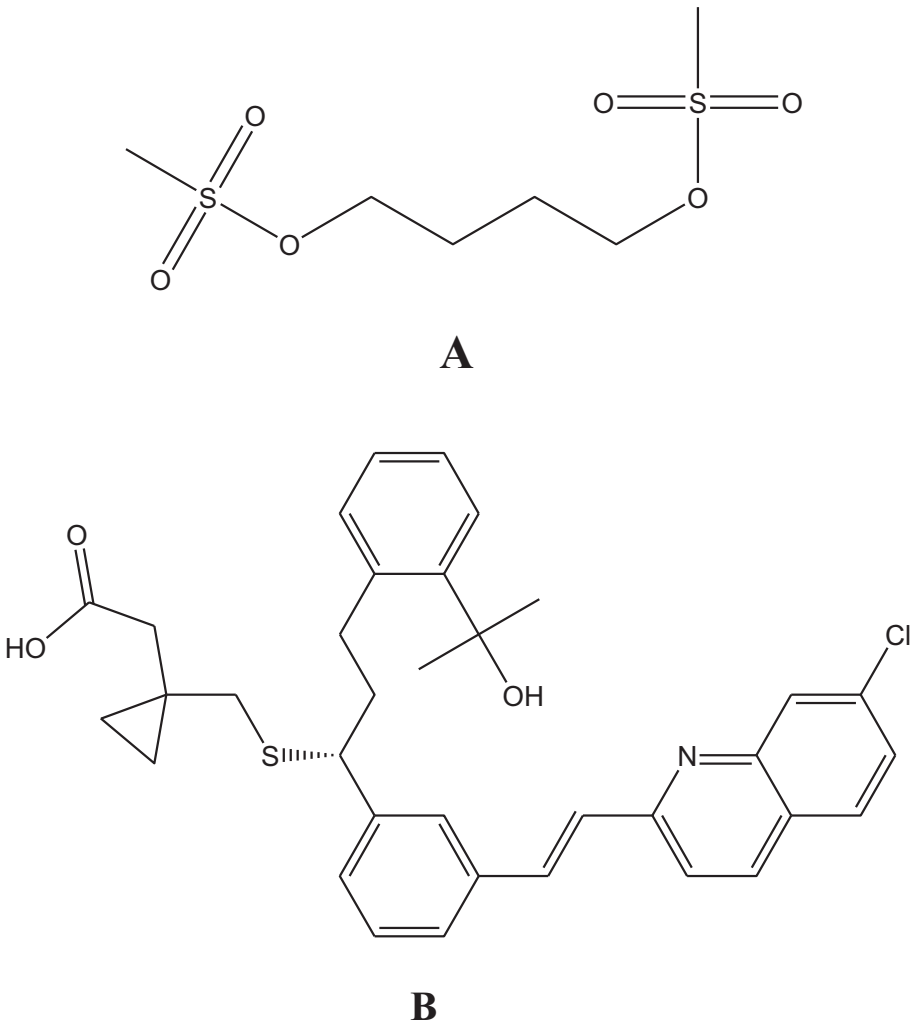


Fig. 1. Busulfan (A) and Muntelukast (B) chemical structures.

amorphous polymeric chains. Also, as long as PHBV can be highly and easily dissolved in chloroform and dichloromethane while it has poor solubility in other solvents, it can readily form nanoparticles or microspheres through the emulsification-solvent evaporation method [21]. In addition, the biodegradation process of PHBV is relatively slow and this qualifies it for long-term drug release applications [22]. Recently, a research review was published on the utilization of PHBV in the very specific field of antitumor applications [23].

There have been many research works reported for utilizing PHBV for drug delivery systems in bone tissue engineering, cancer therapy and many other fields, many of which have used PHBV as microspheres that encapsulate the drugs within their internal cavities. For example, metformin hydrochloride was encapsulated within PHBV as a hydrophilic drug model with encapsulation efficiency of 9.7% [24]. Also, gentamicin was encapsulated into composite microspheres of bioactive wollastonite and PHBV with high encapsulation efficiency of 52%, the encapsulation was achieved by soaking/immersing the PHBV microspheres in the drug solution [25]. In another study, bovine serum albumin was loaded with 18% encapsulation efficiency [26]. Other loaded drugs that were reported include alendronate [27], icariin flavonoid [28,29], vancomycin [30–32], cefuroxime axetil [33], sunitinib [34], mupirocin and ketoprofen [35], 5-fluorouracil and oxaliplatin [36], quercetin [37], hydrocortisone [38], docetaxel [39] and epirubicin [40]. Also, superparamagnetic iron oxide has been encapsulated in PHBV nanoparticles to form magnetically-guided drug delivery systems [41,42].

In this research work, PHBV polyester served as a green, biocompatible and biodegradable material which was biosynthesized by the archaeon *Haloferax mediterranei* from date waste as the carbon source. This green biopolymer is investigated here as potential encapsulating materials for the drugs busulfan and MK separately. The objective was to develop sustained release dosage forms for each of the two drugs separately.

2. Materials and methods

2.1. Materials

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) was produced by *Haloferax mediterranei* via biosynthesis utilizing date waste carbon source as described previously [43,44]. In summary, *H. mediterranei* was obtained as lyophilized sample of strain DSM1411 from Leibniz Institute DSMZ (Germany), and was initially grown in AS-168 nutrient-rich medium. The culture was incubated for 4 days in a rotary shaker (230 rpm, 37 °C). The strain was maintained in vials containing 20% glycerol and 80% AS-168 medium at −80 °C. The stored strain was streaked on salt agar medium and incubated at 37 °C for 48 h. Date waste was extracted by soaking and heating and then used as carbon source to feed the microorganism *H. mediterranei* in a bioreactor fed-batch fermentation process. The produced PHBV was obtained with relatively high 3-hydroxyvalerate (3 HV) content of 18.0 mol%, high molecular weight (746.0 kDa), narrow polydispersity (PDI = 1.5) and a melting point at 148.1 °C. Both drugs of montelukast (MK) and busulfan were provided as active pharmaceutical ingredient (API) from a local pharma company. Polyvinyl alcohol was purchased from Sigma-Aldrich with average molecular weight of 30,000–70,000 Da and 87–90% hydrolyzed.

2.2. Instrumentation

2.2.1. High Resolution/Accurate Mass (HRAM) liquid chromatography mass spectrometry (LCMSMS)

In the release study of busulfan drug, the released concentration of busulfan was measured using high pressure liquid chromatography coupled to high resolution accurate mass spectrometer (Ultimate™ 3000 RSLC coupled to Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer. Thermo Fisher Scientific, USA), which can simultaneously offer high resolution and high sensitivity. The parallel reaction monitoring (PRM) mode was used to quantify the target drug analyte, and this approach is very similar to selected reaction monitoring (SRM) which is commonly used in triple quadrupole technology for targeted quantification. This approach can effectively eliminate matrix and background interferences and increase selectivity. Two ions were monitored through the transition from 264.1 *m/z* to 151.1 *m/z*, where the latest ion was used for the quantification. The operating softwares were Xcaliber™ and Tracefinder™ 4.1. A calibration curve has been established for busulfan standard solutions of different concentrations (0.5, 1.0, 5.0, 10.0, 20.0 ppm).

2.2.2. Scanning Electron Microscope

Images were taken after coating the samples with 10 nm gold layer by sputter coating and the SEM system used was JCM-7000 NeoScope™ Benchtop SEM/Jeol, Japan.

2.2.3. Fourier Transform Infrared (FT-IR)

FT-IR spectra (4000–600 cm^{-1}) were collected in the solid state on Invenio S FT-IR Spectrometer-Bruker, Germany.

2.2.4. X-ray diffraction

The XRD patterns for the drug and its microspheres were recorded using X-Ray Diffractometer with Cu Kα radiation (λ = 0.15418 nm, 45 kV, 40 mA), model Empyrean, Malvern Panalytical, Netherlands.

2.2.5. UV–vis measurement and calibration

To determine the λ_{max} of the MK drug, we have prepared 15 ppm standard solution of MK and measured it in the UV–Vis (Specord 50, Analytica Jena co., Germany) through scanning mode from 190 to 320 nm, and the λ_{max} was found to be 285–286 nm. Then a calibration curve was built at λ = 286 nm by using four different concentration standard solutions of the drug (1, 5, 10, 25) ppm and a blank.

2.2.6. General instrumentation

The sonicator used was from Witeg Co. Germany model WUC-D22H. The oven used was from Binder Co. Germany. Ultra-pure water was obtained from Milli-Q water purification system from Millipore Co. model Direct 8. The centrifuge was model C1015/Centurion. The top loading balance was model NBL 3602i/Adam Co. Analytical balance was model N92/A&D. The magnetic stirrer was model 8P131320-33 from Thermo Fisher Scientific. The pH meter used was model HI 2217 from Hana.

2.3. Preparation of montelukast-encapsulated PHBV microspheres and their release study

Drug-encapsulated PHBV microspheres were fabricated using a water-in-oil-in-water (w/o/w) emulsion/solvent evaporation technique [24]. MK drug (0.25 g) was dissolved in 10 ml (1:1) solution mixture of water and ethanol to form the first aqueous solution (AQ1). Also, 0.5 g of PHBV polymer was dissolved by sonication in 20 ml chloroform to form the organic solution. Poly vinyl alcohol (PVA) solution (1% wt/v) was prepared as aqueous solution 2 (AQ2). The drug aqueous solution (AQ1) was poured into the organic solution and emulsified by stirring with high speed disperser (model L5M-A, Silverson, USA) at 10,000 RPM for 5.0 min. Then the resultant emulsion was poured into 20 ml of the PVA solution (AQ2), and the mixture was stirred for 10 min at 10,000 RPM (model L5M-A, Silverson, USA), then the emulsion was stirred at normal stirrer at 800 RPM (for 4 h) with applying some moderate airflow to help in expediting the evaporation of chloroform and ethanol. Then 15 ml ethanol portion was added, then the resultant suspension was centrifuged at 6000 RPM for 10 min, and the precipitate was washed with water and centrifuged again, then the resultant solid was dried by airflow only at room temperature.

To study the release, 10 mg of the loaded microspheres were inserted

inside a permeable bag and sealed in (a normal green tea bag which was emptied from its contents, washed many times with methanol and then sealed after inserting a known amount of the loaded microspheres in order to be used as a container for the microspheres to keep them from floating around in the solution), and the bag was put in a 50 ml beaker where a 25 ml solution of methanol and phosphate buffer solution PBS (1:1) at pH 7.4 was used as the release solution. Samples were taken at different time durations in the quartz cuvette for UV measurement at λ = 286 nm and they were poured back after each measurement.

2.4. Preparation of busulfan-encapsulated PHBV microspheres and their release study

PHBV microspheres loaded with busulfan drug have been prepared by oil-in-water (o/w) emulsion-solvent evaporation technique. Briefly, 0.25 g of busulfan was dissolved in 40 ml chloroform using sonication for 20 min, then after complete dissolution, 0.5 g PHBV polymer is added to the solution and dissolved as well. A 250 ml aqueous solution of 1.0% *w/v* of poly vinyl alcohol (PVA) was prepared. The organic solution of busulfan was added to the PVA solution to form two layer mixture, and then the mixture was emulsified at 10,000 RPM using a disperser (model L5M-A, Silverson, USA) for 15 min. Then a portion of 8.0 ml isopropanol was added to the formed emulsion. And then the mixture was stirred at 800 RPM with applying some weak airflow on the top of the bottle to expedite the evaporation of chloroform. The resultant suspended microspheres were centrifuged, washed with distilled water and dried under room temperature utilizing slow airflow, and then in the oven at 40 °C. To perform the release study, phosphate buffer solution (PBS) was prepared at pH 7.4, mixed with methanol (1:1), then 5 ml of this solution was used for the release study. 20 mg of the PHBV microspheres loaded with busulfan drug were weighed and transferred into a 10 ml plastic test tube containing 5 ml of the PBS and methanol (1:1). Then the test tube was put onto a rotator shaker (Glas-Col, USA) for gentle shaking. A sample volume of 50 μl was taken every time period of 30, 60, 120, 240, 360, 1440, 2160, 2880, 3000, 3200, 3600, 4000, 4200, 4230 and 4300 min, the pipetted sample was diluted 20 times with mobile phase to get a final volume of 1 ml, and then injected into the Orbitrap™ high resolution/accurate mass LCMSMS system (Ultimate™ 3000 RSLC coupled to Q Exactive™, Thermo Fisher Scientific, USA) with method conditions as described in the supplementary data.

2.5. Drug encapsulation efficiency (EE), loading percentage and kinetic study

To calculate the MK drug loading and encapsulation efficiency, 10 mg of the loaded microspheres were dispersed in 5 ml chloroform and sonicated for 10 min at 70% sonicator strength. Then 5 ml of phosphate buffer solution were added and the turbid suspension was shaken for 5 min and then sonicated for another 3 min. The resultant suspension was centrifuged for 3 min at 6000 RPM and the concentration of the clear solution was measured by UV–Vis. For busulfan drug, the encapsulation efficiency and loading percentage were calculated considering the highest concentration measured in the drug release study. For busulfan, the calculation of drug loading percentage and encapsulation efficiency were calculated by referring to the highest released concentration during the release study.

$$\text{Loading } \% = 100 \times \frac{\text{max.drug released } wt.}{\text{PHBV microspheres } wt.}$$

$$\text{EE} = 100 \times \frac{\text{encapsulated drug } wt.}{\text{total drug } wt.}$$

The drug release kinetics were analyzed using Microsoft Excel software (Microsoft Office 365) by fitting the experimental data to mathematical models of drug release. The mathematical models used for drug release were the zero-order, first order, Higuchi, Hixson-Crowell and Korsmeyer–Peppas models [45].

3. Results and discussion

3.1. Characterization of loaded microspheres of MK and busulfan

The loading of the targeted drugs MK and busulfan within microspheres of PHBV had been clearly confirmed by both FTIR (Invenio S, Bruker, Germany) and XRD (Empyrean, Malvern-Panalytical, Netherlands) analysis of the drugs alone, PHBV polymer alone and the produced loaded-microspheres for each drug.

The FTIR spectrum of the neat PHBV polymer (Fig. 2) showed clearly the characteristic peaks of this polyester; most importantly is the intense peak at 1720 cm^{-1} that corresponds to the ester carbonyl C═O stretching wavenumber, also, wavenumbers at 1379 cm^{-1} and 1453 cm^{-1} correspond to the amorphous part of the polymer [46]. The peaks at 2874 cm^{-1}, 2936 $cm^{-1}$and 2978 cm^{-1} correspond to the different C—H stretching wavenumbers. The FTIR spectrum of busulfan drug (Fig. 3) is in harmony with previously reported values [47]. The wavenumbers at 3020 cm^{-1} and 3032 cm^{-1} are attributed to CH asymmetrical stretching vibrations of –S–CH_3. The peaks in the range 1415 cm^{-1} to 1440 cm^{-1} are assigned to the asymmetrical deformations of the CH_3 group attached to sulfur. The peaks at 2984 cm^{-1}, 2966 cm^{-1} and 2945 cm^{-1} are assigned to asymmetrical stretching modes of the four CH_2 groups. The strong peaks between 1000 cm^{-1} and 700 cm^{-1} are assigned to the S—O stretching of sulfonates. The FTIR spectrum of the busulfan-loaded PHBV microspheres shows characteristic peaks for both busulfan drug and the PHBV polymer indicating the successful encapsulation of the drug within the PHBV polymer microspheres. The combined FTIR spectra are shown in Fig. 3. Also, the FTIR spectrum of MK (Fig. 2) had shown the characteristic absorption peaks of the pure drug as reported in literature [48], with a broad peak at 3300 cm^{-1} assigned to the —OH groups and a peak near 1600 cm^{-1} for the COOH carbonyl. The absorption peaks observed between 2900 cm^{-1} and 3000 cm^{-1} are assigned to the aromatic C—H. The same characteristic peaks of MK and PHBV appear also in the drug-loaded microspheres spectrum (Fig. 2) which indicates the successful loading of the drug within the PHBV polymer, and that there are no functional group changes between the drug alone and in the loaded microspheres (Fig. 2).

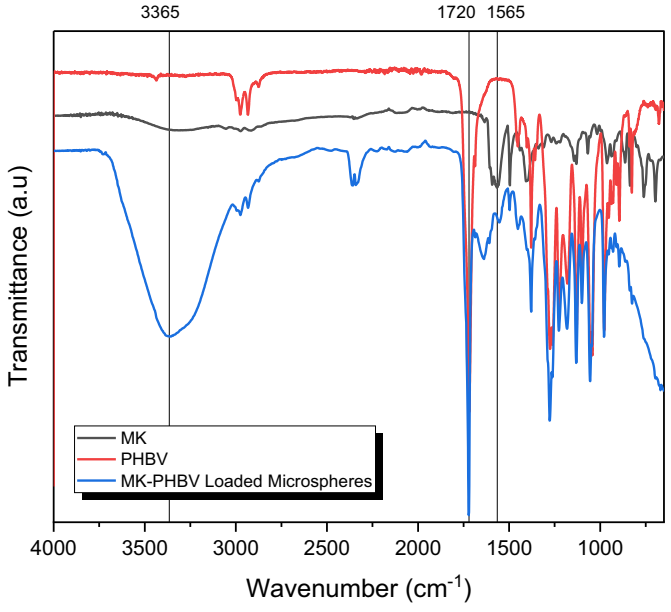


Fig. 2. Combined FTIR spectra of MK, PHBV and MK-PHBV loaded microspheres.

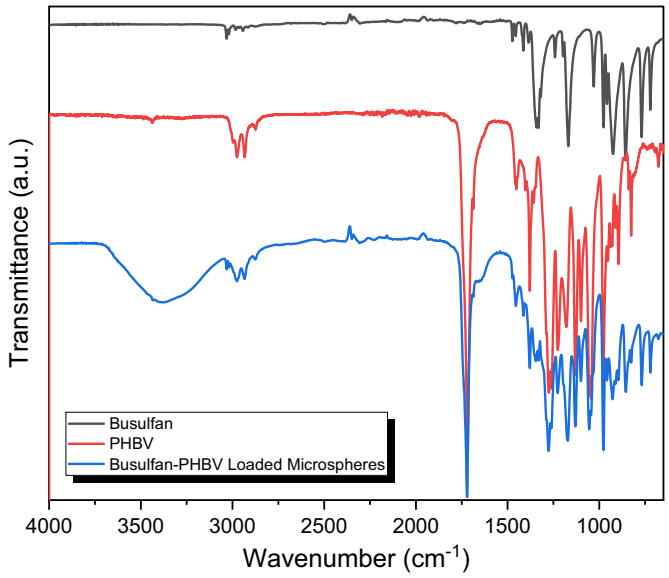


Fig. 3. Overlay of FTIR spectra for busulfan, PHBV and loaded PHBV microspheres.

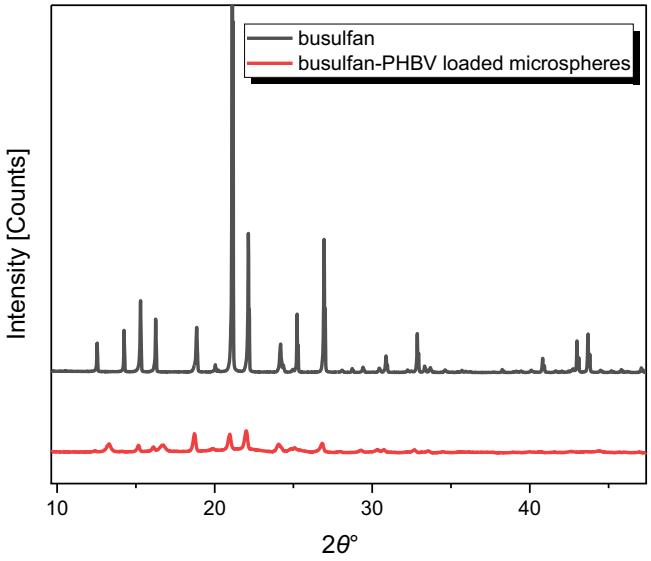


Fig. 5. Overlay of XRD spectra for busulfan and busulfan-PHBV loaded microspheres.

The XRD analyses of the MK, PHBV and the MK-loaded microspheres are shown in Fig. 4. The PHBV polymer exhibited the well-known characteristic peaks at 2θ values of 13.7°, 17.1°, 20.4°, 21.5°, 22.7°, 25.5°, 26.9°, 30.6° which correspond to the typical (020), (110), (021), (101), (111), (121), (040), (200) crystal planes for orthorhombic PHBV, respectively [49]. The intense peaks at 13.7° and 17.1° are connected with the crystalline part of the polymer, while the broad peaks at 2θ =20° to 22° are corresponding to the amorphous phase in the PHBV [46]. The XRD spectrum of the loaded microspheres (Fig. 4) was clearly a combination of both spectra of the neat PHBV and the MK with increased sharpness of the characteristic polymer peaks, indicating that the polymer crystallinity increased throughout the encapsulation process, and also supporting the claim of successful encapsulation of the drug within PHBV. Also, Fig. 5 shows XRD graphs for buslfan and busulfan-loaded PHBV microspheres. The spectrum for busulfan XRD matched previously reported values [50]. The combined XRD of the busulfan drug and the loaded microspheres indicated changes in crystallinity for both the drug and the polyester as a result of the encapsulation process.

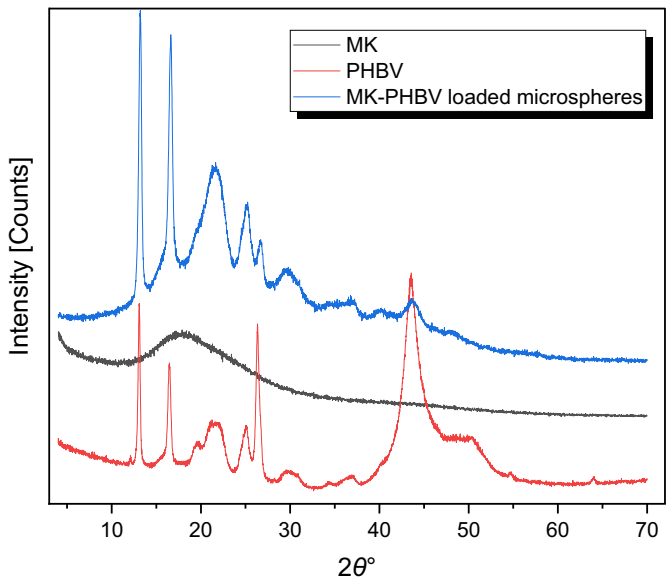


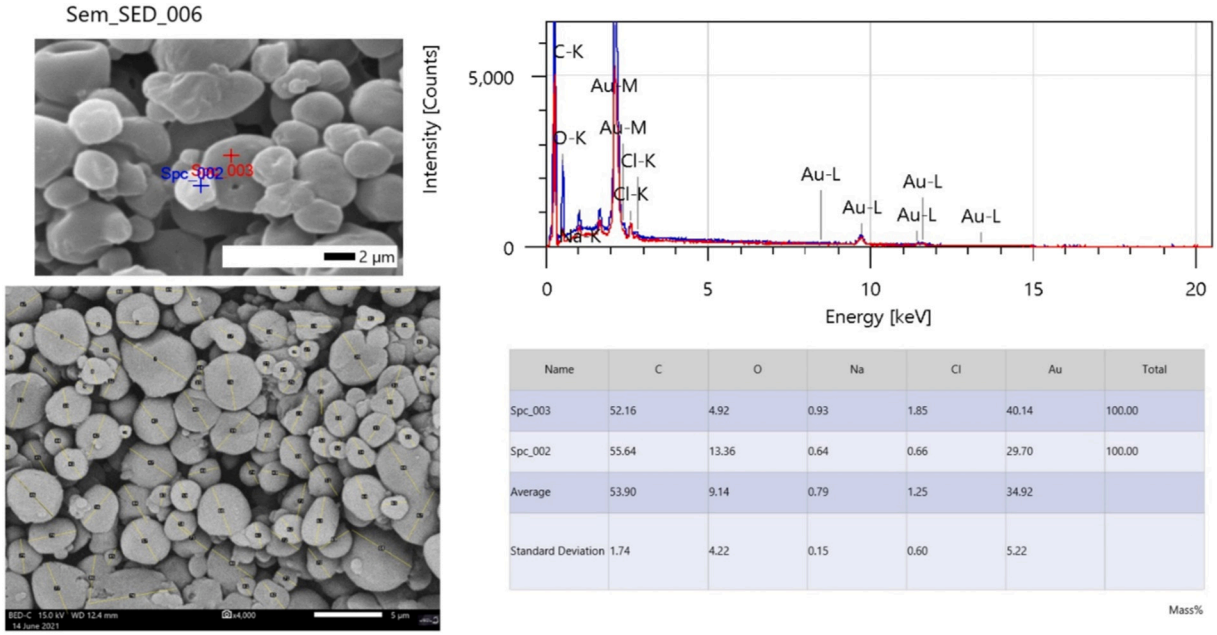
Fig. 4. Combined XRD spectra of MK, PHBV and MK-PHBV loaded microspheres.

The loaded microspheres for both drugs had also been imaged with scanning electron microscope (SEM) (JCM-7000 NeoScope™ Benchtop SEM. Jeol, Japan) after coating with a nano layer of gold. Fig. 6 displays the SEM image with relative diameters for MK-PHBV microspheres and its EDX analyses. The SEM images clearly showed the formation of uniformly spherical microspheres with average diameters of 1.5–3.5 μm for MK and 1.2–2.1 μm for busulfan as can be seen in Fig. 7. This result is very critical in the evaluation process of the new dosage forms. In fact, The United States Pharmacopoeia (USP) defines injectable drug particulate matter as "foreign particles, undissolved, mobile, other than gas bubbles, which are involuntary in these solutions (parenteral formulations)" [51]. Also, the European Pharmacopoeia (EP) has a dedicated chapter on particulate contamination [52]. There are two tests to determine particulate matter in preparations and both count the particles which are larger than 10 and 25 μm with limits according to the volume of the preparation and the particle size [53]. These international guidelines imply that suspensions of less than 10 μm particle size are suitable for intravenous injectable dosage forms, and this becomes meaningful considering that the size of blood vessels range from a 25 mm diameter as in the aorta to only 8 μm in the capillaries.

3.2. *Release profiles for drug-loaded PHBV microspheres*

In order to study the release of MK form the drug loaded PHBV microspheres, the UV–Vis was used as the suitable technique for measuring released concentrations of MK. Scanning the absorption of MK between 190 and 320 nm has shown λ_{max} to be at 286 nm, and a calibration curve was built as shown in Fig. 8.

The MK drug was found to be loaded with a loading percentage of 8.0 ± 0.2% and the encapsulation efficiency was calculated to be 16.0 ± 0.4%. These values of loading percentage and encapsulation efficiency are considered moderate when compared to other drugs examples encapsulated in PHBV [24–40]. The release study of MK-loaded PHBV has shown extended release as seen in Fig. 9. Although there was a noticeable extended release for around 8 h, there was also a burst effect which was seen by the 60% release of the drug within the first hour. The extremely high percentage of release within the first 50 min can be attributed to the existence of holes and discrepancies in the shapes of the microspheres as can be seen in the SEM image Fig. 7. The release profile



Name	C	O	Na	Cl	Au	Total
Spc_003	52.16	4.92	0.93	1.85	40.14	100.00
Spc_002	55.64	13.36	0.64	0.66	29.70	100.00
Average	53.90	9.14	0.79	1.25	34.92	
Standard Deviation	1.74	4.22	0.15	0.60	5.22	

Fig. 6. SEM image showing relative diameters for MK-PHBV loaded microspheres and its related EDX analyses.

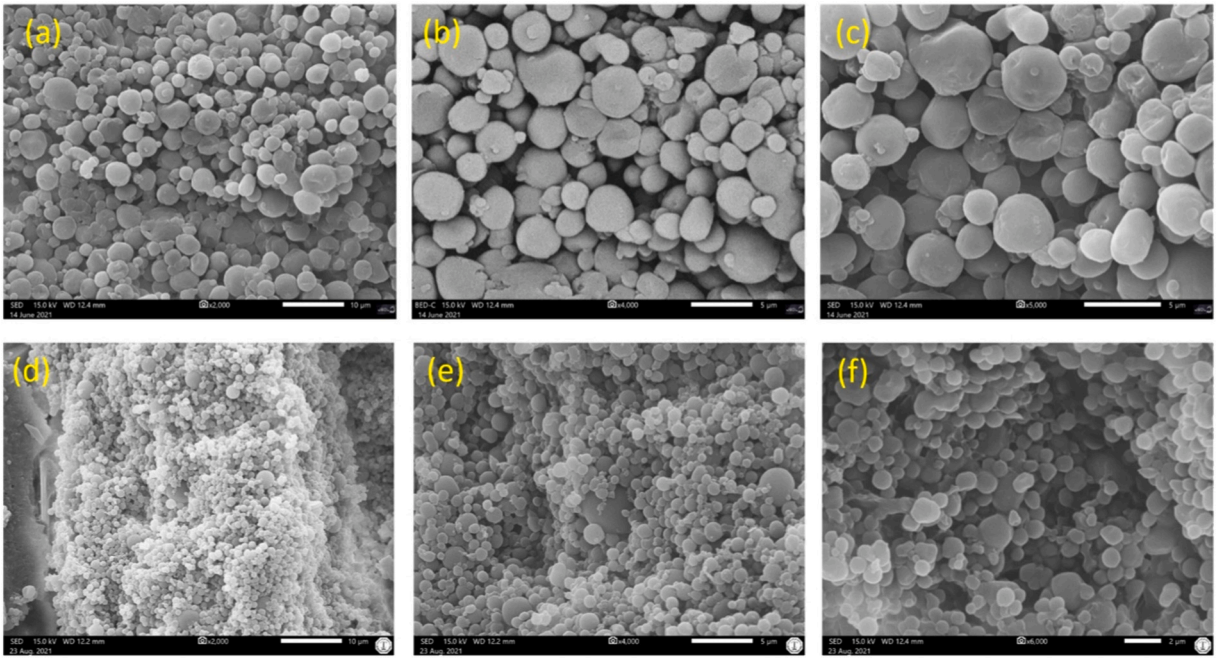


Fig. 7. SEM images for MK-PHBV loaded microspheres (a–c) and busulfan-PHBV loaded microspheres (d–f) at different scales.

suggested the possibility to use the PHBV polyester as an encapsulating biocompatible materials to achieve new dosage form for MK for the purpose of short-term extended release.

For busulfan release study, Fig. 10 shows the release profile as measured by high resolution accurate mass liquid chromatography mass spectrometry system (Orbitrap™ Q Exactive™, Thermo Fisher Scientific, USA). From the release profile curve, it was clearly seen that the complete release of the whole encapsulated drug amount had been achieved after around 60 h. The maximum release was 582 ppm from which we can calculate both the loading percentage and the encapsulation efficiency as 14.6 ± 0.1% and 29.2 ± 0.2%, respectively. The busulfan release profile Figure showed that around 40% of the loaded drug was released within the first 30 min, and that it took the microspheres 2 h to release 53% of the loaded drug and around 30 h to release 70% of the loaded amount. The release of 100% of the loaded drug had been achieved after around 60 h. This release system showed promising results in the sustained release profile and minimized the burst effect.

Considering the release profile for busulfan and the microspheres size and distribution in addition to other characterization outcomes, the newly produced dosage form for busulfan provides many potential advantages when compared both with the reported literature and with the current commercial dosage form Busulfex®. These advantages include the followings: a) this dosage form is composed of a green polymer that is produced from date waste which provides a remarkable advantage when considering bioeconomy, sustainability and carbon footprint. b)

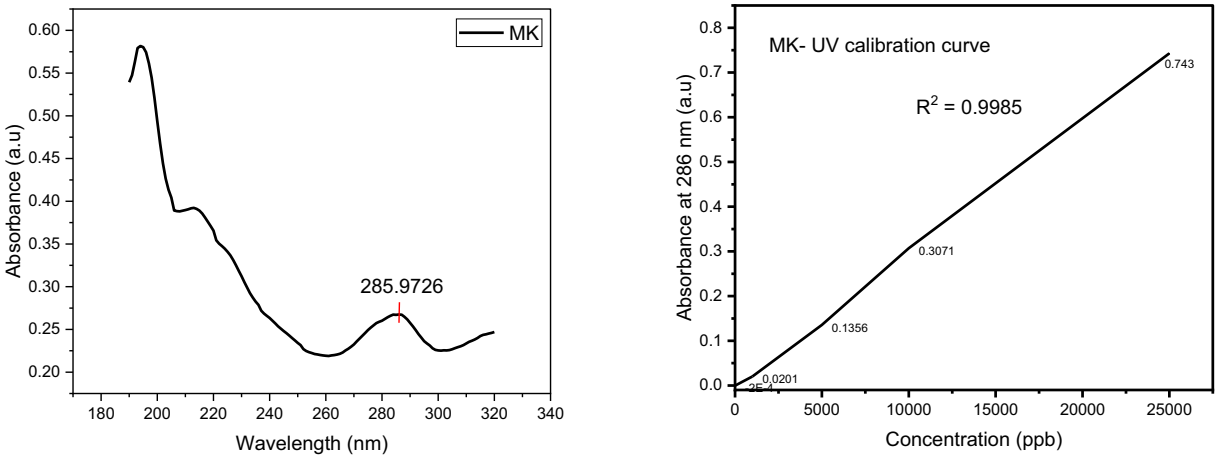


Fig. 8. UV scan spectrum for MK and UV calibration curve (4 points).

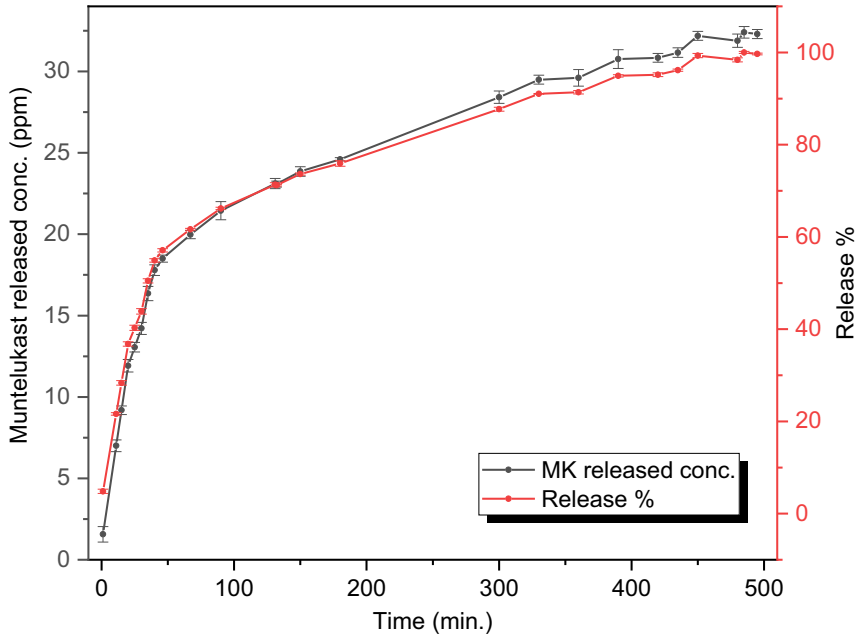


Fig. 9. Release profile for MK from the PHBV loaded microspheres.

Unlike the current commercial drug dosage form Busulfex®, this one doesn't impose the use of any toxic solvents or components. c) Unlike previously reported materials based on MOF [14,15], this dosage form is free from heavy metals, practical to scale up and easy to customize; for example, the release rate can be hugely modified by using different versions of PHBV from different microorganisms and different carbon sources that yield different valerate percentages and hence different crystallinity. Also, microspheres size can be tuned by applying different process parameters during the fabrication. In addition, the polymer can be chemically functionalized and/or blended with other polymers. d) The encapsulation of the drug within the microspheres protects it from degradation and inactivation. e) The drug loading percentage and encapsulation efficiency are considered high when compared to previously reported attempts to encapsulate busulfan in a polymeric material [16,17]. f) The new dosage form is based on an extended slow release which will definitely help minimize the side effects, especially those arising from high systemic exposure to the drug like liver toxicity. It will also decrease the number of doses, and hence time of administration which will help enhance the patient compliance and receptivity to the drug. Also, variations in bioavailability are expected to be lower.

In addition, the long release performance of PHBV microcapsules may suggest the potential of using these materials in medical applications as implants loaded with hormones for contraception or in agricultural applications by loading them with fertilizers and plant nutrients.

3.3. Drug release kinetic study

The study of drug release kinetics gives an understanding of possible release mechanisms via estimation of its rate. The release kinetic data obtained from the batch experiments were analyzed using five common kinetic models. Zero order model, first order model, Higuchi model, Hixson-Crowell model and Korsmeyer-Peppas model [45].

The Zero order model assume the drug dissolution from dosage forms that do not disaggregate and release the drug slowly. The Zero Order rate equation can be expressed as;

$$Q_t = Q_0 + K_0 t$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial

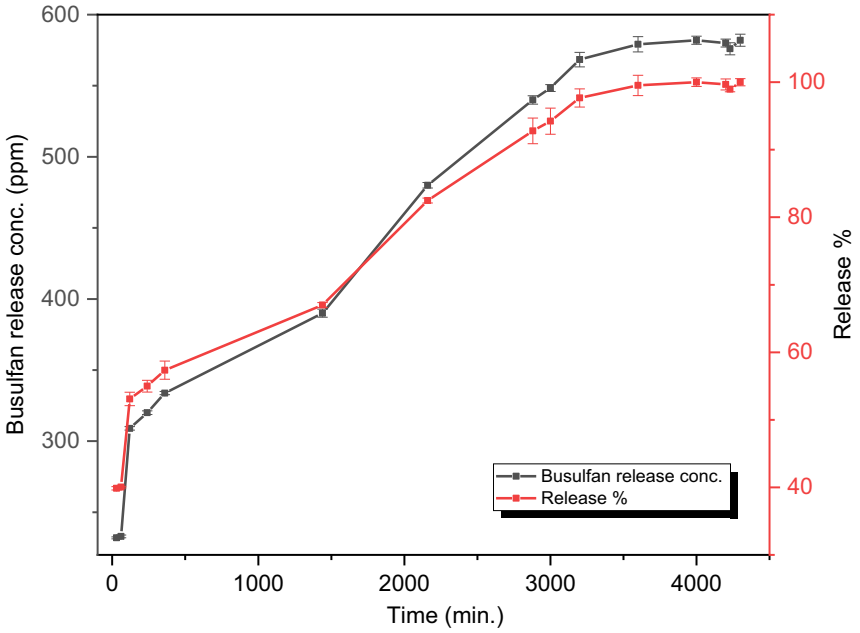


Fig. 10. Release profile for busulfan from the PHBV loaded microspheres.

amount of drug in the solution, and K_0 is the zero-order release constant expressed in units of concentration/time. The drug release studies were plotted as cumulative amount of drug released versus time.

The release of the drug which followed first order kinetics can be expressed by the equation:

$$logC = logC_0 - \frac{Kt}{2.303}$$

where C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of -K/2.303.

Higuchi model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always attained in the release environment.

Higuchi model simplified equation is expressed as;

$$Q = K_H \times t^{1/2}$$

where, K_H is the Higuchi dissolution constant, the data obtained were plotted as cumulative percentage drug release versus square root of time.

Hixson and Crowell recognized that the particles regular area is proportional to the cube root of its volume. They derived the equation;

$$W_0^{\frac{1}{3}} - W_t^{\frac{1}{3}} = kt$$

where W_0 is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and k (kappa) is a constant incorporating the surface volume relation. Data obtained from drug release studies were plotted as cube root of drug percentage remaining in matrix versus time.

Korsmeyer-Peppas derived a simple relationship which described drug release from a polymeric system equation;

$$\frac{M_t}{M_\infty} = K\, t^n$$

where M_t/M_∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. In this model, the value of n characterizes the release mechanism of drug. For the case of cylindrical tablets, $0.45 \leq$ n corresponds to a Fickian diffusion mechanism, $0.45 < n < 0.89$ to non-Fickian transport, $n = 0.89$ to case II (relaxational) transport, and $n > 0.89$ to super case II transport. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

The parameters of the five kinetic models for both drugs are shown in Table 1. From the correlation coefficients, Korsmeyer-Peppas model describes the drug release rates of MK better than other models, and model Higuchi describes the drug release rates of busulfan better than other models.

4. Conclusion

Microcapsules of the naturally biosynthesized PHBV were successfully fabricated by the emulsification-solvent evaporation technique. Both drugs MK and busulfan have been successfully loaded into these microspheres with good loading percentages of 8.0% and 14.6%, respectively. The two drugs served as important examples for hydrophobic and hydrophilic drug models. Both fabricated forms have shown extended drug release which was longer in case of busulfan where it extended up to 3 days. The extended release, the microspheres size, the

Table 1
kinetic models for Busulfan and MK with their coefficient of determination.

Model	Drug	Coefficient of determination	Release rate Constant (K)	Release exponent (n)
Zero order	Montelukast	0.811	0.1389	–
	Busulfan	0.943	0.0134	–
first order	Montelukast	0.9267	0.0078	–
	Busulfan	0.9058	0.0012	–
Huguchi	Montelukast	0.9274	3.766	–
	Busulfan	**0.9769**	1.0002	–
Hixson	Montelukast	0.9357	0.0065	–
	Busulfan	0.9362	0.0008	–
Korsmeyer-Peppas	Montelukast	**0.9696**	9.7×10^{-12}	39.9
	Busulfan	0.782	$4.0 \times 10^{+18}$	20.5

biocompatibility and biodegradability of the used polymer, in addition to the relatively high loading percentage and encapsulation efficiency, all these features qualify the developed busulfan-loaded PHBV microcapsules as a new dosage form that overcome the issues in the current busulfan administration protocol, reduce side effects, minimize cytotoxicity, reduce differences in bioavailability, improve patient compliance and receptivity to the drug. This research can be extended to develop a multilayer and functionalized PHBV encapsulation for even more customized and controlled release of the drugs.

CRediT authorship contribution statement

Mohammad I. Ibrahim: Investigation, Writing – original draft, Methodology. **Diya Alsafadi:** Writing – original draft. **Eyad Safi:** Formal analysis. **Eid Alenazi:** Software, Formal analysis. **Mohamed Aboulsoud:** Software, Methodology. **Mahmoud A. Hussein:** Writing – review & editing, Supervision. **Khalid A. Alamry:** Investigation, Writing – review & editing, Supervision.

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