INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

FORMULATION AND *IN-VITRO* EVALUATION OF GASTRO-RETENTIVE MICROSPHERE CONTAINING *CALENDULA OFFICINALIS* FOR THE TREATMENT OF PEPTIC ULCERS USING OKRA MUCILAGE AND ETHYL CELLULOSE

Rahul Awasthi *, Nisha Thakur, Niladry Shekar Ghosh
Department of Pharmaceutical Sciences, Bahra University Shimla Hills, H.P, India
*Corresponding Author Email: thakur21nisha@gamil.com

Article Received on: 28/09/16 Revised on: 21/10/16 Approved for publication: 03/11/16

DOI: 10.7897/2230-8407.0711125

ABSTRACT

The objective of the research was to formulate and evaluate gastro retentive microspheres containing *Calendula officinalis* for the treatment of Peptic ulcer. An attempt has been made to improve bioavailability and patient compliance by utilizing the concept of microsphere. Microspheres were prepared by solvent evaporation method using ethyl cellulose and okra mucilage. Ethyl cellulose is responsible for sustained release of *Calendula offícnalis* extract whereas okra mucilage aids in mucoadhesion of microsphere. Formulation was optimized using different concentration of polymers and excipients. The Optimized formulation F1 showed 94.31 % release of *Calendula officinalis* and drug content was found to be 88.3% of *Calendula officinalis*. The *in-vitro* studies showed that microspheres were capable of floating up to 12hours in simulated gastric fluid and sustained the release of *Calendula officinalis for* 12 hours. Scanning electron microscopy showed their spherical size, perforated smooth surface and a cavity inside microspheres.

Keywords: *Calendula officinalis*, microsphere, ethyl cellulose, okra mucilage, peptic ulcer

INTRODUCTION

The research on herbal medicinal plants has increased due to their potentials in combating the problem of drug resistance and minimal or no side effects in both developing and developed countries[1].**Oral drug delivery system** is the most convenient and preferred means of any drug delivery to the systematic circulation. Oral controlled release drug delivery releases the drug at a predetermined rate with improved therapeutic result, ease of dosing administration, patient compliance and flexibility in formulation[2]. **Gastro retentive drug delivery system**s are capable of delaying the release rate either by diffusion and erosion of the polymer matrix which retain in the stomach by passing the gastric transit[3]. Microsphere are solid, approximately spherical particles which contain dispersed drug molecules either in solution or in crystalline form and the size ranges from 1 to 1000µm. Microspheres avoid the first pass metabolism and thus have better biocompatibility, controllable biodegradability, and low toxicity and sustains release[4].

Peptic ulcers are the erosion of lining of stomach or the duodenum due to an imbalance between aggressive factors (like acid, pepsin, bile and H.pylori infection) and defensive factors (like gastric mucosa, bicarbonate secretion, prostaglandins, nitric oxide and innate resistance of the mucosal cell)[5]. ***Calendula officinalis*** is herbal aromatic plant which has antiulcer properties. It also possess many pharmacological activities, which include antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral[6]. Various studies showed that calendula also increases cell proliferation and encourages the granulation process of wound healing [7, 8]. Internally it is used for mucous membrane inflammations, peptic and duodenal ulcers, spasms of the GI tract, duodenal and intestinal mucosa, dysmenorrhoea (painful menstruation) especially in nervousoranemic women, splenic and hepatic inflammations[9].

Okra (*Abelmoschus esculentus*) mucilage also known as okra gum is obtained from the fresh fruits of the plant *Abelmoschus esculentus* (family Malvaceae). Okra (*Abelmoschus esculentus*) mucilage as pharmaceutical excipients. Okra gum consists of galactose, galacturonic acid, and rhamnose, with some fractions of glucose, mannose, arabinose and xylose. Various studies suggested that has good binding properties which is directly proportional to the concentration. It can also use as a matrix agent in sustained drug delivery systems [10, 11]. Ethyl cellulose is a derivative of cellulose mainly used as a thin-film. **Ethyl cellulose** is insoluble in water but it can take up water because of its hydrogen bonding capability due to the polarity difference between the oxygen atom and the ethyl group of polymer [12]. The aim of this study was to prepare gastro retentive microspheres containing *Calendula officinalis* to achieve a controlled release profile suitable for oral administration using ethyl cellulose and okra mucilage.

MATERIAL AND METHODS

The plant extract was selected on the basis of their properties and medicinal applications reported in the literature. The authenticated plant extract of *Calendula officinalis,* was purchased from Shri Herbasia Biotec, Amritsar Punjab, India. Ethyl cellulose was purchased from Central Drug House (P) Ltd, India. Acetone, Span 80, Liquid paraffin were purchased from Qualikems, India. All other chemicals were of analytical grade and used without further purification.

PREFORMULATION STUDIES

AUTHENTICATION OF PLANT EXTRACTS: Stock Solution of *Calendula officinalis* was prepared in buffer. This solution was diluted with same solvent to obtain concentration of 100µg/ml. The resultant solution was scanned in the range of 200-400nm on double beam UV-spectrophotometer. The resultant was plotted.

STANDARD CALIBRATION CURVE

Preparation of standard calibration curve of *Calendula officinalis*

In 0.1N HCl: Stock solution was prepared by dissolving 100mg of drug (*Calendula officinalis*) in 100ml of 0.1N HCl solution, which was further diluted to give the solution of concentration of 10, 20, 30, 40 and 50µg/ml respectively. Absorbance of these solutions was measured on UV- spectrophotometer at their respective wavelengths and plotted against concentration.

In Phosphate Buffer pH 6.8: Drug (*Calendula officinalis*) (10 mg) was dissolved in Phosphate Buffer (pH=6.8) and volume was made up to 100ml in 100 ml volumetric flask. This solution (100m g /ml) was further diluted with Phosphate buffer 6.8 pH to obtain solution of 25mg /ml. Absorbance of each solution was measured at their respective wavelengths using UV is double beam spectrophotometer and Phosphate buffer 6.8 pH as reference standard. The standard curve was generated for the entire range from 5 to 25 mcg.

Solubility: The solubility of *Calendula officinalis* drug in various solvents was measured. Solubility was determined by taking 10 mg of drug sample in 10 ml of solvent as Acetone, Methanol, Ethanol, pH buffer 6.8 in small test tubes and well solubilised by shaking. The solution was taken and filtered through Watman filter paper. The filtrate was solubilised in suitable solvent, diluted with the pH 6.8 buffer and the concentration of drug extracts were determined using UV-Visible spectrophotometer at suitable wavelength [13].

EXTRACTION OF MUCILAGE [10]

Step1. Okra (Abelmoschus esculentus) pods are washed and dried for 10 to 15 days till constant weight is achieved. The size is reduced by using a grinder. The resultant powder was then passed through sieve no. #22 to achieve fine powder.

Step2: Then the resultant Powdered was soaked in 500ml of distilled water and heated 60°C with continuous stirring for about 4 hours. The concentrated solution was filtered through muslin cloth and cooled at 4°C-6°C. The mucilage was then further isolated using acetone and then filtered through the muslin cloth. The mucilage was dried and stored in air tight container

FORMULATION OF MICROSPHERE: The microsphere were prepared by solvent evaporation method. Ethyl Cellulose was dissolved in acetone and stirred for few minutes. Then Okra mucilage was added to the mixture and then allowed to stir for 4hours. Then the mixture of liquid paraffin and span80 was added to it drop wise and stirred for 1hours. The extract was added to the above mixture and solvent is allowed to evaporate with continuous stirring. Microsphere was prepared and dried using vacuum pump [14]. The composition of microsphere formulations is shown in the table 1.

Table 1: Composition of Microsphere

Formulation code	*Calendula officinalis* extract	Ethyl Cellulose	Okra mucilage	Acetone	Liquid Paraffin	Span 80
F1	30mg	50mg	10mg	10ml	5ml	5ml
F2	30mg	10mg	50mg	10ml	5ml	5ml
F3	30mg	40mg	20mg	10ml	5ml	5ml
F4	30mg	20mg	40mg	10ml	5ml	5ml
F5	30mg	30mg	30mg	10ml	5ml	5ml

POST FORMULATION STUDIES

ENCAPSULATION EFFICIENCY: - Drug loaded microspheres (100 mg) were suspended in buffer solution followed by sonication for about 20 mins. It was shaken for another 20 mins in a rotary shaker for the complete extraction of drug from the microspheres. The resultant solution was filtered through 0.45 mcg membrane filter. Drug content was determined by UV visible spectrophotometer at 284 nm [15].

PERCENTAGE BUOYANCY: % buoyancy was carried out using 0.1 N HCl containing 1% span 20 as a dispersing medium. Prepared Microspheres were spread over the surface of 500 ml of dispersing medium at 37± 0.5°C. A paddle rotating at 100 rpm agitated the medium. Each fraction of microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighed after drying [12].

% Buoyancy = weight of microspheres floating on the surface / Initial total weight of microspheres

***IN-VITRO* RELEASE STUDY:** The release profiles of the formulations were determined using USP dissolution apparatus. The microspheres were enclosed in a muslin cloth which was then being tied to the lowest part of the paddle. The paddle was then rotated (100 rpm) immersed in pH 1.2 HCl buffer and for 2 hours, and immediately transferred to a phosphate buffer (pH 6.8) medium and tested for 10 hours at 37 ± 0.5°C. Aliquots of 5 ml was withdrawn hourly over a period of 12 h. Drug content was determined spectrophotometrically at 284 nm. The studies were carried out in triplicate, and the release data was obtained [16].

SURFACE MORPHOLOGY AND PARTICLE SIZE [17]

The SEM studies were carried out to get more insight about the morphology of the microsphere By using magnifications from 10X to 100,000X; the size analysis, topographical and elemental information were measured. A concentrated aqueous dispersion of microsphere was finely spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator with a gold layer (20 nm thick). The surface morphology of the nanoparticles was observed by SEM using S-3400N scanning electron microscope

RESULT AND DISCUSSION

Determination of λmax: - λmax of *Calendula officinalis* found to be 284 nm as shown in figure1

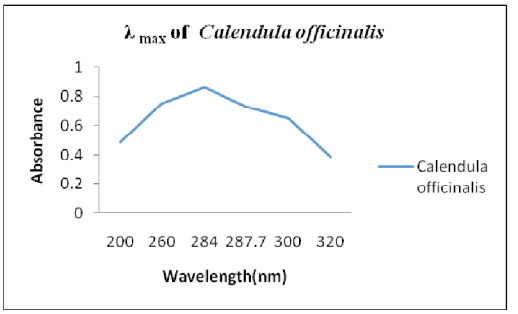

Figure 1: λ max of *Calendula officinalis*

Drug excipient compatibility studies were confirmed by FTIR studies. There was none of the extra peak was found in the graph. Thus FTIR studies showed that there was no drug-excipient interaction. Solubility of *Calendula officinalis* extract drug extracts was found to be highest 429.5µg/ml in Acetone respectively. Thus, acetone was selected as the solvent for the development of the microspheres. Solubility study of herbal extracts in different solutions was given in Table 2.

Table 2: Solubility of *Calendula officinalis*

S No.	Solution	Solubility (µg/ml)
1.	Distilled Water	334.5
2.	Liquid Paraffin	243
3.	Acetone	429.5
4.	Methanol	402.2
6.	Span 20	182
7.	Span 80	203
10.	Isopropyl myristate	156

During the research, the polymer concentrations were gradually increased and decreased. Encapsulation efficiency was found to increase with the increase in the concentration of Ethyl cellulose and decrease with the increase with the concentration of okra mucilage. The percentage of encapsulation efficiency ranged from 80% to 95%. F1 formulation which contains more concentration of ethyl cellulose showed maximum encapsulation efficiency with 95%.

The percentage buoyancies of formulations F1–F5 at the end of 18 hours were found to be 79.21%, 67.24%, 66.46%, and 64.3% as shown table 4. The result indicates that with an increase in the concentration of ethyl cellulose the floating time increases. Formulation F1 of microspheres was found to be the best.

Table 3: Encapsulation Efficiency of *Calendula officinalis*

Formulation code	Encapsulation Efficiency
F1	95%
F2	87%
F3	85%
F4	82%
F5	80%

Table 4: Buoyancy percentage of formulation F1-F5

Formulation code	Buoyancy percentage
F1	79.21%
F2	67.24%
F3	66.46%
F4	64.3%
F5	63.35

Table 5: % CDR release of *Calendula officinalis of* various formulations

Time(min)	F1	F2	F3	F4	F5
15	9.52	6.41	5.12	4.71	4.41
30	23.06	20.17	9.42	6.16	7.32
60	29.5	25.26	11.26	8.41	9.49
120	32.09	29.60	15.19	10.55	11.66
180	32.85	31.40	19.27	12.19	14.79
240	34.36	33.20	21.13	15.32	17.21
300	36.39	35.17	24.96	17.16	19.13
360	38.48	37.80	27.09	19.23	21.19
420	40.57	39.15	31.14	21.41	24.33
480	44.66	42.08	34.27	23.08	26.49
540	50.13	45.62	38.12	25.21	28.53
600	55.31	29.25	41.19	29.32	31.64
660	60.06	52.13	44.92	33.19	33.96
720	65.72	55.63	47.29	35.47	35.19
780	70.31	58.12	50.65	39.77	37.82
840	75.78	60.18	52.11	42.12	39.52
900	80.87	63.41	55.42	45.05	41.14
960	83.54	65.29	57.39	49.32	44.21
1020	88.36	67.65	60.66	51.33	47.67
1080	93.31	70.14	62.32	54.91	49.10
1140	96.96	72.41	66.42	57.62	53.67
1200	94.31	75.67	69.71	59.92	55.19

***In-vitro* drug release** release of *Calendula officinalis* was studied. The percentage cumulative drug release was calculated. The formulation showed drug release up to 20 hrs by performing dissolution studies. The %CDR of *Calendula officinalis* of formulation F1 was found to be maximum i.e. 94.31%. The in-vitro drug release results are shown in table 5. From i*n- vitro* release study, it was concluded that the formulation F1 better than other formulations in respect to percentage cumulative drug release. It indicates that an increase in the concentration of ethyl cellulose the release rate of the drug increases whereas increases in concentration of okra mucilage decrease the release rate as shown in Figure 2.

MECHANISM OF DRUG RELEASE The data obtained for the *in vitro* release were fitted into equations for the zero order, first order, and Higuchi release models. The interpretation of result was based on the value of a resulting regression coefficient. The zero order plot showed maximum regression coefficient value i.e. 0.9755. This show that the drug releases from the microsphere follows zero order release. The microsphere prepared showed that the drug release is independent of drug concentration. The results are shown in Table 6.

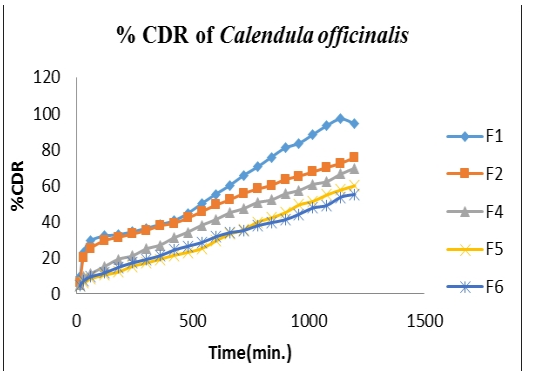

Figure 2: % CDR of *Calendula officinalis*

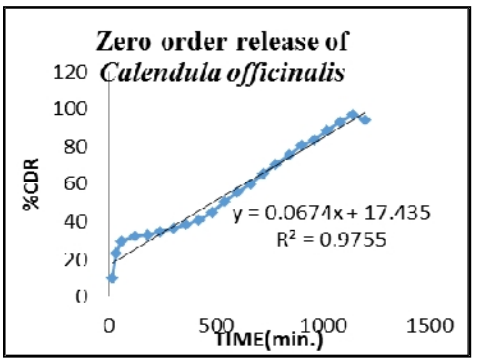

Figure 3: Zero order release of *Calendula officinalis*

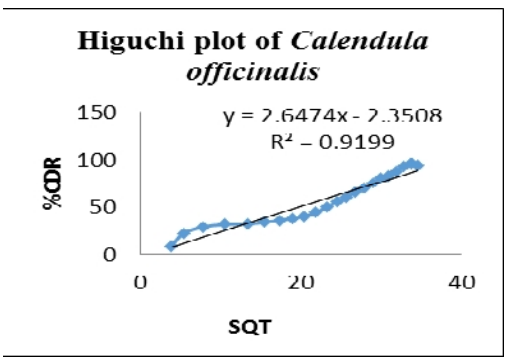

Figure 4: Higuchi plot of *Calendula officinalis*

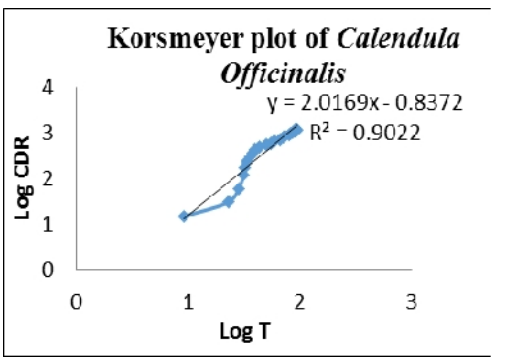

Figure 5: Korsmeyer plot of *Calendula officinalis*

Table 6: Results of model fitting of formulation F1

Model	Parameter	F1
Zero order plot	Slope	0.0674
	Intercept	17.435
	R^2	0.9755
Higuchi plot	Slope	2.647
	Intercept	2.3508
	R^2	0.9199
Kosermeyer – peppas plot	Slope	2.0169
	Intercept	0.8372
	R^2	0.9022

The particle size and surface morphology of the microsphere was determined by using scanning electron microsphere (SEM). The particle size of the microsphere was found to be in the range of 1.88µm- 3.86 µm. The surface of the prepared microspheres were found to be perforated smooth surface and a cavity inside microspheres .Thus the formulated microsphere have the size in micrometre which confirms that the prepared formulation were microspheres.

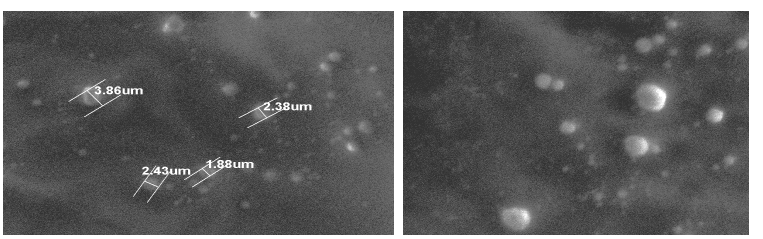

Figure 6: SEM images of *Calendula* loaded microsphere

CONCLUSION

In the present investigation, gastro retentive microsphere of *Calendula officinalis* using ethyl cellulose and Okra mucilage were formulated by Solvent evaporation technique. Formulation was optimized using different concentration of polymers and excipients. The in-vitro drug dissolution studies were carried out for the formulations in pH 0.1N HCL for 2 hours and in phosphate buffer (pH 6.8) for 18hrs. The Optimized formulation F1 showed 94.31 % release of *Calendula officinalis* and drug content was found to be 88.3% of *Calendula officinalis*. Scanning electron microscopy showed their spherical size, perforated smooth surface and a cavity inside microspheres .The particle size of the microsphere was found to be in the range of 1.88µm- 3.86 µm. From the kinetics modelling the drug release from the microsphere follows zero order release. The prepared microsphere showed that the drug release is independent of drug concentration. *In-vivo* evaluation of prepared gastro-retentive microsphere of *Calendula officinalis* can be done to check the effectiveness of the formulation. Clinical studies of this formulation are required to be done.

REFERENCES

1. Kaul Shefali, Dwivedi Sumeet. Indigeneous ayurvedic knowledge of some species in the treatment of human disease and disorders. Int. J. Pharm. Life Sci. 2010; 1(1):44-49.
2. Laura M. Ensign, Richard Cone. Oral Drug Delivery with Polymeric Nanoparticles: The Gastrointestinal Mucus Barriers. Advanced Drug Delivery Reviews. 2012; 64(6). 557–570.
3. Streubel A, Siepmann J, Bodmeier R. Gastroretentive drug delivery systems. Expert Opinion on Drug Delivery. 2006; 3:217-233.
4. S. Freiberg, X.X. Zhu. Polymer microspheres for controlled drug release.International Journal of Pharmaceutics. 2004;282:1–18
5. Kumar Vijay., Pathophysiology of common diseases. 1st edition. Kalyani publisher. New Delhi: 2008; 155-160.
6. Singh mukesh, Pankaj Sahu, K. Nagori, D. Dewangan, T. Kumar. A. Alexander, H. Badwaik and D.K Tripathi. Organoleptic properties in-vitro and in-vivo pharmacological activities of calendula officinalis Linn: An over review. Journal of current pharma research. 2011; 3 (4): 655-663.
7. http://www.theida.com
8. http://www.herbwisdom.com/herbmarigold.html
9. Chakraborthy Guno Sindhu. Phytochemical screening of calendula officinalis linn leaf extract by TLC. international journal of research in ayurveda & pharmacy.2010;1(1):131-134
10. Farooq Uzma, Malviya Rishabha and Kumar Sharma Pramod. Extraction and Characterization of Okra Mucilage as Pharmaceutical Excipient. Academic journal of plant sciences. 2011; 6 (4):168-172.
11. Biswal Biswajit, Biswajit Biswal, Nabin Karna and Rashmika Patel. Okra mucilage act as a potential binder for the preparation of tablet formulation. Der Pharmacia Lettre. 2014; 6 (3): 31-39.
12. Hitesh Kumar, Koshy M.Kymonil, Shubhini. A. Saraf. Gastroretentive Ethyl Cellulose Floating Microspheres containing Ranitidine Hydrochloride. International Journal of Drug Development and Research. 2012;4 (2):315-321
13. K. Prakash,P Narayana Raju , K Shanta kumari and M. Lakshmi narasu. Solubility and Dissolution Rate Determination of Different Antiretroviral Drugs in Different pH Media Using UV Visible Spectrophotometer. E-Journal of Chemistry. 2008; 5(S2):1159-1164.
14. Hardenia Shiv Shankar. Formulation and evaluation of Mucoadhesive microsphere of ciprofloxacin. Journal of Advanced Pharmacy Education & Research. 2011; 1(4):214-224.
15. Prasanth V, Moy V and Chakraborthy A. Microspheres: An overview. International Journal of Research in Pharmaceuticals and Biomedical Sciences. 2011; 2:332-338.
16. Madhurima S Chavan, Dr Surajj Sarode, Prof. Sathe Bhushankumar. Formulation and evaluation of mucoadhesive buccal tablet of flurbiprofen. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(5): 945-962.
17. Nanjwade B. K, J. Varia Pratik kumar, Kadam Vikrant T. et al. Development and Evaluation of Nanoemulsion of Repaglinide. JSM Nanotechology and Nanomedicine. 2013; 1(2): 1-8.

Cite this article as:

Rahul Awasthi, Nisha Thakur, Niladry Shekar Ghosh. Formulation and In-vitro evaluation of gastro-retentive microsphere containing *Calendula officinalis* for the treatment of peptic ulcers using okra mucilage and ethyl cellulose. Int. Res. J. Pharm. 2016;7(11):38-42 http://dx.doi.org/10.7897/2230-8407.0711125

Source of support: Nil, Conflict of interest: None Declared