

Development and Characterization of Amlodipine Besylate-Loaded Microsponges for Topical Wound Management

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Abstract— The present study was undertaken to formulate and evaluate amlodipine besylate-loaded microsponges intended for topical wound management, followed by incorporation of the optimized microsponges into a topical gel. Amlodipine besylate was selected as a model drug based on its reported potential to support wound healing through vasodilatory, angiogenic and tissue-remodelling effects. Microsponges were prepared by the quasi-emulsion solvent diffusion method using ethyl cellulose and Eudragit RS 100 as polymers, with polyvinyl alcohol as the stabilizer. The formulations were optimized by studying the effects of polymer type, drug-to-polymer ratio, internal phase composition and volume, external phase volume, PVA concentration and stirring speed. The prepared microsponges were evaluated for production yield, drug content, entrapment efficiency, particle size, zeta potential, polydispersity index, surface morphology and in-vitro drug release. Among the investigated formulations, the ethyl cellulose formulation containing a 1:2 drug-to-polymer ratio, DCM:ethanol (5:5 mL), 500 mg PVA, 90 mL external phase and 600 rpm stirring speed showed the best overall characteristics, with 77% production yield, 61% drug content, 93% entrapment efficiency, 6.8 μ m particle size, -23 mV zeta potential and 47% drug release. The optimized microsponges were subsequently incorporated into Carbopol 934 gel and evaluated for physical appearance, pH, clarity, spreadability and viscosity. The developed microsphere-based topical gel demonstrated the potential to provide a suitable topical delivery system for amlodipine besylate and may offer advantages of controlled drug release, improved drug retention and ease of application for wound-management applications.

Index Terms— Amlodipine, microsphere, topical delivery, wound healing sustained release.

I. INTRODUCTION

Injury is physical harm that happens when an individual is unexpectedly exposed to energy levels higher than his physiologic tolerance threshold. In the world, injuries are ranked fourth in terms of fatalities, accounting for 11 % of all fatalities, after neoplastic, infectious, and cardiovascular disorders. The main goal of wound management is to keep patients safe from serious infections, speed up wound healing, and reduce scars and pain. Currently, a variety of wound control therapies are available including debridement, autografts, and the application of therapeutic agents. The use of topical drugs, which primarily aim to promote healing and prevent infection, is still essential in the treatment of all types of wounds. As a result, there still a high demand for novel therapeutic agents for topical wound therapy.⁽¹⁾

Amlodipine besylate (ADB), a third-generation dihydropyridine calcium channel blocker, which is widely used in a variety of cardiovascular circumstances, may also play a role in non-cardiac conditions. According to some in-vitro studies, amlodipine was shown to be capable of promoting the healing process by regulation of collagen production, extracellular matrix, re-epithelialization and wound healing through its vasodilation and angiogenic activity. Moreover, Amlodipine was found to improve endothelial NO (nitric oxide) bioavailability by enhancing NO production and extending NO half-life via its antioxidant properties. It was found that, upon using conventional topical formulations such as ointments, creams and gels, the skin functions as a barrier, limiting drug permeation. As a result, conventional topical therapies require a higher dose

for optimal drug concentration delivery into the skin layers, which may probably cause skin irritation. microsponges with loaded active ingredient are of choice, incorporated into gels, creams, lotions, and powders.⁽²⁾

Gels are defined as semi rigid systems in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. Topical gels are more stable than creams and ointments, and can adhere well to the site of application. Thus, the proposed work is intended to provide the advantage of both, microsphere and gel for treating diabetic or burn wounds using amlodipine besylate as model drug.⁽³⁾

II. MATERIALS AND METHODOLOGY:

Chemical and reagents:

Amlodipine was provided by Alembic Research center, Bangalore, Eudragit S-100 and ethyl cellulose by SD fine chemicals Mumbai, polyvinyl alcohol supplied by Essel fine chem, Mumbai, Dichloromethane by Karnataka Fine chem, Bangalore. Ethanol by Karnataka chemicals, carbopol 934 by central drug House (p) Ltd New Delhi, Triethanolamine by T.V Industries estate, Mumbai, propylene glycol by S.D fine Chemical Ltd Mumbai, Methyl paraben supplied by Oxford Lab fine chem LLP, Maharashtra.

Preformulations studies:

Preformulation testing is the first step in the development of dosage form of a drug substance. It can be defined as early -stage scientific investigation that analyze the physical and chemical properties of new drug substance and combined with excipients. preformulation studies encloses a range of investigations, including characterization, stability assessment, and formulation compatibility evaluation.

Determination of melting point:

Determination of melting point gives an idea about purity of the drug. Melting point of Amlodipine was determined by capillary tube method. Fine powder of Amlodipine was filled with glass capillary (previously

sealed on one end). The capillary tube is tied to thermometer and the thermometer was kept in tube containing liquid paraffin. The Assembly was kept on heating temperature was allowed to increase gradually temperature at which the powder melts were noticed.

FTIR study of amlodipine and optimised formulation:

Identification of amlodipine was carried out by FT-IR spectrophotometry. Weighed amount of drug was mixed with IR grade KBr (1:10) under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000-1cm to 500-1cm range. A similar method was adapted for optimized formulation.

Preparation of calibration curve of amlodipine:

Stock solution 1: A standard stock solution was prepared by accurately weighing 50mg of amlodipine besylate and dissolving it in methanol buffer in 50ml volumetric flask containing 0.3ml (0.1N) HCL gives the 500µg/ml.

Stock solution 2: 1ml of stock solution 1 was taken and made up to 10 ml with methanol buffer which contains the concentration of 100µg/ml. From stock 2 solution 0.2, 0.4, 0.6, 0.8 and 1.0ml, were taken and made up to 10 ml with methanol to get the concentration ranges of 2, 4, 6, 8, and 100µg/ml respectively. The absorbance was measured against the methanol as blank at 239 nm using UV spectrophotometer. The study was carried out in triplicate (n=3). Then the calibration curve was plotted taking concentration on X-axis and mean absorbance on Y-axis.⁽⁴⁾

Preparation of amlodipine loaded microsponges by Quasi Emulsion method:

The procedure described here is commonly used to make topical and oral microsponges. The procedure involves the preparation of two phases, the internal phase contains the drug and polymers in a suitable solvents and external phase contains water and the surfactant, the internal phase is slowly added to the external phase under stirring, further the mixture is filtered to separate the microsponges and dried in the hot air oven.

Formulation of drug loaded microsponges:

Batch	Polymer type	Drug: polymer ratio (mg)	Type of internal phase	Volume of internal phase (ml)	Volume of external phase (ml)	Surfactant conc (ml)	Stirring speed (rpm)
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Influence of type of polymer

F1	Eudragit S 100	100:100	DCM:Ethanol	5:5	90	500	600
F2		100:100	DCM:Ethanol	5:5	90	500	600

Influence of polymer ratio

F3	E.C	100:200	DCM:Ethanol	5:5	90	500	600
F4	E.C	100:300	DCM:Ethanol	5:5	90	500	600

Influence of type of internal phase

F5	E.C	100:200	DCM:Acetone	5:5	90	500	600
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Influence of internal phase volume

F6	E.C	100:200	DCM:Ethanol	7.5:7.5	90	500	600
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Influence of external phase volume

F7	E.C	100:200	DCM:Ethanol	5:5	25	500	600
F8	E.C	100:200	DCM:Ethanol	5:5	50	500	600
F9	E.C	100:200	DCM:Ethanol	5:5	75	500	600

Influence of surfactant concentration

F10	E.C	100:200	DCM:Ethanol	5:5	90	750	600
F11	E.C	100:200	DCM:Ethanol	5:5	90	1000	600

Influence of stirring speed

F12	E.C	100:200	DCM:Ethanol	5:5	90	500	1000
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Evaluation of Amlodipine microsponges:

All formulations were evaluated for production yield, drug content, entrapment efficiency, particle size, zeta potential and In vitro drug release studies.

Morphological Characterization: ⁽⁵⁻⁷⁾

Physical appearance: Visual examination of the generated microsponges was done to check the shape, size and colour.

Determination of production yield:

The production yield of the microsponges was determined by calculating accurately the initial weight of the raw materials and the final weight of the microsponges obtained.

Production yield = $\frac{\text{practical mass of microsponges}}{\text{Theoretical mass}} \times 100$

Surface morphology studies

SEM analysis was done on the optimized microsponges' exterior morphology. The powders were imaged by a scanning electron microscope

(SEM) run at an accelerating voltage of 10kV using Hitachi SU 3500. The powder in few μg was fixed on to stub by a double-sided sticky carbon tape and kept inside the SEM chamber and analysed at different magnification such as 60X, 200X, 500X, 1.10X and 2.50X respectively to obtain better clarity on the particle morphology/ topology.

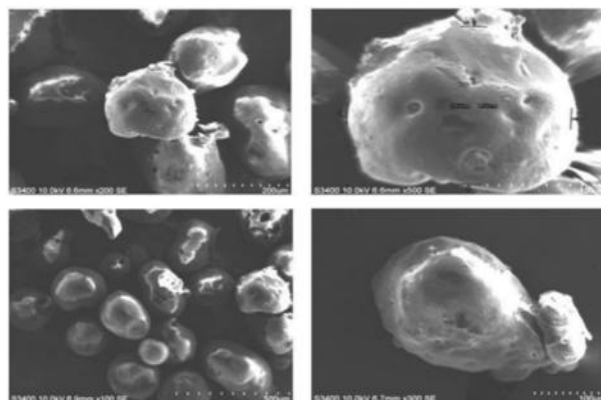


Fig No:01 Scanning Electron microscopic view of microsponges

Drug content:

Amlodipine content in microsponges was assayed by an UV spectrophotometric method. Microsponges equivalent to 10 mg of drug were dissolved in a 10 ml of methanol. After suitable dilution absorbance was measured by UV spectrophotometer against blank at Amax 238 nm and drug content was calculated

$$\% \text{Drug content} = \frac{\text{amount of drug in microsphere}}{\text{total amount of microsphere}} \times 100$$
Entrapment efficiency:

A sample of dried microsponges equivalent to 10 mg was taken into mortar and pestle and add little amount of methanol buffer and allowed to stand for 24 h. Then transfer content into 100 ml volumetric flask and make up volume to 100 ml with methanol buffer. The solution was filtered through Whatman filter paper No.41). From the resulting solution take out 1 ml transferred into 10 ml volumetric flask and then make up the volume to 10 ml by using same solvent. Entrapment efficiency was determined by UV spectrophotometer at 232 nm.

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{amount of drug untrapped}}{\text{total amount of drug}} \times 100$$
Particle size distribution:

Measurement of the particle size distribution and mean diameter of microsponges was carried out with an optical microscope. Stage micrometer was used to calculate the eye piece micrometer. 10 deviations of stage micrometer were matched with the deviation of eye piece micrometer and calibration factor was calculated. The particle size was calculated by multiplying the number of the deviation of the eye piece micrometer occupied by the particle with calibration factor. 30 randomly chosen microsponges taken to measure their individual size.

In vitro study:

In vitro release pattern of microsponges was carried out in dissolution apparatus USP Type-I (basket type dissolution apparatus) using methanol buffer as dissolution medium with a modified basket consisted of 5µm of stainless-steel mesh. Microsponges equivalent to 500 mg of drug was taken in the basket. The speed of the rotation is 50 rpm and temperature of 37± 0.5°C. At fixed intervals, aliquots 5 ml sample were withdrawn periodically and were replaced by

fresh buffer. The samples were assayed by UV spectrophotometer at 232 nm using phosphate buffer pH 7.4 as blank and % CDR was calculated and plotted against time.

Scanning Electron Microscopy:

The powders were imaged by a scanning electron microscope (SEM) run at an accelerating voltage of 10kV using ZEISS EVO 18 SEM. The powder in few µg were fixed on to stub by a double-sided sticky carbon tape and kept inside the SEM chamber and analysed at different magnification such as 60X, 200X, 500X, 1.10X and 2.50X respectively to obtain better clarity on the particle morphology.

Stability study of microsponges:

For the evaluation of stability studies, maintaining the formulations at an ambient condition over a period of one month. The physical appearance, rheological properties, pH value, drug content and drug release studies were determined periodically. Stability is defined as the extent to which a product remains within specified limits throughout its period of storage and use. A drug formulation is said to be stable if it fulfils the following requirements.

- It should contain at least 90 % of the stated active ingredient.
- It should contain an effective concentration of added preservatives, if any
- It should exhibit neither discoloration nor precipitation nor foul odor.
- It should not develop irritation or toxicity.

Stability studies were performed according to ICH guidelines. The optimized formulations were selected for stability studies. They were subjected to short-term stability studies. Formulation was divided into 2 sets of samples and stored at 5±3°C in the refrigerator and stored at room temperature (30°±2°C, 65%±5%RH), and their % Drug content and In vitro release were determined after 30 days

Formulation of microsphere entrapped amlodipine gel:⁽⁸⁻¹²⁾

An optimized microsphere containing amlodipine equivalent to 0.5 % w/w was incorporated into gel base composed of carbopol 934 and purified water.

Procedure

0.125g of carbopol 934 was dissolved in 25 ml of purified water and kept for stirring until the carbopol is dissolved completely to form a soft consistant gel. At the end 0.02% of methyl paraben a preservative is added.

Table no 2:
Formulation of gel

SI NO	Ingredients (percentage)	Gel A	Gel B	Gel C
1.	Carbopol 934(mg)	0.25	0.50	0.75
2.	Purified water (ml)	25±5	25±5	25±5
3.	Methyl paraben(ml)	0.2	0.2	0.2
4.	Gel texture	Low viscous	Soft gel with good texture	Hard gel

Evaluation of microsp sponge topical gel. ⁽¹³⁻¹⁸⁾

Physical characteristics:

The gels were inspected visually for its colour, clarity, consistency, spreadability.

pH measurments:

The pH of microsp sponge formulation was determined by using digital pH meter. 1gm of gel was dissolved in 100ml of distil water and it was placed for 2hr. the measurement of pH of each formulation was done in triplicate and average values were calculated.

Spreadability:

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 125g weight for 5 min. weight (1 g) was added to the pan. The time in which the upper glass slide.

$$\text{Spreadability} = M \times L / T$$

moves over to the lower plate was taken as measure of spread ability.

Where, M = Weight tied to the upper slide

L = Length moved on the glass.

T = Time Taken.

Clarity:

All developed gels were tested for homogeneity by visual inspection after the gels were set in the container and also for presence of any aggregate.

Viscosity measurment:

Brookfield viscometer was used for viscosity determination. The formulation (10g) was taken and it was allowed to calibrate for 5min before measuring the dial reading using spindle no 64 at 20rpm.

Invitro diffusion study:

The diffusion medium used was Phosphate buffer pH 7.4, carried out using Franz diffusion cell. The diffusion cell was placed on the magnetic stirrer; the outlet of the reservoir was maintained at $37 \pm 0.5^\circ\text{C}$. The receptor compartment was filled with Phosphate buffer pH 7.4, prehydrated cellophane paper was used as the membrane to that add 0.6g gel containing an equivalent dose of 500 mg amlodipine microsp sponge was placed on it. The speed of the stirrer was kept constant. With the help of pipette 2ml of sample was taken for specified period of time.

Drug Release Kinetic Data Analysis

Several kinetic models have been proposed to describe the release characteristics of a drug from matrix. The following four equations are commonly used, because of their simplicity and applicability. The zero-order model equation (Plotted as cumulative percentage of drug released vs. time); first-order model equation (Plotted as log percent drug remaining vs. time) to study the kinetics of drug release. Higuchi's square-foot equation (Plotted as cumulative percent drug released vs. square root of time); and the Korsmeyer-Peppas equation (Plotted as log cumulative percent drug released vs log time) to study the mechanism of drug release.

Stability study of gel:

The stability studies of the prepared gel formulations were (F3)) carried out in accordance with ICH guidelines to determine their physical stability. The samples were stored in suitable containers at a temperature ($30 \pm 2^\circ\text{C}$ / $65 \pm 5\%$ RH), for predetermined periods of 1 month. The gel was evaluated for changes in appearance, color, odour, pH, viscosity and spreadability,. Chemical stability was assessed by determining the drug content and percentage drug released. The obtained results were compared with the initial values to identify significant changes.

III. RESULTS AND DISCUSSION

Determination of Melting Point:

Melting point of Amlodipine base was determined by capillary method. The melting point was found to be in the range of 195-201°C. Thus, indicating the purity of the drug. The melting point of Amlodipine was found to be 200°C.

Identification of pure drug:

The IR spectrum of pure drug was found to be similar to the standard spectrum of Amlodipine. The spectrum of Amlodipine shows the functional groups as per the reference peaks.

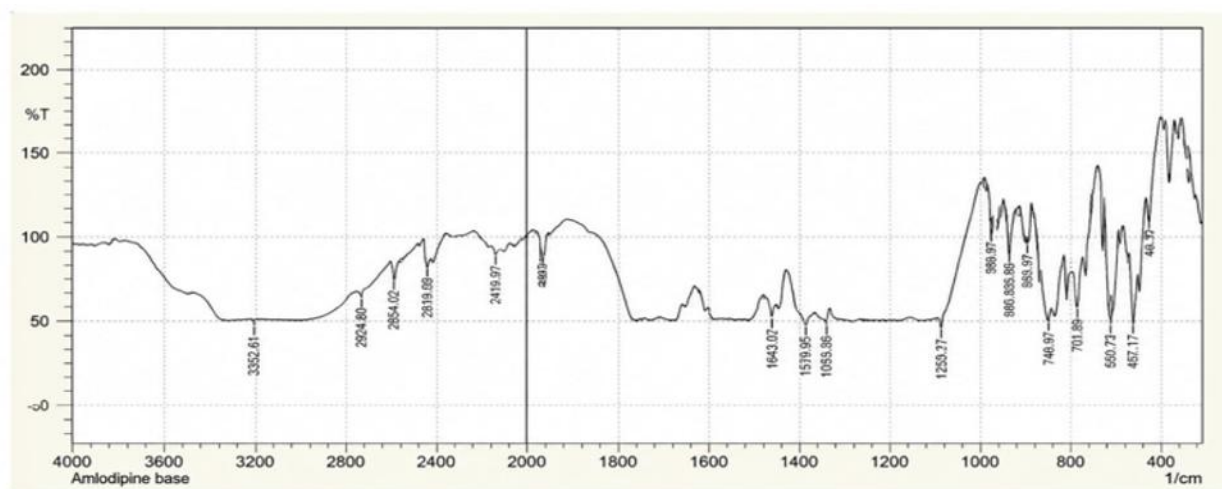


Fig No:02 FTIR spectra of Amlodipine

Identification of Amlodipine was carried out by using Fourier Transform Infra-Red spectroscopy. It was observed that all the characteristic peaks of Amlodipine were shown as of the standard peaks, so it was concluded that the drug is pure.

Preparation of calibration curve Amlodipine in 0.1N HCL Buffer

The calibration curve of amlodipine was developed at this wavelength. The calibration curve was linear between 2-10 µg/ml concentration ranges in pH 6.8 0.1N HCL buffer. Calibration curve was plotted by plotting mean absorbance on Y-axis against concentration on X-axis. The R² and slope were found to be 0.9941 and 0.037 at 238nm respectively.

Calibration Data of Amlodipine pH 6.8 (λ max 238 nm)

Table No. 3: Calibration curve of Amlodipine

Concentration (µg/ml)	Absorbance
0	0
5	0.161
10	0.356

15	0.543
20	0.723
25	0.958

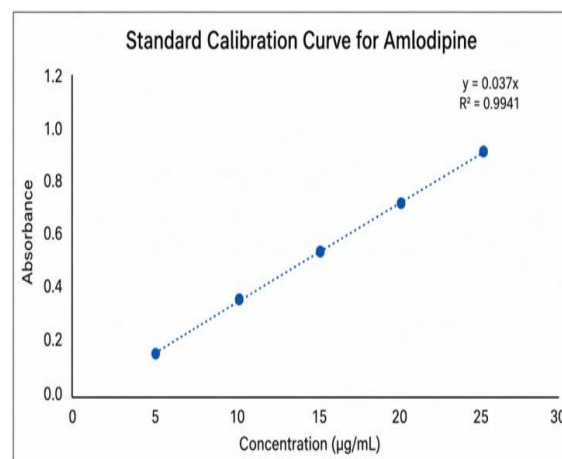


Fig No :03 Calibration curve of amlodipine in 0.1 N HCL Buffer

Formulation of microsponges.

Amlodipine loaded microsponges were successfully prepared by quasi emulsion method.

Evaluated parameters of Amlodipine microsponges
Table No 4: Results for effect of Drug: polymer type

F code	Type of polymer	Drug: Polymer ratio [mg]	IP DCM:ethanol[ml]	PVA conc [mg]	Solvent for E P [ml]	Stirring speed [rpm]	Yield %	D.C %	E.E %	ZP	PDI	PS	% D R
F1	Eud R S 100	1:1	5:5	500	90	600	50	44.3	46.5	-14.8	0.818	3.46	36%
F2	E.C	1:1	5:5	500	90	600	65	58	66	-16	0.885	4.8	46%

Influence of type of polymer

The ethyl cellulose formulation yielded significantly more yield with drug content and entrapment efficiency compared to the eudragit formulation. Ethyl cellulose rapidly precipitates upon contact with water. this quick solidification creates a dense, tight matrix that effectively encapsulated the drug preventing its leakage into the surrounding liquid.

In contrast, Eudragit R S 100 polymer incorporates hydrophilic quaternary ammonium groups. These groups cause the polymer matrix to swells and slow down solidification, allowing more drug to escape during the formation process.

Despite these differences, both formulations achieved acceptable particle size and zeta potential. The ethyl cellulose matrix, owing to its higher viscosity during mixing, resisted breaking down into smaller droplets. The non -inonic surfactant [PVA] Provided an even

coating on the particles,masking the polymers inherent charges and maintaining stable zeta potential values within the desired range for both formulations.

The percentage drug release was found to be 46% for the ethyl cellulose formulation and 36% for the Eudragit RS 100 formulation. The higher drug release observed with ethyl cellulose may be attributed to the formation of a comparatively porous polymeric matrix, which facilitates penetration of the dissolution medium and subsequent diffusion of the entrapped drug. In contrast, Eudragit RS 100 contains hydrophobic polymeric regions and quaternary ammonium groups that form a more diffusion-resistant matrix, thereby restricting drug diffusion and resulting in lower drug release. Thus, ethyl cellulose exhibited higher drug release, whereas Eudragit RS 100 provided a comparatively more sustained drug-release profile. ⁽¹⁹⁾

Influence of polymer ratio
Table No 5: results of Drug: polymer ratio

F code	Polym er type	Drug: Polym er ratio	I P [DCM:Ethanol] ml	PV A mg	EP [wate r] ml	Stirrin g speed [rpm]	Yield %	D. C %	E.E %	Zet a	PDI	Particl e size	%D R
F2	E.C	1:1	5:5	500	90	600	65	58	60	-16	0.885	4.3	46
F3	E.C	1:2	5:5	500	90	600	77	61	93	-23	0.245	6.8	47
F4	E.C	1:3	5:5	500	90	600	73	36	68	-15	0.419	5	38

The highest percentage yield, drug content, and entrapment efficiency were obtained due to the optimal polymer-to-drug ratio, which provided suitable internal phase viscosity and a stable matrix structure. Lower polymer concentration resulted in insufficient viscosity, leading to poor droplet stability

and increased drug leakage, whereas excessive polymer concentration increased viscosity, causing improper droplet breakdown, particle aggregation, and adhesion to the equipment. The optimum polymer concentration facilitated uniform droplet formation, efficient drug encapsulation, and formation of a stable

polymeric matrix, while minimizing drug loss and excessive aggregation. Thus, maintaining an appropriate polymer concentration was essential for achieving higher yield, drug content, entrapment efficiency, uniform particle characteristics, and surface stability.

The initial increase in yield, drug content, entrapment efficiency, and drug release when moving from a 1:1 to a 1:2 drug-to-polymer ratio indicates that an optimal balance was achieved, where the polymer concentration was high enough to build a stable matrix that efficiently captured the drug. However, pushing this ratio further to 1:3 causes a sharp drop across all parameters, which is primarily driven by the excessive viscosity of the internal organic phase. At this higher concentration, the organic solution becomes too thick for the stirring mechanism to break it down effectively into uniform droplets, causing the formulation to clump together and stick to the equipment, which

heavily cuts down the final yield. This high viscosity also stalls internal mass transfer; because the organic solvent diffuses out so slowly, the polymer matrix stays sticky and semi-solid for too long, allowing the drug to leak into the external phase rather than staying trapped inside, which lowers both drug content and entrapment efficiency. Finally, the denser polymer network at the 1:3 ratio creates a much thicker physical barrier and reduces the size of the internal pore channels, which keeps the dissolution medium from easily penetrating the microsponges and slows down the overall drug release rate.

The percentage drug release increased slightly at the optimum polymer concentration due to the formation of a stable matrix that allowed controlled drug diffusion. At higher polymer concentration, drug release decreased because the denser polymeric matrix increased the diffusion path length, thereby retarding drug release.⁽²⁰⁾

Influence of type of internal phase:
Table No 6: Result of drug: type of internal phase

F Cod e	Polym er type	Drug:Poly mer ratio mg]	IP DCM: Ethanol, acetone [ml]	PV A in mg	E P [water] ml	Stirr in g speed [rpm]	Yield %	D.C %	E.E %	Zet a	PDI	Partic le size	% D R
F3	E.C	1:2	5:5 DCM:Etha nol	500	90	600	77	61	93	-23	0.24 5	6.8	4 7
F5	E.C	1:2	5:5 DCM:aceto ne	500	90	600	66	53	89	-22	0.86 5	5.2	4 5

The superior formulation characteristics may be attributed to the difference in solvent properties. Acetone has higher water miscibility and a lower boiling point than ethanol, which promotes faster diffusion and evaporation into aqueous phase. This rapid solvent exchange may cause premature polymer precipitation and drug leakage before stable droplets are formed, resulting in non-uniform particles and reduced drug entrapment and practical yield. It may also affect the integrity and surface characteristics of the polymeric matrix, contributing to a lower zeta potential. In contrast, ethanol provides relatively controlled solvent diffusion and polymer precipitation, favouring the formation of stable and uniform microsponges with better drug entrapment, yield, and surface characteristics.

The ethanol-containing formulation showed slightly higher drug release (47%) compared with the acetone-containing formulation (45%). Ethanol allows controlled solvent diffusion and polymer precipitation, resulting in a more uniform microsphere matrix that facilitates drug diffusion. In contrast, acetone undergoes rapid solvent exchange due to its higher water miscibility and lower boiling point, which may cause faster polymer precipitation and formation of a less uniform matrix. Therefore, the solvent system influenced the matrix structure and drug-release behaviour.

The percentage drug release was found to be 45% and 47% for the formulations prepared with DCM:acetone and DCM:ethanol, respectively. The DCM:ethanol solvent system showed slightly higher drug release

than DCM:acetone. This may be due to better drug solubalisation and diffusion of the drug through the polymeric matrix. The lower drug release observed with DCM:acetone may be attributed to the rapid diffusion of acetone into the external aqueous phase,

leading to faster polymer precipitation and formation of a relatively denser matrix, which can retard drug diffusion. Therefore, DCM:ethanol was considered a more suitable internal solvent system for achieving better drug release from the prepared microsponges.⁽²¹⁾

Influence of internal phase volume:

Table No 7: Results of Drug: Internal phase volume

F Code	Polymer type	Drug: Polymer ratio [mg]	IP DCM:Ethanol [ml]	PVA conc[mg]	EP [water] ml	Stirring speed[rpm]	Yield %	D. C %	E.E %	Zeta	PI	Particle size	%DR
F3	E.C	1:2	5:5	500	90	600	77	61	93	-23	0.245	6.8	47
F6	E.C	1:2	7.5:7.5	500	90	600	61	52	72	-14	0.805	4.23	37

Increase in internal phase volume causes subsequent reduction in viscosity due to dilution. This causes easy diffusion of drug out of the globule into the external phase liquid before the solidification of polymer. As a result, drug content and entrapment efficiency decreases with increase in volume of internal phase.

Furthermore, this large volume of solvent requires a much longer evaporation time leaving the droplets in a unstable, partially formed state for longer, this leads to drug leakage, a loss of surface charge stability [lowering zeta potential] and significantly reduced final product yield.

The percentage drug release was influenced by the volume of the internal phase. The formulation

prepared with 5 mL DCM:5 mL ethanol showed a higher drug release of 47%, whereas increasing the internal phase volume to 7.5 mL DCM:7.5 mL ethanol resulted in a lower drug release of 37%. The reduction in drug release may be attributed to the increased volume of organic solvents, which can alter solvent diffusion and polymer precipitation during microsphere formation. This may result in a denser polymeric structure and greater drug retention within the microspheres, thereby reducing drug diffusion into the dissolution medium. Therefore, the lower internal phase volume was found to be more favorable for achieving higher drug release.⁽²²⁾

Influence of surfactant concentration:

Table No 8: Result of Drug: Surfactant concentration

F code	Polymer type	Drug: polymer ratio[mg]	IP DCM: Ethanol[ml]	PVA conc[mg]	EP [water] ml	Stirring speed [rpm]	Yield%	DC %	EE %	Zeta	PI	Particle size	%DR
F3	E.C	1:2	5:5	500	90	600	77	61	93	-23	0.245	6.8	47
F7	E.C	1:2	5:5	750	90	600	65	59	84	-23	0.634	5.3	37
F8	E.C	1:2	5:5	1000	90	600	61	39.5	64	-12	0.694	4.39	34

Increase in concentration of surfactant (PVA)has resulted in decrease in yield, drug content, entrapment efficiency, particle size. this could be attributed to the excessive stabilization of the emulsion and increased solubalisation of the drug in external aqueous phase. At higher PVA concentration, more drug may diffuse from the internal organic phase into the external

aqueous phase during preparation, resulting in greater drug loss and consequently lower drug content and entrapment efficiency. The excessive PVA may also increase the formation of fine droplets, which are more difficult to recover during filtration and washing, thereby reducing the practical yield.

The reduction in particle size can be explained by the ability of PVA to reduce interfacial tension between the internal and external phases. At higher concentration, PVA provides greater stabilization of the emulsion droplets and allows the internal phase to break into smaller droplets under stirring resulting in smaller microsponges. Further more excess PVA can form a dense protective layer around the microparticles, which may alter their surface characteristics and contribute to a reduction in zeta due to a steric shielding of the surface charge. Therefore, an optimum PVA concentration is essential to achieve adequate yield, drug loading, entrapment efficiency, and desirable particle size.

Simultaneously, the higher PVA concentration reduces interfacial tension, allowing the stirring shear force to break the internal phase into smaller droplets, which decreases the final particle size. This dense layer of nonionic PVA molecules heavily coats the surface of

these smaller micro particles, creating a thick steric barrier that masks the negative surface charges, compresses the electrical double layer, and drops the zeta potential closer to zero.

The percentage drug release decreased with increasing PVA concentration. The drug release was 47% at 500 mg PVA, which decreased to 37% at 750 mg and further to 34% at 1000 mg. This reduction may be attributed to the formation of a more rigid and less permeable polymeric structure with increasing PVA concentration, which can restrict penetration of the dissolution medium and drug diffusion from the micro sponges. Although higher PVA concentration resulted in smaller particle size, the reduced matrix permeability had a greater influence on drug release. Thus, 500 mg PVA provided comparatively higher drug release and was considered more favorable for the formulation.⁽²³⁾

Influence of external phase volume:

Table No 9: Result of Drug: External phase volume

F cod e	Type of polyme r	Drug: polymer ratio[mg]	IP DCM:Eth anol[ml]	PVA conc[mg]	EP [water] ml	Stirring speed[rp m]	Yield %	D.C %	EE %	Zet a	PI	Partic le size	% D R
F3	EC	1:2	5:5	500	90	600	77	61	93	-23	0.24 5	6.8	47
F9	EC	1:2	5:5	500	25	600	70	55	66	-15	0.42 2	5.42	39
F1 0	EC	1:2	5:5	500	50	600	65	52	63	-13	0.62 3	5.29	38. 4
F1 1	EC	1:2	5:5	500	75	600	62	42	52. 2	-15	0.83 2	4.26	37

The non-linear impact of the external phase volume on the physicochemical attributes of the microsponges suggests a critical transition point in the emulsion dynamics and droplet stabilization. The initial decrease in production yield, drug content (DC), entrapment efficiency (EE), particle size, zeta potential, and in vitro drug release observed when scaling the external volume from 25 to 75 mL is likely driven by an enhanced solvent diffusion gradient. This steeper gradient accelerates organic solvent mass transfer and rapidly solidifies the microsponges into smaller, more compact spheres; however, the larger aqueous sink also promotes extensive drug leaching into the continuous phase, thereby depressing Entrapment efficiency, drug content, and subsequent release rates. Conversely, the abrupt reversal and

subsequent increase in all parameters at a 90 mL volume can be attributed to a destabilization threshold. At this extreme volume, the fixed mass of surfactant becomes overly diluted, dropping below the concentration required to fully cover the expanded oil-water interface. This deficiency compromises steric or electrostatic stabilization, leading to droplet coalescence and macro-aggregation during the shearing phase. These larger, irregularly agglomerated matrix structures physically entrap greater fractions of the drug phase—manifesting as an apparent rebound in yield, size, EE, and zeta potential charge density—while simultaneously accelerating in vitro drug release due to structurally altered or poorly consolidated polymeric networks

The percentage drug release was found to be 39%, 38.4%, 37%, and 47% at external aqueous phase volumes of 25, 50, 75, and 90 mL, respectively. A gradual decrease in drug release was observed with an increase in the external phase volume from 25 to 75 mL. This may be attributed to changes in microsponges formation, particle characteristics, and drug entrapment during solvent diffusion and polymer solidification. At 90 mL, the percentage drug release

increased to 47%, which may be due to improved dispersion of the organic phase and more efficient solvent diffusion, resulting in better-developed microsponges structures and greater drug release. Thus, the external aqueous phase volume significantly influenced the drug-release behavior, with 90 mL producing the highest percentage drug release among the studied formulations. ⁽²⁴⁾

Influence of stirring speed:

Table No 10: Drug: stirring speed

F code	Polym er type	Drug:Pol ymer [mg]	IP DCM:Eth anol [ml]	PVA conc[mg]	EP [water] ml	Stirring speed [rpm]	Yield %	DC %	EE %	zet a	PI	Partic le size	%D R
F3	EC	1:2	5:5	600	90	600	77	61	93	- 23	0.23 4	6.8	47
F1 2	EC	1:2	5:5	600	90	1000	60	59	65	- 16	0.43 6	5.44	40

Increasing the stirring speed from 600 rpm to 1000rpm decreased practical yield ,drug content ,and entrapment efficiency due to increased shear forces ,which produced smaller droplets and increased the interfacial area between the organic and aqueous phases .this enhanced drug diffusion into the external phase before complete polymer solidification ,resulting in reduced drug and yield .The decrease in particle size was due to stronger shear forces breaking the organic phase into smaller droplets .The lower zeta potential may be attributed to changes in particle surface characteristics and pva distribution at the particle -aqueous interface under higher agitation.

The percentage drug release decreased from 47% to 40% with an increase in stirring speed from 600 to 1000 rpm. Higher stirring speed generated greater shear forces, producing smaller particles and altering their surface characteristics. The reduced drug content and entrapment efficiency may have contributed to lower drug release. Moreover, faster solvent evaporation at higher speed may have caused premature pore closure and reduced the formation of interconnected internal micro channels, increasing diffusion resistance and restricting drug release. Thus, excessive stirring speed adversely affected the drug-release behaviour of the microsponges. ⁽²⁵⁾

Invitro drug release of optimised formulation

Table No. 8: Results for In vitro Drug Release of Optimized Formulation.

TIME(Hrs)	F-3 (% Drug release)	F-3 (C %D R)
0	11.5	10.29299
1	14.22	26.824
2	15.43	36.273
3	15.4	36.878
4	17.25	53.281
5	20.58	64.171
6	27.5	77.500
7	38	87.490
8	37.53	94.4533

In-vitro drug release profile

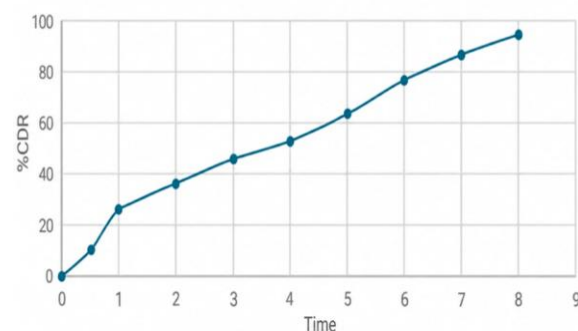


Fig No: 04 In vitro drug release of F3 Formulation

IV. STABILITY STUDIES

Table No: 9 results of stability studies of microsponges

SL.No.	Evaluation parameters	Short term stability (F-3)	
		Initial	After 1 month
1	Physical appearance	Off white	Off white
2	Average particle size	6.55	6.24
3	Drug content	92%	91%
4	Entrapment efficiency	81.2%	80.4%

Results indicated that drug content remained within the acceptable pharmacopeial limits throughout the study period, suggesting that the drug was chemically stable in the lipid matrix. Entrapment efficiency showed no changes with time. Particle size analysis revealed slight growth during storage, which could be due to aggregation or fusion of lipospheres over time. In terms of appearance, no significant color change or phase separation was observed.

Evaluation of microsphere gel

1. Physical appearance: colourless opaque, odourless with smooth appearance.
2. pH: The pH of the formulated gel was found to be 5.75 for F3 formulation respectively.
3. Viscosity: The viscosity of the formulated gel was found to be 3144cp respectively for f3 formulations.
4. Spreadability: The spreadability of the formulated gel was found to 8.23g cm² respectively for F3 formulation.

Invitro diffusion study of gel.

Table No: 10 Results for invitro diffusion study of gel

Time (Hrs)	% Cumulative Drug Release
0	0
0.5	13.8787
1	31.0445
2	35.7925
3	39.4448
4	45.653
5	53.6888
6	62.089
7	79.6201
8	93.6727

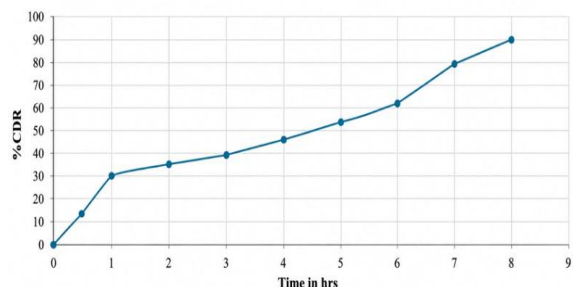


Fig no: 05 In vitro drug release of F3 Formulation

Stability data of microsphere gel

Tests	INITIAL F-3	F-3 AFTER 1 MONTH
pH	6.2±0.006	6.1±0.006
Spreadability	7.2±0.33	7.1±0.33
Viscosity	1143±2	1144±2
% Drug release	46.38	45.49

Two formulations of the microsphere gel were stored at $37 \pm 5^\circ\text{C}$ / ~65% RH for 1 month. Physical appearance, pH, viscosity, entrapment efficiency and drug content limits were evaluated at 0 and 1 month. Both formulations showed minimal changes in all the parameters, no phase separation or color change was observed. These results indicated short-term stability of the formulation at room temperature over 1 month.

V. CONCLUSION

The developed amlodipine besylate microsponges demonstrated satisfactory particle-size uniformity and dispersion stability, with F3 exhibiting the most favorable overall characteristics and consequently being selected as the optimized formulation. Ethyl cellulose provided higher drug content and entrapment efficiency than Eudragit RS 100, along with enhanced drug release, indicating improved drug diffusion through the polymeric matrix. Optimization of the polymer-to-drug ratio revealed that an appropriate polymer concentration favored drug entrapment, whereas excessive polymer concentration adversely affected microsphere formation and drug loading due to increased viscosity. The DCM:ethanol solvent system exhibited better performance than DCM:acetone, while increased internal phase volume and PVA concentration negatively influenced drug content, entrapment efficiency, and drug release. Changes in external phase volume and agitation speed also affected microsphere formation and drug release.

characteristics. Overall, the optimized F3 formulation exhibited satisfactory drug entrapment and controlled drug release, suggesting its potential as a topical microsphere-based delivery system for amlodipine besylate.

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