ARS Pharmaceutica
ISSN: 0004-2927
http://farmacia.ugr.es/ars/

Formulation and evaluation of sustained release microspheres of rosin containing aceclofenac

Lakshmana Prabu S *, Shirwaikar AA, Shirwaikar A, Kumar A

Dept. of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Madhav Nagar, Manipal – 576 104, Karnataka, India

Email: slaxmanvel@gmail.com

ABSTRACT

Aceclofenac was microencapsulated using rosin by o/w emulsion solvent evaporation technique. The effect of three formulation variables including the drug:polymer ratio, emulsifier (polyvinyl alcohol) concentration and organic solvent (dichloromethane) volume were examined. The prepared batches were characterized for microspheres particle size distribution, encapsulation efficiency and in vitro release behavior. The study reveals that drug:polymer ratio had a considerable effect on the entrapment efficiency, however particle size distribution of microspheres was more dependent on the volume of dichloromethane and polyvinyl alcohol concentration rather than on the drug: polymer ratio. Drug, polymer concentrations were varied to obtain optimum release profile for sustaining the action of the drug.

KEYWORDS: Aceclofenac. Rosin. Microspheres. Sustained release.

INTRODUCTION

Conventional oral drug administration does not usually provide rate-controlled release or target specificity. In many cases, conventional drug delivery provides sharp increase in drug concentration often achieving toxic level and following a relatively short period at the therapeutic level of the drug concentration eventually drops off until re-administration. In order to obtain maximum therapeutic efficacy, it becomes necessary to deliver an agent to the target tissue in the optimal amount for the required period of time, thereby causing little toxicity and minimal side effects[1]. Desired drug release can be provided by rate-controlling membranes or by implanted biodegradable polymers containing dispersed medication. Microparticulate drug delivery systems are considered and accepted as a reliable one to deliver the drug to the target site with specificity, to maintain the desired concentration at the site of interest without untoward effects[2]. Microencapsulation is a useful method which

Fecha de Recepción (Date received): 07/04/09
Fecha de Aceptación (Date accepted): 03/08/09

prolongs the duration of drug effect significantly and improves patient compliance. Eventually the total dose and few adverse reactions may be reduced since a steady plasma concentration is maintained[3].

In recent years much research in drug delivery has been focused on degradable polymer microspheres. Administration of medication via such systems is advantageous because microspheres can be ingested or injected, can be tailored for desired release profiles and in some cases it can provide organ-targeted release[4,5,6,7,8]. The meaning of microencapsulation is converting liquids to solids, altering colloidal and surface properties, providing environmental protection and controlling the release characteristics by using the coating materials.

Emulsion solvent[5], phase-separation method[10] and spray drying method[11] are commonly used for the preparation of microspheres. The success of any microencapsulation method depends on many factors such as the drug solubility, partition co-efficiency, polymer composition, molecular weight etc. Among the various microencapsulation methods, emulsion solvent evaporation technique is often widely used to prepare microcapsules of water insoluble drugs (within the water insoluble polymer). Microspheres are formed by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and drug [4; 7; 15; 16; 17].

Aceclofenac is a non-steroidal anti-inflammatory drug, widely used in the management of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. Usual therapeutic dose is 100 mg twice daily and half life is 3-4 hrs; thus it is necessary to be administered frequently in order to maintain the desired concentration. Therefore, aceclofenac is an ideal candidate for sustained release formulation, resulting in more reproducible drug absorption and reducing the risk of local irritations compared to single dosage forms.

The aim of the present work was to encapsulate aceclofenac with biodegradable polymer rosin, the effect of different formulation variables such as concentration of drug, polymer, polyvinyl alcohol and solvent, the effect of these variables on particle size distribution, encapsulation efficiency and its *in vitro* release behavior.

MATERIALS AND METHODS

Materials

Aceclofenac was obtained as gift samples from Micro Labs, Hosur, India; Polyvinyl alcohol (PVA - MW 1,30,000) from SD fine chemicals, Rosin from Yucca Enterprises, Dombivilli, Thane, India were used in the study. Dichloromethane, Potassium dihydrogen phosphate, Sodium hydroxide and Camphor were procured from S.D. Fine Chemicals, Mumbai, India.

Methods

Preparation of microspheres

Microspheres of Aceclofenac were prepared based on o/w emulsion solvent evaporation technique by using rosin as a polymer. Different batches of microspheres were prepared by dissolving the polymer and the drug in dichloromethane and then adding this oil phase in the

aqueous phase (100 ml) containing various percentages of PVA as the emulsifying agent; the mixture was emulsified by constant stirring at 400 rpm for 4 h by using a propeller stirrer (Remi, India). The dispersed drug and polymer solution was immediately transformed into fine droplets, which subsequently solidified into rigid microspheres due to the solvent evaporation. The particles were collected by filtration, washed and dried in vacuum desiccators and characterized.

Drug-Excipients Compatibility Studies

Excipients are integral components of almost all pharmaceutical dosage forms thus it is mandatory to detect any possible physical or chemical interaction of the drug with the excipients since the excipient can affect the bioavailability and stability of the drug. The drug and the excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, easy to administer and safe. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies have a considerable importance. DSC and FTIR techniques were commonly used to investigate the compatibility between the drug and the various excipients used in the formulation.

Sample preparation and analysis by DSC

The samples were prepared by physical mixture of drug and excipients (1:1) using a clean dried glass mortar and pestle. Samples (5-10 mg) were accurately weighed and hermetically sealed in aluminum pans. Thermograms were obtained using Shimadzu (DSC-60) instrument, heating at a constant rate of 10º C/min, over a temperature range of 40 – 300ºC. To maintain on inert atmosphere nitrogen gas was purged at a rate of 20 ml/min.

Sample preparation and analysis by FTIR

FTIR spectra data was taken on a Shimadzu (FTIR-8300) instrument to find out the chemical stability of the drug with excipients. FTIR spectra of drug, polymer and composition of final formulation were obtained by mixing with potassium bromide and converted into pellets by pressing at 1 ton/unit. Spectral scanning was done in the range of $4000 – 400^{-1}$ cm.

Percentage Yield

The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug.

$$\text{Percentage yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer used}} \times 100$$

Particle size and size distribution

The microspheres were suspended in liquid paraffin and examined using an optical microscope. Laser diffraction technique (Malvern Instruments Ltd. Malvern, UK) was used to study the size distribution of the microspheres. The dispersant used was cyclohexane and the average particle size was calculated and expressed in microns.

Drug Content analysis

Ten milligram accurately weighed portion of microspheres were taken in a clean 100 ml volumetric flask and dissolved in about 2 ml of acetone and the volume was made up to the mark with buffer pH 7.4. After filtration and dilution, samples were analyzed spectrophotometrically and the amounts of drug encapsulated in the microspheres were calculated. The drug content of each sample was determined in triplicate and the results were averaged. The entrapment efficiency of microspheres were calculated by dividing the actual drug content to the theoretical drug content of microspheres.

Morphological characterization of microspheres

The surface morphology of microspheres was investigated using scanning electron microscopy (SEM) by mounting on stubs using double-sided adhesive tapes. The stubs were then vacuum-coated with gold-palladium alloy using coat sputter JFC 1100 (JEOL, Japan) and the microspheres were observed and examined using SEM (JEOL JSM 6308).

In-vitro release studies

In vitro release studies were carried out using USP type I apparatus at 37 ± 0.5^{o}C in 900 ml of phosphate buffer solution (pH 7.4) for 24 h. Microspheres equivalent to 20 mg drug was placed into the baskets (tied using muslin cloth), and rotated at 100 rpm. A sample of 5 ml was with drawn at various time intervals like 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h and filtered, analyzed by UV spectrophotometrically at 275 nm using Shimadzu 1610 spectrophotometer.

DISCUSSION

The details of quantities of drug, polymer, stabilizer and solvents used are given in Table 1. Percentage yield of the microspheres and encapsulation efficiency were calculated and the results are shown in Table 2. First order spectroscopy method was used in order to nullify the interference effect due to polymer in the estimation of aceclofenac. First order standard plot of aceclofenac was found to be linear in phosphate buffer pH (7.4) at wavelength 261.4 nm between the concentrations of 4 - 40µg/ml. The linear regression equation was found to be y=0.0006x-0.0001; the correlation coefficient was found to be 0.9986. The UV spectrums are shown in Figure 1 and 2.

TABLE 1. *Composition of aceclofenac loaded microspheres*

Ingredients (mg)	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*
Aceclofenac	*200*	*400*	*600*	*200*	*200*	*200*	*200*
Rosin	*200*	*200*	*200*	*400*	*600*	*400*	*600*
PVA (100ml)	*0.25%*	*0.25%*	*0.25%*	*0.25%*	*0.25%*	*0.25%*	*0.25%*
Dichloromethane	*10 ml*	*10 ml*	*10 ml*	*10 ml*	*10 ml*	*10 ml*	*10 ml*
Water	*100 ml*	*100 ml*	*100 ml*	*100 ml*	*100 ml*	*100 ml*	*100 ml*
Camphor	---	---	---	---	---	*200*	*200*

TABLE 2. Results of percentage yield and encapsulation efficiency

Formulation	Percentage Yield (%)	Encapsulation efficiency (%)
F1	40.0	23.1
F2	46.0	36.2
F3	60.1	53.0
F4	80.0	72.85
F5	88.83	80.24
F6	85.0	73.12
F7	88.5	78.3

FIGURE 1. UV spectrum of aceclofenac and rosin (acetone with buffer pH 7.4)

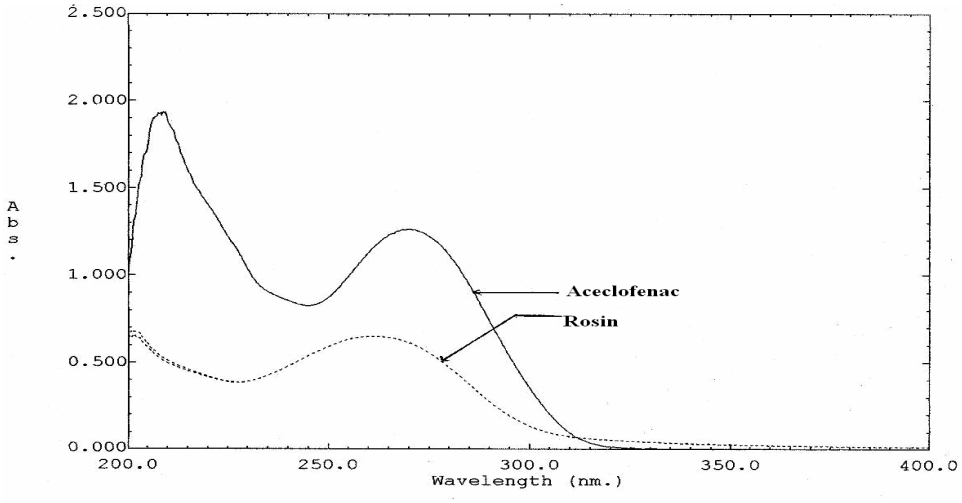
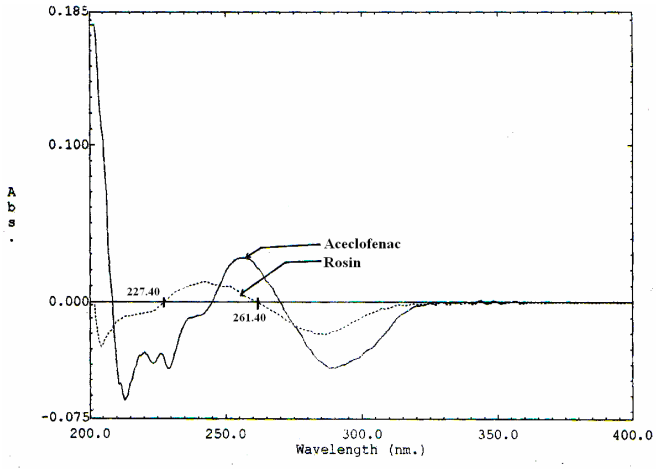


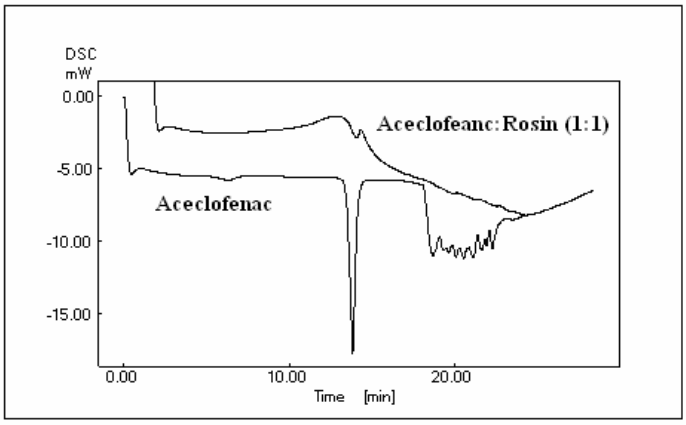
FIGURE 2. First order absorption spectra of aceclofenac and rosin (acetone with phosphate buffer pH 7.4)



Drug-Excipients Compatibility Studies

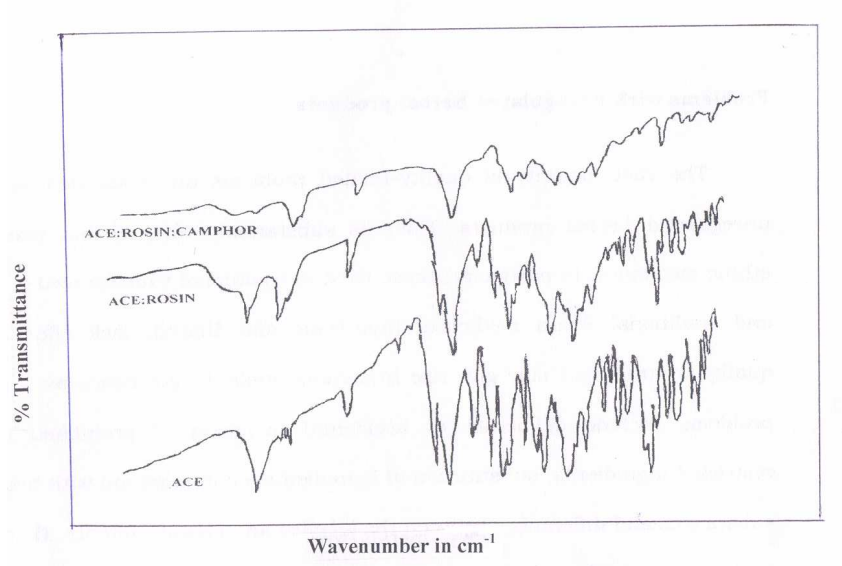
DSC is useful in the investigation of solid-state interactions; hence thermograms were generated for both pure drug and drug excipient mixtures. The DSC thermograms reveal that the physical mixture of aceclofenac with rosin showed superimposition of the thermograms, but a slight preshift was observed at 148.91 **°C** instead of 156.54 °C. There is no considerable change in the melting endotherms of both the drug and excipients as well as there is no other endotherm and exotherm were observed. The thermograms are shown in Figure 3.

FIGURE 3. DSC Thermograms



Aceclofenac contains one carbonyl group, one ester group and one secondary amine which have characteristic band values around 3276, 1770 and 3317 cm^{-1}. Infrared studies reveal that three characteristic bands around 3276, 1770 and 3317 cm^{-1} were present in all spectra while no new bands or shift in characteristic bands were seen in the mixtures. IR spectra are shown in Figure 4. DSC and FTIR results revealed that there is no interaction between the drug and the excipients used in the formulation.

FIGURE 4. IR spectra of aceclofenac and mixture



SEM studies

The scanning electron micrograph of microspheres of formulations F6 and F7 are shown in Figure 5 and 6. It can be seen that microspheres are almost spherical with smooth surface.

FIGURE 5. SEM of formulation F6

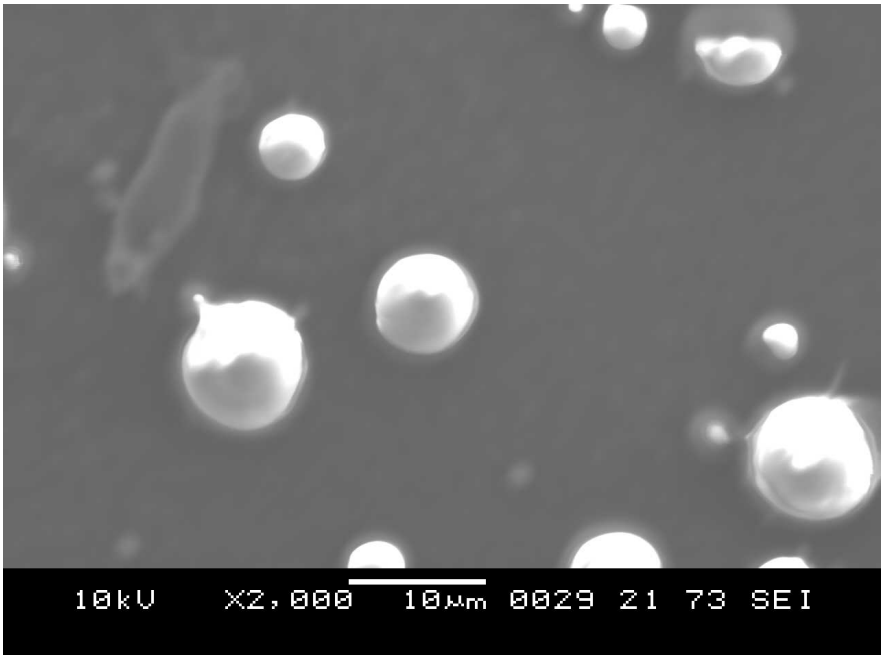
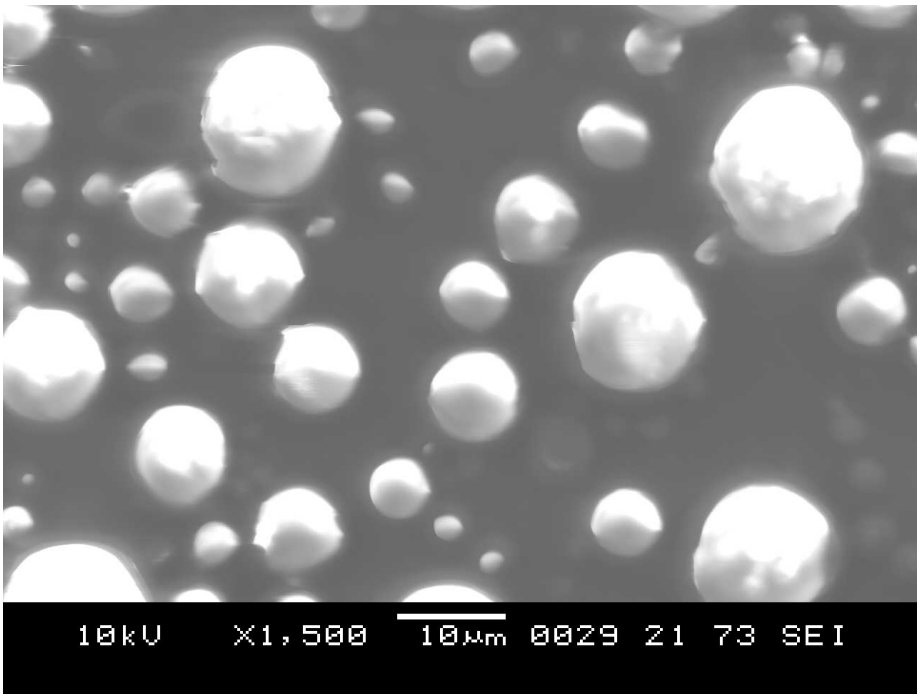


FIGURE 6. SEM of formulation F7

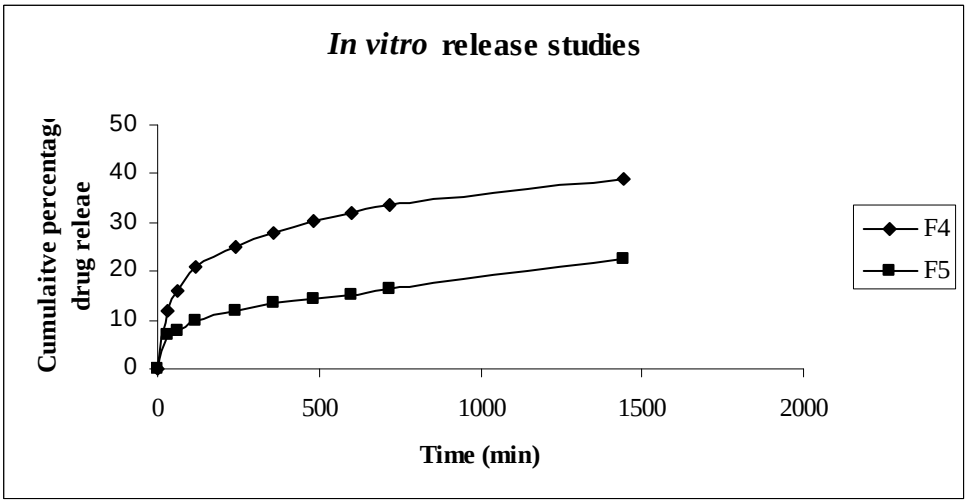


In vitro release studies

Formulation F1, F2 and F3 have comparatively less encapsulation efficiency, thus

formulations F4 and F5 were taken into consideration for *in vitro* release studies. *In vitro* release studies were carried out in phosphate buffer pH 7.4 for a period of 24 h. The cumulative percent release was found to be 38.90% and 22.69% for formulation F4 and F5 respectively. The release results are shown in Figure 7.

FIGURE 7. In vitro release of aceclofenac from F4 and F5 formulations

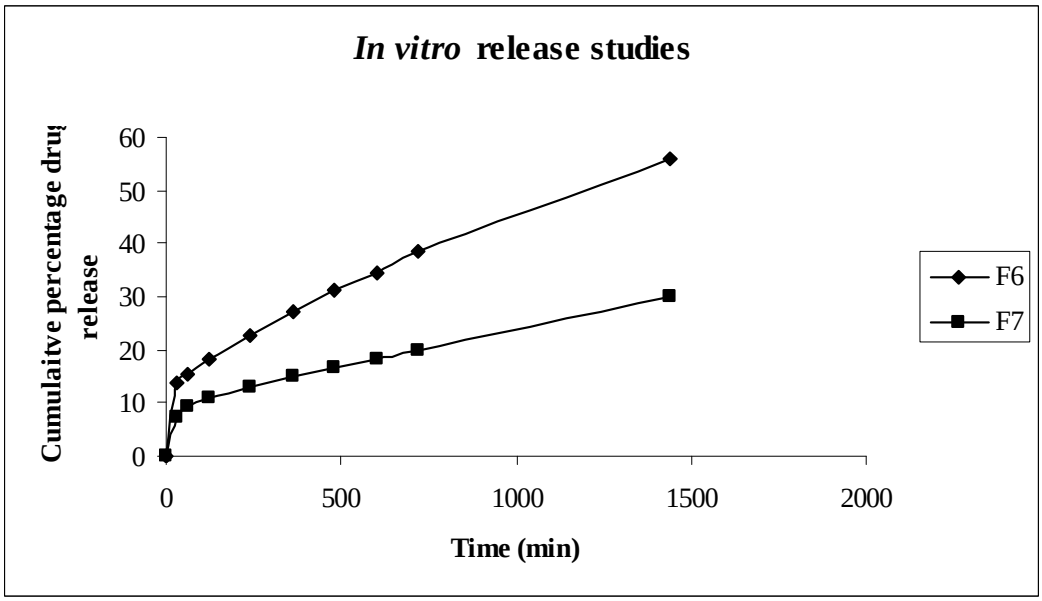


In order to improve the release rate of drug from microspheres, 2.0 % w/v of camphor was included in the formulations. The composition of the formulation is shown in Table 3. Camphor was dissolved in the polymer solution, its forms uniform distribution in the polymer solution. Upon microencapsulation the particles will be getting encapsulated by polymer along with camphor. On drying due to volatile nature camphor may get evaporated and forms pores on the surface of the microspheres, through which drug could easily diffuse to the aqueous phase or dissolution medium. Microspheres were prepared by the emulsion solvent evaporation method and evaluated. The percentage yield was found to be between 85.0 % and 88.5%; encapsulation efficiency was found to be 73.12% and 78.3% for formulation F6 and F7 respectively. *In vitro* release studies for the prepared microspheres were carried out by the above method. The cumulative percentage release after 24 h was found to be 55.76% and 29.83% for formulation F6 and F7 respectively. The release results are shown in Fig. 8. Formulation F6 showed better release rate than F7. The reason for this retarded drug release may be due to the hydrophobic nature of the polymer, which prevents the penetration of the dissolution medium into the microspheres leading to slower dissolution and diffusion of the drug molecules from the microspheres.

TABLE 3. Shape and particle size of rosin loaded microspheres

Formulation	Specific surface area (m^2/g)	Surface weighed mean D[3,2]	Volume weighed mean D[4,3]	Mean particle size d(0.1) (µm)	Mean particle size d(0.5) (µm)	Mean particle size d(0.9) (µm)
F6	0.68	8.826	23.331	6.906	24.144	44.598
F7	0.472	12.708	37.392	10.651	36.955	62.778

FIGURE 8. In vitro release of aceclofenac from F6 and F7 formulations



Evaluation of formulation variables

As mentioned previously, the effect of different formulation variables on microspheres properties including entrapment efficiency and particle size distribution were evaluated. Variables studied in this investigation were as follows. Drug :polymer ratio, volume of dichloromethane and polyvinyl alcohol concentration in the external phase of the emulsion.

Particle size distribution

Particle size and shape parameters like surface weighted mean D [3, 2], volume weighed mean D [4.3] and specific surface area was determined. Particle size distribution parameters like d (0.1), d (0.5) and d (0.9) were analyzed. When increasing the concentration of wall forming material the mean diameter of the particle also increased which may be attributed to the increase in viscosity of the internal phase. The results are given in Table 3.

Effect of drug:polymer ratio

The effects of drug and polymer ratio on the aceclofenac of rosin microspheres were studied, the results revealed that drug loading was observed maximum at drug:polymer ratio at 1:3. On the other hand, there is not any significant difference was observed between particle size distribution of microspheres prepared with drug:polymer ratios of 1:2 and 1:3.

Effect of dichloromethane volume

The effects of dichloromethane volume were studied. The results are shown in Table 4.

TABLE 4. The effect of volume of dichloromethane variables on the drug entrapment efficiency and particle size of rosin microspheres (drug : polymer ratio 1:2)

Volume of Dichlormethane (ml)	Theoretical drug loading (mg)	Actual drug loading (mg)	Encapsulation efficiency (%)	Mean particle size d(0.5) (µm)
9.0	200	145.76	72.88	23.68
9.25	200	146.12	73.06	23.76
9.5	200	145.42	72.71	23.91
9.75	200	145.18	72.59	24.02
10.0	200	146.30	73.15	24.14
10.25	200	146.40	73.20	24.30

The results revealed that the drug content of microspheres was not affected by the volume of dichloromethane, but the particle sizes were found to change significantly. This may also be due to the increase in the volume of dichloromethane leads to decrease in viscosity of the internal phase could be an effective factor in the droplet size of the emulsion in the aqueous medium. In this case, it seems that the shear effect of the propeller is able to break the large droplets into smaller ones, which are solidified into microspheres on solvent evaporation.

Effect of PVA concentration

The effect of PVA as an emulsifying agent in the external phase of the emulsion was investigated. The results are shown in Table 5. The amount of PVA as an emulsifying agent did not influence the drug loading and entrapment efficiency of microspheres however the particle size of microspheres is seen to be dependent on the PVA concentration in the continuous phase. The results revealed that on increasing PVA concentration, more PVA molecules may overlay the surface of the droplets, providing an increased protection of the droplets against coalescence resulting in the production of small emulsion droplets. Since microspheres were formed from emulsion droplets after solvent evaporation, their size was dependent on the size of emulsion droplets.

TABLE 5. The effect of PVA concentration on drug entrapment and particle size of rosin microspheres

Concentration of PVA as continuous phase	Theoretical drug loading (mg)	Actual drug loading (mg)	Encapsulation efficiency (%)	Mean particle size d(0.5) (µm)
0.1	200	145.26	72.63	30.28
0.15	200	145.82	72.91	28.54
0.2	200	146.22	73.11	26.43
0.25	200	146.48	73.24	24.14

CONCLUSION

This study shows that o/w emulsion solvent evaporation can be used as a simple method to prepare Aceclofenac sustained release microspheres by suing rosin as an encapsulating polymer. The drug entrapment efficiency of prepared microspheres were affected only by the drug:polymer ratio. The emulsifier concentration and organic phase volume influenced the particle size distribution of microspheres. Based on the above findings, it was observed that formulation F6 showed optimum release characteristics. The release rate of drug from the microspheres could be properly controlled for about 24h. Appropriate variation in the proportions of drug; polymer and stabilizer can lead to a product with the desired controlled release features.

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