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Formulation and Characterization of Hydralazine Hydrochloride Biodegraded Microspheres for Intramuscular Administration

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Abstract

Objective: To prepare and characterize Albumin microspheres of hydralazine hydrochloride for the treatment of hypertension.

Methods: Albumin microspheres of antihypertensive drug hydralazine hydrochloride were prepared by emulsion cross-linking method by using glutaraldehyde as cross-linking agent. Drug and polymer compatibility was determined by Fourier-Transform Infrared spectroscopy. To determine the effect of polymer concentration and amount of glutaraldehyde, formulations were characterized for their entrapment efficiency, particle size, surface morphology and release behavior. In vivo study was carried out on hypertensive wistar rats.

Key findings: Maximum percentage entrapment efficiency (%EE) was found to be 68.20±1.03 %. Laser particle size analyzer confirmed mean particle size in the range of 31.7 to 39.6µm. *In vitro* drug release studies showed a biphasic release pattern for all formulations with an initial burst effect followed by slow release for almost 24 hrs.

Conclusion: *In vivo* study to determine antihypertensive effect of selected formulation strongly correlates with *in vitro* drug release behavior. The release behavior was significantly regulated by polymer concentration and volume of glutaraldehyde. The study revealed that hydralazine hydrochloride loaded albumin microspheres exhibited prolonged reduction of systolic and diastolic arterial pressure compared to hydralazine hydrochloride solution.

Keywords: Hydralazine hydrochloride; Antihypertensive; Albumin microspheres; Intramuscular administration; Glutaraldehyde

Abbreviations: %EE: Percent Entrapment Efficiency; i.v.: Intravenous ; i.m.: Intramuscular; s.c.: Subcutaneous; BSA: Bovine Serum Albumin; DOCA: Deoxycorticosterone acetate; FT-IR: Fourier Transform Infrared; SEM: Scanning Electron Microscopy

Introduction

Unlike mucosal & transdermal drug delivery, in which the systemic bioavailability of a drug is always limited by its permeability across a permeation barrier (epithelial membrane or stratum corneum) and oral drug delivery, in which the systemic bioavailability of a drug is often subjected to variations in gastrointestinal transit and biotransformation in the liver by "first-pass" metabolism, parenteral drug delivery, can give easy access to the systemic circulation with complete drug absorption and therefore reach systemic circulation rapidly[1]. It is followed by rapid decline in blood drug level. As a result, therapeutic response is of short duration with parenteral administration and also initial peak levels might provoke severe side effects mainly in i.v. administration. So for that, continuous i.v. infusion is currently acknowledged as the most reliable means of maintaining any desired drug blood concentration at a constant therapeutic level long enough for successful treatment of a pathological condition[2,3]. Unfortunately, the potential drawbacks associated with i.v. infusion necessitate close medical supervision (and especially hospitalization) during therapy. So, efforts in developing controlled/sustained release parenteral dosage forms have been concentrated on the intramuscular (i.m.) and subcutaneous (s.c.) drug delivery systems[2]. Several pharmaceutical formulation approaches may be applied to the development of parenteral controlled-release or sustained-release formulations, polymeric microspheres is one of them.

The term 'microsphere' describes a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles. Microspheres can be prepared in a wide range of sizes e.g. from nanometer (nanosphere) up to hundreds of micrometers (microsphere) [4]. In particular, there is much interest currently in the use of biodegradable polymers for the preparation of microspheres containing a wide range of therapeutic agents which can, of course, be used for parenteral administration. Solid biodegradable microspheres incorporating a drug dispersed throughout the particle matrix have the potential for the controlled release of the drug from this system after i.m. injection. Microspheres designed for parenteral drug delivery can be composed of a variety of materials with different physical characteristics such as biocompatible, biodegradable, injectable, sterile, compatible with diluents, and pharmaceutically stable [3].

Albumin is a major plasma protein constituent, accounting for ~55% of the total protein in human plasma [5]. Ever since its introduction by Kramer, [6] albumin microspheres have been extensively investigated in controlled release systems as vehicles for the delivery of therapeutic agents. Albumin is used for preparation of microspheres because it is non-antigenic, biodegradable, free from toxicity and readily available [7]. Two methods have been developed for the preparation of albumin microspheres which include heat stabilization and chemical cross-linking by using glutaraldehyde.[8] Size of particles, degree of stabilization, and site of metabolism are the main factors influencing the extent of metabolism of albumin microspheres in the body. Drug release from the microspheres can be controlled by the extent and nature of cross-linking, size, and drug incorporation level

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in the microspheres. More than 100 therapeutic and diagnostic agents have been incorporated into albumin microspheres and drugs of various therapeutic categories such as anti-inflammatory drugs,[9,10] nifedipine,[11] vancomycin,[12] and anti-cancer drugs[13,14] have been investigated previously.

Hydralazine hydrochloride is a direct-acting smooth muscle relaxant used to treat hypertension by acting as a vasodilator primarily on arteries and arterioles. By relaxing vascular smooth muscle, vasodilators act to decrease peripheral resistance, thereby lowering blood pressure.[15] Hydralazine hydrochloride is a water soluble drug and is highly plasma protein bound. Its average plasma half-life is 2-4 hrs and undergoes considerable first-pass metabolism by acetylation. Moreover, its small dose requirement makes it suitable for this study.[16] Adverse effects associated with the use of hydralazine hydrochloride are facial flushing, palpitation, fluid retention, constipation and muscle cramps [15,16].

Thus in this study, hydralazine hydrochloride-loaded bovine serum albumin (BSA) microspheres were prepared and characterized for entrapment efficiency, particle size, surface morphology, *in vitro* release study and *in vivo* study to investigate the effect of polymer concentration and amount of glutaraldehyde.

Materials and Methods

Materials

Hydralazine hydrochloride was procured from Sigma Chemical Company (St. Louis, MO). Bovine serum albumin (Fraction-V) was purchased from S.D. Fine-Chem Ltd. (Mumbai, India). Deoxycorticosterone acetate (DOCA) was purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals and reagents were of analytical grade.

Methods

Preparation of microspheres: Albumin microspheres were prepared using emulsion polymerization technique [17] / emulsion cross-linking method. [18] Microspheres were prepared from different concentrations of BSA and various amounts of glutaraldehyde as shown in Table 1. A weighed amount of BSA was dissolved in distilled water. Hydralazine hydrochloride (50mg) was dissolved in prepared BSA solution. One ml of this solution was added dropwise to liquid paraffin (40 ml) containing 1% w/v of span 80 as a surfactant while stirring the whole system at 2500 rpm. After 10-15 minutes of stirring, glutaraldehyde was added (Table 1) into the emulsion to cross-link the albumin present in the internal phase of the emulsion. Stirring was continued for the required cross-linking duration (4hrs). Microspheres formed were then separated from the oil phase by filtration and were washed with n – hexane (75 ml) to remove the excess oil. The microspheres were then suspended in 10 ml of 5% w/v sodium bisulphite solution and stirred for 10 minutes to remove residual glutaraldehyde. Finally, they were washed with water and then dried at room temperature.

Aseptic microsphere production: Syringes, glasswares, and dismantled stirrer rod of propeller were autoclaved, while remaining parts of the propeller were surface disinfected with 70% ethanol. The equipment was assembled in a laminar air-flow cabinet and finally purged with 70% ethanol. BSA solution was sterile filtered over 0.2 µm filter paper before using for the preparation of microspheres. The final product was sterilized by effective UV radiation [19].

Drug-polymer compatibility study: FT-IR spectroscopy was carried out to check the compatibility between drug and polymer after the microsphere formation. The FT-IR spectrum of formulation was compared with the standard FT-IR spectrum of the pure drug [20].

% Practical yield: Microspheres were collected and weighed to determine practical yield (PY) from the following equation.

$$PY\ (\%) = \frac{\text{Practical Mass (Microspheres)}}{\text{Theoretical Mass (Polymer + Drug)}} \times 100 \quad (1)$$

The prepared microspheres were then characterized for their various properties.

Entrapment efficiency

To determine the amount of drug encapsulated in microspheres, 50 mg of microspheres were suspended into screw-capped vials with 0.1N HCl (50 ml) and digested for 24 hrs on a magnetic stirrer in order to extract the entrapped drug completely. The absorbance was noted at 263 nm using a double-beam UV spectrophotometer after diluting suitably with distilled water. [9] The percentage of encapsulation efficiency was calculated by the following formula.

$$EE\ (\%) = \frac{ED}{AD} \times 100 \quad (2)$$

Where %EE is the percentage encapsulation efficiency; ED is the amount of encapsulated drug; and AD is the amount of added drug.

Surface morphology

Scanning electron microscopy (SEM) of the albumin microspheres was performed to examine the surface morphology. The microspheres were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. The photographs were taken using a Jeol scanning electron microscope.

Particle size analysis

The particle size distribution of the microspheres was determined by laser particle size analyzer using n-hexane as dispersant. The microspheres dispersions were added to the sample dispersion unit containing stirrer and stirred to reduce the aggregation between the microspheres. The average volume-mean particle size was measured after performing the experiment in triplicate.

Sterility test

In order to ensure the sterility of the finished products, formulation F6 was subjected to sterility test. The microspheres were mixed with casein-peptone-soypeptone (CASO) broath, incubated for 24h at ambient condition, and the broath mixture was filtered using Milliflex 100 system. One part of the Milliflex filter was incubated with tryptic soy broath (TSB) at 30-35°C for 14 days to test for bacterial growth, and the another one at 20-25°C for 14 days to test for growth of yeasts and moulds. The filters were checked daily for colony growth [19].

In vitro drug release studies

Drug release from the microspheres was determined using

Formulation Code	Drug (mg)	BSA Conc. (%w/w)	Span 80 (%w/v)	Vol. of Glutaraldehyde (ml)
F1	50	10	1	0.5
F2	50	10	1	1
F3	50	10	1	2
F4	50	20	1	0.5
F5	50	20	1	1
F6	50	20	1	2

Table 1: Composition of hydralazine hydrochloride loaded BSA microspheres.

phosphate buffer (pH 7.4) as the release medium. A weighed amount of microspheres, equivalent to 5 mg of drug, were suspended in 50 ml of the dissolution medium and stirred on a magnetic stirrer at 50 rpm in a thermostated bath at 37°C. One ml sample was withdrawn at appropriate time intervals and absorbance was measured at 263 nm after suitable dilution in a double-beam UV spectrophotometer using the dissolution medium as blank [10].

In vivo studies

This study was carried out to evaluate the efficiency of drug loaded microspheres to decrease hypertension in hypertensive Wistar rats weighing about 250 - 300 g. All the experiments were carried out in accordance with the protocols approved by the institutional animal ethics committee.

Dose of Hydralazine hydrochloride to be administered to rats was calculated according to body surface area ratio of rats to human being [21].

$$\text{Dose (mg/200 gm of rat)} = \text{Human dose (mg)} \times \text{conversion factor} \quad (3)$$

Animals were divided into 4 groups, each containing 4 rats. Cardiovascular parameters were measured by the tail cuff method. Hypertension was induced by treating the animals subcutaneously with deoxycorticosterone acetate (12.5 mg kg^{-1} per week) and 0.9% NaCl drinking solution *ad libitum* for 30 days [22].

Group-I was kept as control, Group-II was treated with only DOCA salt, while Group-III and Group-IV were treated with DOCA salt + Formulation and DOCA salt + pure drug, respectively. On thirty-first day after the beginning of DOCA salt treatment, when animals became hypertensive, Group-III and IV were treated intramuscularly with 1.8 mg/ kg equivalent Hydralazine hydrochloride contained F6 formulation in the suspension form and pure drug as solution in pH 7.0 phosphate buffer solution, respectively. The arterial blood pressure was measured at 0, 1, 2, 6, 12 and 24 hrs with the help of tail cuff method.

Statistical analysis

The results are expressed as mean ± SD and in vivo study was analyzed by One-way Analysis of Variance followed by Tukey's Multiple Comparison Test at the significant level of $P < 0.05$ and $P < 0.001$.

Results

Compatibility study

FT-IR spectroscopy was carried out to study the compatibility of pure drug hydralazine hydrochloride with bovine serum albumin after the formulation of microspheres. The individual IR spectrum of the pure drug hydralazine hydrochloride was also found to be similar to that of its standard spectrum. [20] The characteristic peaks of the pure drug as N-H stretch (3217.1 cm^{-1}), aromatic C-H stretch (3028.1 cm^{-1}), C=C stretch (1591.4 cm^{-1}) and out of plane bending, adjacent H atoms on an aromatic ring (786.6 cm^{-1}) were present with the peaks obtained in the microspheres formulation spectrum. So, it shows the presence of the drug in the microspheres formulations and confirms the compatibility of drug with the polymer.

Entrapment efficiency

The values of entrapment efficiency are shown in Table 2. The maximum entrapment efficiency was found for F6 (68.20±1.03%) while it was lowest for F1 (57.39±1.48%). Formulation F1, F2 and F3 with different volume of glutaraldehyde showed entrapment efficiency as 57.39±1.48, 59.78±1.43 and 62.60±2.03 respectively. This demonstrates that an increase in the amount of glutaraldehyde results in an increase in entrapment efficiency. Similar kinds of results were obtained for the formulations F4, F5 and F6. Results showed that the concentration of polymer also significantly affects the entrapment efficiency. There is an increase in the entrapment efficiency as polymer concentration increased from 10% w/w to 20% w/w.

Surface morphology

Surface topography and morphology of the microspheres were investigated with a scanning electron microscope. SEM is one of the common methods used owing to the simplicity of sample preparation and ease of operation. Scanning electron photomicrographs of the formulation F6 is shown in Figure 1. SEM analysis of the samples revealed that all microspheres prepared were spherical in shape. Figure 1 also represents the morphology of F6, which shows smooth surface of microspheres.

Particle size analysis

The particle size distribution was determined by laser particle size analysis and results are shown in Table 2. The particle size was found to be mainly dependent on the albumin concentration. An increase in the albumin concentration from 10% w/w to 20% w/w led to significant increase in the particle size, while amount of glutaraldehyde had not much influence on the particle size of the formulations. Figure 2 describes effect of polymer concentration and amount of glutaraldehyde on mean particle size of microspheres.

Sterility test

The results obtained after 14 days of incubation showed no growth of organisms on the filters. This indicates that, the formulation is sterile and passes the test for sterility.

In vitro drug release studies

The release profile of an entrapped drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behavior. All the six formulations of microspheres were subjected to *in vitro* release studies. These studies were carried out using pH 7.4

Formulation code	% Practical yield ± SD	% Entrapment efficiency ± SD	Mean Particle Size (µm) ± SD
F1	81.33 ± 1.35	57.39 ± 1.48	31.70 ± 1.47
F2	79.33 ± 0.88	59.78 ± 1.43	32.91 ± 0.45
F3	82.66 ± 1.91	62.60 ± 2.03	34.53 ± 0.84
F4	87.00 ± 1.71	65.88 ± 0.94	36.11 ± 0.86
F5	81.40 ± 1.27	66.35 ± 1.13	39.80 ± 0.99
F6	84.20 ± 0.92	68.20 ± 1.03	39.62 ± 0.78

*Data are expressed as mean ± SD (n=3)

Table 2: % Practical yield, % Entrapment efficiency and Mean particle size of microspheres.

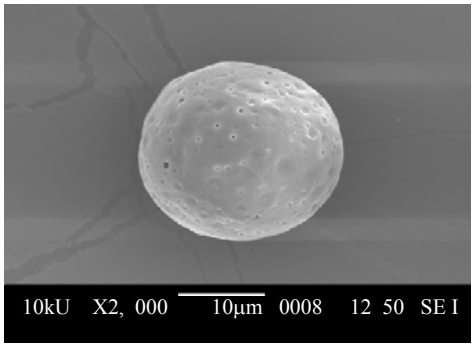

Figure 1: Scanning Electron Photomicrograph of albumin microspheres formulation F6.

phosphate buffer saline as dissolution medium in 100 ml glass vials. The *in vitro* drug release data for each of the formulations is shown in Figure 3. The cumulative percent drug release after 24 hrs was found to be 97.51%, 96.13%, 95.48%, 97.18%, 96.37% and 95.69%, respectively for the formulations F1 to F6. Plot of cumulative percent drug release as a function of time showed that almost all the formulations except F1 exhibited a prolonged release for almost 24 hrs, while F1 released 90% drug in the 8th hour of *in vitro* release process. For the other formulations as the glutaraldehyde concentration increases, a decrease in the release was observed. The initial dose release of 48.59%, 43.59%, 35.98%, 46.14%, 42.14% and 27.88% during the initial 1 hr for F1 to F6 formulations was noted followed by slower continuous release.

Polymer concentration also affects the release behavior, as increase in the polymer concentration retards the drug release. Formulation F4, F5 and F6 shows slight decrease in the release pattern as increase in the concentration of polymer as compared to the formulation F1, F2 and F3.

To obtain the values of the release constant and to understand the release mechanism the release data was fitted to various mathematical models such as Korsmeyer-Peppas, Higuchi Matrix and Hixson-Crowell. The correlation coefficients (r^2) for the different drug release kinetic models are shown in Table 3. Models with the highest correlation coefficient were judged to be the most appropriate model for the *in vitro* release study. For the formulation F6, the best fitting linear parameter is Higuchi matrix model and their correlation coefficient is 0.9910. This indicates that the drug release is controlled by diffusion of the drug through the pores. On the other hand, the formulations F1 to F5 best fitted into the Korsmeyer-Peppas model. The 'n' value could be used

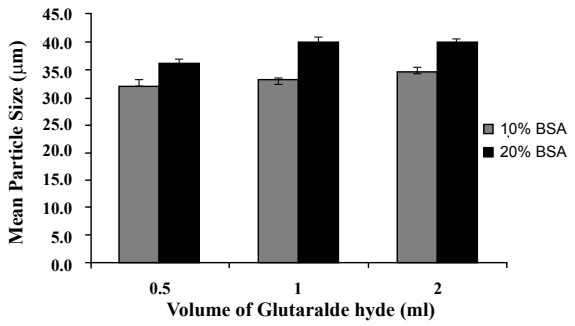

Figure 2: Effect of polymer concentration and volume of gluta raldehyde on mean particle size of hydralazine hydrochloride microspheres. BSA – Bovine serum albumin. Error bars in the graph represents the SD (n=3) values.

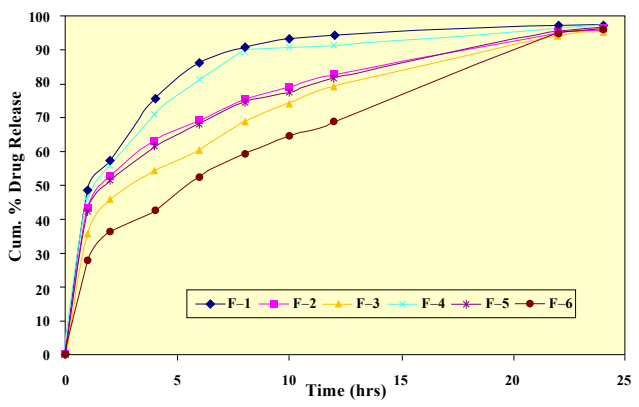

Figure 3: In vitro release behavior of hydralazine hydrochloride microspheres.

Formulation	Higuchi		Korsmeyer-Peppas		Best fit Model	Mechanism of Drug Release
	r^2	Slope (k)	r^2	Slope (n)		
F1	0.778	17.62	0.907	0.228	Korsmeyer-Peppas	Fickian
F2	0.890	17.28	0.998	0.249	Korsmeyer-Peppas	Fickian
F3	0.949	18.03	0.997	0.309	Korsmeyer-Peppas	Fickian
F4	0.809	17.71	0.932	0.242	Korsmeyer-Peppas	Fickian
F5	0.906	17.54	0.999	0.261	Korsmeyer-Peppas	Fickian
F6	0.991	18.82	0.990	0.392	Higuchi	Diffusion

Table 3: Values of r^2, k and n for all the formulations.

to characterize different release mechanisms for Korsmeyer-Peppas model. The 'n' values indicate that the formulations F1, F2, F3, F4 and F5 exhibited Fickian release.

In vivo studies

Formulation F6 having optimal particle size, high entrapment efficiency and satisfactory *in vitro* drug release was selected for *in vivo* studies. The study was done to compare *in vivo* antihypertensive effect of prepared formulation F6 and pure drug (Hydralazine HCl) and the results are presented in Table 4.

The baseline systolic and diastolic arterial pressure in control group (Group-I) was 120±0.40 and 91±0.7 mmHg. Because of the administration of DOCA in other Groups- II, III and IV for thirty days, systolic and diastolic arterial pressure was increased upto 162±1.35 and 119±1.08 mmHg in DOCA salt group, 164±1.08 and 118±0.82 mmHg in DOCA salt + formulation group, and 163±0.71 and 119±0.42 mmHg in DOCA salt + pure drug group. On day 31 after 1 hr, a sudden decrease in the systolic and diastolic pressure was observed in Group-IV (142±1.08 and 108±1.07 mmHg respectively) and Group-III reduced up to 153±1.58 and 115±0.91 mmHg. But at 12 hrs, Group-IV showed slight increase in blood pressure 137±1.0 and 100±1.04 mmHg, while Group-III reduces blood pressure to 129±0.91 and 97±1.23 mmHg. And after 24 hrs of study, Group-III maintained the systolic and diastolic arterial pressure to 126±0.41 and 94±1.08 mmHg that is near to baseline arterial pressure of Group-I while Group-IV showed 139±0.87 and 101±0.85 mmHg.

Discussion

Albumin microspheres have been extensively investigated in controlled release systems as vehicles for the delivery of therapeutic agents. There are numerous ways of preparing albumin particles. These can involve drug incorporation either during the process or after formation of particles. The first involves either thermal denaturation at elevated temperatures or chemical cross-linking in either vegetable oil, isooctane emulsions etc. The method used is called as emulsion cross-linking or emulsion polymerization technique. A one more reason for emulsion cross-linking method used to prepare microspheres is because BSA and Hydralazine hydrochloride are water soluble. Albumin and drug were dissolved in distilled water, and this solution acted as the aqueous phase. The method involved the formation of small droplets of aqueous albumin in immiscible liquid, light liquid paraffin. The water-in-oil emulsion was stabilized by a lipophilic surfactant, Span 80. Being a soluble polymer, albumin has to be chemically cross-linked to become insoluble at 37 °C. Glutaraldehyde is used as a cross-linking agent to obtain rigid microspheres in this study. Glutaraldehyde was added after 15 minutes of stirring the system at 2500 rpm. Fifteen minutes was given for the droplet formation, which is a dynamic process that approaches a steady-state droplet size distribution within a period of several minutes depending on various parameters of the system. An important feature of this technique is that the albumin droplets are converted to swollen particle of the same size. The individuality of the initially formed droplets is maintained by performing the cross-linking

Time (hours)	Animal Groups							
	Control (Group I)		DOCA salt (Group II)		DOCA salt + Formulation (Group III)		DOCA salt + Pure Drug (Group IV)	
	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP	Systolic BP	Diastolic BP
0 hr	120 ± 0.40	91 ± 0.7	162 ± 1.35*	119 ± 1.08*	164 ± 1.08#	118 ± 0.82#	163 ± 0.71##	119 ± 0.42##
1 hr	119 ± 0.82	92 ± 1.23	164 ± 0.81*	120 ± 0.58*	153 ± 1.58#	115 ± 0.91#	142 ± 1.08##	108 ± 1.07##
2 hr	121 ± 0.7	90 ± 1.08	162 ± 1.23*	121 ± 0.71*	148 ± 0.41#	109 ± 0.912#	131 ± 1.59##	102 ± 0.41##
6 hr	120 ±0.91	91 ± 0.40	163 ± 2.34*	120 ± 1.47*	139 ± 1.23#	103 ± 0.57#	132 ± 1.68##	101 ± 0.71##
12 hr	122 ± 1.08	91 ± 1.22	162 ± 0.41*	119 ± 1.68*	129 ± 0.91#	97 ± 1.23#	137 ± 1.0##	100 ± 1.04##
24 hr	120 ± 1.0	92 ± 0.82	163 ± 1*	119 ± 1.15*	126 ± 0.41#	94 ± 1.08#	139 ± 0.87##	101 ± 0.85##

BP- Blood pressure, DOCA- Deoxycorticosterone acetate.
Data expressed in Mean ± SEM. n = 4. Statistical analysis is done by One-way Analysis of Variance followed by Tukey's Multiple Comparison Test. *P<0.001: when compared with Control Group; #P<0.05: When compared with DOCA Treated group; ##P<0.001: when compared with DOCA Treated group.

Table 4: *In vivo* studies of hydralazine hydrochloride microspheres (formulation F6) for antihypertensive effect.

reaction at constant stirring and in the presence of lipophilic surfactant Span 80. Initially formed albumin microspheres are hardened with the chemical cross-linking agent glutaraldehyde. The cross-linking reaction was initiated after the droplet formation process was complete. The excess oil was removed by washing with n-hexane in order to prevent agglomeration of the prepared microparticles. Long term exposure to 100 ppb glutaraldehyde vapor (5 days a week for 78 days) causes respiratory tract lesions including hyperplasia of squamous epithelium, necrosis and exfoliation of epithelial cells and granulocytes. [23] Therefore, it is important to remove excess glutaraldehyde from the microspheres to avoid any toxic reactions associated with it. In the present study, microspheres were washed with sodium bisulphite to remove the excess glutaraldehyde. The percentage yield is given in Table 2. The effect of different factors on percentage yield was not clear, possibly as improper recovery of microspheres from the filter paper.

As shown in the Table 2, an increase in the polymer concentration led to a significant increase in the entrapment efficiency. It may be due to the formation of more viscous solutions that can more efficiently prevent the dissolution of hydralazine hydrochloride in the external phase of the emulsion. The SEM photomicrographs reveals that microspheres were spherical with smooth surface. The result obtained was may be due to the low viscosity of light liquid paraffin which was used as external phase.

One of the aims of this study was to formulate microspheres which would be administered through intramuscular route; hence the particle size range obtained would be suitable for this purpose. This study showed, however, that there was no significant difference in particle size with change in cross-linking agent volume used in the formulation, but as polymer concentration increased there was significant increase in the particle size. Furthermore, considering that microspheres obtained were within the size range of 1-60 μm, so are suitable for the intramuscular administration.

All six formulations were evaluated for in vitro drug release for 24 hrs in 100 ml glass vials containing 50 ml of dissolution medium. The immediate release of drug from F-1 may be due to the low proportion of the polymer (10%) and reduced amount of glutaraldehyde (0.5ml) for cross-linking. For the other formulations as the glutaraldehyde concentration increases, the extent of denaturation increases and this makes albumin microspheres insoluble. This will lead to slower release of the drug from microspheres. The release pattern of hydralazine hydrochloride from BSA microspheres showed a biphasic drug release pattern, characterized by an initial burst effect followed by slow release. The initial burst effect of hydralazine hydrochloride release from microspheres was mainly seen due to the portion of the drug located on the surface of the microspheres followed by the diffusion of the drug through the cross-linked BSA matrix. Polymer concentration significantly affects the drug release by retarding the release of drug from the microspheres. It is because of the formation of denser polymer cross-links leading to an increase in the diffusional path length that the drug molecules have to transverse. This is in accordance with the earlier reports [24].

Antihypertensive effect of formulation F6 and pure drug hydralazine hydrochloride was determined by using tail-cuff method to measure arterial blood pressure. These results revealed that, Group-III with the drug loaded albumin microspheres (formulation F6) maintains reduced systolic and diastolic arterial pressure upto 24 hrs, while an increase in the pressure after 6 hrs was seen in Group-IV with pure drug. These results are strongly correlating with *in vitro* drug release behavior of formulation F6.

Conclusion

The results of the present study suggest that the entrapment efficiency, particle size distribution, *in vitro* release pattern and *in vivo* anti hypertensive effects of the hydralazine hydrochloride loaded albumin microspheres can be controlled by varying polymer concentration and volume of glutaraldehyde. Considering the obtained results of particle size distribution, *in vitro* release and *in vivo* study; the prepared BSA microspheres can be used for intramuscular administration. So, it may be concluded that developed microspheres could be useful for once-a-day antihypertensive therapy. Further, pharmacokinetic and pharmacodynamic studies and detailed stability studies are required to confirm the application of these microspheres for IM administration.

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