Original Article

Formulation of Silk Fibroin-based Single Polymeric Floating Microspheres for Sustained Release of Lafutidine

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ABSTRACT

Purpose: The present study was aimed at the formulation of lafutidine-loaded silk fibroin-based floating microspheres (LAFU-SF-Microspheres) for the site-specific sustained release of the drug. **Materials and Methods:** Briefly, the single polymeric system comprising SF was selected to prepare LAFU-SF-Microspheres by employing the emulsion solvent evaporation method. Subsequently, the obtained LAFU-SF-Microspheres were assessed for particle size, zeta potential, percent entrapment efficiency (%EE), percentage drug content (%DC), micromeritics, floating profile, *in-vitro* drug release, spectroscopical analysis, and accelerated stability study. **Results:** The particle size and zeta potential of the LAFU-SF-Microsphere were found to be 2.3-6.8μm and -21.93mV respectively whilst % EE and % DC of LAFU-SF-Microspheres were found to be 86.83 ± 3.46% and 93.89 ± 3.98% respectively. Moreover, it demonstrated the adequate angle of repose (26.50 ± 1.06°) and Carr's Compressibility Index (CI) confirming the excellent flow properties. In view of the floating profile, LAFU-SF-Microspheres showed floating lag time (FLT) between 9-13sec and total floating time (TFT) more than 12hr. Moreover, the % buoyancy was found to be 97.62 ± 4.78%. LAFU-SF-Microspheres showed *in-vitro* % drug release up to 92.41 ± 4.29% adopting the first-order model. The FTIR indicated successful incorporation of LAFU in LAFU-SF-Microspheres. The DSC and PXRD indicated the disrupted crystallinity of LAFU in LAFU-SF-Microspheres. The SEM images of microspheres displayed spherical shapes with smooth textures. **Conclusion:** SF microspheres can be fruitfully applied for customized floating and release patterns of drugs with distinct solubility classes.

Key words: Lafutidine, Silk fibroin, Microsphere, Floating drug delivery, Sustained release.

Submission Date: 23-05-2021;
Revision Date: 09-01-2022;
Accepted Date: 28-01-2022.

DOI: 10.5530/ijper.56.2.59

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INTRODUCTION

Despite tremendous advancements in drug delivery approaches, the oral drug delivery system still contributes the major share owing to its versatility, convenience, patient compliance, and cost-effectiveness. Considering recent developments, modified oral dosage forms can prominently offer targeted drug delivery.[1] In this context, the gastroretentive drug delivery system has gained much attention from researchers specifically for actives that act locally and exhibit absorption windows in the upper part of the gastrointestinal tract (GIT).[2,3] Interestingly, various approaches have been developed to achieve gastro retention which includes swelling and expanding system, floating system, bio(muco) adhesive system, etc. by incorporation of excipient that can modify density, shape, size, and adhesion ability of dosage form.[4] Amongst them, the floating drug delivery system has been widely explored by researchers due to the simplicity and feasibility of formulation design.[5,6]

Lafutidine (LAFU); a recent H2- receptor antagonist (second generation) is widely advised for the treatment of gastric ulcers.[7] LAFU demonstrates high receptor binding affinity (2-80 folds) compared to the

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others.[7] Interestingly, it inhibits gastric acid secretion in daytime specifically postprandial and nighttime as well.[8] Reportedly it can be also employed in the treatment of gastroesophageal reflux disease (GERD), acute and chronic gastritis, etc.[7,9] Despite this, its therapeutic application is limited due to low aqueous solubility and short elimination half-life (1.5-2hr) and thus low bioavailability is a key concern.[10] Principally, it suffers from degradation in alkaline pH whereas it shows excellent stability in acidic pH.[2] To overcome these limitations, we thought to adopt a floating drug delivery approach for this active.

The use of polymer composites for the design of floating dosage forms has suffered critically from non-reproducible response which can be endorsed to the involvement of discrete material composite-related parameters.[10] Interestingly, the microspheres are gaining enormous interest in the designing of the oral drug delivery systems as suitable carriers.[11] Out of this, floating microspheres have been widely reported for drug delivery applications.[12] In this context, the use of low-density materials composites can offer excellent floating profiles.[13]

Thus, we selected Silk Fibroin (SF) as a polymer. SF is a low-density natural polymer obtained from a variety of insects and spiders.[14] Literature survey suggested that the use of SF in dosage form design offers customized and predefined drug release. Moreover, SF is biocompatible and biodegradable.[10] Thus, we can prominently use the SF as an alternative substituent for designing dosage forms. Interestingly, it exhibits low density as compared to the gastric fluids that affirm the suitability of SF in the design of floating dosage forms.[10,13] As far as we are aware, no reports are available on a single SF-based floating microsphere for targeted delivery of LAFU.

This study was attempted to formulate the single polymer (SF)-based floating LAFU-Microspheres for site-specific drug release. Accordingly, it was emphasized to enhance the gastric retention time of LAFU that will subsequently help to improve solubility, dissolution, and thus the bioavailability. The freeze-dried SF was utilized as low-density material that provided the floating ability to the microspheres.

MATERIALS AND METHODS

Materials

LAFU was purchased from Swapnroop Drug and Pharmaceuticals, Aurangabad, Maharashtra, India. Silk cocoons were collected from Government Silk Processing Center, Islampur, Maharashtra, India. Dialysis membrane-110 was procured from Himedia Lab. Pvt. Ltd. Mumbai, Maharashtra, India. Lithium bromide (LiBr) was purchased from Molychem, Mumbai, Maharashtra, India. Analytical grade reagents and solvents were used for experimentation.

Methods

Isolation and Characterization SF

Completely dried cocoons were treated with steaming (80°C) 0.5% sodium carbonate solution (aq) for 25min accompanied with physical stirring. Later, the resultant mass was washed with double distilled water (DDW) repeatedly for complete removal of sericin. Subsequently, 10g of degummed silk was dispersed in 9.3M LiBr solution (70°C, 2.5 hr). Then, this SF solution was dialyzed using a dialysis cassette using distilled water (DW) for consecutive 3 days, along with simultaneous replacement of the DDW after every 6h for removal of LiBr. The concentrated SF solution was stored at 25°C and further processed for freeze-drying by a laboratory freeze dryer (Freezone12USA). Briefly, the solution was exposed to pre-freezing at -33°C (24hr). Then, the primary drying was accomplished at -53°C and 0.016 mBar for 24h followed by the secondary drying at 10°C for 8hr. Subsequently, continuous drying was carried out at 25°C for 4h with the application of a steady amplifying of temperature (1°C/min). Then, the cold trap temperature was kept constant at –53°C till the complete drying was ensured.[13,15]

Preparation and Characterization of LAFU-SF-Microspheres

Formulation of LAFU-SF-Microspheres

LAFU-SF-Microspheres were formulated by using the emulsion solvent evaporation method.[16] Initially, a suitable amount (1g) of the LAFU was dissolved in 50mL chloroform. Then, an optimized amount (5g) of freeze-dried SF was added to the abovementioned drug-chloroform solution i.e. organic phase. 0.2% of polyvinyl alcohol (PVA) was dissolved in DDW. This solution was utilized as an aqueous phase. Subsequently, the LAFU-SF solution was dispersed in the aqueous phase at a 500rpm stirring rate. Further, chloroform from the microsphere mixture was evaporated using a propeller (speed-350rpm, time-1h at Room Temperature). Finally, the mixture was washed with deionized water and filtered using vaccum filtration for triplicate. The collected microspheres were dried at RT for 12h and the percentage yield was calculated by using the following formula.[13]

$$\text{Percentage yield} = \frac{\text{Actual weight of the product}}{\text{Total weightk of drug and polymer}} \quad (1)$$

Particle Size and Zeta Potential analysis

Both evaluation parameters of LAFU-SF-Microspheres were analyzed using Nanoplus 3, micrometrics, USA. Briefly, analysis was completed below 25°C wherein DW was utilized as dilution medium for particle size distribution and polydispersity index (PDI) measurement (RI: 1.33, Scattering Intensity: 26024 cps).

Percent Entrapment Efficiency (% EE) and Percentage Drug Content (%DC)

To calculate %EE, initially, the LAFU-SF-Microspheres (n=3) weighing 50mg were dispersed in 50mL of hydrochloric acid buffer (pH 1.2) with a subsequent stirring at 50rpm for 5h at Rt. Then, the obtained mixture was subjected to sonication (Time-15min, Temperature-30°C). Then, the solution was exposed to centrifugation (Remi, India) at 6000rpm for 20min. Later supernatant was separated using Whatman filter paper (0.22µm). Finally, the obtained clear filtrate was diluted and analyzed using a UV-visible spectrophotometer (Shimadzu UV Spectrophotometer) at 283nm in triplicates. The %EE of all samples was calculated using the formula.[13,17]

$$\text{Percent Entrapment Efficiency} = \frac{\text{Total quantity of drug} - \text{Free untrapped drug}}{\text{Total quantity of drug}} \times 100 \quad (2)$$

To determine the percentage drug content, LAFU-SF-Microspheres equivalent to 10mg LAFU were added to 100mL hydrochloric acid buffers (pH 1.2) and stirred constantly at 50rpm for 24h at RT (n=3). Then, the obtained solutions were sonicated, filtered, diluted suitably using the same buffer, and analyzed at 283nm by using a UV spectrophotometer (Shimadzu UV Spectrophotometer) in triplicates and percentage drug content was calculated.[13,17]

Micromeritic Properties

Angle of repose

The angle of repose was determined using the following formula (n=3).[13]

$$\text{Tan}\,\theta = \frac{h}{r} \quad (3)$$

Wherein, 'θ' is the angle of repose. The 'h' represents the height whilst 'r' represents the radius of the pile.

Carr's compressibility Index (CI)

Percent CI of prepared LAFU-SF-Microspheres was calculated by using the previously reported method.[13] Initially, the bulk density and the tapped density of the LAFU-SF-Microspheres were measured. Then, the CI was measured using the following formula.[18]

$$\text{Carr's compressibility index (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \quad (4)$$

Evaluation of Floating Parameters

In brief, 10mg equivalent LAFU-SF-Microspheres were weighed accurately and then added into 150mL of pH 1.2 simulated gastric fluids. The time interval between introduction and floating of LAFU-SF-Microspheres on the surface of simulated gastric fluid was recorded in triplicate as floating lag time (FLT).[19] The total floating time (TFT) of LAFU-SF-Microspheres was measured using USP dissolution apparatus type II (paddle) containing 900mL of simulated gastric fluid (pH1.2) at 37± 0.5°C. Herein, paddle rotation speed was maintained at 50rpm and the total time for which LAFU-SF-Microspheres floated on the surface of simulated gastric fluid was noted.[20] For measurement of % buoyancy of LAFU-SF-Microspheres, 50mg of LAFU-SF-Microspheres were added into dissolution apparatus (USP Type II) containing 900mL of freshly prepared simulated gastric fluids (pH 1.2) and stirred at 100rpm for 12hr. Later, both the floating and settled portions were separated, dried, and weighed. At last, the percent buoyancy of LAFU-SF-Microspheres was calculated using the following formula.[17]

$$\text{Percent buoyancy} = \frac{\text{The total amount of Microsphere float (mg)}}{\text{The total amount of microsphere added (mg)}} \times 100 \quad (5)$$

In-vitro Drug Release Study

In this study, *the in-vitro* release profile of LAFU from LAFU-SF-Microspheres was evaluated using USP apparatus Type-I (basket apparatus) at the rotation speed of 100rpm. In brief, the LAFU-SF-Microspheres equivalent to 10mg of LAFU were placed separately in the baskets immersed into a vessel containing 900mL of hydrochloric acid buffer (pH 1.2) wherein temperature was kept constant at 37±0.5°C. Then, 5mL of the sample was withdrawn from each vessel at predefined time intervals. Simultaneously, the same volume of hydrochloric acid buffer (pH 1.2) was added to baskets to sustain the sink condition. The drug release was calculated using a UV-visible spectrophotometer (Shimadzu UV Spectrophotometer) at 283nm. Finally, the dissolution profile was analyzed using PCP-Disso-V3.08 software.[17]

Spectroscoptical Characterizations

Initially, SF, LAFU, and LAFU-SF-Microspheres were analyzed by an Infrared spectrophotometer (Jasco-V-530 model). Spectra were recorded with a resolution of 4cm^{-1} and an absorption range between 4000-400cm^{-1}. Thermal behavior of was analyzed by DSC (Shimadzu, SDT 2960, USA) over a temperature range of 30–370°C (heating rate of 10°C/min). Throughout the process, an inert environment was sustained by purging nitrogen gas at a flow rate of 50mL/min. PXRD patterns were recorded at R$_t$ by X-ray diffractometer (Philips analytical XRD, PW 3710) with CuKα radiation (1.54Å), at 40 kV voltage, 40mA current, 0.01° step size, and 1sec step time over an angular range of 5 to 40°. The LAFU-SF-Microspheres were characterized for morphology assessment using SEM (LEO 435 VP, Eindhoven, and The Netherlands). The stubs were coated with gold = (thickness-~300 Å) by a gold sputter coater in a high-vacuum evaporator under an argon atmosphere (condition- 0.1 torrs, 90sec, 15kV).

Accelerated Stability Study of LAFU-SF Microspheres

Accelerated stability study of the LAFU-SF-Microspheres has performed wherein the temperature and percent relative humidity (% RH) conditions were maintained constant at 40°C ± 2°C and 75% ± 5% respectively. After predefined intervals of 1, 2, and 3 months, the LAFU-SF-Microspheres were withdrawn and assessed for percent drug content, floating profile, and % DR with the same method as aforementioned.

RESULTS AND DISCUSSION

Preparation and Characterization of LAFU-SF-Microspheres

Spherical LAFU-SF-Microspheres were successfully prepared using SF wherein the percentage yield was found 86.23±4.13% w/w. Due to adequate polymeric concentration, it provides a better practical yield.

Particle Size and Zeta Potential analysis

Figure 1A represents the particle size analysis of LAFU-SF-Microspheres. The particle size was observed to be in the range of 2.3µm-6.8µm, which is optimum, and within the acceptable limit. The good viscosity of the SF solution offers a good range of particle sizes. The PDI was found to be 0.75 ensuring homogeneity of particle size distribution into the dispersion. As depicted in Figure 1B, the zeta potential of the LAFU-SF-Microsphere was found to be -21.93mV that may

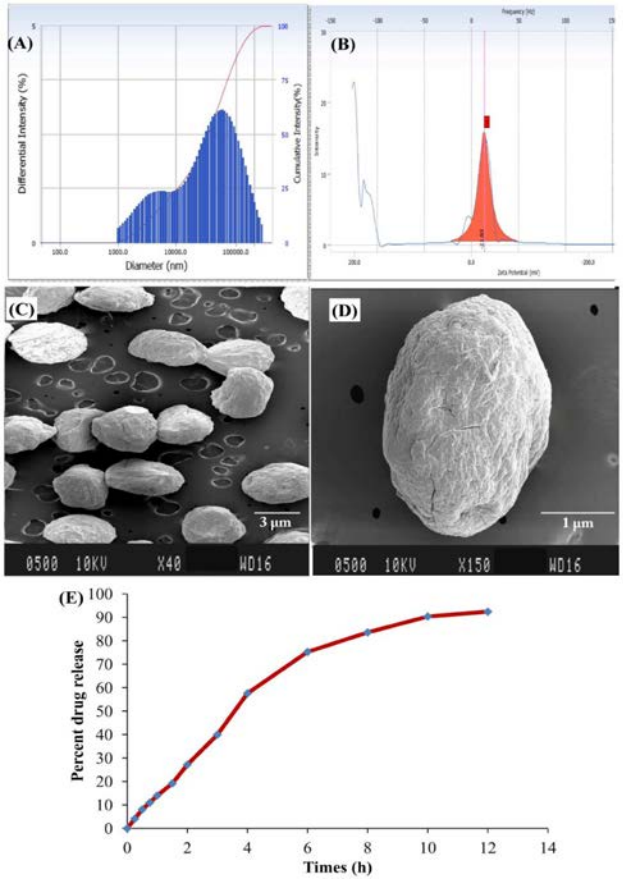

Figure 1: (A) particle size analysis and (B) Zeta potential of LAFU-SF-Microspheres. (C and D) SEM images of LAFU-SF-Microspheres, (E) Dissolution profile of LAFU in 0.1 N hydrochloric acid buffers from LAFU-SF-Microspheres

be because of the oxygen-based functionality in SF. It assured the stability of microspheres in dispersion.

%EE and % DC

Principally, %EE conveys the amount of active entrapped into microspheres compared to its total amount used in the formulation. In this case, % EE was found to be 86.83±3.46% EE (Y=0.018X-0.001; R^2=0.999) whilst the %DC was found to be 93.89 ± 3.98%. Both the values affirmed optimum % EE and %DC. Herein, the optimum polymeric concentration furnishes an adequate amount of %EE and %DC.

Micromeritic Properties

In this study, the bulk density and tapped density of LAFU-SF-Microspheres were found to be 0.60g/mL and 0.71g/mL, respectively. Interestingly, the density of prepared drug-loaded microspheres was less as compared to the stomach dissolution fluid. The low density of SF helps to float the dosage form for a longer duration. The angle of repose of LAFU-SF-Microspheres was found to be 26.50 ± 1.06°, which expressed the excellent flow

property. It concludes the formation of non-aggregated drug-loaded single polymeric microspheres. The % compressibility index of 14.25± 0.42% was indicative of LAFU-SF-Microspheres exhibiting good flow-ability and compressibility also. Possibly, the single polymer (SF) phenomenon helps to develop spherical-shaped microspheres.

Evaluation of Floating Parameters

FLT, TFT, and % Buoyancy

It was observed that during experimental studies LAFU-SF-Microspheres floated within 9-13sec. Moreover, the TFT of LAFU-SF-Microspheres was found to be more than 12hr. Moreover, % buoyancy of LAFU-SF-Microspheres was found to be 97.62 ± 4.78%. Probably, because of the low bulk density and porous nature of LAFU-SF-Microspheres, it showed excellent *in-vitro* floatability. Herein, utilization of low-density SF furnishes the self floating potential to dosage from. This confirmed the ability of LAFU-SF-Microspheres as an excellent carrier for the design of floating dosage forms.

SEM

As shown in Figures 1 C and D, the SEM images of LAFU-SF-Microspheres exhibited nearly spherical and almost smooth surface morphology. Herein, a few microspheres demonstrated the rough surface that may be because of drug loading on the surface. It was also responsible for initial burst release from microspheres.

In-vitro Drug Release Study

As we know, SF contains a significant quantity of β-sheet, which imparts the crystalline nature to the SF protein. It is worthy to mention that this crystalline nature of SF can act like a mechanical barrier that hinders drug release from the nanocarriers. In the case of SF microspheres, the LAFU is entrapped into the microsphere that diffused out systematically from crystalline sheets of SF microsphere. It helps to sustain the release of LAFU from microspheres. The LAFU release from LAFU-SF-Microspheres was studied in 0.1 N hydrochloric acid buffers (pH1.2) for 12hr. During *the in-vitro* release study, the LAFU-SF-Microspheres were started to float within 10sec and the entire quantity of microspheres floated within 24sec on the surface of dissolution media. In the case of LAFU-SF-Microspheres, LAFU release was found to be 92.41 ± 4.29% at the end of 12h (Figure 1E). As we know, the SF is a type of protein composed of several amino acids that can be degraded by stomach fluid containing enzymes, and accordingly, it enhances the solubility of SF. Additionally, SF can easily dissolve at high acidic pH. Therefore, the highly acidic pH of stomach fluids also helps to increase the solubility of SF carriers into dissolution media. In conclusion, LAFU release from LAFU-SF-Microspheres can be influenced by the pH and enzymes presents in dissolution media. Table 1 shows the interpretation of the diffusion mechanism. In brief, the release of LAFU was found to follow the first-order model in pH 1.2 hydrochloric acid buffers. In addition, it demonstrates an anomalous transport drug transport mechanism (n=0.84). In conclusion, dissolution studies of the microsphere accomplished better drug dissolution and sustained drug release along with good matrix integrity during the dissolution study.

ATR-FTIR

Figure 2A represents the FTIR spectra of SF. The characteristic vibration band around 1637cm^{-1} was assigned to the peptide backbone of amide I containing C =O stretching vibration, whereas 1509cm^{-1} to amide II containing N-H bending vibration, 1232cm^{-1} to amide III containing C-N stretching vibration, and 729cm^{-1} to amide IV. All these characteristic bands designated the presence of a hydrogen-bonded NH group. Figure 2B depicts the FTIR spectrum of LAFU. In brief, the characteristic peaks at 3324cm^{-1}, 3324cm^{-1}, 1635cm^{-1}, 1286cm^{-1}, 1039cm^{-1}, and 727cm^{-1} indicated the presence of aliphatic N-H stretching vibration, aromatic C-H stretching vibration, -C=N stretching vibration, C-N stretching vibration, S=O stretching vibration, C-S stretching vibration, respectively representing the presence of functional groups present in the LAFU structure thus assuring its purity. Figure 2C demonstrates the FTIR spectra of LAFU-SF-Microspheres. It showed the presence of aliphatic N–H stretching vibration at 3323cm^{-1}, aromatic C–H stretching vibration at 2919cm^{-1}, C=N stretching vibration at 1635cm^{-1}, C–N stretching vibration at 1280cm^{-1}, S=O stretching vibration at 1038.95cm^{-1}, and -C-S stretching vibration at 727cm^{-1}. Overall, it was confirmed that LAFU was successfully included in the SF-microsphere as its characteristic FTIR peaks could be well differentiated from pure dried SF.

DSC

Figure 3A shows the thermogram of freeze-dried SF powder. Briefly, the thermogram of SF showed the presence of two broad endothermic peaks out of which the initial endothermic peak was observed at 82°C and later at 291.64°C. Principally, wide low- temperature endothermic peak present at 82°C corresponded to the presence of moisture content in SF. Furthermore, the high-temperature endothermic peak at 291.64°C

Table 1: Kinetic data of LAFU-SF-Microspheres.

Dissolution medium	Zero-order (R)	First-order (R)	Matrix model (R)	Peppas model (R)	Hixson Crowell (R)	Release exponent (n)	Drug transport mechanism	Best fit model	% drug release
0.1N hydrochloric acid buffer	0.9467	0.9956	0.9634	0.9936	0.9917	0.84	Anomalous transport	Zero-order	92.41 %

'±'=SD, *n*=3

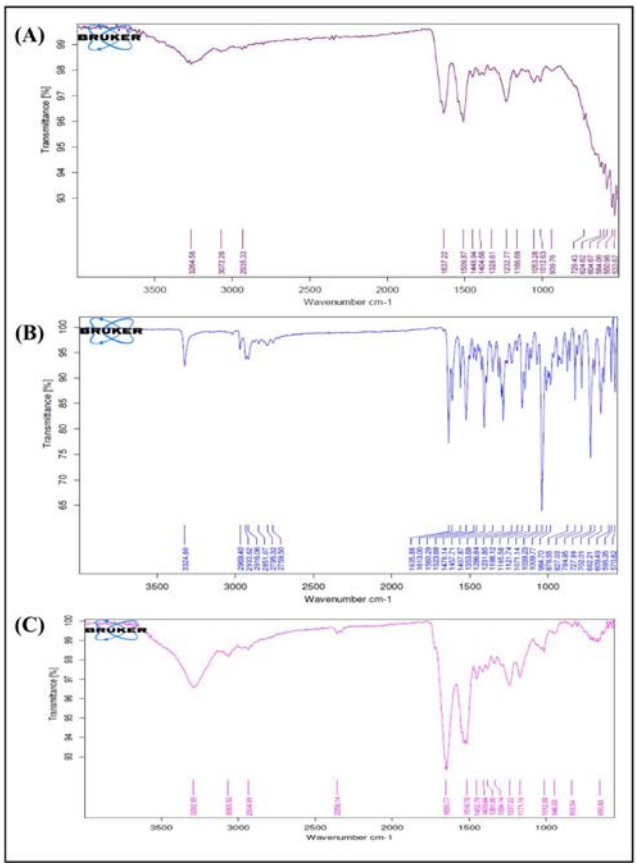

Figure 2: FTIR spectra of (A) SF; (B) LAFU and (C) LAFU-SF-Microspheres.

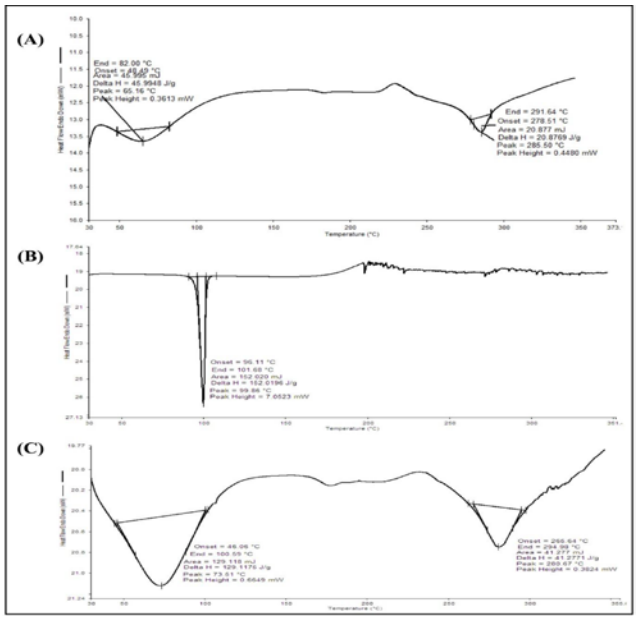

Figure 3: Thermogram of (A) SF; (B) LAFU and (C) LAFU-SF-Microspheres.

might be attributed to the random coil into the β-sheet conversion and thermal-based decomposition. The amorphous nature of SF was confirmed by analyzing the broad nature of endothermic peaks.

Figure 3B depicts the thermogram of LAFU. Principally, it exhibited the single sharp high-temperature endothermic peak at 101.68°C. Hence, it is obvious that LAFU is present in the crystalline form. Figure 3C demonstrates the thermal profile of LAFU-SF-Microspheres. The DSC thermal profile of LAFU-SF-Microspheres showed an overlapping peak for LAFU at 100.59°C indicating successful entrapment of LAFU in the microspheres. Probably, the absence of individual peaks of LAFU in the microsphere maybe because of the molecular dispersion form of LAFU in LAFU-SF-Microspheres. In this context, the ΔH value for pure LAFU (ΔH =152.14J/g) was found to be more than the LAFU-SF-Microspheres (ΔH=41.27J/g) that indicated LAFU has been entrapped inside the microspheres in form of molecular dispersion.

PXRD

Figure 4A depicts the diffractogram of SF wherein the maximum low-intensity peaks was observed. Principally, diffraction peaks were observed at 2θ values of 13.42°, 20.54°, 24.13°, 31.26°, and 35.7°. The lack of high-intensity peaks indicated the amorphous nature of SF. Figure 4B represents the diffractogram of LAFU. It demonstrates high-intensity peaks at 11.08°, 20.63°, 23.15°, and 25.30°, etc. which indicated crystalline nature. Figure 4C shows the diffractogram of LAFU-SF-Microspheres. It displayed a few characteristic diffraction peaks of LAFU with relatively low intensity. Probably, the interaction of SF functional groups to LF resulted in the conversion of crystalline LF to an amorphous

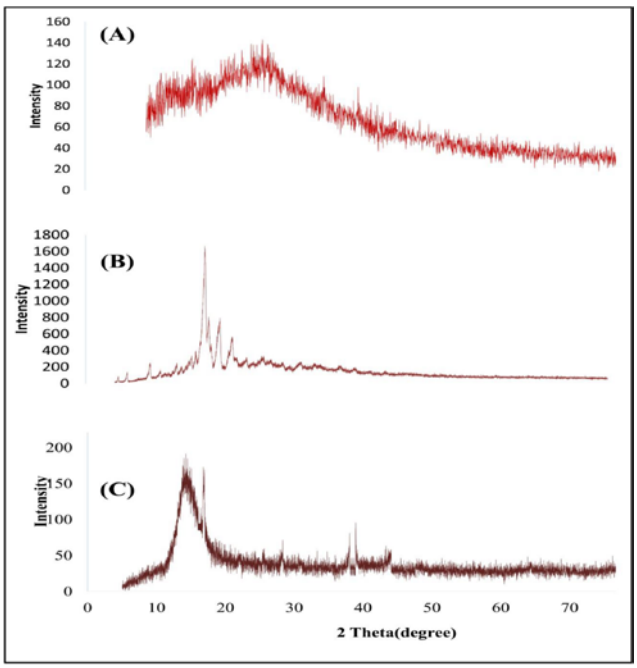

Figure 4: Diffractogram of (A) SF; (B) LAFU and (C) LAFU-SF-Microspheres.

state. Thus, a PXRD spectrum revealed crystallinity disruption of LAFU in LAFU-SF-Microspheres.

Accelerated Stability Study of LAFU-SF-Microspheres

Table 2 shows the accelerated stability study profile of LAFU-SF-Microspheres. Briefly, the stability study ensured that there were no significant changes observed in the floating profile of LAFU-SF-Microspheres. In addition, the %DC and *in-vitro* drug release were found to be optimum. Hence, the microspheres were found to be exceptionally stable for 3 months at fixed stability stations i.e. 40°C± 2°C of temperature and 75 ±5% of RH. Herein, the obtained results were close to the initial results. Notably, the minuscule alteration in the microspheres results after three months might be due to its porous arrangement. In conclusion, drug-loaded SF Microspheres are stable at a constant temperature and relative humidity.

CONCLUSION

The current work has succeeded in preparing single polymeric floating LAFU microspheres using SF by the emulsion solvent evaporation method. The formulation of a sustained-release floating drug delivery system can improve the oral bioavailability of drugs like LAFU. Here, a promising floating profile of LAFU-SF-Microspheres was observed i.e. FLT between 9-13s, TFT more than 12h, and % buoyancy up to

Table 2: LAFU-SF-Microspheres accelerated stability profile.

Evaluation parameters	Stability results			
	0 Months	1 Months	2 Months	3 Months
% DC	93.89 ± 3.98	93.65 ± 3.17	92.87 ± 3.12	92.43 ± 3.01
FLT (sec)	9-13	10-15	10-15	11-17
Floating buoyancy (%)	97.62 ± 4.78	97.12± 4.51	96.92 ± 4.39	96.56 ± 4.07
TFT (h)	12 ± 1.02	12 ± 0.93	12 ± 0.91	12 ± 0.92
In-vitro drug release (%)	92.41 ± 4.29	91.47 ± 4.12	90.20 ± 3.71	90.09 ± 3.25

'±'=SD, *n*=3

97.62 ± 4.78%. The *in vitro* drug dissolution kinetics of LAFU-SF-Microspheres showed 92.41±4.29% drug release within 12h following the first-order model. PXRD, DSC, FTIR revealed crystallinity disruption of LAFU in formulated microspheres. The SEM images of LAFU-SF-Microspheres indicated nearly spherical and with smooth surface morphology. Moreover, an accelerated stability study confirmed the stability of LAFU-SF-Microspheres. Concisely, prepared single polymeric microspheres demonstrated outstanding floating ability and sustained drug release. Therefore, biocompatible, biodegradable, and cost-effective SF-based floating microspheres can be fruitfully included for customized floating and release patterns of drugs with different BCS classes.

ACKNOWLEDGEMENT

The authors acknowledge H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur for providing research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LAFU-SF-Microspheres: Lafutidine-loaded silk fibroin-based floating microspheres; **% EE:** Percent entrapment efficiency; **% DC:** Percentage drug content; **SEM:** scanning electron microscopy; **FTIR:** Fourier-transform infrared spectroscopy; **DSC:** Differential scanning calorimetry; **PXRD:** Powder X-ray diffraction; **FLT:** Floating lag time; **TFT:** Total floating time; **GIT:** Gastrointestinal tract; **LAFU:** Lafutidine; **GERD:** Gastroesophageal reflux disease; **DDW:** Double distilled water; **DW**: Distilled water; **RT:** Room Temperature;

CI: Carr's compressibility index; **SF:** Silk fibroin; **ICH:** International Conference on Harmonization.

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SUMMARY

In the current work, floating microspheres of LAFU were formulated by employing a low density, biocompatible and biodegradable polymer i.e. SF using the emulsion solvent evaporation method for site-specific drug release. The formulated microspheres were analyzed for particle size, zeta potential, % EE, % DC, micromeritics, floating profile, *in-vitro* drug release, spectroscopical analysis, and accelerated stability study. The particle size, zeta potential, %EE and %DC of the LAFU-SF-Microsphere were found to be 2.3-6.8 µm, -21.93 mV 86.83±3.46% and 93.89±3.98% respectively. Moreover, it demonstrated the adequate angle of repose (26.50±1.06°) and CI (14.25±0.42%) confirming the excellent flow properties. In addition, LAFU-SF-Microspheres showed FLT between 9-13 sec and TFT more than 12hr. The % buoyancy was found to be 97.62± 4.78 %. LAFU-SF-Microspheres showed *in-vitro* % drug release up to 92.41±4.29 % adopting the first-order model. The FTIR indicated successful incorporation of LAFU in LAFU-SF-Microspheres. The DSC and PXRD indicated the disrupted crystallinity of LAFU in LAFU-SF-Microspheres. The SEM images of microspheres displayed spherical shapes with smooth textures. Finally, an accelerated stability study confirmed the stability of LAFU-SF-Microspheres. Thus, biocompatible, biodegradable, and cost-effective SF-based floating microspheres can be fruitfully included for customized floating and release patterns of drugs with different BCS classes.

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Cite this article: Pantwalawalkar J, Nangare S. Formulation of Silk Fibroin-based Single Polymeric Floating Microspheres for Sustained Release of Lafutidine. Indian J of Pharmaceutical Education and Research. 2022;56(2):396-404.