Electrospinning Fibres Mucoadhesive Buccal Tablet using Atorvastatin Calcium Trihydrate as modeling drug: Preparation and Characterization

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Abstract:

Atorvastatin (ATV) is a synthetic lipid-lowering agent. According to the Biopharmaceutical Classification System (BCS), ATV is classified as a Class II compound characterized by low solubility and high permeability. Its oral bioavailability is relatively low, at 14%, due primarily to pre-systemic clearance within the gastrointestinal mucosa as well as extensive first-pass metabolism in the liver. So, this research aimed to develop mucoadhesive tablets containing atorvastatin as fibres via the electrospinning technique for buccal application, aiming to reduce gastric irritation, first-pass metabolism, and enhance bioavailability. Then, three fiber formulations with constant amounts of atorvastatin (ATV) and polyvinyl pyrrolidine, which represent one part and four parts, respectively, and different ratios of soluplus (1:4:1, 1:4:2, 1:4:3) were prepared. They underwent different characterization tests such as Fourier transform infrared spectroscopy, differential scanning calorimetry, optical microscopic imaging, and scanning electron microscopy. The F1 fiber with a ratio of 1:4:1 formulation was further investigated and formulated into mucoadhesive buccal tablets. These tablets underwent various tests, showing satisfactory results. Fourier transform infrared spectroscopy and differential scanning calorimetry studies were harmonized by revealing the change that affirmed the hydrogen bonds more likely to be between ATV and polymers, which assured the amorphous form of fibers by differential scanning calorimetry. F1 fibers achieved 99.6% release of ATV, while F2 and F3 reached 49% and 38.97%, respectively. Also, fibers were examined under an optical microscope, and a scanning electron microscope showed successful fiber formulation and smooth surfaced fibers without any beads or drug crystals on the surface, respectively. Using a controlled flow pump, the buccal tablets (B1 and B2) containing fibers equivalent to 20 mg of ATV showed a slow release of 2.78 mg and 4.39 mg, respectively. In contrast, dissolution by a conventional method, B2 tablet, reached 11.28 mg within 4 hours. In conclusion, drug-loaded ATV fibers with the more stable amorphous form were successfully formulated and characterized, as demonstrated by FTIR and DSC. SEM images exposed fibers with a smooth surface without solid particles, and the successful formation of unidirectional buccal tablets.

Keywords: Atorvastatin, Polyvinyl pyrrolidine, Fibers, Electrospinning, Buccal tablet.

تحضير الألياف المجهزة بتقنية الغزل الكهربائي لاستخدامها في أقراص لاصقة للفم باستخدام عقار أتورفاستاتين كالسيوم ترايهيدرات كنموذج: التحضير والتوصيف

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خلاصة

الاتورفستاتين هو دواء مصنع لمعالجة ارتفاع نسبة الدهون في الدم وفقا لنظام التصنيف الحيوي الصيدلاني، ينتمي الاتورفستاتين الى الفئة الثانية المتمثلة بانخفاض الذوبانية وارتفاع النفاذية، ولديه ايضا انخفاض بالامتصاص الفموي بنسبة تقدر ب 14%, ذلك بسبب التخلص القبل نظامي في النسيج المعوي والايض الاولي الواسع في الكبد. هذا البحث يهدف إلى تطوير أقراص لاصقة مخاطية تحتوي على الأتورفاستاتين كألياف عبر تقنية الغزل الكهربائي للتطبيق في الفم، بهدف تقليل التهيج المعدي، والأيض في المرور الأول، وتعزيز الامتصاص الحيوي. تم تحضير ثلاث تركيبات من الألياف بكميات ثابتة من الأتورفاستاتين ، بوليفينيل بيروليدين، ونسب مختلفة من سولوبلس (1:4:1، 1:4:2، 1:4:3) وخضعت لاختبارات توصيف مختلفة مثل الطيف الأشعة تحت الحمراء بالتحويل الفوري، والكالوريمترية التفاضلية للمسح الحراري، وتصوير المجهر البصري. وتمت متابعة تركيبة الالياف F1 بشكل اعمق وصنعت بشكل اقراص مخاطية لاصقة. خضعت هذه الاقراص الى لاختبارات متنوعة، واظهرت نتائج مرضية. تم مزامنة الدراسات الطيفية للأشعة تحت الحمراء بالتحويل الفوري والكالوريمترية من خلال الكشف عن التغيير الذي أكد على أن الروابط الهيدروجينية على الأرجح بين الاتورفستاتين والبوليمرات التي ضمنت الشكل البلوري للألياف من خلال الكالوريمترية التفاضلية للمسح الحراري. حققت الالياف F1 من إطلاق 90% من الدواء، بينما حققت F2 و F3 37.9% و 23.07% على التوالي، كما تم فحص الألياف تحت المجهر البصري، والمجهر الإلكتروني الماسح ولوحظ نجاح تكوين الألياف والألياف ذات سطح املس دون وجود أي حبيبات أو بلورات دوائية عليه على التوالي. الاقراص (B2,B1)التي تحتوي على ألياف ما يعادل 20 ملغ من الاتورفاستاتين باستخدام مضخة تدفق متحكمة أظهرت إطلاقًا بطيئًا يمثل 2.78 مغم و 5.66مغم على التوالي ، بينما وصل إطلاق الذوبان بالطريقة التقليدية لاقراص B2 الى 11.28 مغم خلال 4 ساعات. في الختام، تم تصيغ وتوصيف ألياف الأتورفاستاتين المحملة بالدواء بالشكل البلوري الأمورفي الأكثر استقرارا بنجاح، كما تبين من خلال التحليل بالأشعة تحت الحمراء الفوق البنفسجية والتحليل الحراري التفاضلي. كما أظهرت صور المجهر الالكتروني الماسح أليافًا بسطح أملس بدون جسيمات صلبة ونجاح تشكيل القرص الفموي ذو الاتجاه الواحد.

الكلمات المفتاحية: اتورفاستاتين، بوليفنيل بايرولدين، الالياف، الغزل الالكتروني، الاقراص المخاطية.

Introduction

Swallowing insufficiency is common and affects not only nutrition but also the ingestion of medicines; hence, dysphagia is emerging as a notable challenge in administering medication and providing therapy (1), so an alternative to the oral swallowable solid dosage forms is needed to overcome the difficulty of intaking associated with dysphagia.

However, the oral cavity is the most established drug delivery route; thus, avoiding swallowing, the buccal region or route is an alternative to other traditional oral systemic drug administration. This method offers advantages like direct entry into the bloodstream, bypassing liver metabolism, and gastrointestinal degradation. Moreover, the oral cavity is easily accessible for self-administration and allows for quick termination of drug absorption if needed to prevent toxicity by removing the dosage form from the buccal cavity(2). Indeed, the buccal dosage forms should be designed as mucoadhesive to obtain the full advantages of this route. As historically, there was a growing recognition of mucoadhesion as an innovative strategy to enhance the effectiveness of various drugs, and it was then implemented in different drug delivery systems (3). Since the bio-adhesion is an interfacial phenomenon of two materials, at least one being the biological membrane that adheres and remains connected for an

extended period (4) This adhesion is achieved through non-covalent bonds like hydrogen bonds, Van der Waal's forces, electrostatic double-layer forces, and ionic interactions between the mucus gel layer and polymers (5). This approach aims to release the drug near the target site, enhancing its bioavailability and supporting either a localized or systemic therapeutic effect (6).

This work focused on both atorvastatin calcium trihydrate (ATV), a synthetic lipid-lowering agent categorized as a BCS class-II drug, with low solubility and high permeability, and also has a low oral bioavailability, which is 14%, primarily because of pre-systemic clearance in the gastrointestinal mucosa and extensive first-pass metabolism in the liver. And the electrospinning technique was utilized to create fibres loaded with ATV. Actually, electrospinning is a process by which fibers are produced by applying high voltage to a viscous polymeric solution. This voltage disrupts the surface tension, leading to solvent evaporation and forming a fibrous mat (7). Electrospinning is a simple, rapid, cost-efficient, and scalable technology capable of generating single-layer or multi-layered fibers, achieved by employing mono or multi-axial nozzle systems, respectively (7, 8). Electrospinning fibers possess a high surface-area-to-volume ratio, high porosity, and significant drug-loading capacity (9).

This research aimed to create and formulate mucoadhesive unidirectional buccal tablets containing ATV as fibres that were fabricated by an electrospinning process using polyvinyl pyrrolidine and soluplus polymers assisting in gaining the amorphous state, as this was proved via the electrospun fibres of ketoprofen presented this transition in material status (2) . This method aids in bypassing the initial metabolism, thereby boosting the drug's availability in the body.

The combination of polyvinyl pyrrolidine and soluplus was previously used in the preparation of electrospun fibres of fast-dissolving nifedipine and atorvastatin film (10), and also in the enhancement of poorly water-soluble spironolactone dissolution of the electrospun formulation of an immediate-release solid dosage form (11). These tablets were intended to coat the circumference, and one tablet side was used for buccal mucosa application to enable the unidirectional release. This approach aimed to reduce and bypass the first pass effect to enhance the drug's bioavailability.

2. Materials and methods

Materials

Atorvastatin calcium trihydrate (ATV) was purchased from Energy Chemical, China; Ethanol (99%) was bought from Honeywell, Germany. Ethyl cellulose (EC), Hydroxypropyl methylcellulose K4M(HPMC), Mannitol, and Magnesium stearate (MS) were all purchased from Macklin, China. Also, Lactose was obtained from Alpha Chemica, India, and Microcrystalline cellulose (MCC) was obtained from Changsha Goomo Chemical Technology, China. At the same time, polyvinyl pyrrolidine K90(PVP), soluplus (SP), and Sodium alginate were earned from Glentham Life Science, UK, BASF Pharmaceutical Industries, Germany, and Alpha Chemica, India, respectively.

Methods

2.2.1 preparation of electrospinning solution

Soluplus (SP) was dissolved in 25 ml of ethanol and stirred at room temperature on a magnetic stirrer for at least 15 minutes. After that, PVP was included and stirred for another 60 minutes until fully dissolved. ATV was introduced into the polymeric

solution as a final step while continuously stirring for 60 minutes to achieve a homogeneous electrospinning solution after a total stirring time of 135 minutes (10). The spinning solution was prepared in three formulations with varying ratios of SP, constant ATV, and PVP, as outlined in Table 1.

Table 1: Electrospinning solution formulations

Ingredients	F1	F2	F3
ATV-SP-PVP ratio	1:1:4	1:2:4	1:3:4
Soluplus	500 mg	1 gm	1.5 gm
Ethanol	25 ml	25 ml	25 ml

*** ATV and PVP were added in a constant amount in all formulations, as 500 mg and 2 gm, respectively.**

2.2.2 Determination of electrospinning solution viscosity

Before applying the prepared solutions to the electrospinning process, the viscosity of the prepared electrospinning solutions was measured at room temperature with a viscometer (63s spindle) at different shear rates (2) . The viscosity was measured by taking 15 ml of each spinning solution at different rotation speeds (20, 30, 50, 60, and 100) rpm.

2.2.3 Electrospinning process

The electrospinning process was facilitated using a high-voltage direct current power supply generator with a maximum voltage of 10 kV. The drug-polymer solutions were filled into 10 mL plastic disposable syringes fitted with a tip of 22G that connected to flat needles mounted on a horizontally positioned syringe pump, at a distance between the needle and collector was 12 cm, and the flow rate was 1 mL/min. The fibers were deposited on aluminum foil covering the rotating drum collector at a rotation speed of 200 rpm. The temperature and relative humidity were set at 25 ± 2 °C and 35 ± 1%, respectively;

2.2.4 Characterization of spinning fibres

2.2.4.1 Physical appearance

The fiber's appearance depended on visual observation of accumulated fibers on the aluminum foil on the electrospinning collector.

2.2.4.2 Optical microscope

The fiber morphology was investigated using an optical microscope (Optika Microscope, Italy). A thin portion of the fiber mat was examined under optical microscopy at 20X magnification, and an image was captured using an Optika microscopy digital camera equipped with software version 2.13.

2.2.4.3 Fourier transform infrared spectroscopy

Samples of about 2 mg ATV, PVP, SP, and ATV fibers were mixed with 200 mg of pure potassium bromide powder and compressed into disks. The samples were analyzed using FTIR (Shimadzu 8400S, Japan) from 400 to 4000 cm^{-1} at room temperature(2) .

2.2.4.4 Differential scanning calorimetry

Thermograms of samples were recorded using a differential scanning calorimeter (DSC 131 evo, Setaram, France). Each sample (2-5mg) was placed in an aluminum pan and hermetically sealed. All measurements were performed at 21 °C as the starting temperature and a heating rate of 1.5 °C /minute, and ended at 280 °C above the melting point of ATV and polymers, as the nitrogen was purged at 50 ml/min (12).

2.2.4.5 Determination of drug loading (DL%), entrapment efficiency (EE%), and fiber yield (Y) of the drug-loaded fibers

2.2.4.5.1 Drug loading and entrapment efficiency

The amount of ATV entrapped within the fibers was determined by dissolving the electrospun fibers equivalent to 20 mg of ATV in 10 ml of 99% ethanol (13) Using a magnetic stirrer for 15 minutes until the complete dissolution of the fibers. The drug was analyzed at 247 nm using a UV spectrophotometer after filtration and suitable sample dilution. The unknown concentration was determined using a preconstructed, validated calibration curve equation in ethanol (y=0.0226 x - 0.0059). Drug loading and entrapment efficiency were determined using the following equations (2) .

$$DL\% = \frac{\text{weight of the drug in fibres}}{\text{Weight of fibres}} x100 \ldots\ldots 1$$

$$EE\% = \frac{\text{Weight of drug in fibres}}{\text{Theoretical weight of the drug in fibres}} x100 \ldots\ldots 2$$

2.2.4.5.2 Fibers yield

The theoretical amount of electrospun fibers was calculated based on the amount of solid content in the fibers (polymer and drugs) in the total volume of the spun solution.

The yield (Y) of the fibers was also measured using the following equation (10):

$$Y\% = \frac{\text{The actual amount of fibres}}{\text{The theoretical amount of the fibres}} x100 \ldots\ldots 3$$

2.2.4.6 Disintegration time of fibres

The disintegration of drug-loaded fibers was assessed using a modified method of the Petri dish assay described in Tawfik et al (14, 15). Three mg of each fiber formulation was placed into a Petri dish containing 10 ml pre-warmed phosphate buffer (pH 6.8) at 37°C under gentle stirring using a shaking water bath until complete disintegration of fibers.

2.2.4.7 *In vitro* release of fibres

The *in vitro* ATV-loaded fibers release studies were carried out in 900 ml of phosphate buffer solution, pH 6.8, for four hours using a dissolution basket apparatus (Vanguard, USA) to prevent fibers from floating on the surface at 100 rpm and 37 °C ± 0.5 (16). Drug release was evaluated using a UV-spectrophotometer by measuring the absorbance at 241 nm at predetermined times (5, 15, 30, 60, 90, 120, 150,180, and 240) minutes. The reading was compared against a calibration curve equation (y = 0.01 x - 0.0261) produced using standard samples of ATV in buffer solution pH 6.8. A total of 5 mL aliquot of the solution was withdrawn at each time point for UV analysis, and an equal volume of fresh buffer solution was added to maintain sink conditions (17).

2.2.4.7 Scanning Electron Microscopy

The fiber morphologies were assessed using a scanning electron microscope (Axia, ChemiSEM-Thermo Scientific, USA). Before an examination, the samples were gold sputter-coated under argon to render

them electrically conductive. Images were then recorded at an excitation voltage of 30 kV. The average fiber size was determined by measuring their diameters in SEM images using the ImageJ software (18).

2.2.5 Preparation of unidirectional buccal tablet

The unidirectional buccal tablet was created using the direct compression method. The fibers, equivalent to 20 mg of ATV, were cut into tiny pieces, and the excipients were weighed. All the ingredients were mixed, excluding the ethyl cellulose, as illustrated in Table 2. The core tablets were formed using an 8 mm diameter single-punch tablet machine. Ethyl cellulose was used as a backing layer to make the unidirectional buccal tablet, and it was filled into a round die with a 10 mm diameter on the tablet machine. The core tablet was then placed in the center above the ethyl cellulose and compressed to create the unidirectional buccal tablet (19).

Table 2: Ingredients of unidirectional buccal tablet formulation

Ingredients	Formulation	
	B1	B2
Hydroxypropyl methylcellulose	20 mg	10 mg
Microcrystalline cellulose	15 mg	-
Magnesium stearate	3 mg	3 mg
Mannitol	30 mg	-
Sodium alginate	-	40 mg
Lactose	22	37 mg
Total weight	200 mg	200 mg

*** 60 mg and 50 mg of ATV as fibers and ethylcellulose were added, respectively, to all tablet formulations**

2.2.6 Characterization of unidirectional buccal tablet

2.2.6.1 Weight Variation Test

A weight variation assessment was conducted on three tablets taken from every batch, employing an electronic balance known as the Kern ALS 220-4N, made in Germany. Subsequently, the results were used to calculate average values (20).

2.2.6.2 Thickness and hardness tests

The thickness and hardness of mucoadhesive tablets were assessed using a digital vernier caliper (Mitutoyo Corporation, Japan) and an electronic hardness tester (YD-1, China), respectively (21, 22). Three tablets were chosen to be examined in each test.

2.2.6.3 Surface pH

An electrode pH meter was utilized to determine the surface pH. The tablet was placed in contact with 10 mL of pH 6.8 phosphate buffer for 2 hours at room temperature to allow it to swell. The pH was then measured by bringing the electrode in contact with the tablet's surface and allowing it to reach equilibrium for 1 minute (23, 24).

2.2.6.4 Swelling index

To determine the swelling index (SI), tablets were first weighed and attached to glass slides measuring 2x2 cm. These prepared slides were then submerged in Petri dishes filled with 10 mL of phosphate buffer at pH 6.8 (20). A consistent temperature of 37 °C ± 0.5 °C was maintained throughout the study

using a hot plate. At specific time intervals (1, 2, 4, 6, and 8) hours, the tablets were taken out, any excess surface water was removed using filter papers, and the expanded tablets were precisely reweighed. SI was then calculated using the following equation (25, 26).

$$SI = \frac{Wt - W0}{W0} * 100 \dots \dots 4$$

The Wt corresponds to the buccal tablet's weight at a particular time, and W0 signifies the tablet's initial weight.

2.2.6.5 Disintegration test

The examination was conducted on buccal tablets without a backing layer material. Three tablets were randomly chosen from each batch and inserted into disintegration apparatus baskets (Karl Kolb, Germany). These tablets were subjected to a disintegration process until complete disintegration occurred. Afterward, the baskets were removed from the solution, and the tablets were observed to ensure complete disintegration (20).

2.2.6.6 Mucoadhesion strength

The mucoadhesion strength was determined by a modified balance method [18]. Shortly after slaughter, sheep buccal mucosa was obtained from a local slaughterhouse and separated by removing fat and loose tissues. The membrane underwent washing with distilled water and phosphate buffer pH 6.8 at 37 °C (27).

The experimental setup comprised a double-beam physical balance with a pan on the right side and a string on the left, holding a glass slide with a sectioned buccal tablet. The sheep buccal mucosa was positioned on an inverted 50 mL beaker inside a 500 mL beaker filled with pH 6.8 phosphate buffer at 37 ºC. Five grams of weight were initially added to the right pan, and after placing the tablet on the mucosa, the weight was removed. The tablet remained in contact with the mucosa for 5 minutes. To measure bioadhesive strength, weights were added to the right side, and the accumulated weight (minus the initial 5 g) determined the tablet's adhesion force. The bioadhesive force was calculated using a specific equation (28).

$$N = \frac{W*g}{1000} \dots\dots\dots 5$$

N represents bioadhesive force, W signifies the necessary weight for tablet detachment from sheep buccal mucosa in grams, and g is the acceleration due to gravity at 9.81 m/sec² (28).

2.2.6.7 *In vitro* release of unidirectional buccal tablet using a controlled flow rate pump.

The drug release process was conducted using the transfer method with a peristaltic pump at a consistent flow rate of 1 ml /minute for four hours. The typical salivary flow rate is approximately 1 ml /minute, with a maximum value reaching 7 ml/minute (29, 30). This investigation aimed to simulate the salivary flow rate in the oral cavity. The phosphate buffer (pH 6.8) was placed in a beaker over a hotplate to maintain the buffer temperature at 37 °C ± 0.5.

The flow rate of the phosphate buffer was regulated using a peristaltic pump (Ditron Technology Co., Ltd, China) maintained at 1 mL per minute for both B1and B2 (31), and the B2 tablet performed release at 5ml per minute too. A tablet was affixed to a glass slide using a drop of buffer solution. After cutting its upper side, this slide was placed inside a plastic pipette and inserted into a glass tube, as shown in Figure 1. Samples were collected every 15 minutes and measured using a UV-visible spectrophotometer (Shimadzu, Japan) at 241 nm. The concentration was determined for each time point, and the cumulative amount of ATV was calculated by adding the released amount to the previous time point.

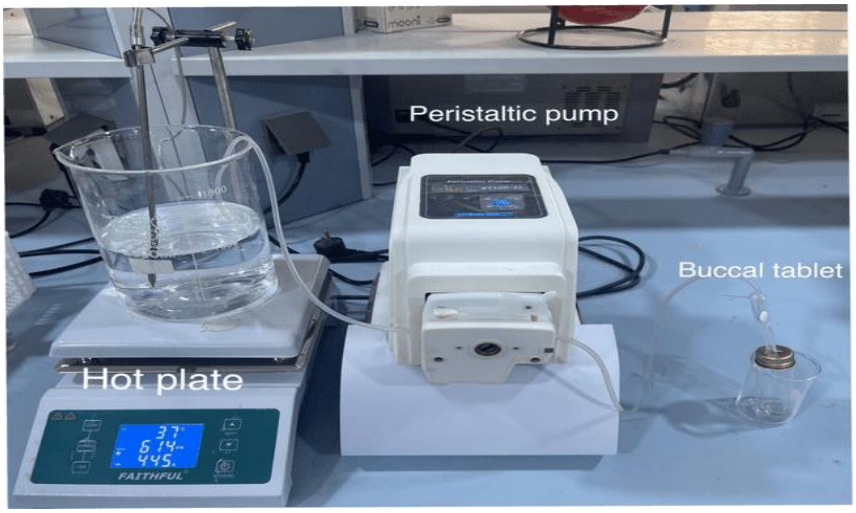

Figure 1: The release of a unidirectional buccal tablet by the peristaltic pump.

2.2.6.8 *In vitro* release of buccal tablet by dissolution apparatus

The USP dissolution test apparatus, apparatus II paddle type (Vanguard, USA), was utilized to assess the tablet drug release. The dissolution medium consisted of 500 ml of phosphate buffer with a pH of 6.8. The dissolution occurred at 37 °C ± 0.5 °C, with the paddle rotating at 50 rpm. To affix the backing layer of the buccal tablet, instant adhesive was used to attach it to a glass slide, which was then positioned at the bottom of the dissolution vessel. Samples of 5 ml were withdrawn at specific time intervals (5,10, 15, 30, 60, 90, 120, 150, 180, and 240) minutes, and replaced with fresh medium. These samples were filtered through a 0.45 µm syringe filter and analyzed via a UV spectrophotometer (Shimadzu, Japan) at 241 nm (32).

3. Results and Discussion

3.1 Determination of electrospinning solutions viscosity

The assessment of the electrospinning solution viscosity aimed to demonstrate how various polymers and their ratios influence the solution to be injected into the pump of the electrospinning process. The concentration of ATV remained constant across all spinning solutions. The results outlined in Table 3 and the prepared solutions were viscous enough to be pumped into the syringe of the electrospinning apparatus; the presence of PVP and increasing SP polymer

amounts in the spinning solution tends to elevate its viscosity. This finding was similar to that of nifedipine spinning solutions prepared with increasing amounts of Eudragit, reflecting the increasing viscosity (33). The increase in the rate of the viscometer resulted in a lower viscosity, which pointed to a non-Newtonian solution. To conclude, the viscosities of all prepared electrospinning solutions did not hinder the electrospinning process.

Table 3: The viscosity of electrospinning solutions

Formulas	Viscosity (cp)				
	100 rpm	60 rpm	50 rpm	30 rpm	20 rpm
F1	396	420	522	624	954
F2	615	805	920	1000	1375
F3	919	1133	1231	1624	1856

3.2 Characterization of spinning fibers

3.2.1 Physical appearance of fibers

The outcome of the electrospinning process investigation on the collector of the device is essential and a clue to the successful preparation. All formulations presented a fiber mat positioned onto the aluminum foil. As illustrated in Figure 2, the visual appearance and the tactile qualities of ATV-loaded fibers resembled cleaning disposable tissue (34).

Figure 2: The physical appearance of ATV-loaded fibres

3.2.2 Optical microscopic image

To prove the fabrication of the fiber by the electrospinning process, microscopic examinations were done for all obtained fibers, as shown in Figure 3; all formulations showed fibers as evidence of the successful formulation proportions, which agreed with the previous research that showed starch fiber smooth surfaces, and the fibers were essentially continuous (34). In summary, the microscopic images of the prepared electrospinning fibers evidenced the constitution of the fiber mat.

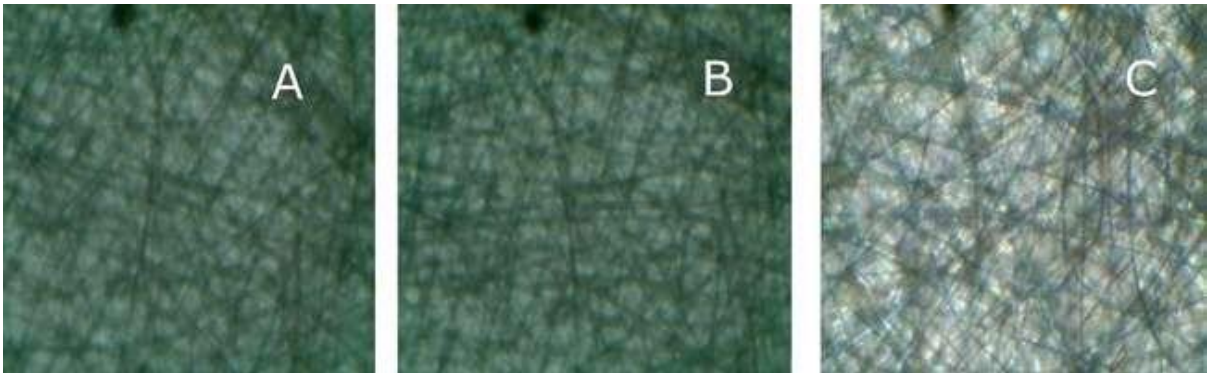

Figure 3: The microscopic images display electrospinning fibers observed through an optical microscope, labeled as follows: A) F1, B) F2, and C) F3.

3.2.3 Fourier transform infrared spectroscopy

FTIR spectroscopy analysis investigated any interaction between ATV, PVP, and SP (35). As illustrated in Figure 4, the ATV spectrogram exhibited distinct peaks at 3362 cm^{-1}, 3230 cm^{-1}, and 1650 cm^{-1} correlated to stretching N-H, O-H, and C=O groups, while the PVP spectrogram showed the prominently featured peaks, which were 1650 cm^{-1} that related to the C=O and the clear broad peak that was associated with the hydroxyl group between 3200 cm^{-1} and 3600 cm^{-1} (36-38). The broad hydroxyl peak of SP and the peaks at 1735 cm^{-1} and 1620 cm^{-1} are associated with the C=O stretching (39, 40). F1, F2, and F3 spectrograms exhibited the carbonyl group shifting from 1650 cm^{-1} to 1670 cm^{-1} and 1663 cm^{-1}, respectively, pointed to the hydrogen bondings between the carbonyl groups of ATV, PVP, and SP with other functional groups (41).

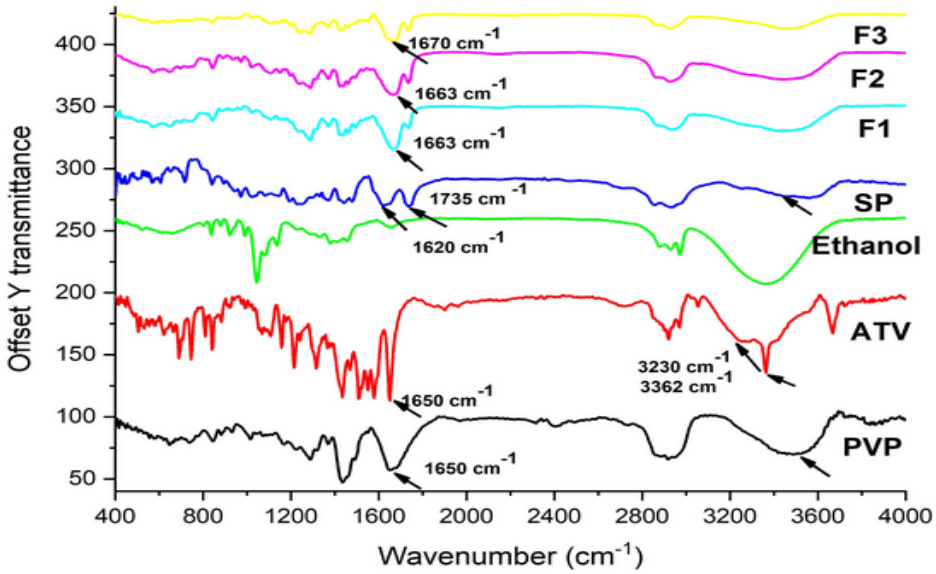

Figure 4: FTIR spectrograms within wavenumber ranges (400 to 4000 cm^{-1}) of ATV, PVP, SP, and fiber formulations.

3.2.4 Differential scanning calorimetry

DSC is essential in detecting the status changes in the compounds thermally; hence, DSC was applied and clarified in Figure 5, and all formulations clearly did not show the ATV peak at 169°C, with the appearance of a melting point peak of SP at 107°C. SP exhibited a lower melting point than 120°C, reported due to SP incorporation with any drug (42). The ATV peak disappearance indicated an amorphous ATV that was part of the fabrication with the electrospun fibers. In Lopez *et al* study, indomethacin and griseofulvin exhibited an amorphous status after the formulation of electrospun fibers using PVP polymers (43).

FTIR and DSC studies were harmonized by revealing the change that affirmed the hydrogen bonds more likely to be between ATV and polymers, which assured the amorphous form of fibers by DSC.

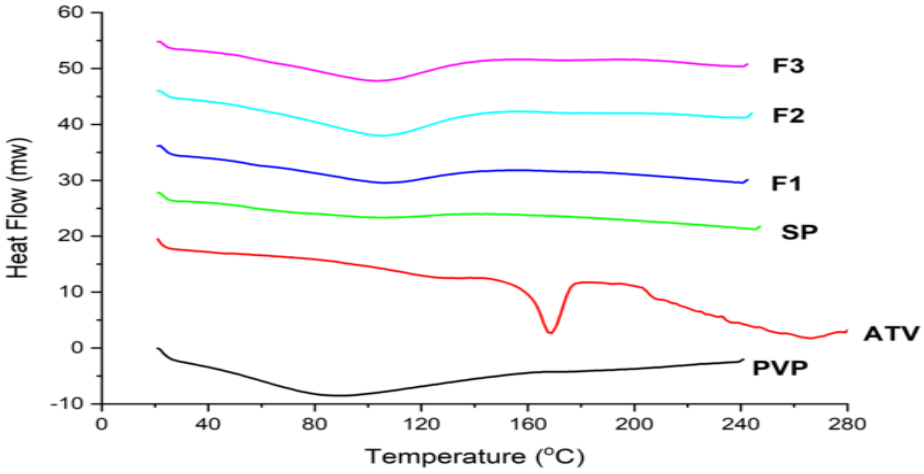

Figure 5: The DSC thermogram of ATV, PVP, SP, and fibre formulations.

3.2.5 Determination of drug loading (DL%), entrapment efficiency (EE%), and fibers yield (Y%) of the drug-loaded fibers.

These tests are essential to know the amount of drug entrapped and the yield of the electrospinning fibers. The drug loading, entrapment efficiency, and fiber yield are illustrated in Table 4, which shows that the DL% decreased in value in fiber formulations as the polymer amount increased and with the addition of SP to the fiber formulations. This finding was similar to a study that prepared electrospun fibers of ornidazole with increasing PVP amount, which decreased DL% (44). Furthermore, all the formulations exhibited a high entrapment efficiency, consistent with the findings of Reda et al. 's research. This alignment was anticipated due to the pervasive surface area of the fibers and the passive method of incorporating the drug into the polymeric solution, which subsequently solidified during the electrospinning process, securely encapsulating the drug within the polymer fiber matrix. Consequently, the risk of drug loss during this procedure was minimal (2). At the same time, the fiber yield depends on the quantity of threads that reach the aluminium foil surrounding the collector. This was found in the research of Alshaya *et al*, which showed a high atorvastatin-nifedipine fiber yield of about 99 % (10).

To sum up, the ATV DL% was decreased with the increasing polymer amounts and showed good entrapment efficiency with yield.

Table 4: Drug loading, entrapment efficiency, and yield of ATV-loaded fibers

Number of electrospun fiber formulations	DL% ±SD	EE%±SD	Yield%
F1	16.49±0.432	98.99±2.59	87.5
F2	13.74±0.35	96.19±2.46	94.6
F3	11.72±0.13	93.83±1.11	91.87

3.2.6 Disintegration time of fibres

The commonly employed disintegration apparatus could simulate the volume and peristaltic movement in the gastrointestinal tract; however, it poorly mimics the oral cavity (12). Thus, the petri dish method is used to determine the disintegration time of fibers. In this test, the endpoint was defined as the complete disappearance of threads from the Petri dish's surface, and the corresponding time was recorded. Figure 6, which exhibited F1 that was randomly selected, showed that the subsequent disintegration occurred until the complete disappearance of fibers within 3.5 minutes, where the disintegration time is directly related to the amount of polymer within all formulations of fibers, as outlined in Table 5.

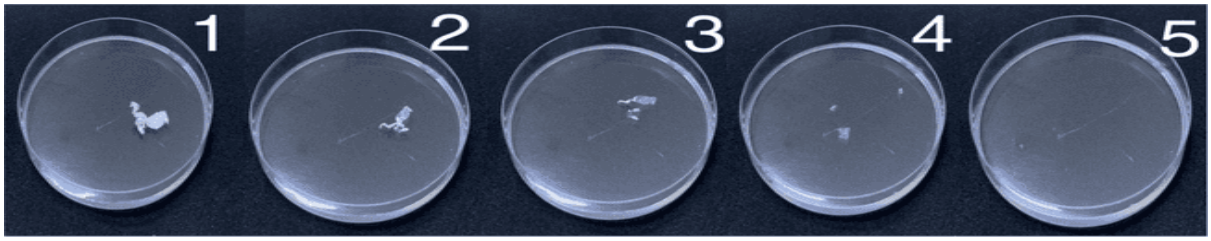

Figure 6: Disintegration of electrospun ATV-loaded F1 fiber formulation by the petri dish method lasts 3.5 minutes.

The time of complete disintegration for all electrospun fibers was comparable to the 3-minute disintegration time of sildenafil electrospun fibers fabricated from PVA and PVP (45).

In summary, the disintegration time is directly proportional to the number of polymers added to the fibre formulation.

Table 5: Disintegration time of drug-loaded fibers obtained from the disintegration test.

Formula	Disintegration time (Min)
F1	3.5
F2	3.8
F3	4

3.2.7 *In vitro* release of fibres

The release pattern of ATV from fiber formulations with an equal amount of 20 mg of ATV is presented in Figure 7. In this Figure, the SP and PVP impact varied according to the ratio used, displaying an inverse relationship with the drug release from fibre formulations. As a result, F1, which contains a lower quantity of SP, achieves a 99.6% release after 90 minutes, whereas F2 and F3 only reach 49% and 38.97% release, respectively. This outcome can be attributed to the highest viscosity of 1856 cp at 20 rpm for F3 of the combined polymeric PVP and SP solution (46), leading to the lowest ATV release percentage. In conclusion, the SP ratio has an inverse effect on ATV release.

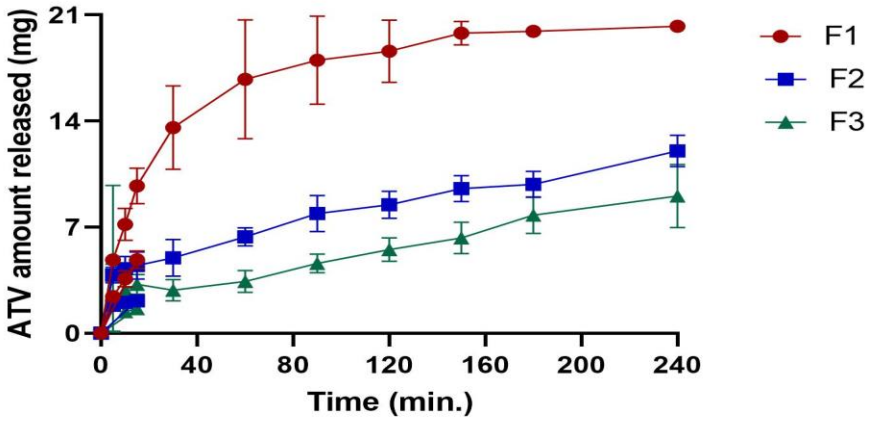

Figure 7: *In vitro* release of ATV-loaded fibres, as this study was done in triplicate (n=3)

The fiber release study resulted in F1 being selected for further investigations, including SEM and tablet formation, since this formulation showed a faster release than other fiber formulations.

3.2.8 Scanning Electron Microscopy (SEM)

The SEM investigation, as depicted in Figure 8, showed images that scaled against 5µm and 40 µm respectively, a very high magnification to reveal the fiber diameter, which varies between 1.944 µm and 3.427 µm with a few fibers within the nano size, and the 40 µm image showed that this formulation produced smooth-surfaced fibers without any beads and visible crystals on the surface, indicating a suitable concentration of polymer which has high viscoelastic properties that resist the formation of beads, and positive interaction with even distribution of atorvastatin and polymers, respectively. Our result was similar to Reda *et al* who found that a suitable ratio of polymers resulted in fibers with smooth surfaces and free from beads (2) .

In summary, the SEM images exhibited smooth fibers without free particles on their surfaces, proving the productive fabrication during the fiber processing in this work.

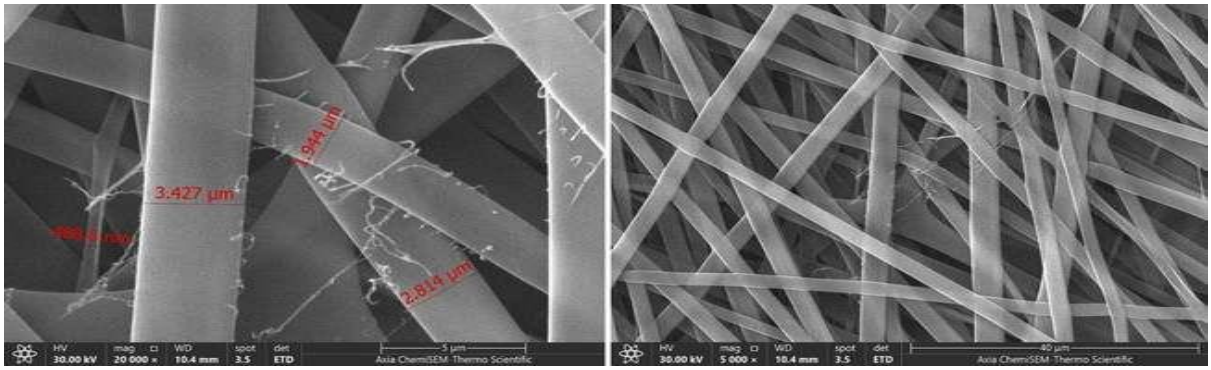

Figure 8: SEM image shows the diameter and morphology of ATV-loaded fiber of F1 formulation.

3.3 ATV- buccal tablet characterization

3.3.1 Weight Variation

The variation in tablet formulations' weight, as shown in Table 6, was (199.7-199.8) mg ± (0.264 - 0.680). These values were within the USP limit for tablet weight between 130 and 324 mg, which is considered to be 7.5%.

3.3.2 Thickness and hardness

The hardness was 4.05 to 4.35 ± (0.015 to 0.05), close to that of the nitroglycerin tablet (47), while the thickness was 3.40 to 3.41 ± (0.0057 to 0.02), which agreed with the result of the salbutamol buccal tablet (48).

3.3.3 Surface pH

Table 6 indicates the formulations' surface pH, which aligns with the previous work of Seham et al, in which all the formulations of Mebeverine hydrochloride buccal tablet surface pH were in agreement with normal salivary pH 5.5 to 7.8 (49).

3.3.4 Swelling index

The swelling index of B1 was 93.5 %, while that for B2 was 77.5% after 6 and 2 hours, from the total time of the swelling index test, which is 8 hours, respectively; the swelling occurred in the tablet core rather than the impermeable backing layer. These results were close to those of an ATV mucoadhesive tablet (22).

3.3.5 Mucoadhesive strength

The force required for tablet detachment from the mucosa depends on the polymer concentration in the tablet formulations the HPMC, and the MCC. For B1, which includes both HPMC and MCC, the mucoadhesive force was 0.637, while in B2, containing only HPMC, it was 0.588; these results agreed with those of ATV buccal tablets, in which tablets of a higher concentration of polymers (HPMC, MCC, and carbapol) gave higher mucoadhesive force (22).

Table 6: Mucoadhesive buccal tablet properties

Formula	Weight variation (mg) ±SD	Thickness (mm) ±SD	Hardness Kg/cm^2 ± SD	Surface pH ±SD	Swelling index %	Mucoadhesive Strength (N)
B1	199.8±0.264	3.4±0.0057	4.35±0.015	6.5±0.1	93.5	0.637
B2	199.7± 0.680	3.41± 0.02	4.05± 0.05	6.76± 0.057	77.5	0.588

3.3.2 Disintegration test

The disintegration times were 80 and 75 minutes, respectively. This finding was harmonized with buccal tablet disintegration time within 4 hours and was close to that of the tizanidine hydrochloride buccal tablet (20).

3.3.3 *In vitro* release of unidirectional buccal tablet using a controlled flow rate

The in vitro release of ATV from buccal tablets reveals that, within 4 hours, B1 and B2 at a flow rate of 1m/minute exhibited releases of 2.78 mg and 4.39 mg, respectively, as shown in Figure 9 (A), B1 showed a slower release, despite it has a higher swelling index, since the release by the controlled flow pump depends on the part of tablet that hydrated by the buffer, while during the swelling test the tablet remained in 10 ml buffer, for the entire test time.B2 tablet achieved 5.66 mg of ATV at 5 ml/minute, as illustrated in Figure 9 (B). This implies a slow liberation of ATVs from their dosage form, and by increasing the flow rate, the percentage of ATV release also increased.

3.3.4 *In vitro* release of buccal tablet by using conventional dissolution method

In Figure 9 (C), the release of the B2 tablet (selected for this study) formulation using the conventional dissolution apparatus reached 11.28 mg within 4 hours. In contrast, with the controlled flow rate pump, the B2 formulation achieved only 4.39 mg release (as seen in Figure 9 (A) and 5.66 mg (as shown in Figure 9 (B) at a flow rate of 1ml/minute and 5ml/minute, respectively. This discrepancy in release rates was attributed to differences in the release procedure. Unlike the controlled flow pump, which employs a flow rate mimicking salivary flow, since the volume of the conventional method does not accurately simulate the buccal cavity.

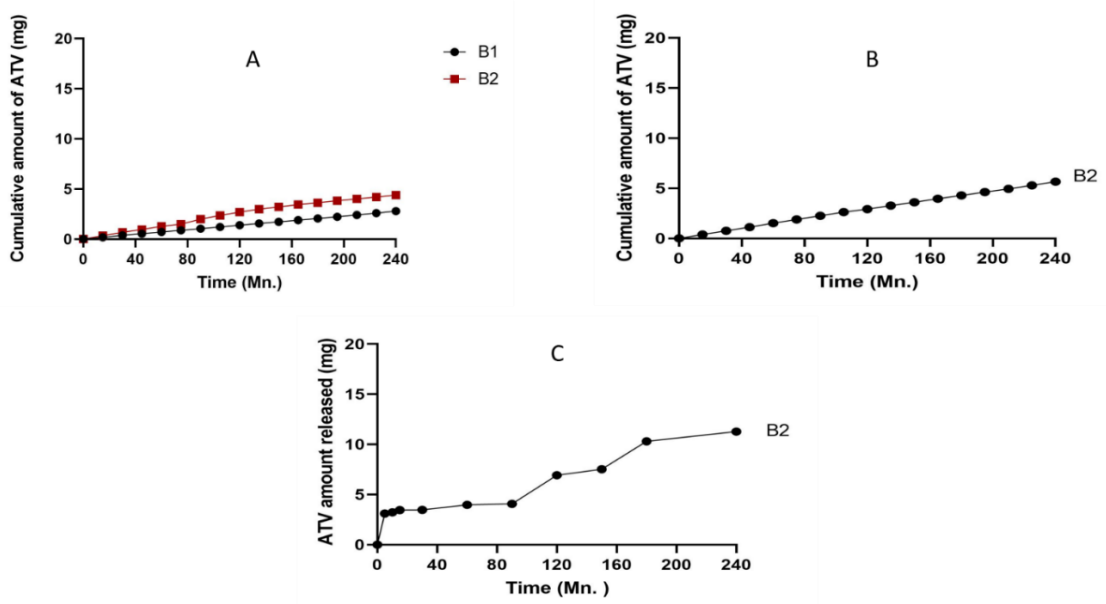

Figure 9*: In vitro* release of (A) B1 and B2 tablets by controlled flow pump at a rate of 1ml/min, (B) B2 tablet at a rate of 5ml/min, and (C) B2 tablet release by conventional dissolution method.

4. Conclusion

In summary, fibers loaded with ATV were effectively formulated and showed an amorphous ATV, evidenced by FTIR and DSC. SEM images reveal fibers with a smooth surface devoid of solid particles. The electrospinning F1 fibers released 99.6% of ATV within 90 minutes. However, the tablets that contained fibers liberated the ATV slowly as the B2 tablet via the controlled transfer method at a flow rate of 1ml/min and 5ml/min gave 4.39 mg and 5.66 mg, within 4 hours, respectively, as the conventional method released ATV at around 11.28 mg, This indicates a slow liberation of ATV from its dosage form, and by increasing the flow rate, the released amount of ATV also increased.

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