ORIGINAL ARTICLE

Comparison of Microemulsion and Solvent Evaporation Technique for Solubility Enhancement of Amlodipine Besylate

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ABSTRACT

Introduction: Amlodipine besylate is a calcium channel blocker indicated for hypertension and angina. It is described as slightly soluble in water and due to its limited solubility, it may result in poor bioavailability. The aim of this study is to enhance the solubility of amlodipine besylate using solvent evaporation method and microemulsion technique and to compare the two methods. **Method:** Solid dispersions (SD) of amlodipine besylate were developed by employing solvent evaporation method. PEG6000 was the polymer of choice and different drug:polymer ratios were used. Evaluation of the prepared SDs include solubility studies, dissolution studies and scanning electron microscopy (SEM). As for the microemulsion technique, microemulsions were prepared by phase titration method and the optimized microemulsion formulation was then characterized for solubility studies and dissolution studies. **Results:** SD3 with drug:polymer ratio of 1:4 achieved the highest solubility which was 96.97 mg/ml ± 0.92 whereas the solubility of the optimized microemulsion was found to be 112.54 mg/ml ± 0.92. In solvent evaporation method, as the drug:polymer ratio increases, the solubility and dissolution rate of SDs increases. **Conclusion:** The two methods had significantly enhance the solubility of amlodipine besylate however the microemulsion technique showed better solubility profile.
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Keywords: Amlodipine Besylate, Solvent Evaporation Method, Microemulsion, Solubility Enhancement

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INTRODUCTION

Solubility is definediin terms of the amount of solvent parts necessary to dissolve a part by volume of a liquid or one part by weight of a solid according to pharmacopoeias (1). Poor drug solubility will result in a low dissolution rate resulting in a low bioavailability of orally administered drugs. The degree and rate at which a drug's active component enters the systemic circulation and allowing the drug to get to the action site, is known as bioavailability. When the bioavailability of a drug is low, it will result in a therapeutic potential that is minimal leading to unsatisfactory clinical results (2).

To accomplish an enhanced solubility of weakly soluble drugs, a myriad of different techniques have been utilized including chemical and physical modification of the drug such as micronization, hot-melt extrusion, solvent evaporation, supercritical fluid processes, crystal engineering and so. Choosing the right method to be used for solubility enhancement of a drug is influenced by the drug's absorption site, the drug's properties and the necessary characteristics of dosage form (1).

Amlodipine besylate is an antihypertensive drug that is classified as dihydropyridine calcium channel blockers. It has an experimental solubility of 75.3mg/L which is quite limited and it is also described as slightly soluble in water (3). Amlodipine besylate is a great candidate for solubility enhancement studies due to its limited solubility. Some of the techniques that can be used to improve its solubility are the solvent evaporation method and the microemulsion technique. Solvent evaporation method is one of the method that can be used to prepare solid dispersions. Solvent evaporation method dissolves both the carrier and the drug in the same solvent and when the solvent is evaporated, it produces the solid dispersions. When solvent evaporation method is employed, it prevents the decomposition of drugs or

carriers by heat due to the low temperature needed for the organic solvents to evaporate which is a major advantage when using this method (4).

On the other hand, microemulsions are mixtures of oil, surfactant and water that form spontaneously in contrast to ordinary emulsions which require the high shear conditions. One of the advantages of microemulsions is that they are thermodynamically stable and the extremely low interfacial tension of the microemulsion systems facilitates its formation. This results in the formation of the dispersed phase to be extremely small droplets. These microemulsions can take a variety of forms such as oil microemulsion, water microemulsion and oil/water bicontinuous microemulsions (5). The objectives of study was to determine and compare the solubility of amlodipine besylate processed by solvent evaporation method and microemulsion technique.

MATERIALS AND METHODS

Materials

Amlodipine besylate was a gift sample given by IKOP, IIUM (Malaysia), PEG 6000, Chloroform, Dichloromethane and Propylene glycol were purchased from R&M Chemicals (Malaysia), Tween 20 and Oleic acid were purchased from Pro Prima Enterprise (Malaysia).

Solvent Evaporation Method

Solid dispersions formulations are presented in Table I. Solid dispersion of amlodipine besylate in PEG 6000 in three different ratios (1:2, 1:3 and 1:4 w/w) were developed by employing the method of solvent evaporation. Respective amount of the polymer PEG 6000 was dissolved in 40 ml of chloroform and 60 ml of dichloromethane with a magnetic stirrer. Then, the respective quantity of amlodipine besylate was weighed and carefully added to the mixture while constantly stirring until the drug has dissolved completely. Then, the solvent was evaporated using a hotplate with stirrer in a fume hood. The resulting residue was dried using an oven at 40°C and stored overnight in a desiccator. The dried mass obtained was then sieved after they were crushed and ground using a mortar and pestle (6).

Microemulsion Technique

Selection of the oil phase was based on the maximum solubility of the drug in the oil phase. Different oils such as oleic acid, isopropyl myristate and olive oil were screened as well as several surfactants including Tween 20 and Tween-60. The co-surfactants were then selected based on their capability to form a stable microemulsion with the surfactants. Microemulsion containing amlodipine besylate was prepared by phase titration method using four components which are oleic acid as the oil phase, Tween 20 as surfactant, propylene glycol as co-surfactant and distilled water as the aqueous phase. In this study, different surfactant–cosurfactant ratios (S/Cos ratio) were tried. The construction of a pseudoternary phase diagrams was done to study the effect of different ratios of surfactant to cosurfactant on the area of microemulsion existence. If a phase separation follows the appearance of turbidity, then the samples will be deemed biphasic. However, if after stirring, a transparent and monophasic mixtures are observed, the samples is identified as points in the phase diagram. The region of microemulsion existence is defined as the region being covered with these points. The microemulsion formulation was then optimized for evaluation (5).

Table I: Formula of Amlodipine Besylate Solid Dispersions

Formula code	Amlodipine Besylate (mg)	PEG 6000 (mg)
SD1 (1:2)	1000	2000
SD2 (1:3)	1000	3000
SD3 (1:4)	1000	4000

Percentage Yield

The percentage yield was calculated using the following formula:

% Yield = [Practical mass / Theoretical mass] x 100

Drug Content Uniformity

The prepared solid dispersions were analyzed for uniformity of drug content. Solid dispersions from each formulation containing amlodipine besylate equivalent to 20 mg were obtained and dissolved using ethanol in a 100 ml volumetric flask which was then filtered. The filtered solution was then diluted suitably and the drug content of the solid dispersions were analyzed using UviLine 9400 Spectrophotometer AHS Laboratory Supplies, Malaysia (7).

Solubility Studies

To determine solubility of the solid dispersions, optimized microemulsions and the pure drug, the shake flask method was used. The sample equivalent to 20 mg were added to 2 ml of distilled water and the suspension obtained was shaken using a rotary shaker at a constant temperature of 37°±0.5°C for 24 hours to reach equilibrium solubility. Then, the saturated solutions were quantified using UviLine 9400 Spectrophotometer AHS Laboratory Supplies, Malaysia (8).

In vitro Dissolution Test

Dissolution studies of solid dispersions, optimized microemulsions and pure drug were performed. The paddle's speed was set to 50 rpm in phosphate buffer pH 6.8 and the temperature maintained at 37°C. Solid dispersions and microemulsion containing 10 mg of amlodipine besylate were obtained for dissolution. At selected time intervals, aliquots of 5 ml were extracted. The absorbance at 239 nm was measured to estimate the drug quantity released using UviLine 9400 Spectrophotometer AHS Laboratory Supplies, Malaysia (9).

Scanning Electron Microscope
The surface morphology of amlodipine besylate solid dispersions were analyzed using SEM. The solid dispersion were placed on a double-sided adhesive tape and deposited with thin layer of gold palladium and the morphology of the surface of the samples were analyzed using an SEM (10).

Visual Inspection
After each water addition to the oil and mixture of surfactants, microemulsions were inspected visually and were then characterized (11).

Statistical Analysis
Data collected was analysed using One-way ANOVA and the Statistical Package for Social Sciences (SPSS) Version 20.0 was used. The results was expressed as the mean ± standard error of mean (SEM) and a value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Solvent Evaporation Method

Percentage Yield
The results of the percentage yield of amlodipine besylate solid dispersions prepared by the method of solvent evaporation are presented in Table II. The result of the One-Way ANOVA test was significant ($p < 0.05$) to suggest that at least one pair were significantly different.

The results showed that the percentage yield of solid dispersions ranges from 88.87% ± 0.85 to 92.82% ± 0.51. Moreover, the results showed that the solid dispersions prepared using drug:polymer ratio of 1:4 has a higher percentage yield of 92.82% ± 0.51 than the other formulations prepared.

Drug Content
The results of the drug content of amlodipine besylate solid dispersions prepared by the method of solvent evaporation are presented in Table II. The result of the One-Way ANOVA test was significant ($p < 0.05$) to suggest that at least one pair were significantly different. A standard curve that range from 10 – 300 µg/mL was prepared. The standard curve has a good linearity with $r^2 = 0.999$. The results showed that the drug content of solid dispersions was uniform and ranges from 94.9% ± 0.60 to 97.3% ± 0.45. Moreover, the results showed that the solid dispersions prepared using drug:polymer ratio of 1:4 showed higher drug content that is 97.3% ± 0.45 than the other formulations prepared.

Solubility studies
The results of the solubility profile of amlodipine besylate solid dispersions prepared by the method of solvent evaporation are presented in Table 2. The result of the One-Way ANOVA test was significant ($p < 0.05$) to suggest that at least one pair were significantly different.

The results showed that the solubility profile of solid dispersions ranges from 88.15 mg/ml ± 0.83 to 96.97 mg/ml ± 0.92. Moreover, the results showed that the solid dispersions prepared have better solubility profile than the pure drug and solid dispersion using drug:polymer ratio of 1:4 showed higher solubility that is 96.97 mg/ml ± 0.92 than the other formulations prepared.

In Vitro Dissolution Studies
The results of the dissolution profile of amlodipine besylate solid dispersions prepared by the method of solvent evaporation are presented in Fig 1. Preparation of solid dispersions of amlodipine besylate using PEG 6000 by the method of solvent evaporation resulted in a significant enhancement in the dissolution rate in comparison to the pure drug. The increase in the rate of dissolution of the solid dispersions is influenced by the concentration of polymer in the solid dispersion. Hence, SD3 which uses drug:polymer ratio of 1:4 showed higher dissolution than the other formulations prepared which was 89.36% ± 0.67.

SEM
Figure 2 (A) is a micrograph of pure amlodipine besylate that showed its crystalline nature and (B) is a micrograph of SD3 which appears to be in amorphous state.

Table II: Results of percentage yield of, drug content uniformity and solubility of amlodipine besylate solid dispersions

Formulation	Parameter	Mean (%) ± SD	ANOVA	Post-hoc (Tukey)*
SD1	Percentage yield	88.87 ± 0.85	$p < 0.05$	• SD1 & SD3 • SD2 & SD3
SD2		89.74 ± 0.73		
SD3		1.82 ± 0.51		
SD1	Drug Content Uniformity	94.9 ± 0.60	$p < 0.05$	• SD1 & SD3 • SD2 & SD3
SD2		96.6 ± 0.27		
SD3		97.3 ± 0.45		
Pure Drug	Solubility	31.43 ± 0.67	$p < 0.05$	• Pure drug & SD1 • Pure drug & SD2 • Pure drug & SD3 • SD1 & SD2 • SD1 & SD3 • SD2 & SD3
SD1		88.15 ± 0.83		
SD2		94.28± 0.21		
SD3		96.97 ± 0.92		

One-Way ANOVA test
*Pair wise showing the significant difference between the formulations

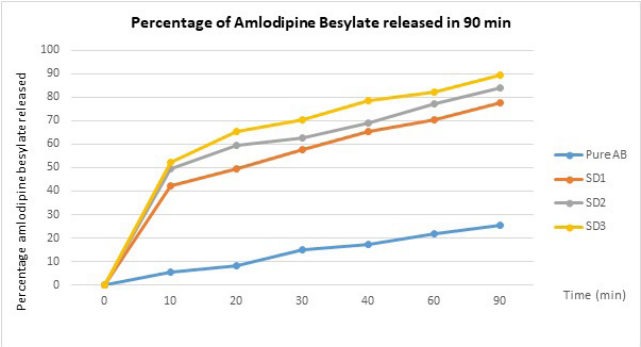

Figure 1: Dissolution profile Pure Anlodipine Besylate and SD1-3 in phosphate buffer pH 6.8. The results are presented in mean ± SD, N=3

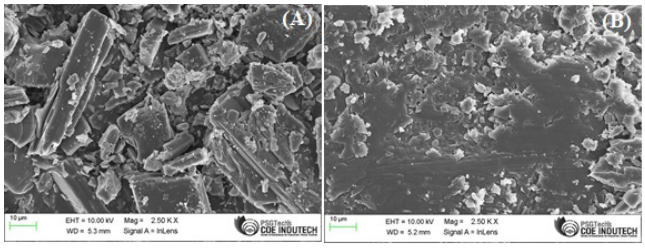

Figure 2: Scanning electron micrograph of (A) Pure Amlodipine Besylate (B) SD3

Microemulsion

Visual Inspection of Microemulsions

The results of visual inspection of microemulsion with S/Cos ratio of 1:1 and 2:1 are presented in Table III.

Solubility studies

The results of the solubility studies for pure drug was 31.43 mg/ml ± 0.67 whereas the solubility studies for microemulsion was 112.54 mg/ml ± 0.92. This shows that the optimized microemulsion containing amlodipine besylate prepared has a better solubility profile than the pure drug.

In vitro Dissolution Studies

The results of the dissolution profile of optimized microemulsion containing amlodipine besylate are presented in Fig 3. Preparation of optimized microemulsion resulted in a significant increase in the rate of dissolution compared to the pure drug.

Comparison of Solvent Evaporation Method and Microemulsion Method

The comparison of results between pure drug and the best formulation prepared using both methods are presented in Table IV. The formulation chosen for solvent evaporation method is SD3 which has a drug:polymer ratio of 1:4.

The results showed that the SD3 and the optimized microemulsion has a better solubility profile and dissolution profile than the pure amlodipine besylate. The solid dispersion prepared using solvent evaporation method had enhance the solubility of the pure amlodipine besylate by 3 folds whereas the optimized microemulsion had enhance the solubility of pure amlodipine besylate by close to 4 folds. However, comparing between the two methods showed that the optimized microemulsion has a better solubility and dissolution profile than the solid dispersion prepared using solvent evaporation method.

Table III: Visual Inspection of Amlodipine besylate microemulsion S/Cos ratio (1:1) and (2:1)

Formula Code	Smix (%)	Oil (%)	Water (%)	Appearance	
				S/Cos ratio (1:1)	S/Cos ratio (2:1)
A1	9	1	90	Transparent	Transparent
A2	18	2	80	Transparent	Transparent
A3	27	3	70	Translucent	Transparent
A4	36	4	60	Translucent	Translucent
A5	45	5	50	Translucent	Translucent
A6	54	6	40	Cloudy	Translucent
A7	63	7	30	Cloudy	Cloudy
A8	72	8	20	Cloudy	Cloudy
A9	81	9	10	Cloudy	Cloudy
A10	90	10	0	Cloudy	Cloudy

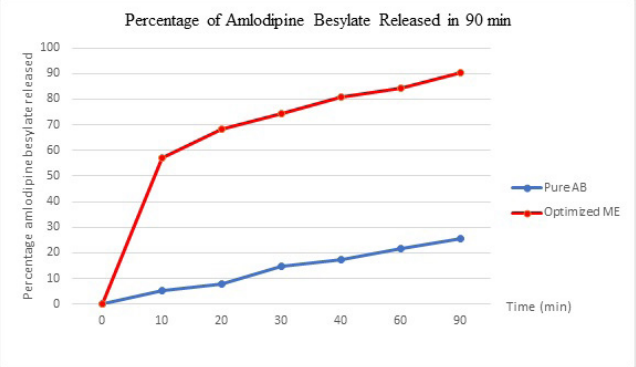

Figure 3: Dissolution profile Pure Amlodipine Besylate and optimized microemulsion in phosphate buffer pH 6.8. The results are presented in in mean ± SD, N=3

Table IV : Comparison of solubility studies of pure drug and best formulation

Parameters	Pure Drug	SD3	Optimized Microemulsion
Mean Solubility (mg/ml) ± SD	31.43 ± 0.67	96.97 ± 0.92	112.54 ± 0.92
Mean Percentage of amlodipine besylate released at 90 min (%) ± SD	25.67 ± 0.96	89.36 ± 0.67	90.15 ± 0.94

DISCUSSION

Solvent Evaporation Method and Mechanism of Solubility Enhancement

The solid dispersions containing amlodipine besylate prepared using solvent evaporation method demonstrated improved solubility profile in comparison

to the pure amlodipine besylate (12). The enhancement of the solubility and dissolution profile can be explained by a plethora of factors such as particle size reduction, wettability improvement of drug particles and changing a drug's crystalline form into its amorphous form (13).

Particle Size Reduction

A drug's solubility is related intrinsinctly to the particle size. When the particle size of a drug reduces, the surface area to volume ratio of the drug increases hence resulting in enhanced solubility and dissolution. This is due to the fact that a greater ratio of surface area to volume allows the drug to interact better with the solvent. Solid dispersions prepared by the method of solvent evaporation in this study applied this principle which leads to an increased solubility and dissolution rate which consequently may improve its bioavailability (13).

Improved Wettability

Reduction in the particle size of a drug also improves its wettability. One of the major contribution in enhancing a drug's solubility is related to the improved wettability of the drug which has been demonstrated in the solid dispersions that have been prepared in this study. Using a higher percentage of polymer also helps to increase the porosity of the solid dispersions which helps to improve the wettability of the drug. This is the reason SD3 which uses a drug to polymer ratio of 1:4 showed better solubility than the other solid dispersions prepared in this study (13).

Amorphous State of Solid Dispersions

Solid dispersions also change a drug into its amorphous form from a crystalline form which helps to enhance its solubility. So, when the solid dispersions were prepared, it appears to be in its amorphous form which leads to less energy needed in breaking apart the crystal lattice of the amlodipine besylate hence resulting in an enhanced solubility of amlodipine besylate. Using a higher percentage of polymer also helps to reduce the crystallinity of the drug. This is also another reason SD3 with a drug to polymer ratio of 1:4 showed better solubility than the other solid dispersions prepared in this study (13).

Microemulsion and Mechanism of Solubility Enhancement

The optimized microemulsion formulation containing amlodipine besylate showed improved solubility profile in comparison to the pure drug. The enhancement of the solubility and dissolution profile can be associated with several factors such as droplet size reduction, presence of surfactant and improved wettability (14).

Droplet Size Reduction

Similarly to solid dispersions prepared, the optimized microemulsion prepared in this study also experienced a reduction in the droplet size which increases the surface area to volume ratio of the drug. An increased surface area enhances the solubility and dissolution of the drug as it allows for a greater interaction with the solvent (14).

Presence of Surfactant

Preparation of a microemulsion consists of surfactants which decreases the surface tension and enhance the rate of dissolution in aqueous medium of drugs that are lipophilic. So in this study, the use of Tween 20 as surfactant and propylene glycol as cosurfactant results in the formation of a dual film of surfactant-cosurfactant at the interface leading to a decrease in oil and water tension to values that are significantly low which helps to enhance the rate of dissolution of amlodipine besylate (14).

Improved Wettability

The surface tension of the optimized microemulsion decreases due to the utilization of surfactant in preparing the microemulsions which helps to improve the wettability of the optimized microemulsion containing amlodipine besylate. Hence resulting in a better dissolution and solubility profile of the drug which can be seen with the optimized microemulsion prepared in this study (14).

Comparison between Solvent Evaporation Method and Microemulsion Method

The reason for the difference in the solubility profile of both formulations can be due to the mechanism in which they enhanced solubility. As have discussed earlier, the solubility of a drug is related intrinsically to its particle size. In general, the particle size of a solid dispersion appears to be in the range of 1 to 300 000 nm whereas the particle size of a microemulsion ranges between 1 to 100 nm. This explains the reason behind microemulsion having a better solubility profile as its smaller particle size results in a greater surface area to volume ratio allows the drug to interact better with the solvent. The greater reduction of the particle size of microemulsions also allows a better wettability of the drug which results in a better solubility profile of microemulsion when compared to solid dispersions (15).

CONCLUSION

This study focuses on enhancing the solubility of amlodipine besylate using solvent evaporation method and microemulsion method. Based on the results and findings of the study, both methods had significantly enhance the solubility of amlodipine besylate. The solubility of the optimized microemulsion was found to be 112.54 mg/ml ± 0.92 whereas the highest solubility achieved with solvent evaporation method was found to be 96.97 mg/ml ± 0.92. This showed that the microemulsion method produced a better solubility profile because it had enhance the solubility of pure amlodipine besylate which was 31.43 mg/ml ± 0.67 by close to 4 folds whereas by employing the

solvent evaporation method, it managed to enhance the solubility of amlodipine besylate by only 3 folds.

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