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FORMLATION AND EVALUATION OF MICROPARTICLES OF CLOPIDOGREL BISULPHATE USING NATURAL MUCILAGES

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Abstract:
The aim of the present research work was to develop the microparticles of Clopidogrel bisulphate by using natural polymers, Prosopis juliflora seed mucilage, Chia seed mucilage and sodium alginate. Prosopis juliflora seed mucilage and Chia seed mucilage were isolated and purified using ethanol. Ionotrophic gelation technique was used to prepare microparticles of Clopidogrel bisulphate. 5 % w/v Calcium Chloride was used as crosslinking agent. The prepared microparticles were evaluated for compatibility study, entrapment efficiency, in vitro dissolution and surface morphology study. Developed formulations were found compatible, determined by Fourier Transform Infrared spectroscopy method. Prepared microparticles were shown 79.3 to 94.26% and 76.92 to 96.38 % drug entrapment efficiency for Prosopis juliflora seed mucilage and Chia seed mucilage respectively. In-vitro drug release study showed that the drug release was extended up to 12 hours using Chia seed mucilage at 98.63 % concentration in prepared microparticles. Prepared micropaticulate drug delivery system followed zero order drug release kinetics with non-fickian type diffusion. Chia seed mucilage was shown better sustained drug release than Prosopis juliflora seed mucilage. Scanning electron microscopy study showed the spherical and porous nature of the prepared micro-spheres.

Keywords: *Ionotropic gelation, Microparticle, Clopidorel bisulphate, Prosopis seed mucilage, Chia seed mucilage.*

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INTRODUCTION:

Microencapsulation is a process by which solids, liquids or even gases may be enclosed in microscopic particles formation of thin coatings of wall material around the substances [1, 2].

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects.

Microparticulate drug delivery system provides the sustained & controlled delivery of drug for long duration. They are small particles of solids or small droplets of liquids surrounded by walls of natural & synthetic polymer films of varying thickness & degree of permeability acting as a release rate controlling substance & have a diameter upto the range of 0.1µm-200µm [1]. In 1974, Kramer initially used albumin microspheres in drug delivery system. In 1997, Java Krishna & Catha introduced the use of microspheres as sustained release vehicles.[2] Microparticles are also a beneficial to deliver APIs which are pharmacologically active but are difficult to deliver due to limited solubility in water. In such drugs the attainment of high Cmax, Tmax, and Area under the curve is problematic [3].

Coronary heart disease (CHD) is a major cause of mortality and morbidity all over the world. According to a report of World Health Organization (WHO) in 2005, cardiovascular disease (CVD) caused 17.5 million (30%) of the 58 million deaths that occurred worldwide [4].

Clopidogrel bisulfate is an oral, thienopyridine class antiplateletagent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Clopidogrel is a pro-drug of carboxyl clopidogrel activated in the liver by cytochrome P450 and CYP2C19 enzyme. The active metabolite has an elimination half-life of about 7-8 hrs and acts by forming a disulfide bridge with the platelet ADP [5].

Following oral administration, Clopidogrel bisulphate shows bioavailability of about only 50% due to poor water solubility and first pass metabolism to large portion of inactive metabolite. The main side effect of the drug is gastric bleeding. A sustained release microparticle of clopidogrel bisulphate formulation may be preferred over conventional film coated tablet for improving the bioavailability, to minimize gastric bleeding and better patient compliance [6].

The main objective is to prepare the sustained release microparticles of clopidogrel using different natural polymers- *Prosopis juliflora* seed mucilage, Chia seed mucilage and sodium alginate.

MATERIALS AND METHODS:

Materials

Clopidogrel was obtained as a gift sample fromResearch-lab fine chem industries, Mumbai. Sodium alginate was obtained form Vishal-chem, Mumbai, Prosopis seed was obtained from Fields of Malegaon,Chia seedwas obtained form Go India Organic (New Delhi India), All other chemicals and reagents used in the study were of analytical grade

Methods

Preparation of Microspheres

Microspheres of Clopidogrel bisulphate were prepared by ionotropic gelation method using Sodium alginate, Prosopis seed Mucilage, Chia seed mucilage and calcium chloride. Weighed quantity of drug and polymer were added to 30 ml of sodium alginate solution with stirring at about 300 rpm. The resultant solution was then added drop wise using 24 gauge syringe to 100 ml of calcium chloride solution under continuous stirring. Stirring was continued for 30 minutes. The obtained microspheres were filtered and washed with purified water and then dried for 6 hours at 40°C.

Evaluation of Microspheres [7-9]

Percentage yield

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculatedusing formula given below.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}}$$

Drug entrapment efficiency

Drug loaded micro capsules (100 mg) were powdered and suspended in0.1 N HCl. Then the contents suspended in the water were kept for sonication for about 20 minutes and shaking using mechanical shaker for about 20 mts for the complete extraction of drug from the microcapsules. The resultant solution was filtered through 0.45 µm membrane filter. Drug content was determined by UV- visible

spectrophotometer at 220 nm. The percent entrapment was calculated by using the following formula

$$\%\ \text{drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{theoretical drug content}}$$

Morphology

Morphological characterization of microsphere was done using scanning electron microscope.The external surface morphology of optimized batch of microparticles were obtained by scanning electron microscope under vacuum. The samples for SEM were prepared by mounting dried microparticles on a double adhesive tape stuck to an aluminium stub. The stubs were then coated with platinum to a thickness of about 10°A under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Afterwards, the stubs containing the coated samples were placed in the scanning electron

Swelling Index of Microspheres

For estimating the swelling index, weighed 100 mg of microspheres were allowed to swell in 0.1 N HCL for 24 h. The excess surface adhered liquid drops were removed by blotting and swollen microspheres were weighed by using microbalance. The degree of swelling was calculated by following formula.

$$\%\ \text{Swelling index} = \frac{(Wg - W0)}{W0} \times 100$$

Where,

W_o=Initial weight of microparticles

W_g = Weight of swelled microparticles in the medium after 8hr.

Determination of IR spectrum

The infrared spectrum of Prosopis seed mucilage, Chia seed mucilage, Sodim alginate was recorded by KBr pellet technique in which mixture of polymer and potassium bromide was compressed and an infrared spectrum was recorded using FTIR Spectrophotometer (FTIR-8400S, Shimadzu). The identified peaks were compared with the principle peaks of reported IR spectrum of respective polymer and the samples were authenticated.

***In vitro* Dissolution studies**

In vitro dissolution study was performed using USP dissolution test apparatus-I (basket assembly). The dissolution was performed using 900 ml of 0.1 N HCl solution for 1 hr. and after 1 hr. 0.1 N HCl was replace by phosphate buffer solution (pH 6.8) for 11 hr. as dissolution media maintained at 37 ± 0.5°C and 50 rpm. Samples (5ml) were withdrawn at regular intervals and volume were replaced with fresh dissolution medium to maintain sink condition. Samples were filtered through filter paper and assayed spectrophotometrically on UV-visible spectrophotometer at 220 nm wavelength for 0.1 N HCl (pH 1.2) and at 222 nm for phosphate buffer solution (pH 6.8). For each formulation, the release was repeated in triplicate, results are expressed as a mean ±S.D.

Micromeritic properties

It is clear that no single and simple test method can adequately characterize the flow properties of pharmaceutical powders. So, the microspheres and drug were characterized in terms of commonly employed test methods that are valuable during pharmaceutical development.

Angle of repose (θ):

Angle of repose of different formulations was measured according to fixed funnel standing method. The height of funnel was kept fixed at 2cm. The micro particles were allowed to flow out of the funnel orifice on a plane paper kept on the horizontal surface and the diameter of pile was measured. The angle of repose was calculated by substituting the values of base radius 'r' and pile height 'h' in the following equation,

$\tan\theta = h/r$

The flow properties of the microparticles were evaluated from the changes in the volume due to rearrangement and packing occurring during tapping in a graduated measuring cylinder.

Bulk density

The bulk density determined by small quantity of microsphere (m) samples is carefully introduced into 10ml graduated cylinder, without compacting, read the unsettled apparent volume (V0) to nearest graduated unit. Calculate the bulk density in g/ml by the formula.

$$\text{Bulk density (Db)} = \frac{\text{Weight of powder}}{\text{Volume of packing before tapping}}$$

Tapped density

Tapped density determined by taking small quantity of microsphere sample carefully introduced into 10 ml graduated cylinder. Cylinder was dropped at 2 sec.

intervals on hard wood surface 100 times from height 1 inch. Tapped density of each sample was obtained by dividing weight of sample in gm. By final tapped volume in cm3 of sample contain in cylinder.

$$\text{tapped density (Dt)} = \frac{\text{Weight of powder}}{\text{Volume of packing after tapping}}$$

Carr's compressibility index

TheCarr's compressibility Index was calculated by substituting the values of tapped density and bulk density in the following equation,

$$\text{Carrs compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \text{ x100}$$

Hausner ratio (HR)

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Stability Studies

The microspheres prepared in the present study were filled in the hard gelatin capsules and then filled in HDPE containers and stored at the following conditions like 40°C/75 RH for 3 about months as per ICH guidelines. The samples were characterized for % drug content.

Table 1: formulations of clopidogrel bisulphate microspheres

Ingredient (quantities in gm)	Formulation Code							
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
ClopidogrelBisulphate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Prosopis seed mucilage	1	1.25	1.5	1.75	---	---	---	---
Chia seed mucilage	---	---	---	---	1	1.25	1.5	1.75
Sodium alginate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

RESULTS AND DISCUSSION:

Drug-polymer interactions were studied by FTIR spectrophotometry and no interaction was found. Clopidogrel bisulphate loaded microparticles were formulated by using ionotropic gelation technique.The effects of different concentration of polymer on microparticles were studied.The formulated microparticles were evaluated for sphericity, microparticles size, % entrapment efficiency, % swelling index, flow properties and *In vitro* % drug release.

Prosopis seed mucilage and chia seed mucilage having very weak cross-linking ability in calcium chloride solution. Hence, sodium alginate in small concentration was selected in combination with prosopis and chia seed mucilage and it results in formation of highly cross-linked microparticles.Concentration of sodium alginate was constant (1%) for all F1-F8 formulations and concentration of prosopis seed mucilage and chia seed mucilage was varied from 2%-3.5% for F1-F4 and F5-F8 formulations respectively. The % drug release data of microparticles of both prosopis seed mucilage and Chia seed mucilage showed that the microparticles of chia seed mucilage having high sustained effect as compared to microparticles of prosopis seed mucilage.

Table no. 2 Evaluation parameters for Clopidogrel bisulphate microparticles

Batch Code	% Entrapment Efficiency*	% Swelling Index (After 8 hr.)*	% Yield*
F1	79.3± 076.	72±0.99	85± 1.92
F2	83.3± 0.64	77±0.87	89± 3.41
F3	90.47± 0.52	82±0.75	93.2± 1.29
F4	94.26± 0.38	83±0.76	94.18± 1.9
F5	76.92± 0.48	67±0.87	84.5± 1.51
F6	86.95± 0.68	80±0.79	93.77± 2.39
F7	90± 0.56	81±0.95	95.6± 2.9
F8	96.38± 0.87	85±0.87	98.8± 1.11

*All values are expressed as Average ± SD, n = 3

FTIR Studies of mixture of drug and polymers (Prosopis seed mucilage+Chia seed mucilage+Na-alginate):
The IR spectra of drug and polymers were recorded in combination with each other.

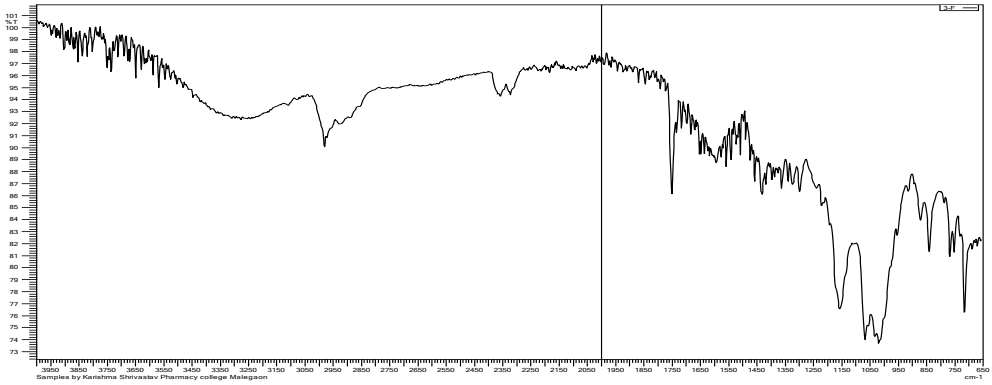

Figure 1: FTIR Spectra for compatibility study: mixture of drug+polymers (Prosopis seed mucilage+Chia seed mucilage + Na-alginate)

Dissolution study:

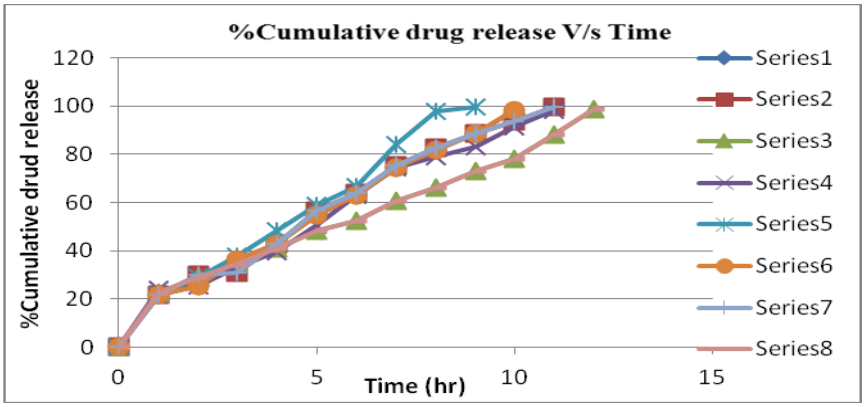

Figure 2: Dissolution profile of formulation F1-F8

Drug release kinetic study:

Table no.3: drug release kinetic study

Formulation Code	Zero order (R^2)	First order(R)	Higuchi Model (R^2)	Korsemeyer peppas	
				(R^2)	(n)
F1	0.9894	0.7689	0.9366	0.9736	0.747
F2	0.9891	0.6925	0.9509	0.974	0.688
F3	0.9829	0.8269	0.9583	0.9583	0.680
F4	0.896	0.4942	0.8426	0.8426	0.516
F5	0.9796	0.7407	0.9155	0.9155	0.734
F6	0.9901	0.8021	0.9491	0.9659	0.711
F7	0.9799	0.9173	0.955	0.9595	0.694
F8	0.9794	0.7001	0.9582	0.9679	0.597

Flow properties of Microparticles:

Table no.4: flow properties study of formulations F1-F8

Batch Code	Angle of Repose	Bulk Density* ($g\ cm^3$)±S.D.	Tapped Density* ($g\ cm^{-3}$)±S.D.	Carr'sIn dex	Hausner Ratio (HR)	Flowability
F1	28	0.58±0.03	0.68±0.03	14	1.17	Excellent
F2	26	0.56±0.34	0.64±0.05	10.9	1.14	Excellent
F3	25	0.55±0.06	0.58±0.08	5	1.05	Excellent
F4	21	0.52±0.04	0.54±0.13	5.5	1.03	Excellent
F5	29	0.58±0.05	0.60±0.09	5.2	1.03	Excellent
F6	27	0.55±0.03	0.58±0.07	5.1	1.05	Excellent
F7	26	0.54±0.04	0.57±0.05	5.5	1.05	Excellent
F8	22	0.51±0.06	0.54±0.32	5.1	1.05	Excellent

*All values are expressed as Average ±SD, n = 3

Stability studies:

Table no.5: stability study data of optimized F8 formulation

Sr. No.	Parameter	40°C ± 2°C/75% RH ± 5% RH		
		30 days*	60 days*	90 days*
1	% Drug content	95.81 ± 0.26	95.76 ± 0.15	95.71 ± 0.10
2	*In-vitro* Dissolution	98.73±0.45	98.5±0.31	98.24±0.21

*values are expressed as Average ±SD, n = 6

CONCLUSION:

Clopidogrel bisulphate microparticles using Prosopis seed mucilage and Chia seed mucilage were successfully prepared by ionotropic gelation method. Drug and polymers were compatible with each other as indicated by FT-IR study. Among the different formulations prepared in this study, formulation F8 was shown better results for percentage drug entrapment efficiency (96.38%), sphericity, *in-vitro* drug release (98.63 %) up to 12 hours, which contains chia seed mucilage of 3.5 %. The coefficient of regression indicated that the release data was best fitted with zero order kinetics and Higuchi equation and Non Fickian type diffusion (n Value ranges from 0.5 to 0.7). These sustained release microparticle are thus suitable for oral controlled release of Clopidogrel

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