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Original Article

AMLODIPINE BESYLATE LOADED POLYMERIC NANOPARTICLES: PREPARATION AND *IN VITRO* CHARACTERISATION

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ABSTRACT

Objective: The present investigation aims to formulate the ideal drug formulation using different surfactants and optimize the amlodipine-loaded polymeric nanoparticles.

Methods: The present work was to formulate the drug-loaded polymeric nanoparticles to enhance the dissolution rate of a poorly water-soluble drug, amlodipine besylate, using the anti-solvent precipitation method. The Characterisation studies include particle size (nm), Zeta potential (mV), polydispersity index, Drug entrapment efficiency (%), *in vitro* release drug release, and surface morphological studies like SEM and XRD.

Results: The drug-loaded Polymeric nanoparticles of F3 containing PLGA and PVA shows the desired smaller particle size is 198.8±5.25, maximum zeta potential is-24.76±2.54 mV and the stable polydispersity index of 0.957±0.45. The drug entrapment efficiency is 93%, and the controlled dissolution of the ideal formulation pattern is about 94.88±2.45 in 24h.

Conclusion: The release pattern observed that PNs significantly improved the dissolution character of amlodipine besylate. PNs have a controlled drug release pattern and can be used as a suitable drug delivery carrier for low solubility and poorly bioavailable drugs like amlodipine to improve its dissolution rate.

Keywords: Polymeric nanoparticles, Optimization, *In vitro* drug release, Amlodipine besylate

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INTRODUCTION

Demand for the creation of dosage forms that may be easily handled and chosen by many patients has increased in recent years due to lifestyle changes. In India, hypertension is a significant health concern, and its prevalence is rising among urban and rural populations. The use of polymeric delivery systems as carriers for small and large molecules is receiving increased attention in drug delivery [1, 2]. Drug delivery and formulation using polymers for difficult-to-formulate reagents is no longer a novel concept, altering the drug's future. Polymeric drug delivery methods drastically change drug pharmacokinetic and pharmacodynamic properties [3].

Nanotechnology engineering processes have recently seen fast growth in pharmaceutical applications to improve solubility and bioavailability. The route of absorption through the highly vascular zed buccal mucosa allows the drug to enter the bloodstream directly. As a result, direct systemic medical and sublingual drug administration is used in the fields of cardiovascular medications, steroids, barbiturates, and enzymes. Because the buccal mucosa primarily acts as a barrier, comparable to the skin, not all substances are permeable and accessible to it. Various nanoparticle engineering processes have been developed and used in pharmaceutical applications [4]. Nanoparticle engineering formulates poorly soluble drugs into particles alone or combined with pharmaceutical excipients. These can reduce the particle size from a micron to a nanometer on a scale, and it was found that there is a significant increase in the surface area and related dissolution rate [5, 6]. When these tablets are placed on the tongue, they will disintegrate instantaneously, releasing the drug, which dissolves or disperses in saliva. Some medications are absorbed from the mouth, pharynx, and esophagus as the saliva passes down into the stomach [7, 8]. Polymeric nanoparticles (NPs) are one of the most commonly employed nanomaterials in nanomedicine because they can deliver a drug to a specific region of an organ with a lower dose, hence increasing drug bioavailability at the desired target [9]. Amlodipine is a dihydropyridine calcium channel antagonist introduced for treating hypertension, coronary heart failure, and certain types of angina. It is extensively (about 90%) converted to inactive metabolites via hepatic metabolism, with 10% of the parent compound and 60% of the metabolites excreted through urine [10]. The present invention provides a process for preparing amlodipine besylate nanoparticles formulation by using the anti-solvent evaporation method to enhance the dissolution rate with improved solubility. It is another object of the present invention to characterize the solubility and bioavailability of Amlodipine besylate in nanoparticulate form.

MATERIALS AND METHODS

Chemicals involved

Amlodipine besylate, poly D,L-lactic-co-glycolic (PLGA), Poloxamer, L-Argine, Polystrene, Polyvinyl alcohol, Tween 80 were purchased from Sigma Aldrich and used without any further purification. Double Distilled water was used throughout the study.

Formulation of Amlodipine besylate nanoparticles

A laboratory scale apparatus was used to identify a suitable stabilizer for preparing nanosuspensions using the solvent evaporation method. The apparatus comprised of 100 ml beaker; in this process, the drug substance was dispersed uniformly in an aqueous medium containing dissolved stabilizers [25] in the milling chamber using a magnetic stirrer at 500rpm. The total volume of slurry (drug substance+stabilizer+water) was 50 ml; the batch size for these development trials was 50 ml. The suspension temperature was maintained at 20–25 °C on a magnetic stirrer for an hour to allow the volatile solvent to evaporate. Add the organic solvents using a syringe drop by drop positioned with the needle directly into the surfactant-containing water. The ratio of the drug to the surfactant used in the study was 1:2 for 15 formulae (F1-F15) prepared by this technique, demonstrated in table 1 with their composition. Generally, the choice and concentrations of stabilizers not only depend on their ability to facilitate particle size reduction but also on their ability to produce suspensions with good physical stability [11]. Typically, a combination of surfactants and electrostatic stabilization is most effective for nano-suspension stability. The composition of surfactant and polymeric stabilizers used for producing Amlodipine nanosuspension is summarized in table 1.

Table 1: Formulation design of amlodipine besylate (AB) nanosuspension using different stabilizers

Materials (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
AB	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
PLGA	35	45	55	65	-	-	-	-	-	-	70	-	-	-	-
Polystyrene	35	-	-	-	45	55	65	-	-	-	-	80	-	-	-
L-Arginine	-	35	-	-	45	-	-	55	65	-	-	-	90	-	-
PVA	-	-	35	-	-	45	-	55	-	65	-	-	-	100	-
Tween 80	-	-	-	0.1	-	-	0.1	-	0.1	0.1	-	-	-	-	0.2
Methanol	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Water	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Characterization of nanoparticles

Drug-excipient compatibility studies by using FTIR

The Fourier transform infrared spectroscopy (FT-IR) spectrum was studied to detect any sign of interaction or complexation between Amlodipine besylate and stabilizers used in the preparation and with the excipients used in the preparation. The spectrum was obtained using FT-IR Shimadzu 8300 Japan. Physical mixtures of the pure drug (1:1) and PLGA, respectively, drug and Polystyrene, drug and L Arginine. All these samples were grounded and mixed thoroughly with potassium bromide at a 1:5 (sample: Potassium bromide) weight ratio [12]. The spectrum obtained was between the wave number of 4000-400 cm^{-1}.

Differential scanning calorimetry (DSC)

DSC can be used to determine the compatibility between the drug and excipients and to evaluate the drug's crystalline state, especially when converted to nanoparticles [13]. Accurately weighed samples (5 mg) were placed in non-hermetically aluminum pans and heated at the rate of 10 °C/minute against an empty aluminum pan as a reference covering a temperature range of 40 °C to 300 °C

Particle size analysis

Particle size and size distribution of the suspension before, during (at different milling times), and following milling were determined using the laser diffraction (LD) method with a wet sampling system (Mastersizer S, Malvern Instruments, Worcestershire, UK). The particle diameters reported were calculated using volume distribution of the particle size obtained with the different stabilizer compositions and their physical stability upon storage. Based on particle size distribution obtained following milling and suspension stability, a formula composition comprising PLGA as the primary stabilizer and Polystyrene as the secondary stabilizer was chosen for scale-up trials using the media-milling machine [14].

Determination of drug entrapment efficiency (DEE) of nanoparticles

The freshly prepared nanoparticles were centrifuged at 20,000 rpm for 20 min using an ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of a diluted 25 ml of the supernatant solution at 256 nm using a UV spectrophotometer. %DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of medication taken. The experiment was performed in triplicate [15, 16].

The following equation achieved drug entrapment efficiency (%DEE):

$$\text{Entrapment efficiency \%} = \frac{\text{W initial drug} - \text{Wfree drug}}{\text{W initial drug}} \times 100$$

***In vitro* dissolution study**

Dissolution rates of Amlodipine formulas were determined according to a method described with minor modifications. A USP dissolution apparatus (Model: DISSO 2000, Labindia, Mumbai, India) type II (paddle method) with a paddle operating at 50 rpm was used for dissolution studies [17]. All dissolution tests were performed on 10 mg of Amlodipine besylate (in a suspension state). Acetate buffer, pH 6.8, was used as a dissolution medium. The volume and temperature of the dissolution medium were 500 ml and 37 °C, respectively. The freshly prepared amlodipine nanoparticles (10 ml) were added to a dialysis bag and fitted to the paddle, samples (5 ml) were withdrawn at regular intervals of every 10 min up to 24 h and replaced with fresh dissolution medium to maintain sink condition. Samples were filtered through ashless filter paper and assayed in a UV-VISIBLE spectrophotometer [18, 19].

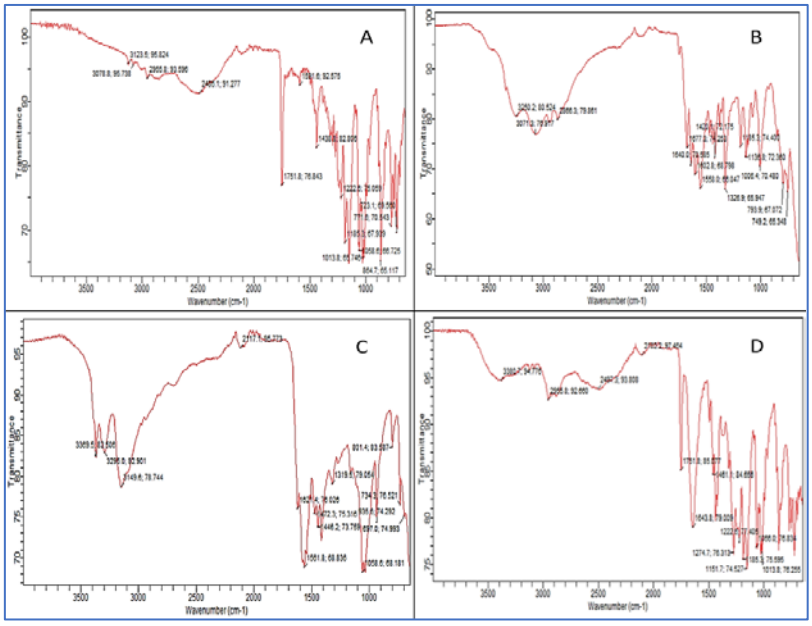

Fig. 1: (A) Compatibility studies by using FTIR of Amlodipine; (B) Compatibility studies by using FTIR of Amlodipine and PLGA; (C) Compatibility studies by using FTIR of Amlodipine and L-Arginine; (D) Compatibility studies by using FTIR Amlodipine with PVA

Scanning electron microscopy (SEM)

The morphology of raw drug and amlodipine nanoparticles F3 were examined by scanning electron microscopy. The morphology of the optimized drug was done by direct deposition of powder on double-sided carbon tape and coated with gold [20, 21].

Powder X-ray diffraction

X-ray diffraction is used to study the atomic and molecular structure of crystalline substances such as drugs and excipients. X-ray diffraction patterns (diffract grams) can be used to confirm the crystalline nature of a sample. Therefore, this information is used to verify whether the substances are crystalline or amorphous [22, 23].

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy (FT-IR) spectrum was studied to detect any sign of interaction or complexation between amlodipine besylate, polymer chooses, and the surfactants. The spectrum of pure drugs, physical mixture of drugs, and excipients were obtained using FT-IR Shimadzu 8300 Japan. From the IR graphs it was inferred that the main functional group wavenumbers were effectively reproducible in a physical mixture along with polymers. So it was infers that the drug and polymer are compatible to each other and ideal for formulating nanoparticles. For the optimized formulation, the composition was examined for possible incompatibility of the drug with excipients compatibility studies of the drug [17].

Differential scanning calorimetry (DSC)

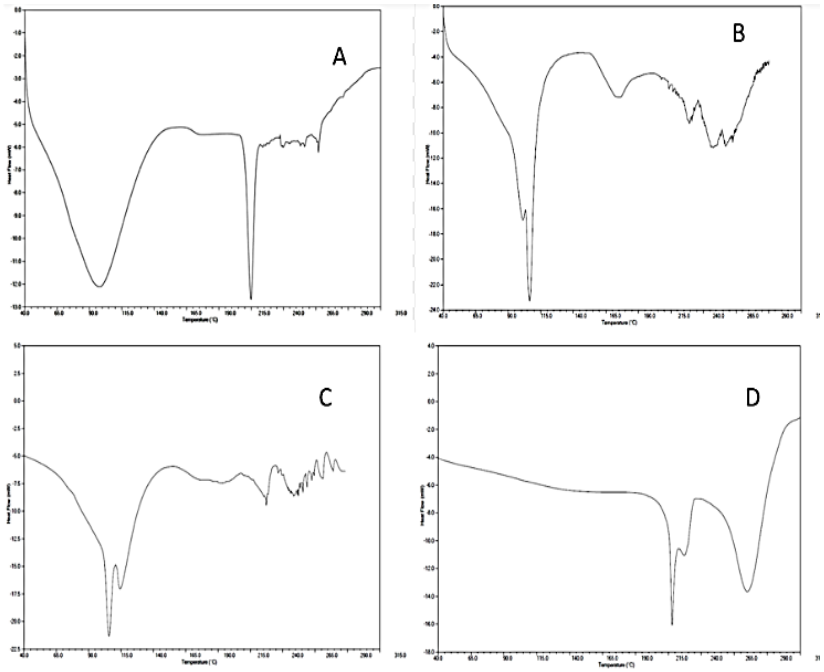

Fig. 2: (A) DSC of the pure drug (Amlodipine Besylate); (B) DSC of drug and PLGA; (C) DSC of drug and L-Arginine; (D) DSC **of drug and PVA**

The antisolvent precipitation process may change the physical state of Amlodipine. The DSC curves as fig. 2 of raw Amlodipine besylate showed a single sharp endothermic peak at 199 °C, corresponding to its melting point. The peak melting of amlodipine, PLGA, Polystyrene, and L-Arginine at a ratio (1:1) exhibited an endothermic peak of raw Amlodipine at 199 °C, which indicates that stabilizers do not change the physical state of amlodipine in the physical mixture. There is no chemical interaction between the drug and the stabilizers.

Particle size analysis, polydispersity index (PI), zeta potential, and drug entrapment efficiency

Table 2: Particle size, PI, zeta potential, and DEE amlodipine nanoparticles

S. No.	Batch number	Particle size diameter (nm)	Polydispersity index (PDI)	Zeta potential (mV)	% DEE
1.	F1	384.8±5.34	0.820±0.01	-6.1±2.42	72
2.	F2	532.6±3.14	0.412±0.32	-7.21±1.53	56
3.	F3	198.8±5.25	0.957±0.45	-24.76±2.54	93
4.	F4	232.6±4.24	0.616±0.32	-9.7±2.12	78
5.	F5	653.5±3.42	0.235±0.24	-19.6±2.43	62
6.	F6	113.0±5.26	0.843±0.31	-12.38±3.13	69
7.	F7	504.6±4.32	0.819±0.14	-5.9±3.12	89
8.	F8	642.4±3.48	0.925±0.25	-26.9±1.32	58
9.	F9	545.0±4.12	0.438±0.16	-22.43±3.32	69
10.	F10	154.4±3.18	0.526±0.19	-10.1±2.14	75
11.	F11	113.7±2.73	0.216±0.21	-7.8±2.42	45
12.	F12	391.7±5.32	0.387±0.28	-11.7±3.14	67
13.	F13	430.5±4.24	0.234±0.22	-19.6±1.32	69
14.	F14	212.7±3.41	0.912±0.15	-9.6±1.96	82
15.	F15	347.6±4.56	0.663±0.16	-8.7±2.42	86

(All values are expressed in mean±SD; n=3)

Effects of the stabilizer's types

The particle size and the polydispersity index (PI) of the prepared nanoparticles were measured by an ABT-9000 Nanolaser particle size analyzer. Particle size was expressed by the volume moment mean diameter, as this mean is quite sensitive to the presence of large particles and therefore considered the most suitable for comparing different nanoparticle formulations. The average particle size of amlodipine nanoparticles and all the formulae were found to be in the range of 113.0±5.26 nm to 642.4±3.48 nm, summarized in table 3. Water-soluble polymers and surfactants have been used as stabilizers to inhibit the particles' aggregation and improve the drug's physicochemical properties to reduce the drug's size effectively.

Using one stabilizer

The formulae (F11-F15) that contain one stabilizer yield particles sizes ranging from 113.7±2.73 nm to 430.5±4.24 nm, using PLGA, Polystyrene, L-arginine, PVA [26], and Tween 80 [24] as primary stabilizers PVA are polymeric non-ionic stabilizers for Nanosuspensions they stabilize the system by steric stabilization which is achieved by adsorbing polymers onto the drug particles surface through an anchor segment that strongly interacts with the dispersed particles. At the same time, the other well-solvated tail segment extends into the bulk medium while arginine HCL (cationic amino acid) and tween 80 (non-ionic surfactant) are electrostatic stabilizers that stabilize the nanoparticles by countering Vander Waals attractions between particles, additionally promoting wetting and dispersion of the drug particles, which is usually very hydrophobic.

The formulae (F1-F10) using a combination of two stabilizers yield particle sizes ranging from 113.0±5.26-653.5±3.42 nm. The most significant effect ($p<0.01$) of the combination was shown in the formulae that contain PLGA and PVA with another stabilizer; in F3, at a ratio of 1:2, when PLGA was used as the primary stabilizer, the particle size was 198.8±5.25 nm and the PDI having 0.957±0.45. The Zeta Potential of the optimized formulation is-24.76±2.54. Still, in F1, F2, and F4 (PLGA used with Polystyrene, L-Arginine, and tween 80), respectively, the particle sizes were 384.8±5.34 nm, 532.6±3.14, 232.6±4.24, that means the combination has a good surface affinity and could form a substantial mechanical and thermodynamic barrier at the interface of the drug molecule.

L-Arginine HCl as a secondary stabilizer is not always given small particle size, the particle sizes F2, F8, and F9 still have a larger particle size. In addition, the particle size of F5 containing L-Arginine HCl and Polystyrene as stabilizers are 653.5±3.42 nm, which the larger particle size ($p<0.01$) indicates a poor stabilization, and their combination was not appropriate for amlodipine nanoparticles.

The particle size of F3 stabilized with PLGA and PVA is significantly stabilized ($p<0.01$) and greater than other stabilizers. PVA contains a few OH groups which have formed hydrogen bonds with the solvent resulting in an increased dispersion viscosity. However, suppose there is no affinity between the particle's surface and the polymer. In that case, the attractive forces between the two particles become dominant due to polymer depletion from the gap between the two particles (depletion force).

Drug entrapment efficacy

The drug entrapment efficiency of the formulations is shown in the range of 62% to 93%. The results have been displayed in table 2. From the data it was infer that on the decrease in particle size it shows good entrapment efficiency.

Scanning electron microscopy (SEM)

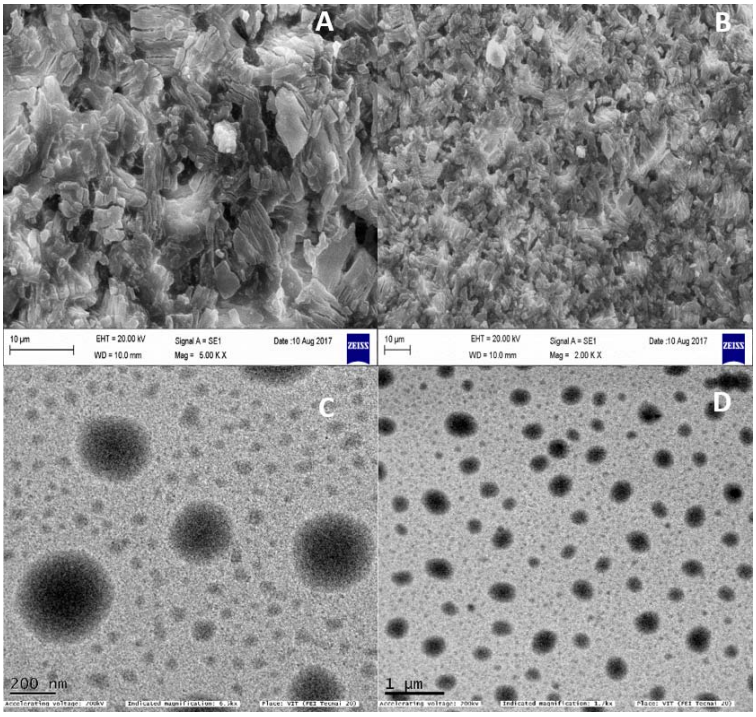

Fig. 3: SEM images (a) Raw Amlodipine; (b) Sieved and screened physical mixture (c) Amlodipine nanoparticle showing drug entrapped in Spherical shaped Micelles at 100X; (d) Group of amlodipine nanoparticle at 10X showing more spherical shaped nanoparticles

Fig. 3 describes the morphology of raw material and prepared nanoparticle formulations. Raw Amlodipine possesses a clumsy irregular-shaped agglomerated coarse particle (fig. 3a). Fig. 3b shows a sieved and screened physical mixture with irregular coarse particles with smaller diameter ranges of 1–2 µm. After the Nano process, amlodipine nanoparticle (fig. 3c and d) was transformed into monodisperse spherical-shaped drug-entangled micelles. This surface character has been found to enhance the entrapment efficiency, uniformity in distribution and drug content uniformity in surface area. As a result, the space available for interaction with adjacent particles is limited, and the powder cohesion is condensed, so aggregation will not be there, leading to good particle stability.

XRD spectra of amlodipine nanoparticle

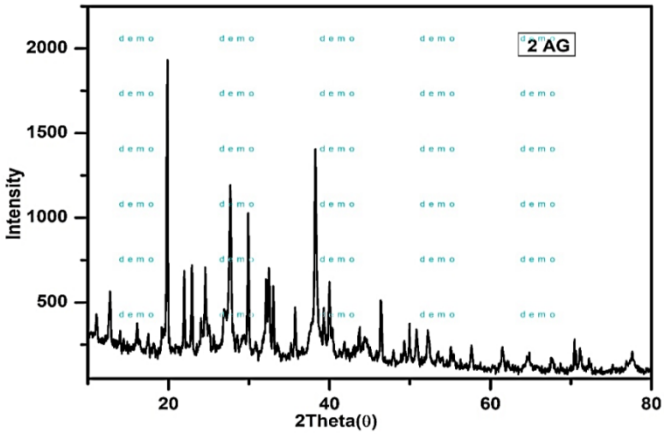

Fig. 4: XRD spectra of amlodipine nanoparticle

The results obtained from DCS reasonably agreed with the results obtained by XRD, the change in the crystalline state of the dried amlodipine nanoparticles was further confirmed by X-ray diffraction. The X-ray patterns of the Amlodipine besylate powder displayed the presence of numerous narrow and symmetrical characteristic diffraction peaks, the strongest 3peaks are 11°, 18° and 21° at 2θ and with high intensity this indicated the crystalline structure of the drug [14]. The crystalline nature of the Amlodipine nanoparticle was evaluated by a powder X-ray diffractometer (PXRD). It could be seen from X-ray diffractograms that Amlodipine nanoparticles possess high amorphous, and some specific peaks could distinguish between the salt form and the base form. For Amlodipine, the drug was converted to an amorphous state by nanoparticles. PXRD detected no signs of crystallinity. It seems reasonable that Amlodipine existed in the amorphous form in nanoparticles, different from the solid particles of pure amlodipine. The results are shown in fig. 4.

Comparative *in vitro* drug dissolution studies of Amlo press tablet Vs. F3

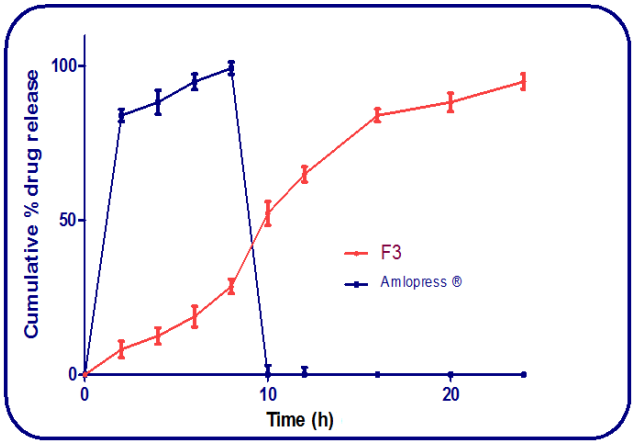

Fig. 5: Comparative *In vitro* drug dissolution studies of Amlo press tablet Vs. F3 Amlodipine nanoparticle (*mean±SD; n=3)*

F3 Amlodipine nanoparticle vs. Marketed Amlo press Tablet comparative *in vitro* drug dissolution investigations were graphed in fig. 11. The F3 Amlodipine nanoparticle (F3) drug release pattern was found to be 94.88±2.45% at 24h, and the commercialized Amlopres® tablet was found to be 92.82±2.48 percent at 6thhr. The drug release data concluded that the F3 Amlodipine nanoparticle displayed a unique controlled drug release pattern. This could be attributed to the excellent entrapment efficiency effect. The release of drugs from nanoparticles obeys zero-order release kinetic design. The higher entrapment efficiency of amlodipine in nanoparticles leads to the enhanced drug content entangled in it; on the other hand, it leads to a controlled release pattern, possibly because of its high viscous network between the drug and polymer. From this data, it was inferred that the F3 nanoparticle was deemed to be a more promising drug carrier for amlodipine than the marketed Amlopress®Tablet.

CONCLUSION

In this work, amlodipine besylate-loaded polymeric nanoparticles were successfully prepared by an emulsion solvent evaporation method using biodegradable polymer PLGA. Different preparation variables include sonication time, polymer amount, surfactant concentration, and volume of organic solvent. Analysis of morphology confirmed that the nanoparticles were spherical with a nearly smooth surface. The preferred formulation, F3, has a particle size of 198.8±5.25 nm, 0.957±0.45 PDI, and-24.76±2.54 Zeta potential as 93% drug entrapment efficiency and process yield. *In vitro* release studies indicated a non-fiction or anomalous type of transport for releasing amlodipine besylate from the nanoparticles. Further *in vivo* studies are necessary to support the findings.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest concerning the article's research, authorship, and publication.

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