Evaluation and Comparison of the Formulations of Amlodipine-Loaded Bovine Serum Albumin and Egg Albumin Microspheres

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Abstract: The main aim of this research is to compare amlodipine-loaded bovine serum albumin (BSA) and egg albumin microspheres, to obtain the optimal formulation of amlodipine microspheres for the treatment of hypertension. The ability to load various drugs, higher cell uptake and controlled kinetics for drug release are exceptional benefits offered by this system. In addition, the rate of chemical degradation in the solid matrix can also be modified, and offers delivery of chemically–sensitive lipophilic ingredients. On account of these benefits, this study was designed for the development of amlodipine microspheres for oral administration, and will be a beneficial approach to achieving increased bioavailability. Microspheres can be prepared by industrially-scalable techniques. Bovine serum albumin (BSA) microspheres containing amlodipine besylate were prepared by using emulsification cross-linking method. In this method, glutaraldehyde is used as a cross-linking agent. Egg albumin microspheres containing amlodipine besylate were prepared by using an emulsification heat denaturation method. The parameters studied in this research include particle size analysis, entrapment efficiency, in-vitro drug release studies, SEM analysis and Fourier Transform Infra Red Spectroscopy (FTIR) studies. The results indicate that the amlodipine-loaded BSA microspheres provide more prolongated action when compared to that of amlodipine-loaded egg albumin microspheres.

Keywords: amlodipine, microspheres, bovine serum albumin, scanning electron microscopy, FTIR.

氨氯地平负载牛血清白蛋白和卵白蛋白微球制剂的评价和比较

摘要：本研究的主要目的是比较载有氨氯地平的牛血清白蛋白（BSA）和卵白蛋白微球，以获得氨氯地平微球治疗高血压的最佳配方。装载各种药物的能力、更高的细胞摄取和药物释放的受控动力学是该系统提供的特殊优势。此外，固体基质中的化学降解速率也可以改变，并提供化学敏感的亲脂性成分。考虑到这些益处，本研究旨在开发用于口服给药的氨氯地平微球，并将成为提高生物利用度的有益方法。微球可以通过工业上可扩展的技术来制备。采用乳化交联法制备了含有苯磺酸氨氯地平的牛血清白蛋白（BSA）微球。在该方法中，戊二醛用作交联剂。采用乳化热变性法制备含有苯磺酸氨氯地平的蛋清微球。本研究中研究的参数包括粒度分析、包封率、体外药物释放研究、扫描电镜分析和傅里叶变换红外光谱(红外光谱)研究。结果表明，与载有氨氯地平的蛋清蛋白微球相比，载有氨氯地平的BSA微球的作用更持久。

关键词： 氨氯地平、微球、牛血清白蛋白、扫描电子显微镜、红外光谱。

1. Introduction

A novel drug delivery system focuses on delivery of a drug to the targeted site in order to obtain maximum therapeutic efficacy, and to overcome the problems caused by conventional drug delivery systems [1], [2]. There are different ways to deliver a therapeutic substance to the target site in a sustained release

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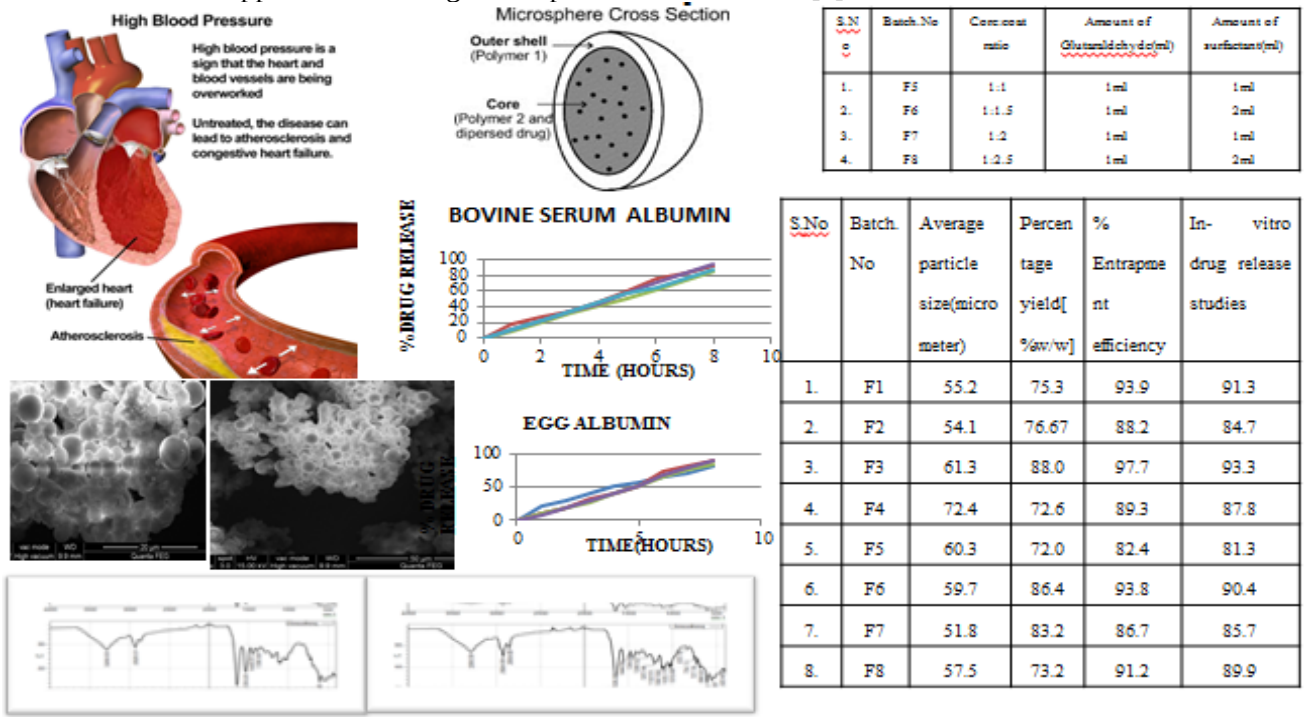
fashion. One of such approaches is using microspheres as carriers [3].



S.No	Batch.No	Concentration ratio	Amount of Glutaraldehyde(ml)	Amount of surfactant(ml)
1.	F5	1:1	1ml	1ml
2.	F6	1:1.5	1ml	2ml
3.	F7	1:2	1ml	1ml
4.	F8	1:2.5	1ml	2ml

S.No	Batch. No	Average particle size(micro meter)	Percentage yield[%w/w]	% Entrapment efficiency	In- vitro drug release studies
1.	F1	55.2	75.3	93.9	91.3
2.	F2	54.1	76.67	88.2	84.7
3.	F3	61.3	88.0	97.7	93.3
4.	F4	72.4	72.6	89.3	87.8
5.	F5	60.3	72.0	82.4	81.3
6.	F6	59.7	86.4	93.8	90.4
7.	F7	51.8	83.2	86.7	85.7
8.	F8	57.5	73.2	91.2	89.9

Fig. 1 Evaluation and comparison for the formulations of amlodipine-loaded bovine serum albumin and egg albumin microspheres

Microspheres constitute a vital part of this novel drug delivery system, by virtue of their small size and efficient carrying capacity. Microspheres are typically free-flowing powders comprised of proteins or synthetic polymers, with particles of varied size (ranging from 1-1000 μm), which are biodegradable in nature [4], [5], [6], [7]. In some cases, microspheres are also known as micro-particles [8], [9]. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through a particle matrix have the potential for the controlled release of drugs. They are comprised of polymeric, waxy, or other protective materials; that is, biodegradable synthetic polymers and modified natural products.

Microspheres can be obtained from several natural polymers such as BSA [10], [11], [12], egg albumin [13], [14], [15], chitosan [16], [17], starch [18], [19], poly dextran [20], [21], and synthetic polymeric materials such as polylactic acid [22], [23], polyadipic anhydride [24]. There are various techniques used for microsphere preparation: single-emulsion [25], [26], double-emulsion [27], [28], polymerization [29], [30], phase separation coacervation [31], [32], spray drying and spray congealing [33], [34], and solvent extraction [35], [36].

Amlodipine besylate is a calcium channel blocker that dilates blood vessels and improves blood flow, which is why it is used in the treatment of hypertension [37], [38], [39]. Hypertension is a condition that causes the blood pressure in the arteries to be persistently raised. High blood pressure does not typically cause symptoms, although in the long term it is a major risk factor for coronary artery disease [41], stroke [42], and heart failure [40, 43]. The microsphere formulation has shown a decreased frequency in dosing by improving patient compliance. The formulation of microsphere is economical and has also shown a reduction in side effects and decreased toxicity.

The aim of the present study was to compare amlodipine-loaded bovine serum albumin (BSA) microspheres and egg albumin microspheres as well as to determine the formulation that results in the best bioavailability of the drug to treat hypertension.

2. Material and Methods

2.1. Materials

The amlodipine besylate used in this study was provided free of charge by Madras Pharmaceuticals Pvt. Ltd. (Chennai, India). The bovine serum albumin (Southern India Scientific Corporation, Chennai, India), egg albumin (Southern India Scientific Corporation), glutaraldehyde (Southern India Scientific Corporation), liquid paraffin (Southern India Scientific Corporation), Tween80 (Southern India Scientific Corporation), and Span80 (Southern India Scientific Corporation) were all obtained from the manufacturer. The other ingredients like potassium dihydrogen phosphate, sodium hydroxide, ethanol, and hydrochloric acid were obtained from local suppliers.

2.1.1. Preparation of Amlodipine-Besylate-Loaded Bovine Serum Albumin Microspheres [12]

The BSA microspheres containing amlodipine besylate were prepared using the emulsion cross-linking method. The desired quantity of the drug (200 mg) was weighed and dissolved in BSA solution in water (20%, 2 ml) in a beaker. The drug-polymer

mixture was poured dropwise into a beaker containing 50 ml of liquid paraffin, which was kept on a magnetic stirrer and stirred at a speed of approximately 2500 rpm. Then, 1% wt/vol Span80 was added as a surfactant to the oil phase. After 15 mins of stirring, glutaraldehyde was added as a cross-linking agent and stirring was continued for 4–6 hrs. Next, the stirring was stopped and the albumin microspheres were filtered and cleaned using n-hexane to remove any excess oil. This procedure was followed for all four batches of amlodipine microspheres containing BSA, which used different concentrations of BSA and Span80 (F1, F2, F3, and F4). The compositions of these formulations are given in Table 1.

Table 1 Composition of amlodipine microspheres by using bovine serum albumin

S. No	Batch. No	Core: Coat ratio	Amount of cross-linking agent (ml)	Amount of surfactant (ml)
1.	F1	1:2	1 ml	1 ml
2.	F2	1:2	1 ml	2 ml
3.	F3	1:4	1 ml	1 ml
4.	F4	1:4	1 ml	2 ml

2.1.2. Preparation of Amlodipine-Besylate-Loaded Egg Albumin Microspheres [15]

The egg albumin microspheres containing amlodipine besylate were prepared using the emulsification and heat denaturation method. A suitable quantity of the drug was weighed (0.5 g) and dissolved in egg albumin solution in water (12.5 ml). This solution was added to 50 ml liquid paraffin, which was preheated to 60ºC. Tween80 was then used as an emulsifying agent, and the contents were stirred for approximately 1 hr using a magnetic stirrer. The temperature of the contents was reduced to 40ºC for 25 minutes to facilitate the hardening of microspheres. The resulting microspheres were stabilized using glutaraldehyde (25% v/v) solution for approximately 15 mins. The microspheres were then collected by means of decantation, cleaned using n-hexane, and dried at room temperature. This procedure was followed for all four batches of egg albumin microspheres containing amlodipine, which used different concentrations of egg albumin and Tween 80 (F5, F6, F7, and F8). The compositions of these formulations are given in Table 2.

Table 2 Composition of amlodipine microspheres by using egg albumin

S. No	Batch. No	Core: coat ratio	Amount of Glutaraldehyde (ml)	Amount of surfactant (ml)
1.	F5	1:1	1ml	1 ml
2.	F6	1:1.5	1 ml	2 ml
3.	F7	1:2	1 ml	1 ml
4.	F8	1:2.5	1 ml	2 ml

2.1.3. Construction of a Standard Calibration Curve for Amlodipine

Some 100 mg of amlodipine was accurately weighed and transferred into a 100 ml standard flask. A small quantity of ethanol was added to solubilize the drug. Then, the volume was made up to 100 ml with ethanol. This gave the stock solution. The stock solution was diluted with a phosphate buffer (pH 6.8) to give concentrations of 5, 10, 15, 20, and 25 µg/ml. Next, the absorbance of the solution was measured at 239 nm using an ultraviolet (UV)-visible spectrometer. A graph was plotted by taking the concentration on the x-axis and the absorbance on the y-axis to obtain a standard curve. The standard curve was used to determine the amount of amlodipine in the present study.

2.2. Evaluation of the Amlodipine Microspheres

2.2.1. Particle Size Analysis

The particle size analysis was performed by means of optical microscopy. Approximately 200 microspheres were randomly selected and their sizes determined using an optical microscope fitted with a standard micrometer scale.

2.2.2. Determination of the Drug Content of the Microspheres

About 50 mg of microspheres were accurately weighed and then crushed in a pestle and mortar. The resulting powder was transferred to a 50 ml standard flask and dissolved in 0.1 N hydrochloric acid, and the volume was made up to 50 ml. The solution was then filtered using Whatman filter paper. Next, 1 ml of filtered solution was added to a 10 ml standard flask and diluted to 10 ml using 0.1 N hydrochloric acid. The absorbance of the solution was measured at 240 nm and compared with standard amlodipine.

2.2.3. Percentage of Entrapment Efficiency

The percentage of entrapment efficiency was calculated using the following formula:

$$Percent\ entrapment\ efficiency = \frac{Entrapped\ drug}{Total\ drug} \times 100$$

2.2.4. Shape and Surface Morphology

The shape and surface characteristics of the microspheres (i.e., roundness, smoothness, and formation aggregates) were assessed using a scanning electron microscopy (SEM), while the size distribution of the microspheres was determined using an optical microscopy.

2.2.5. FTIR Studies

FTIR studies were conducted to determine the interaction between the drug and the polymer.

2.2.6. In-Vitro Drug-Release Studies of Amlodipine-Loaded Bovine Serum Albumin and Egg Albumin Microspheres

The amlodipine-loaded BSA and egg albumin microspheres were evaluated with regard to their *in-vitro* drug release. The drug release analysis of the microspheres was performed using USP Dissolution Apparatus-I (Basket Apparatus) with 0.1 N hydrochloric acid used as the dissolution medium. The dissolution was carried out at 100 rpm and 37±0.5ºC using 900 ml of dissolution medium. From each batch, microspheres equivalent to 50 mg were accurately weighed and added to hard gelatin capsules. The capsules were placed in the basket and then immersed in 900 ml of dissolution medium. After immersion, the dissolution process was carried out. Some 5 ml of sample solution was withdrawn from the dissolution apparatus at a fixed interval of 1 hr and the same volume of fresh 0.1 N hydrochloric acid was used to replace it. This process was carried out for 8 hrs. The withdrawn samples were assessed with regard to their amlodipine content at 240 nm using a UV-visible spectrometer and the amount of drug release was interpreted using the standard calibration curve. The percentages of the drug released at various time intervals were calculated and plotted against time vs. % drug release.

The standard curve of amlodipine is depicted in Fig. 2.

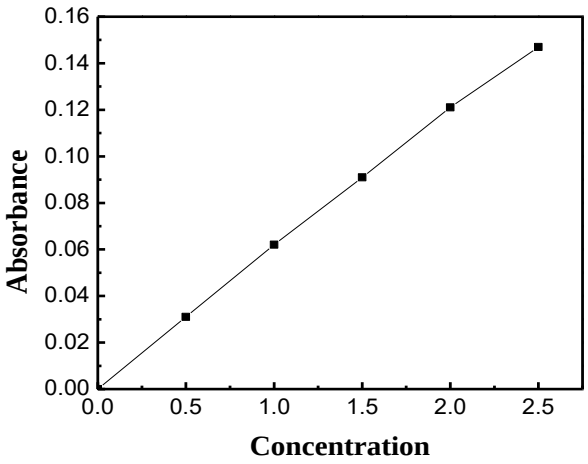


Fig. 2 Calibration curve of amlodipine

3.1. FTIR Studies

Compatibility studies between drug (amlodipine) and polymers can be obtained by FTIR Analysis. From Fig. 3-9, we have found identical peaks between the IR spectra of amlodipine and different combined samples. Thus, there is no interaction found between the drug and excipients in the preparation of amlodipine microspheres.

3. Results and Discussion

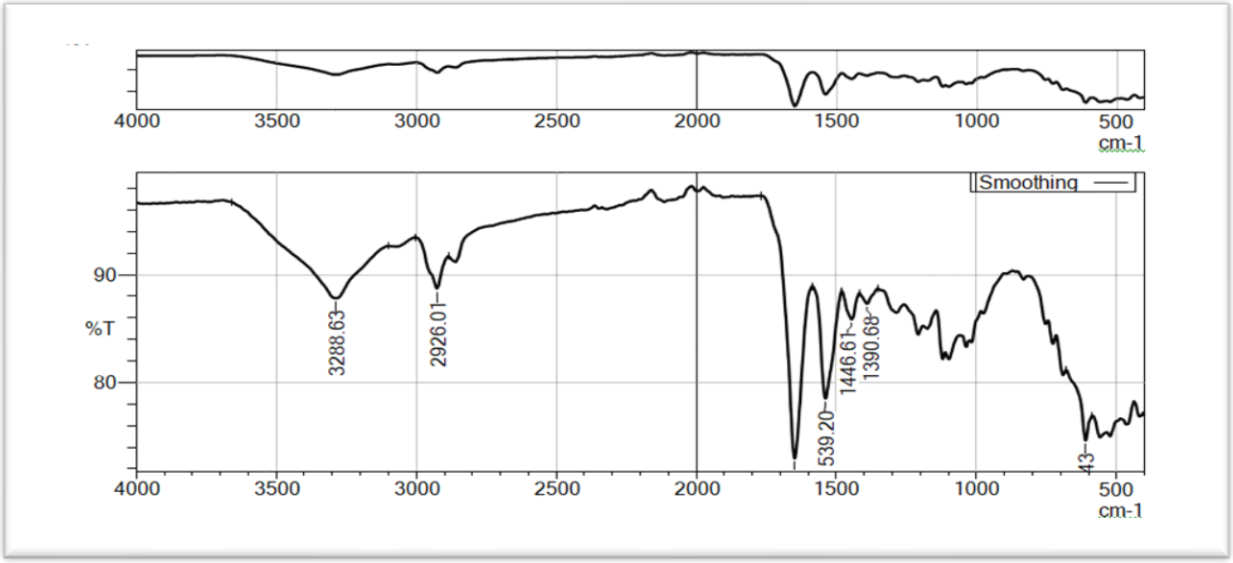


Fig. 3 FTIR of amlodipine (pure drug)

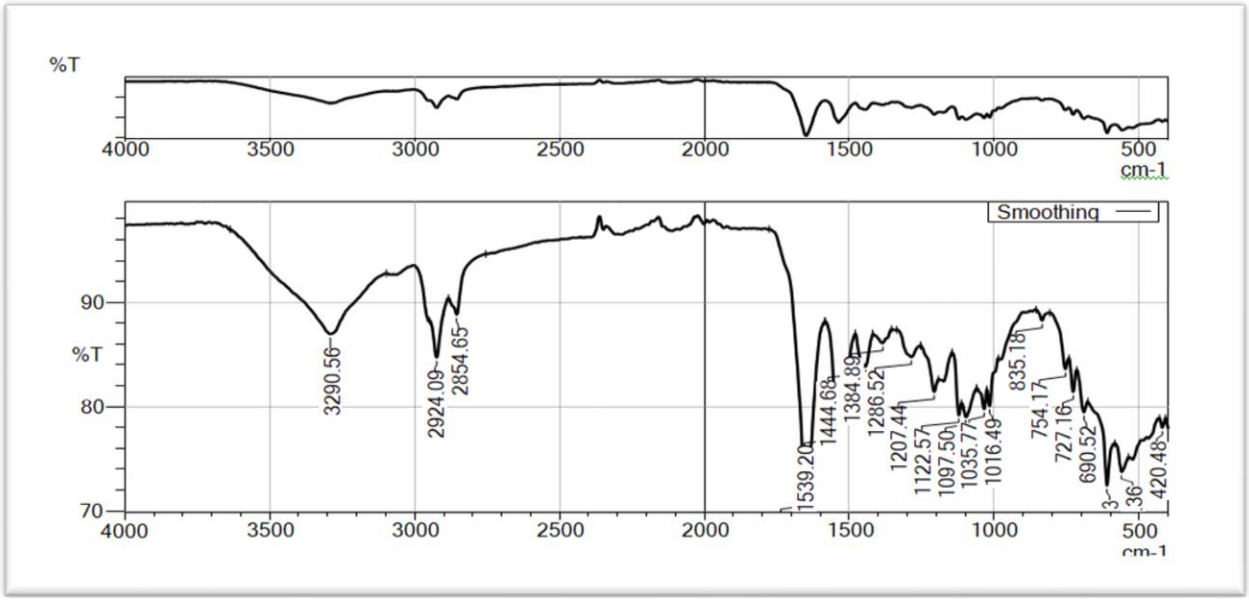


Fig. 4 FTIR of BSA (raw material)

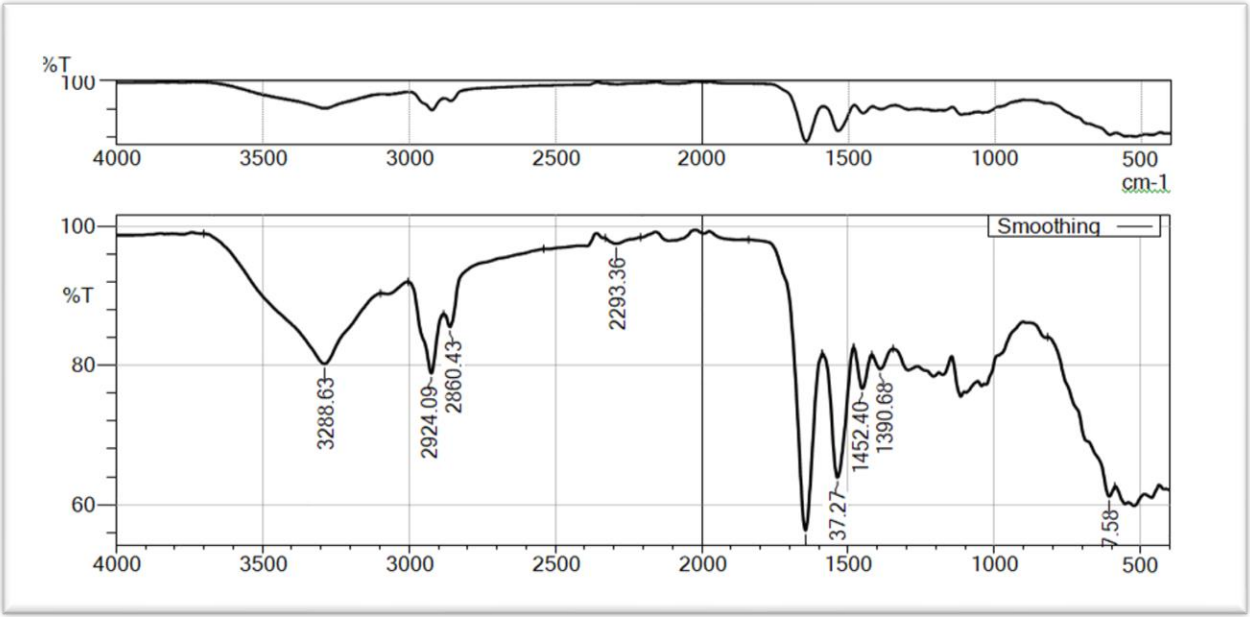


Fig. 5 FTIR of amlodipine + BSA (raw material)

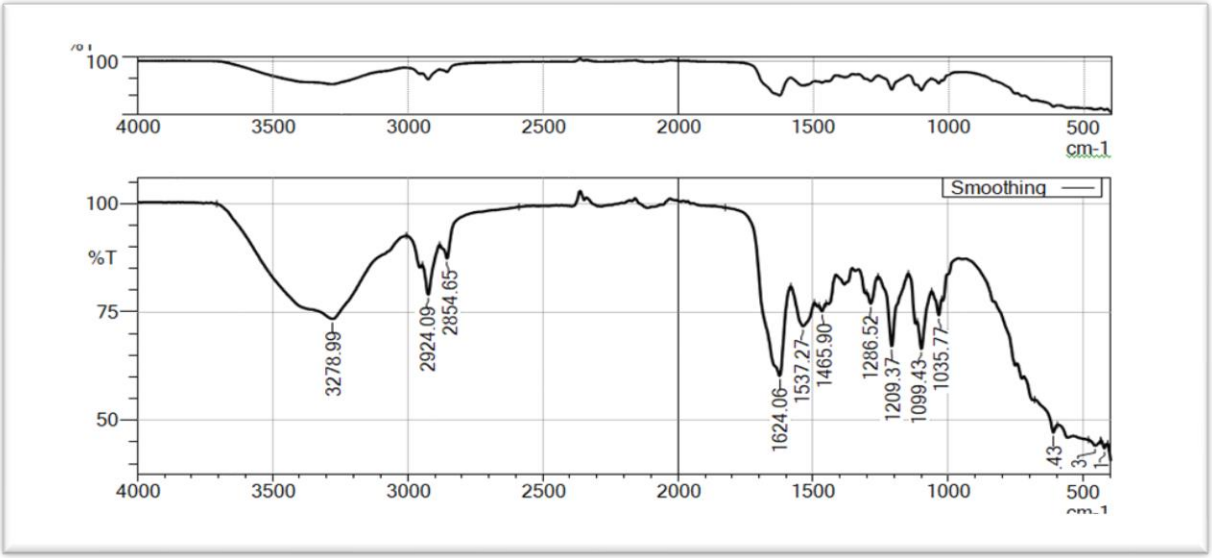


Fig. 6 FTIR of amlodipine-BSA microspheres

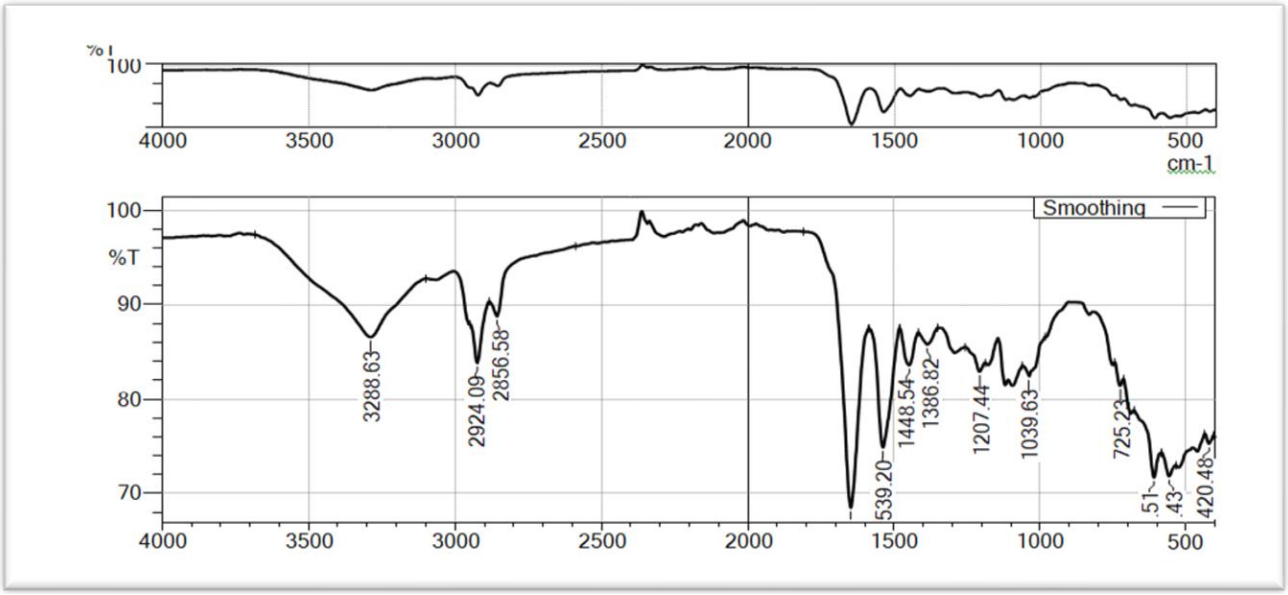


Fig. 7 FTIR of egg albumin (raw material)

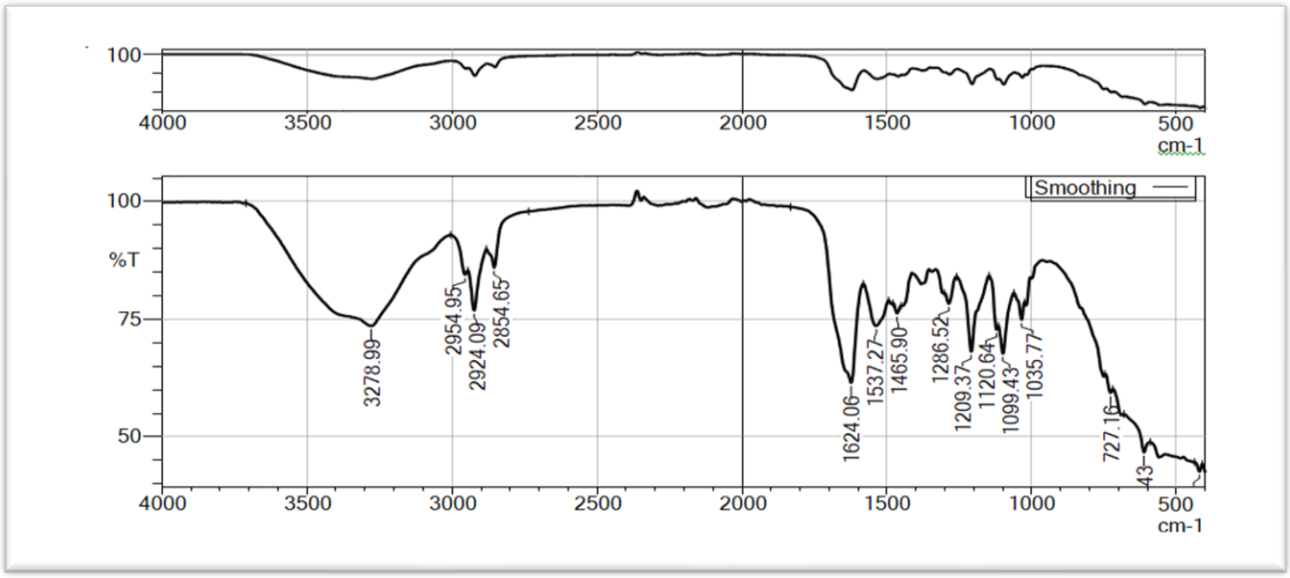


Fig. 8 FTIR of amlodipine + egg albumin (raw material)

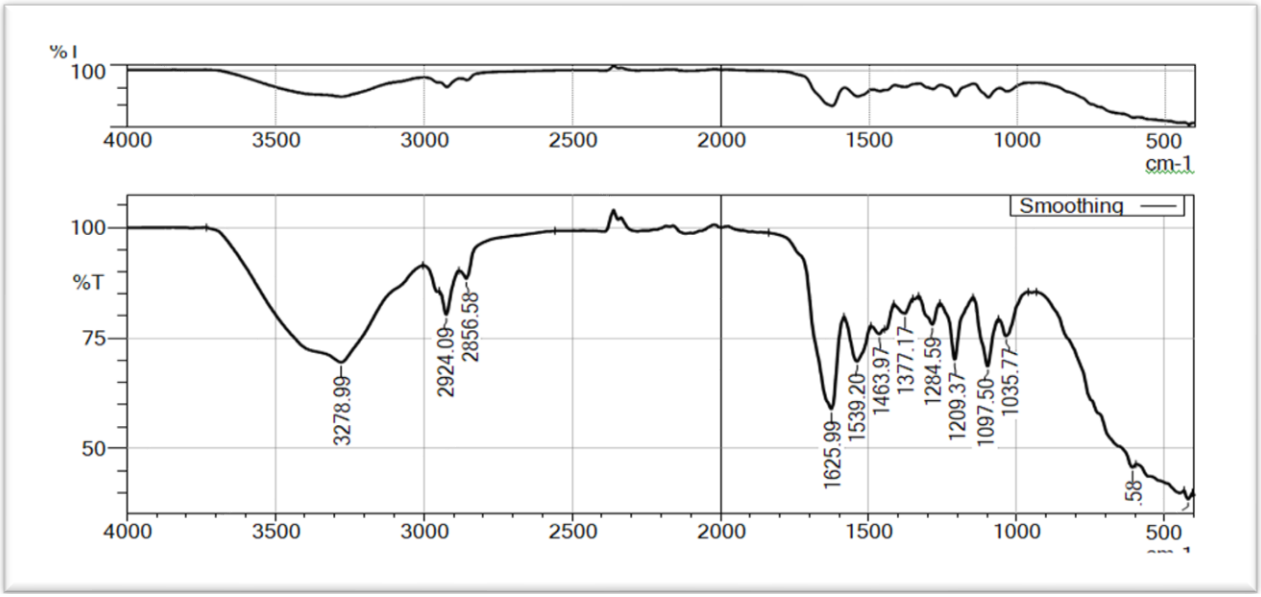


Fig. 9 FTIR of amlodipine - egg albumin microspheres

3.2. Particle Size Analysis

The particle size of eight batches of amlodipine microspheres was performed by optical microscope, and the results were given the Table 3. The particle size of the formulated microspheres was found to be within the range of 50-75 µm. The particle size distribution of the microparticles is presented in Table 3. The mean particle size (n = 50) of the microparticles ranged from 50.05 ± 8.82 µm to 72.40 ± 10.43 µm. Microparticles formulated with F3 formulation consisting of BSA give smallest particle size.

Table 3 Evaluation of microspheres

S. No	Batch. No	Average particle size (micrometer)	Percentage yield [% w/w]	% Entrapment efficiency	In-vitro drug release studies
1.	F1	55.2 ± 10.55	75.3 ± 1.35	93.9 ± 4.55	91.3 ± 1.41
2.	F2	54.1 ± 11.50	76.67 ± 3.08	88.2 ± 3.58	84.7 ± 2.52
3.	F3	61.3 ± 5.55	88.0 ± 2.55	97.7 ± 1.50	93.3 ± 1.87
4.	F4	72.4 ± 9.55	72.6 ± 1.02	89.3 ± 3.55	87.8 ± 2.04
5.	F5	60.3 ± 8.34	72.0 ± 2.08	82.4 ± 1.04	81.3 ± 3.55
6.	F6	59.7 ± 6.50	86.4 ± 5.42	83.8 ± 1.57	85.4 ± 5.44
7.	F7	51.8 ± 12.05	83.2 ± 1.58	86.7 ± 3.59	85.7 ± 2.46
8.	F8	57.5 ± 10.46	73.2 ± 1.25	8 1.2 ± 1.44	79.9 ± 2.33

3.3. Percentage Yield

The percentage yield of these formulations ranges from 72% to 88%, while the F3 formulation gives the maximum yield of 88%. The results presented in Table 1 show that the microparticle yield (percentage yield) was high. Particularly noteworthy is the batch that had the highest yield of 88 %. This occurred in microspheres containing BSA.

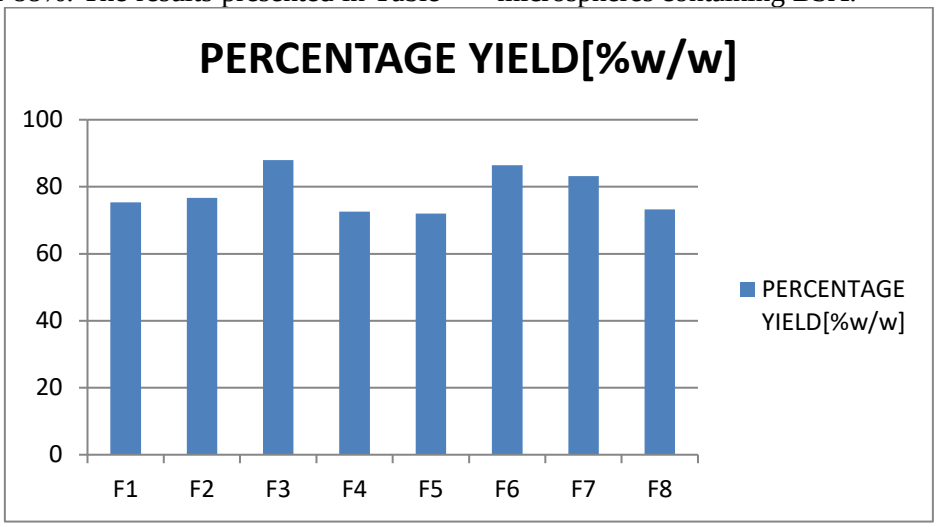


Fig. 10 Percentage yield

3.4. Percentage of Drug Entrapment Efficiency

Entrapment efficiency of amlodipine-loaded BSA and Egg albumin microspheres are given in Table 3 and Fig. 11 and 12, respectively. In case of BSA formulations, F3 gives the maximum entrapment efficacy, while in case of Egg albumin formulations F6 gives the maximum entrapment efficacy of 86.7%. But in comparison between these two formulations, F3 formulation consisting of BSA gives the highest entrapment efficacy of about 97.7%. The general pattern was that drug entrapment decreased with increasing proportions of the BSA used in preparing the microspheres.

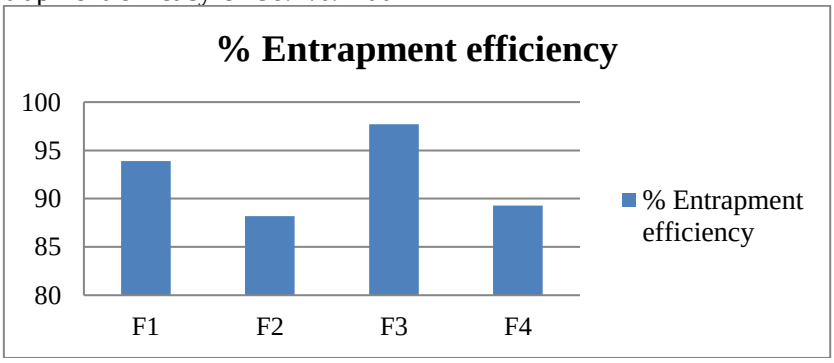


Fig. 11 The entrapment efficiency of amlodipine-loaded BSA microspheres

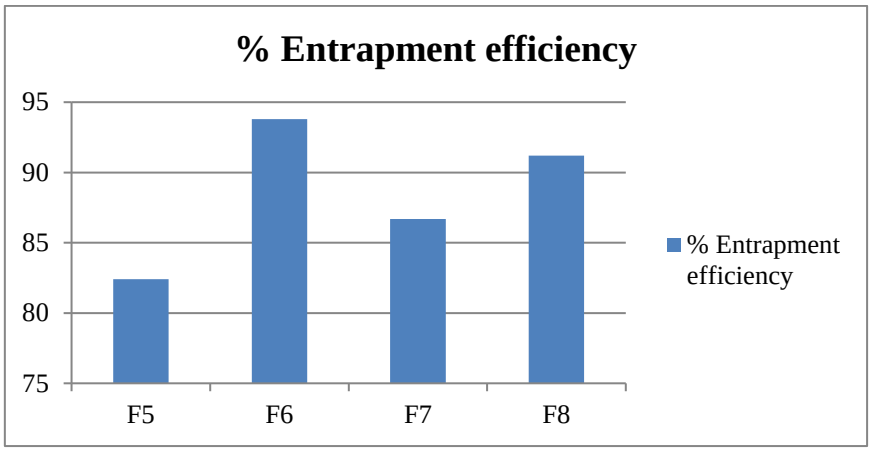


Fig. 12 The entrapment efficiency of amlodipine-loaded egg albumin microspheres

3.5. In-Vitro Release Studies for Amlodipine Microspheres by Using Bovine Serum Albumin and Egg Albumin

The in-vitro drug release studies of Amlodipine loaded BSA formulations (F1 to F4) and Egg albumin formulations (F5 to F8) are given in the Table 3 and Fig. 13 and 14. When we observe the in-vitro drug release of BSA formulations, F3 formulation showed the maximum release of 93.3% at the end of 8hrs, while in case of Egg albumin microspheres, F6 formulation showed the maximum release of 85.4% at the end of 8hrs. In comparison of F3 BSA formulation and F6 Egg albumin formulation, F3 showed the highest in-vitro drug release which is evident from Fig. 13. So, F3 formulation made up of BSA polymer was found to be the optimized formulation which has a composition of a core: coat ratio of 1:4 and a surfactant: crosslinking agent ratio of 1:1. The release profiles of Amlodipine from the microspheres are graphically represented in Fig. 13 and 14. There was a sustained release of the drug from most of the microspheres. However, there was an initial rapid release of amlodipine from the formulation of F3 BSA formulation and F6 egg albumin formulation within 2 h. A characteristic feature of the release profile of F3 BSA formulation and F6 egg albumin formulation batches is a biphasic pattern of release.

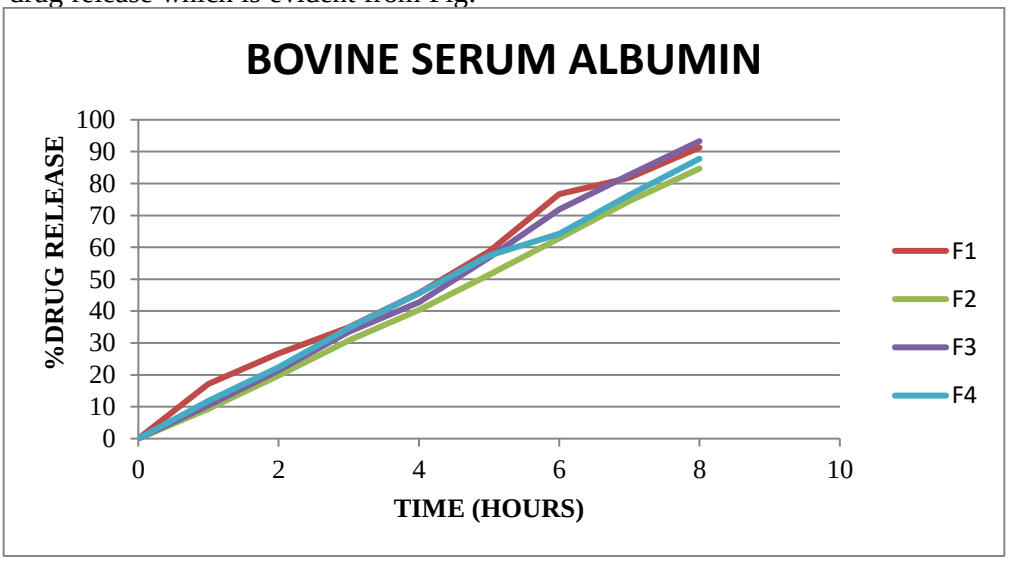


Fig. 13 In-vitro drug release profile of amlodipine-loaded BSA microspheres

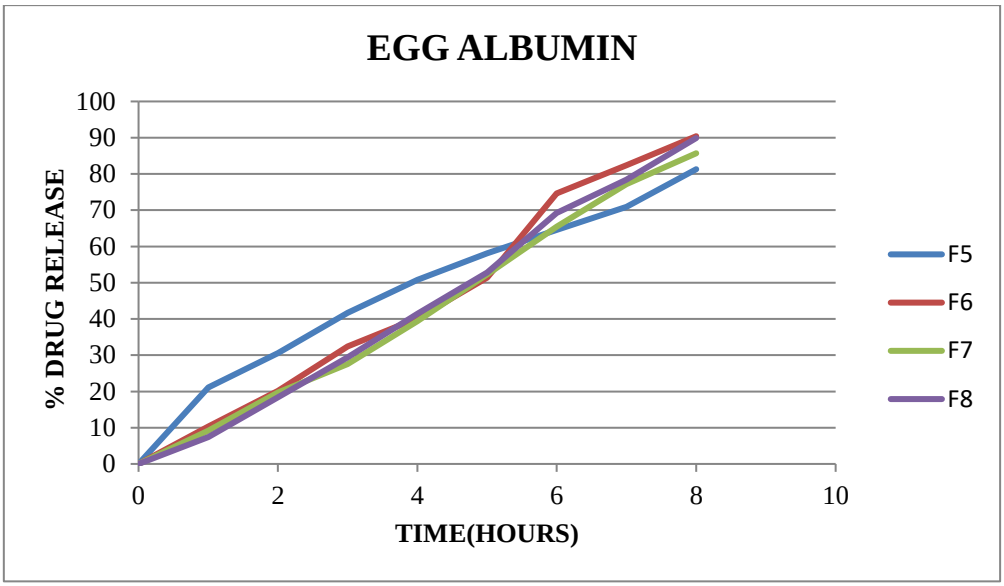


Fig. 14 In-vitro drug release profile of amlodipine-loaded egg albumin microspheres

3.6. Scanning Electron Microscopy

The SEM photographs of the microspheres F3 and F6 were examined using SEM-JEOL, JSM-840. Comparing the F3 and F6 formulations, the F3 formulation made up of BSA was found to be more spherical and smoother, which is identified as the optimised formulation. The SEM photomicrographs of the F3 BSA formulation and the F6 egg albumin formulation showed that the microspheres had a brownish and spherical appearance except for some batches that appeared to have some degree of hydration and irregular shapes.

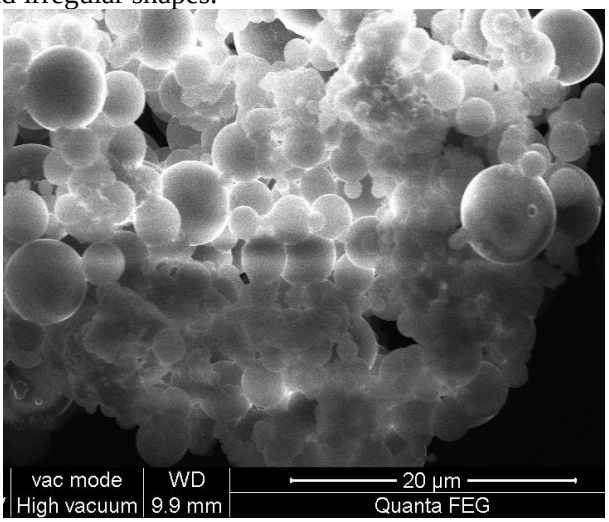


Fig. 15 SEM of F3

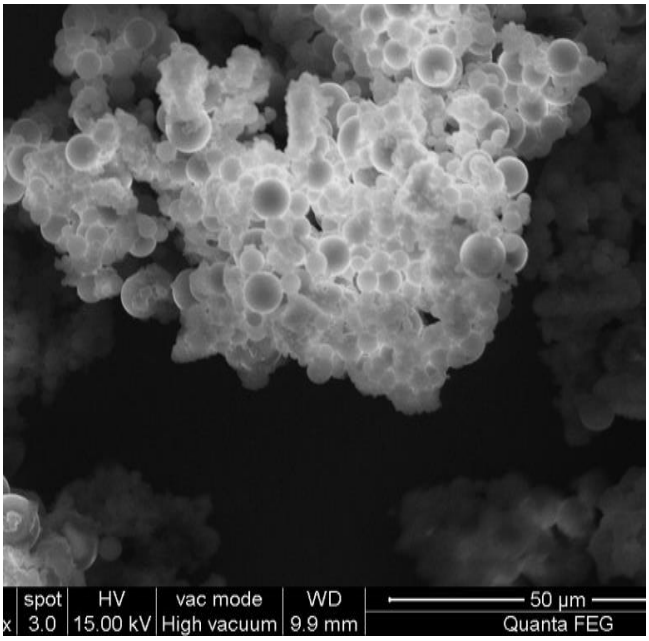


Fig. 16 SEM of F6

4. Discussion

The sizes of the microspheres were all within the micrometer range, indicating that the production process was able to achieve the intended endpoint. It appears that the average size of the microspheres increased with an increase in the proportion of the cross-linking agent and the polymers employed. However, the microspheres formulated with egg albumin were larger than the other batches, especially microspheres formulated with bovine serum albumin. The microspheres evaluated in this study are intended for oral administration and particle size would influence the rate of drug release and followed by pharmacokinetics . The microspheres' yield was high, an indication that the formulation technique adopted was reliable. There was no evidence of correlation between the proportion of cross-linking agent used in the formulation of microspheres and the microsphere

yield. The various factors that influence the swelling of polymers when prepared by cross-linking techniques are the time taken for cross-linking and the concentration of the cross-linking agent. By implication, encapsulation of amlodipine in bovine serum albumin (BSA) cross-linked Span 80 microspheres would cause a tiny amount of the drug to be released into the stomach and further enhance targeting of the intestines, where the desired sustained release effects would be achieved. This would likely be the optimum amount of the cross-linking agent needed to produce microspheres for the sustained release dosage form. Drug entrapment efficiency is an important variable for assessing the drug loading capacity of microspheres and their drug release profiles, thus suggesting that the drug would be released in a sustained manner. The rapid release of amlodipine from batches F3 and F6 during the first 30 minutes is possibly due to a burst effect caused by the leaching out of the unentrapped drug adhering to the surface of the microspheres after the initial rapid hydration and swelling. Burst release resulting in a biphasic release pattern may be utilized in the therapeutic design of dosage forms. This has been observed in microspheres prepared using bovine serum albumin. In this case, it may be an advantage because it would lead to a high initial blood concentration of the drug and a gradual release of the remaining medication. This indicates that once the drug adhering to the microspheric surface has leached, the drug release becomes diffusion-controlled. Based on the observations, the formulated microspheres of amlodipine could be widely accepted, physiologically safe, and capable of exhibiting sustained properties.

5. Conclusion

Amlodipine-loaded microspheres were successfully prepared using BSA with the emulsification cross-linking technique and egg albumin microspheres using the emulsification heat denaturation method. In this study, glutaraldehyde-saturated toluene is used as the cross-linking agent. The present study concentrated on obtaining optimized formulation by comparing amlodipine microspheres prepared using different biodegradable polymers. Results obtained conclude that the F3 formulation made with BSA is the best compared to the F6 formulation made with egg albumin. The formulated microsphere of amlodipine could be widely accepted, and physiologically safe polymers could exhibit sustained-release properties.

In conclusion, the microspheres of amlodipine designed in this work were shown to be efficient drug delivery systems for poorly soluble drugs. It can be concluded that the application of the microspheres of amlodipine in the clinical setup may be useful in achieving the complete therapeutic advantage of the calcium channel blockers and can effectively control hypertension. Hence, the bio-polymeric carrier formulated with BSA for sustained-release delivery of amlodipine was found to render acceptable drug entrapment efficiency (97.7% w/v). The percentage drug release rate was found to be 93.3% w/v. Thus, the novel formulation of BSA-amlodipine gives a newer prolongation of action, which can be used to treat hypertension.

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