FORMULATION AND EVALUATION OF NIFEDIPINE MICROSPHERES OF CONTROLLED RELEASE USING NATURAL POLYMERS

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ABSTARCT

To develop and evaluate Nifedipine microspheres using natural polymers like Chitosan, Gelatin, and Sodium Alginate. Microspheres of Nifedipine were prepared by emulsification, suspension polymerization and emulsification solvent evaporation techniques by using polymers like Chitosan, Gelatin and Sodium Alginate respectively. Various evaluation parameters were assessed, with a view to obtain oral controlled release of Nifedipine. In the present study nine formulations were formulated by using Chitosan, Gelatin, Sodium Alginate in various proportions. The prepared Nifedipine microspheres were then subjected to particle size and size distribution, % yield, drug content, entrapment efficiency, in vitro dissolution studies, release kinetics. The FTIR Spectras revealed that, there was no interaction between polymers and Nifedipine. Nifedipine microspheres was spherical in nature, which was confirmed by SEM. Nifedipine microspheres with normal frequency distribution were obtained. A maximum of 66.5%, 93.90% and 84.20% drug entrapment efficiency was obtained in the Nifedipine microspheres which were prepared by using Chitosan, Gelatin and Sodium Alginate respectively. The in vitro performance of Nifedipine microspheres showed controlled release depends on the polymer concentration. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model were found to be Non-Fickian. The present study conclusively demonstrates the feasibility of effectively encapsulating Nifedipine into Chitosan, Gelatin and Sodium Alginate microspheres to form potential controlled release drug delivery system.

Keywords: Nifedipine, Controlled release formulation, Microspheres, Natural polymers.

INTRODUCTION

The oral route of drug administration is the more usual, convenient and comfortable route for active drug delivery to the body. Oral controlled release systems continue to be the most popular drug delivery systems as it offers various advantages over the conventional drug delivery system like:

- Improve patient's compliance and convenience due to less frequent dosing of drug.
- Decrease in fluctuation of steady state blood plasma level which helps in better control of disease condition.
- Maximum utilization of drug which reduces the total amount of dose administered.
- Improved drug therapy, shorter treatment period and less frequency of dosing[1, 2].

There are various approaches in delivering a drug substance to the target site in a controlled release fashion. One such approach is developing microspheres as carriers for drugs. However, these formulations have to be injected either subcutaneously or intravenously, which in general is not acceptable. Hence, it is necessary to develop an oral drug delivery system that is convenient for patients. Various natural polymers like Chitosan, Gelatin and Sodium alginate have been used to develop drug delivery systems for entrapping and delivering drugs orally[3]. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is combined with a drug in such a manner that the drug is released from the material in a predetermined manner. The purpose of controlled drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing. Various characteristics of drug molecule that render it unsuitable for controlled release dosing:

- Narrow therapeutic index
- Short/long elimination half life
- Poor absorption
- Low aqueous solubility
- More first pass metabolism
- Incompatible pharmacological effects

Controlled-Release mechanisms: The major mechanisms by which active agents can be released from a delivery system: diffusion, degradation, and swelling followed by diffusion. Any or all of these mechanisms may occur in a given release system.

Microencapsulation: It is a technology that used to entrapping solids, liquids, or gases inside one or more polymeric coatings[14]. Microencapsulation helps to separate a core material from its environment until it is released. It protects the unstable core from its

environment, thereby improving its stability, extends the core's shelf life and provides a sustained and controlled release[15, 16]. There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microspheres as carriers for drugs. Microspheres are characteristically free flowing powders containing proteins or synthetic polymers which are biodegradable in nature. Those microspheres are having a particle size less than 200 m[17]. Biodegradable microspheres can be prepared from certain synthetic as well as natural polymers. Some of the important methods used for the preparation of microspheres are: Single emulsion technique, Double emulsion technique, Polymerization techniques, and Solvent evaporation.

Treatment of Hypertension[23]: Most people with hypertension are treated with antihypertensive medications. Hypertension is commonly treated with drugs that decrease cardiac output[3]. These drugs either block beta-adrenoceptors on the heart (i.e., beta-blockers) or L-type calcium channels (i.e., calcium-channel blockers), which decreases cardiac output by decreasing heart rate and contractility (inotropy).

Calcium Channel Blockers[25]: Calcium Channel blockers (like Nifedipine) are emerging as a very important group of drugs for the treatment of Hypertension and Angina Pectoris. These agents are calcium influx inhibitors. They inhibit calcium ionic entry through select voltage sensitive areas called as 'Slow channels' across the cell membranes .By reducing intercellular calcium concentration in cardiac and vascular smooth muscles, they block coronary smooth muscles, they block coronary arteries, Peripheral arterioles and may reduce heart rate, decrease myocardial contractility and slow atrioventricular nodal contraction.

MATERIALS AND METHODS

Materials: Nifedipine was collected from Nivedita chemicals, IPCA, Ratlam. Chitosan, Gelatin were collected from Spectrum labs Hyderabad. Glacial acetic acid, Paraffin liquid light, Paraffin liquid heavy, Sunflower oil, Glutaraldehyde, Toluene, Methanol, hexane, Acetone and Hydrochloric acid were collected from SD fine-chemical limited, Mumbai. Sodium alginate was collected from Shreeji chemicals. Span 80 were collected from Central drug house (p) Ltd.

Methods:

Preparation of microspheres of Nifedipine using chitosan polymer by emulsification method: In this technique, a 4% w/v chitosan solution was prepared in 5% aqueous acetic acid and nifedipine was dispersed in that solution. Now this solution was dispersed in 50 ml of 1:1 of light and heavy liquid paraffin mixture containing 0.15 g of span80 in a beaker. Then the dispersion was stirred at 1000 rpm for 2 min. Add 1 ml glutaraldehyde saturated toluene solution and continued stirring. After a stipulated stirring

time, the microspheres were centrifuged, washed many times with hexane, methanol and lastly with acetone. Then the microspheres were dried at 50°C. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are prepared.

Table 1: Formulation formula for Nifedipine microspheres using Chitosan

Sl. No	Batch code	Drug: Polymer Ratio
1	A-1	1:1
2	A-2	1:2
3	A-3	1:3

Preparation of microspheres of Nifedipine using gelatin polymer by suspension polymerization method: Suspension polymerization technique was used to prepare gelatin microspheres of Nifedipine by taking glutaraldehyde as the cross-linking agent. 100 mg Nifedipine and 100 mg Gelatin were dissolved in 10 ml water which acts as the internal phase. The solution was added drop by drop to 50 ml sunflower oil which acts as the external phase and stirred the mixture by using an overhead stirrer at 500 rpm. The microspheres were hardened by addition of 20 ml glutaraldehyde saturated toluene. Than these were washed with acetone, filtered and dried overnight. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are prepared.

Table 2: Formulation formula for Nifedipine microspheres using Gelatin

Sl.no	Batch code	Drug: Polymer Ratio
1	B-1	1:1
2	B-2	1:2
3	B-3	1:3

Preparation of Nifedipine microspheres using Sodium Alginate Polymer by water-in-oil (w/o) emulsification solvent evaporation method: Another technique to prepare the microspheres was water-in-oil (w/o) emulsification solvent evaporation method. Nifedipine was dissolved in Sodium Alginate polymer aqueous solutions. The solutions were poured in to 200 g of sunflower oil containing 0.5% span 20, acts as an emulsifying agent. The aqueous phase was added into the oily phase by continuous stirring in a 500 ml beaker. Stirring was continued at 2000 rpm by using mechanical stirrer. Then the beaker and content were heated at 80°. Stirring and heating were continued for 4.5 h until the aqueous phase was completely evaporated. The mineral oil was decanted and the microspheres were collected and washed 3 times with 100 ml aliquots of n-hexane. Then those were filtered through Whatman filter paper, dried in an oven at 50°C for 2 h and stored in desiccators at room temperature. Three different formulations with drug: polymer ratios (1:1, 1:2, 1:3) are

prepared.

Table 3: Formulation formula for Nifedipine microspheres using Sodium alginate

Sl.no	Batch code	Drug: Polymer Ratio
1	C-1	1:1
2	C-2	1:2
3	C-3	1:3

Evaluation of Nifedipine Microspheres:

Frequency distribution analysis: The average particle size of nifedipine microspheres was determined by optical microscopy. A minute quantity of Nifedipine microspheres was spread on a glass slide and average size of 300 Nifedipine microspheres was determined from each batch. In order to define a size distribution or compare the characteristics of particles with different diameters, the size distribution can be broken down into different size ranges and presented in the form of a histogram.

Percentage yield: Percentage practical yield is determined to know the percentage yield or efficiency of any method. The percentage yield of Nifedipine microspheres was determined by using the formula:

Percentage yield= (Practical wield x Theoretical yield) x 100

Percentage drug entrapment efficiency: The efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment as per the following formula:

Percentage yield= (Practical drug content x Theoretical drug content) x 100

Theoretical drug content was determined by calculation assuming that the entire Nifedipine present in the polymer solution used gets entrapped in Nifedipine microspheres, and no loss occurs at any stage of preparation of Nifedipine microspheres. Practical drug content was analyzed by taking weighed amount of Nifedipine microspheres equivalent to 100 mg of Nifedipine was dissolved in 100 ml of distilled water. This solution was kept overnight for the complete dissolution of the Nifedipine in water. This solution was filtered and further diluted to make a conc. of 10 µ g/ml solution. The absorbance of the solutions was measured at 235 nm using double beam UV-Visible spectrophotometer against distilled water as blank and calculated for the percentage of drug present in the sample.

Dissolution Studies: Dissolution studies done in dissolution test apparatus (USPXXIII) by in stimulated gastric fluid pH 1.2 for 2 h and in phosphate buffer pH 7.4 for remaining 10 h. The dissolution media were maintained at a temperature of 37 ± 5°C. The speed of rotation of basket was adjusted at 50 rpm. The samples were withdrawn at 30 min intervals and analyzed. The amount of drug released was determined by UV absorption spectroscopy at 235 nm.

Kinetics of drug release: To examine the drug release kinetics and mechanism, the results obtained from in vitro release studies were plotted in 4 kinetics models as follows:

- Zero order rate kinetics: Cumulative percentage drug release Vs. Time
- First order rate kinetics: Log cumulative percentage drug retained Vs. Time
- Higuchi's classical diffusion equation: Cumulative percentage drug release Vs. Time
- Peppas exponential equation: Log of cumulative percentage drug release Vs. log Time

RESULTS & DISCUSSION

Determination of Average particle size

Table 4: Average diameter of Nifedipine microspheres

Formulation Code	Average size (µm)±SEM
A1	100±6.75
A2	121±5.41
A3	214±7.63
B1	143±9.26
B2	265±8.35
B3	327±4.33
C1	291±8.27
C2	333±7.34
C3	424±8.19

Frequency distribution analysis

Table 5: Frequency distribution data of Nifedipine microspheres

Size range (µm)	Number of particles								
	A1	A2	A3	B1	B2	B3	C1	C2	C3
0-50	60	45	35	40					
50-100	85	60	45	70					
100-150	60	90	60	90	25				
150-200	50	60	85	65	45	20	30	20	
200-250	45	45	55	35	50	30	50	40	
250-300			20		85	65	85	60	25
300-350					60	85	60	80	30
350-400					35	60	50	65	60
400-450						40	25	20	85
450-450								15	65
500-550									35

Percentage drug entrapment efficiency

Table 6: Drug entrapment efficiency of Nifedipine microspheres

Sl. no	Formulation code	Percentage yield	Drug content (%)	Entrapment Efficiency (%)
1	A1	53.00	17.86	36.8
2	A2	65.00	15.73	48.78
3	A3	75.70	17.63	67.74
4	B1	83.70	42.96	88.63
5	B2	83.70	29.52	92.86
6	B3	97.00	24.84	93.86
7	C1	73.60	19.42	36.72
8	C2	93.00	16.15	62.73
9	C3	96.00	27.51	84.35

In-Vitro Dissolution Studies

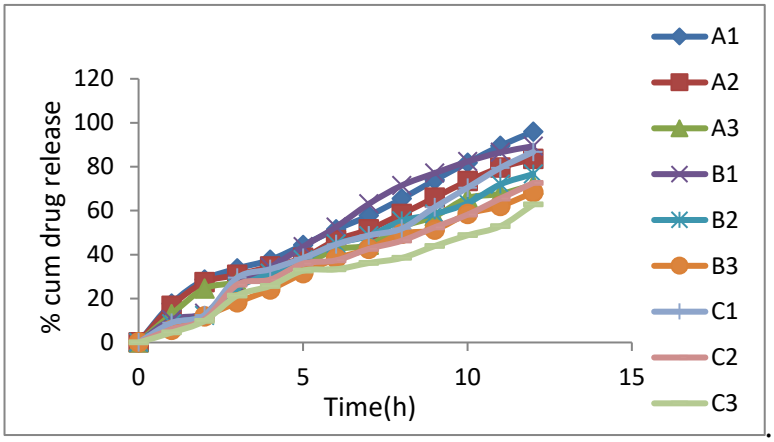

Fig. 1: Comparative in vitro release profile of Nifedipine microspheres

Release kinetics of Nifedipine Microspheres

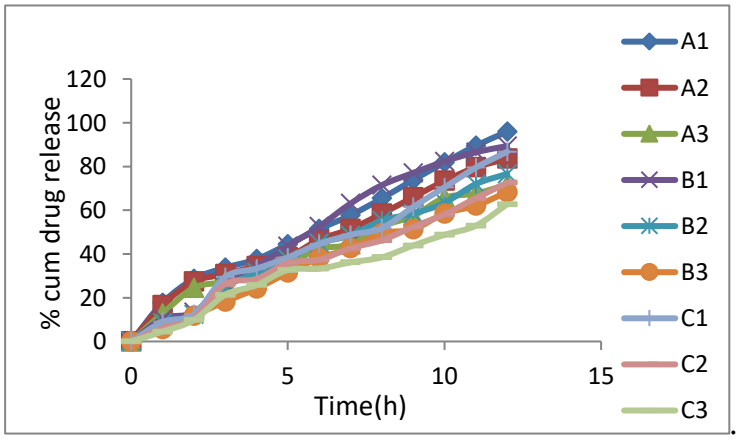

Fig. 2: Release kinetics profile of Nifedipine microspheres according to zero order

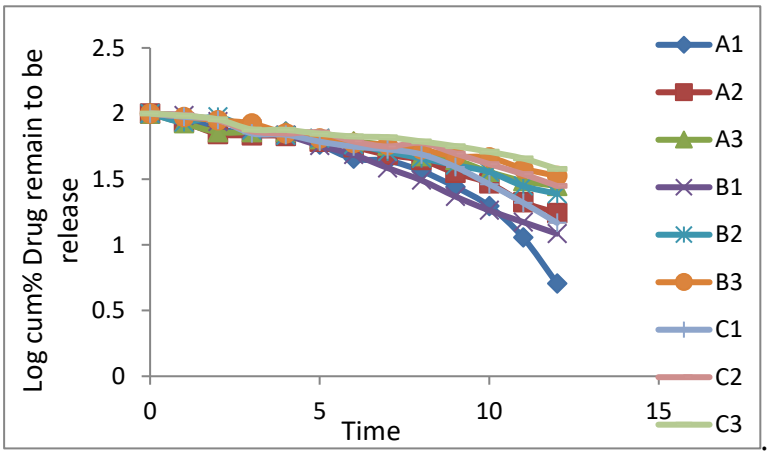

Fig. 3: Release kinetics profile of Nifedipine microspheres according to First order

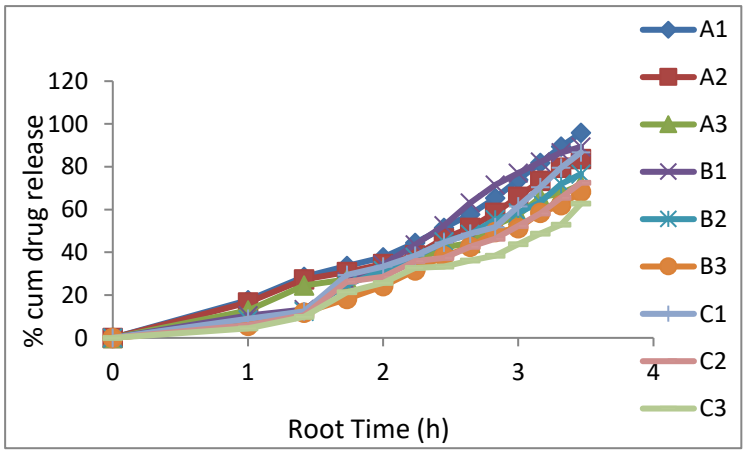

Fig. 4: Release kinetics profile of Nifedipine microspheres according to Higuchi matrix diffusion

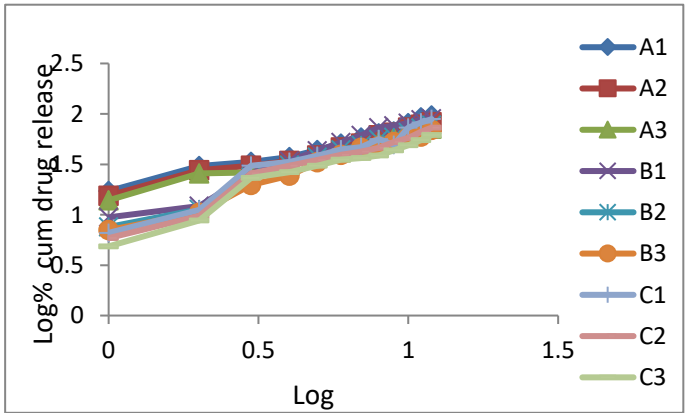

Fig. 5: Release kinetics profile of Nifedipine microspheres according to Peppas mode

Table 7: Regression co-efficient (r^2) values of different kinetic models and diffusion exponent (n) of Peppas model for Nifedipine microspheres

Formulation	Zero order	First order	Higuchi Matrix	Peppas plot	
				(r2) values	'n' value
A1	0.9866±0.0002	0.8578±0.05	0.9455±0.0005	0.9723±0.005	0.6684±0.03
A2	0.9836±0.0005	0.9467±0.06	0.9544±0.0002	0.9767±0.008	0.6531±0.03
A3	0.9785±0.0008	0.9764±0.04	0.9486±0.0006	0.9754±0.005	0.6411±0.03
B1	0.9793±0.0003	0.9788±0.08	0.9845±0.0008	0.9776±0.004	0.9882±0.04
B2	0.9878±0.0006	0.9887±0.05	0.9922±0.0005	0.9856±0.008	0.9618±0.03
B3	0.9892±0.0004	0.9884±0.02	0.9888±0.0007	0.9884±0.007	0.9587±0.03
C1	0.9826±0.0005	0.9034±0.04	0.9585±0.0004	0.9732±0.009	0.9748±0.05
C2	0.9778±0.0004	0.9606±0.06	0.9756±0.0001	0.9626±0.007	0.9957±0.06
C3	0.9645±0.0002	0.9608±0.05	0.9706±0.0004	0.9522±0.003	0.9754±0.06

SD=Standard deviation (n=3) The difference in mean of Zero order, First order, Higuchi kinetics, Peppas Equation between batch series 'A, B, C' were significant ($p < 0.0001$)

Microspheres of Nifedipine were prepared by emulsification, suspension polymerization and emulsification solvent evaporation techniques by using polymers like Chitosan, Gelatin and Sodium Alginate respectively. Various evaluation parameters were assessed, with a view to obtain oral controlled release of Nifedipine. In the present work, total nine formulations were prepared and the detailed composition is shown in Table 1, 2 and 3. The prepared Nifedipine microspheres were then subjected to particle size, size distribution, % yield, drug content, entrapment efficiency, dissolution, release kinetics.

Frequency Distribution Analysis

As the Nifedipine to polymer ratio was increased, the mean particle size of Nifedipine microspheres was also increased Table 4. The significant increase may be because of the increase in the viscosity of the droplets (may be due to the increase in conc. of polymer solution). Nifedipine microspheres having a size range of 10 to 550 µm Table 5 with normal frequency distribution was obtained.

Percentage Yield

The percentage yield for Nifedipine microspheres were 53%, 65%, 75.6%, 83.6%,83.6%, 97%, 73.5%, 93% and 96% for formulation A1, A2, A3, B1, B2, B3,C1, C2 and C3 respectively are given in Table 6.

Percentage drug Entrapment Efficiency

Entrapment efficiency increases with increase in the polymer conc. From the results it can be inferred that there is a proper distribution of Nifedipine in the microspheres and the deviation is within the acceptable limits. The percent of drug content in the formulations was found to be in the range of 17.86% to 29.52%. The percentage entrapment efficiency was found to be

36.78% to 84.20%. The results obtained are given in Table 6. A maximum of 67.74%, 93.86% and 84.35% drug entrapment efficiency was obtained in the Nifedipine microspheres which were prepared by using Chitosan, Gelatin and Sodium Alginate respectively. It was further observed that the drug entrapment was proportional to the Nifedipine: polymer ratio and size of the Nifedipine microspheres. By increasing the polymer conc., the encapsulation efficiency was increased.

***In-Vitro* Dissolution Studies**

The performance of Nifedipine microspheres showed prolonged and controlled release of Nifedipine. The results of the dissolution studies of formulations A1 to C3 are shown in Fig. 1.

Release Kinetics of Nifedipine Microspheres

The plots of Cumulative percentage drug release V/s. Time, Log Cumulative percent drug retained V/s. Time, Cumulative percent drug release V/s. root Time, and Log Cumulative percent drug release V/s. Log Time were drawn and represented graphically as shown in Fig. 2 to Fig. 5. The slopes and the regression co-efficient of determinations (r^2) were listed in Table 7. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 for the Nifedipine microspheres prepared with Chitosan, Gelatin and Sodium Alginate indicating Non- Fickian of drug through Nifedipine microspheres.

CONCLUSION

From the above experimental results it can be concluded that oral controlled release of Nifedipine can be achieved by emulsification, suspension polymerization and emulsification solvent evaporation techniques by using polymers like Chitosan, Gelatin and Sodium alginate respectively. As the drug to polymer ratio was increased, the mean particle size of Nifedipine microspheres was also increased. Nifedipine microspheres with normal frequency distribution were obtained. The entrapment efficiency was increasing with increase in the polymer concentration. From the results it can be inferred that there was a proper distribution of Nifedipine in the microspheres and the deviation was within the acceptable limits. When the concentration increases, the drug release decreases. The in-vitro performance of Nifedipine microspheres showed prolonged and controlled release of drug. The co-efficient of determination indicated that the release data was best fitted with zero order kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of Korsemeyer-Peppas model was found to be in the range of 0.5 to 1 for the Nifedipine microspheres prepared with Chitosan, Gelatin and Sodium alginate indicating

Non-Fickian of drug through Nifedipine microspheres. From the study it is evident that promising controlled release microspheres of Nifedipine may be developed by emulsification, suspension polymerization and emulsification solvent evaporation techniques by using polymers like Chitosan, Gelatin and Na Alginate respectively.

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