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Sustained Release Microspheres of Diclofenac Sodium Using PEGylated Rosin Derivatives

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The PEGylated derivatives of rosin-PD-1 and PD-2 synthesized and characterized earlier (Nande et al., 2006) were investigated as potential materials for sustained release microsphere prepared by emulsion solvent evaporation method using diclofenac sodium (DCS) as model drug. All the microspheres exhibited smooth surfaces intercepted by pores; their sizes (d_{90}) ranged between 11–24 μm. The entrapment efficiency (< 80%) of the microspheres increased proportionally with derivative concentration. Presence of solvent like isopropyl alcohol or dichloromethane rendered the microspheres with large sizes but with reduced drug entrapment. Microspheres with small size were obtained at an optimum viscosity of liquid paraffin; any change lead to increase in the particle size. Magnesium stearate was found to be most suitable detackifier in the present system. The drug release was directly related to the particle size—small sized microspheres released drug at a faster rate. The dissolution data complied with Higuchi equation while the mechanism of drug release was Fickian diffusion (n ~ 0.5). Controlled inhibition of edema, as tested by hind paw edema method, was observed for 10 h when the microspheres were administered intraperitoneally. The present study found the derivatives as promising materials for preparing microspheres for sustained delivery of DCS.

Keywords PEGylated derivatives; microspheres; entrapment efficiency; solvent

INTRODUCTION

Microparticulate drug delivery system includes both microspheres and microcapsules wherein the drug is either dispersed throughout a polymer matrix or is coated by a thin polymer coat respectively. These systems when used for oral drug delivery system offer advantages over the conventional single-unit systems like low variability in gastric emptying transit time, better drug dispersion, lower incidence of drug dumping and improved drug absorption (Florence, 1994; Follonier, 1992). The idea of microcapsules as controlled release drug delivery systems was first explored about four decades back (Chang, 1964). Since then many polymers have been evaluated for their property to form microspheres for controlled drug delivery. The different synthetic polymers investigated include cellulose derivatives like ethyl cellulose, sodium carboxymethylcellulose, cellulose acetate phthalate (Billon, 2000; Nokhodchi, 2002; Sheorey, 1991), poly lactic/glycolic acid polymers (Benita, 1984; Tuncay, 2000), polyanhydrides (Tabata, 1993), polycarbonates (Joseph, 2002), and acrylic acid polymers (Barkai, 1990; Jiao, 2002). These polymeric materials were successfully used to produce microspheres containing drugs with varying solubility parameters.

Apart from these synthetic and semisynthetic polymers, naturally occurring materials have also found favor as release-retarding components in microsphere preparation. Some of the materials taken from nature for these formulations include lipids and waxes (Adeyeye, 1991; Karasulu, 2003), proteins like albumin and gelatin (Giunchedi, 1994; Saparia, 2001), polysaccharides like alginate and chitosan (Bregni, 2000; Wang, 1996).

Rosin or colophony is a clear, pale yellow to dark amber, thermoplastic solid resin obtained from the trees of Pinus species (Family:*Pinaceae*). The major components of rosin include resin acids represented by general formula $C_{20}H_{30}O_2$ that are present as free acids or dimmers or as anhydrides (Seymour, 1992). They are monocarboxylic acids of alkylated hydrophenanthrene; abietic acid constitutes the principal resin acid (Enos, 1968; Finar, 1975). The oral toxicity studies on animals have proved rosin to be practically nontoxic. The minimum dose that killed 50% of animals tested (LD_{50}) showed an increasing trend with derivatization of rosin; the maleic anhydride-pentaeryhtritol ester of rosin being four times safer than the parent material in guinea pigs (Stonecipher, 1976).

Rosin and its derivatives have been investigated as matrix-forming agents (Pathak and Dorle, 1990; Shirwaikar, 2005),

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coating materials (Mandaogade, 2002; Satturwar, 2004), and chewing gums (Synosky and Reed, 1994; Wolf, 2000; Yong, 1992). Derivatives of rosin and abietic acid have also been successfully used as materials for microsphere formation (Fulzele, 2004; Pathak, 1985; Pathak, 1986; Puranik, 1991; Sheorey, 1994).

All the earlier reported derivatives of rosin were prepared by reacting rosin with a number of reactants taken together. The exact nature of these derivatives was therefore difficult to determine due to possibility of formation of a number of products. Also no correlation between the types of excipients used, their chemical nature and the final product could be made from these studies. In our earlier study (Nande, 2006), rosin was reacted sequentially with polyethylene glycol 200 (PEG 200) (7.5, 15, and 25% w/w) and maleic anhydride (7.5% w/w) at elevated temperatures in presence of zinc dust to form ester-adduct derivatives PD-1, PD-2, and PD-3, respectively. These derivatives were characterized for their physico-chemical properties like molecular weight, acid number, solubility, glass transition temperature, film-forming property, etc. Free and applied films of these derivatives exhibited extremely low water vapor transmission rate. The release of diclofenac sodium and propranolol hydrochloride was retarded for 8 h when these derivatives were evaluated as sustained release matrix former (Nande, 2006). The release rate of these model drugs was further retarded when matrix tablets were prepared by melt granulation technique (Communicated to Drug Development and Industrial Pharmacy, 2006). The derivatives PD-1 and PD-2 also exhibited good sustained release film-forming property. Tablets coated with these derivatives sustained the release of diclofenac sodium and propranolol hydrochloride. The release from these coated systems was by channel formation in the film coat and diffusion of drug through them (Communicated to Drug Development and Industrial Pharmacy, 2006).

In view of the drug release-retarding property exhibited by the derivatives the present study was undertaken to evaluate these derivatives as materials for microencapsulation. The derivatives PD-1 and PD-2 with glass transition temperatures (T_g) of 42.42°C and 38.10°C respectively were used in this study. PD-3 with T_g of 26.74 yielded microspheres that were soft at room temperature and fused on standing; it was therefore not investigated further. Microspheres were prepared by emulsion solvent evaporation method using acetone/ liquid paraffin system; diclofenac sodium was used as model drug. The microspheres prepared were evaluated for their size distribution, drug entrapment, and surface morphology. Effects of drug: derivative ratio, propeller speed, solvent system used and viscosity of the dispersion media were also investigated.

MATERIALS AND METHODS

Materials

Polyethylene glycol derivatives PD-1 and PD-2 were synthesized and characterized in our laboratory (Nande, 2006). Diclofenac sodium (DCS) was received as gift sample from Zim Laboratories, Nagpur, India. Carrageenan was purchased from Sigma Chemicals Co., St Louis. All the other chemicals and reagents used were of analytical grade and used as received.

Preparation of Microspheres

The microspheres were prepared by emulsion solvent evaporation method. The drug, DCS, was dissolved in 5 mL derivative solution in acetone (Table 1). Magnesium stearate (10% w/w) was added to this solution and stirred for 5 min using a magnetic stirrer. This mixture was poured into contentiously stirred (propeller speed = 1200 rpm) liquid paraffin (160 mL, 90 cps) contained in a beaker (250 mL); the solvent was allowed to evaporate at room temperature. After 8 h microspheres were collected by filtration and washed three times with 25 mL aliquots of petroleum ether (60–80°C) to remove any adhered paraffin. The microspheres were stored in desiccators maintained at relative humidity < 10%.

Propranolol hydrochloride, a highly water soluble drug, was also used in microsphere preparation using these derivatives. The irregular microparticles obtained had entrapment efficiency < 5%; large numbers of drug crystals were found in the dispersion media. These particles were therefore not investigated further.

Effect of Drug: Derivative Ratio

Microspheres were prepared with the drug: derivative ratios of 1:1.5, 1:2.0, and 1:2.5. Microspheres prepared with these ratios were evaluated for drug entrapment and size distribution.

Effect of Solvent and Solvent Systems

Methylene chloride and isopropyl alcohol were investigated as the alternative solvents for these systems. Microspheres were also prepared using these solvents in combination with

TABLE 1
Formula of Microspheres with DCS Prepared Using Acetone/Liquid Paraffin System

	g/Batch		
Derivative Type	DCS	Derivative	Magnesium Stearate
PD-1	1.00	1.50	0.15
	1.00	2.00	0.15
	1.00	2.50	0.15
PD-2	1.00	1.50	0.15
	1.00	2.00	0.15
	1.00	2.50	0.15

acetone in the ratios of 25:75, 50:50, and 75:25; 5 mL of solvent system was used for each trials.

Effect of Detackifier Type and Concentration

Glycerol monostearate, magnesium stearate, and talc were investigated as suitable detackifying agents. These agents were used at concentrations of 5, 10, and 15% w/w. The trials were undertaken with the drug: derivative ratio of 1:2.0.

Effect of Propeller Speed

The propeller was rotated at 800, 1200, and 2000 rpm to study the effect of speed on microsphere preparation.

Effect of Viscosity of Dispersion Media

Microspheres were prepared with liquid paraffin having viscosities of 90, 140, and 180 cps at 25°C. The viscosity of liquid paraffin was adjusted by adding light and heavy liquid paraffin at different ratios. However the volume of liquid paraffin was kept constant at 160 mL.

Entrapment Efficiency

Measured quantities of microspheres were crushed in a mortar-pestle. This powder was added to approximately 25 mL of phosphate buffer pH 6.8 contained in a 100 mL volumetric flask. The mortar-pestle was carefully washed three times with 15 mL aliquots of the buffer and the wash added to the flask. The suspension was sonicated for 5 min, filtered, and the volume adjusted with buffer. After suitable dilutions the drug content was measured spectrophotometrically at 276 nm.

Entrapment efficiency was determined as

$$\text{Entrapment efficiency} = \frac{W_p}{W_t} \times 100$$

where, W_p is the practical drug content and W_t is the theoretical drug content in the microspheres.

Surface Morphology

Microspheres of selected batches were sputter coated with gold under vacuum and viewed under scanning electron microscope (SEM) (LEO 435VP, LEO Electron Microscopy Ltd., England).

Particle Size Distribution

Particle size of microspheres was measured using a Bright field microscope (Carl-Zeiss, Germany). Diameters of about 150 particles were measured for every batch and their d_{90} calculated. The results provided a realistic picture of size distribution of microspheres as against the average diameter.

In Vitro Drug Release

The in vitro drug release profile from microspheres was studied using USP XXVI dissolution apparatus type II (paddle; Veego Scientific, Mumbai, India). Accurately weighed microspheres (equivalent to 25 mg DCS) were taken in muslin cloth and tied to the paddle set at rotation speed of 75 rpm. The release of drug from the microspheres was tested in 750 mL phosphate buffer pH 6.8 at 37 ± 0.5°C. Aliquots of the release medium were removed at predetermined time intervals and assayed spectrophotometrically (UV-150-02, Schimadzu, Japan) for the released amount of DCS at 276 nm. All the experiments were carried out in triplicate.

Drug Release Kinetics

The mechanism of drug release from the delivery devices was determined by using the release models described in literature (Costa, 2001; Sood, 1998). The equations describing zero order (Eq. 1), first order (Eq. 2), Higuchi model (Eq. 3) and Hixon-Crowell model (Eq. 4) are given below.

$$Q_t = k_{0 \cdot t} \tag{1}$$

$$\ln Q_t = Q_0 - k_{1 \cdot t} \tag{2}$$

$$Q_t = k_{H \cdot} \sqrt{t} \tag{3}$$

$$\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = k_{HC \cdot} t \tag{4}$$

where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in microsphere, S is the surface area of microsphere, and k_0,k_1,k_H and k_{HC} are release rate constants for zero order, first order, Higuchi and Hixon–Crowell rate equations respectively.

In order to define a model that will represent the best-fit model for the formulations, the dissolution data were further analyzed using Peppas and Korsenmeyer equation:

$$M_t / M_\alpha = k \cdot t^n$$

where, M_t is the amount of drug released at time t and M_α is the amount released at time $t = \alpha$. Therefore M_t/M_α is the fraction of drug released at time t, k is kinetic constant, and n is the diffusional exponent. The value of n describes the mechanism for solvent penetration and drug release.

In Vivo Study

Prior permission was taken from the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Sciences, Nagpur (Proposal number 17; Approval date 11/08/

2005) to carry out experiments on animals. No animal rights were violated during Animal handling and the experiments were performed within the prescribed guidelines. Carrageenan-induced paw edema method (Winter, 1965) was used to evaluate the anti-inflammatory activity of the microspheres. Rats (Sprague-Dawley, either sex) weighing 200–250 g were fasted overnight and divided in groups of five animals each. The animals were treated orally with free DCS (10 mg/kg), DCS-loaded microspheres (≈ DCS 10 mg/kg; dispersed in sodium chloride saline with the aid of acacia) and vehicle (containing microspheres without drug in sodium chloride saline) by intra peritoneal route; one hour before the subplanter injection of 0.1 mL of 1% carrageenan (Sigma Chemicals). Paw volumes were measured using plethysmometer (Ugo Basile, Italy) immediately (measured within 60 sec and referred as initial paw volume) and 1, 4, 6, and 10 h after carrageenan injection. The amount of paw swelling was expressed as percent edema relative to the initial paw volume. The percent inhibition of edema for the treated groups was calculated by following formula:

$$\%\text{Inhibition} = \frac{\%\text{Edema (control)} - \%\text{Edema (drug)}}{\%\text{Edema (control)}} \times 100$$

RESULTS AND DISCUSSION

Preparation of Microspheres and Entrapment Efficiency

The microspheres obtained were light yellow colored, fairly spherical in shape with smooth surface. The concentration of derivative in acetone for microsphere formation played a vital role in the process. The minimum drug: derivative ratio of 1.0:1.5 was chosen based on the experimental observations—the resultant drug-derivative-solvent should provide a clear transparent solution. This end point was essential because in the absence of sufficient quantity of derivative, the DCS remained suspended in the quantity of acetone taken. Presence of soluble and insoluble drug particles would lead to formation of microspheres and microcapsules respectively of different sizes. Further addition of derivatives to this suspension increases the solubility of drug into the solution. Since the mixture was clear and transparent, size variation in the microspheres due to variations in particle size of drug was avoided.

Microspheres of all the batches showed adhered magnesium stearate particles and presence of minute pinholes on the surfaces (Figure 1). The presence of magnesium stearate particles at the surface prevents agglomeration of the dispersed phase droplets. The pinholes were formed by the evaporation of solvent from the microspheres. Evaporation of solvent from the dispersed phase results in formation of a hard coat surrounding a viscous drug-derivative gel. Upon continued drying, the solvent from the core is lost by its transport to the surface. This migration of solvent from the interior to the surface results in formation of pinholes or holes depending upon the polymer, solvent system, and drying conditions. In the present case acetone was used as solvent and was allowed to evaporate at room temperature for 6–8 h. This slow drying rate resulted in microspheres with smooth surfaces interrupted at places with pinholes left by the evaporated solvent; no acicular crystals of DCS could be seen on the surface.

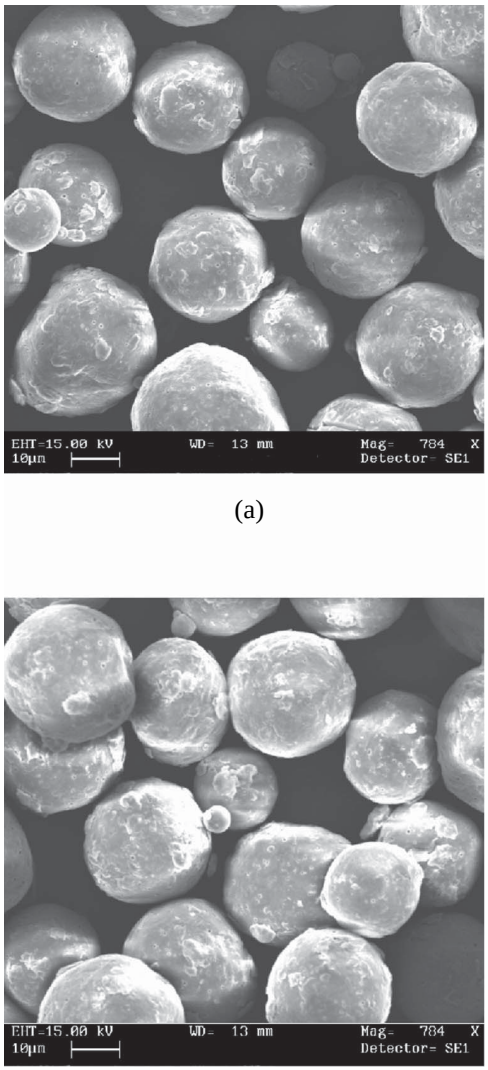


FIGURE 1. Scanning electron photomicrographs of DCS microspheres prepared from (a) PD-1 and (b) PD-2.

The microspheres formed were small in sizes; the d_{90} for microspheres of all formulations ranged from 11.8 to 24.5 µm (Table 2). All microsphere formulations prepared from PD-2 were smaller than corresponding batches prepared from PD-1. The smaller particle size of microspheres of all formulations with PD-2 than the corresponding PD-1 microspheres could be

TABLE 2
Entrapment Efficiency and Particle Size of Microspheres

	PD-1		PD-2	
Variables	Entrapment Efficiency (%)	d_{90}(μm)	Entrapment Efficiency (%)	d_{90}(μm)
DCS: Derivative, 1:1.5	67.81 (2.3)	15.3 (4.1)	61.44 (2.1)	14.7 (3.8)
DCS: Derivative, 1:2.0[#]	74.64 (1.7)	16.5 (1.1)	66.47 (1.4)	15.8 (2.1)
DCS: Derivative, 1:2.5	77.42 (1.4)	17.3 (3.4)	69.22 (2.3)	16.7 (3.6)
Liquid paraffin, 140cps*	70.45 (2.7)	15.7 (2.1)	62.14 (2.2)	14.1 (3.3)
Liquid paraffin, 180cps*	72.15 (2.8)	19.8 (1.7)	66.16 (3.5)	19.8 (2.5)
Propeller speed, 800rpm*	79.94 (1.5)	20.2 (2.1)	70.09 (3.2)	18.9 (2.2)
Propeller speed, 2000rpm*	63.11 (1.5)	15.2 (1.5)	54.21 (1.9)	13.7 (1.9)
Solvent, Act: IPA*	65.71 (1.4)	23.6 (2.2)	54.19 (1.4)	21.5 (2.1)
Solvent, Act: DCM*	72.45 (2.0)	21.1 (5.2)	64.07 (1.6)	19.2 (5.8)
Solvent, Acetone, 10ml*	72.15 (2.2)	15.2 (2.6)	62.13 (4.2)	13.6 (2.4)
Solvent, Act: IPA, 10ml*	66.54 (2.6)	14.5 (1.5)	58.63 (2.5)	13.5 (1.8)
Solvent, Act: DCM, 10ml*	62.18 (3.1)	13.7 (2.5)	56.49 (1.6)	11.8 (2.2)
Magnesium stearate 5%*	65.12 (2.5)	22.4 (2.8)	52.33 (1.9)	20.4 (2.9)

[#]Microspheres prepared with liquid paraffin 90 cps, propeller speed 1200 rpm, magnesium stearate 10% and acetone 5 mL; *DCS: Derivative ratio of 1:2.0; $n = 3$, *SD* in parenthesis.

attributed to their chemical composition. These derivatives were prepared by esterification of resin acids by polyethylene glycol 200 (PEG 200) at elevated temperatures. The concentration of PEG 200 used in the preparation of PD-2 was higher (15% w/w of rosin) than that in PD-1 (7.5% w/w of rosin). This 'internal plasticization' of rosin was carried out to improve its physicochemical properties like film formation. Presence of higher concentrations of PEG esters in PD-2 allows it to form smaller spherical droplets than of PD-1 when its solution is dispersed in continues phase. Smaller particles have been reported in literature when emulsifiers were added in the dispersion media (Hariharan, 2002; Jalil, 1990). In the thermogravimetric analysis of microspheres the drug peak was either very small or absent indicating solubility of drug into the derivative matrix (data not shown). The results also indicate that some fraction of the drug was present in the derivative matrix as crystalline form. However the results could not be quantified at this level of study.

Microspheres prepared from PD-1 had higher entrapment efficiency than the corresponding formulations with PD-2. DCS with octanol-water partition coefficient of 13.4 and is sparingly soluble in water. PD-1 contains less concentration of the hydrophilic PEG esters of resin acids, thereby providing higher hydrophobic domains amongst the two derivatives. These hydrophobic domains provide suitable environment for DCS to reside. PD-2 on the other hand, with higher ester content, provides the matrix hydrophilic nature that does not favor entrapment of DCS. Higher concentration of esters might also aid in partitioning of drug into liquid paraffin. The higher concentration of derivative provides greater chance for the drug molecules to entrap into the microspheres, thereby improving the entrapment efficiency (Gadheri, 1996). Increase in the derivative concentration raises the viscosity of the drug-derivative solution. This solution when poured into dispersion phase produces droplets with high gel strength. These droplets could not be broken into fine droplets owing to their high viscosities; particle size of microspheres therefore increases with the derivative concentration (Gadheri, 1996; Lin, 2000). The shape of the microspheres however remained spherical at all the ratios studied.

Effect of Solvent System and Solvent Volume

Microspheres with lower entrapment efficiency and higher d_{90} were obtained when the solvent was changed from acetone to isopropyl alcohol or dichloromethane. The following discussion is limited to cases where the drug and polymer are soluble in the solvent used for microsphere preparation as in our case. Microsphere preparation by solvent evaporation comprises of a mixture of processes occurring sequentially. As the solvent evaporates from the liquid droplets, it leaves behind a gel having progressively increasing viscosity with time. Some part of the drug gets leached out into the dispersion media along with the solvent during drying. The remaining part of the drug gets entrapped into the viscous gel from which it cannot escape. This gel upon complete loss of solvent hardens into solid microsphere particles. This system however becomes more complex when a combination of solvent is used.

After the solvent with low boiling point has been evaporated, the polymeric gel contains predominantly the other

solvent. The type of microsphere formed then depends upon the solubility of the drug in the polymer gel of the other solvent. If the drug were soluble in the polymeric gel, the evaporation of solvent would result in co-precipitation of drug and polymer. Since the solvent has affinity for both the drug and the polymer, only a part of it would be available to either. Presence of small fractions of solvent to the drug molecules distributed into polymer gel prevents precipitation of drug into large crystals. These crystals once formed are entrapped into the polymeric matrix, thereby yielding microspheres with high entrapment efficiency.

The polymeric gel offering low solubility to drug would however present a different picture. Owing to their poor solubility, the drug particles together with the fraction of solvent associated with them would segregate in the gel. This portion of gel upon solvent evaporation results in large crystals. Depending upon their location and size, these crystals may precipitate in the core, at the surface or outside the microsphere surface resulting in microcapsules, microspheres with high surface bound drug or microspheres with low entrapment efficiency respectively.

The solubility of DCS in various solvent systems is shown in Table 3. When the solubility was measured in pure solvents, DCS showed highest solubility in acetone (ACT) than in any other solvent. When used in combination with acetone, the solubility of DCS in IPA and DCM increased by two times than when in individual solvents. This trend was extrapolated to solubility of DCS in derivative solutions. It is worth mentioning that the solubility of DCS in the derivative gel (upon solvent evaporation) was lower than the solubility in the solution.

When acetone alone was used as solvent for microsphere preparation at room temperature, it resulted in uniform spherical matrices of derivative with drug embedded in them. In case of acetone: IPA system acetone evaporated early, thereby leaving behind derivative solution or gel in IPA. As indicated in Table 3, the solubility of DCS in this solution was less than in acetone. The drug particles therefore tend to form crystals, some of which migrated into the dispersion media (as observed under microscope) that resulted in reduction of entrapment efficiency, Figure 2 (a). The slight increase in d_{90} of these particles was attributed to the increased surface roughness and irregularities due to the differences in evaporation rates of the two solvents.

The entrapment efficiency of microspheres prepared with ACT: DCM system were similar to those prepared with ACT alone. The derivative gel rich in ACT formed after DCM evaporation allowed greater entrapment of DCS. However this fast removal of DCM resulted in microspheres having large pores with a derivative gel entrapped under a hard coat, Figures 2(b) and 2(c). The system formed under these conditions was therefore matrices covered with thin coat of the same material, that is, walled microspheres. Polymers with varying rates of precipitation from the organic solvents under controlled conditions result in double-walled microspheres (Leach, 1999; Pekarek, 1994). However the exact reasons for such observations in the present case could not be figured. It must be noted here that these results were seen in a section of microparticles and that the majority of the microparticles were microspheres where the drug was distributed throughout the derivative. Presence of these microcapsules, however, resulted in greater retardation of drug release. The surface irregularities of the microspheres were also attributed to their higher d_{90}. Increase in the solvent volume resulted in decreased viscosity of the drug-derivative solution. The decrease in viscosity of this solution facilitated reduction of liquid drops to smaller size that upon drying yielded smaller particles. However smaller microspheres prepared from the solvent systems of ACT: DCM or ACT:IPA were unable to hold all crystals of DCS in their matrices. High affinity of DCS for derivative solutions in acetone prevented drug loss even when the microsphere size was reduced. The microspheres obtained therefore were similar in size distribution and entrapment efficiency. Formulations with PD-1 showed higher entrapment efficiency and particle size than the corresponding preparations with PD-2. The derivative PD-1 being more hydrophobic forms better matrix with the sparingly water-soluble drug diclofenac sodium. The increase in the volume of solvent system resulted in microspheres with smaller particle size and reduced drug entrapment. The microscopic observations revealed presence of precipitated derivatives and free drug crystals in the dispersion media. When acetone was used alone, the particle size decreased marginally while the entrapment efficiency remained unchanged.

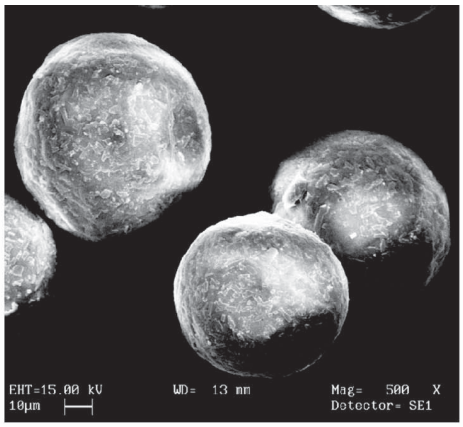
TABLE 3
Solubility of DCS in Different Solvent Systems

Solvent System	Solubility (mg/mL)*	Solubility in Solvent(s) Containing 30% w/w Derivative, (mg/mL)*
Acetone (ACT)	33.1 (1.2)	216 (1.7)
Isopropyl alcohol (IPA)	9.7 (1.6)	87.6 (2.1)
Dichloromethane (DCM)	10.2 (1.1)	94.5 (1.3)
Acetone: IPA	21.5 (3.5)	148.2 (2.1)
Acetone: DCM	23.1 (2.8)	156.7 (3.6)

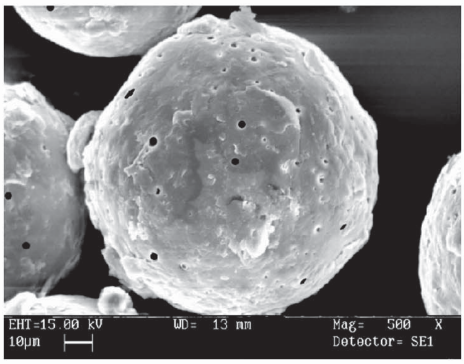
*Measured at 25 ± 1°C; $n = 5$, *SD* in parenthesis; # equivalent to the derivative concentration used in microsphere preparation.

Effect of Detackifier Type and Concentration

Of the three detackifiers evaluated, only magnesium stearate produced microspheres with spherical discrete particles. Magnesium stearate remained suspended in the drug-derivative solutions. This suspension when poured into paraffin resulted in liquid droplets containing suspended particles of magnesium



(a)



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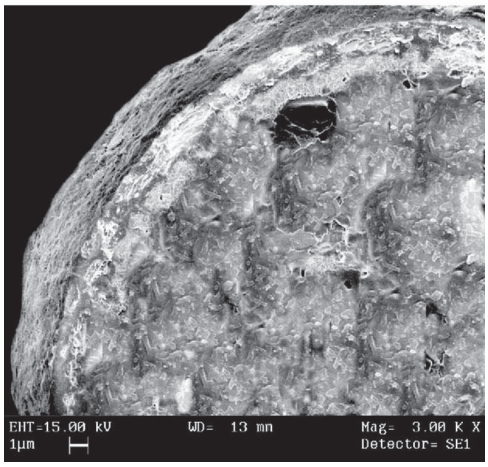

(c)

FIGURE 2. Scanning electron photomicrographs of microspheres (a) Prepared from IPA (b) Prepared from DCM (c) A part of sectioned microsphere prepared from DCM.

stearate. A part of these suspended particles remained aligned at the surface. The presence of the detackifier at the surface prevented agglomeration of droplets. However the entrapment efficiency and particle size of the microspheres remained unchanged. The results differ from those in literature that increment in magnesium stearate concentration results in reduced particle size with higher drug entrapment (Bogataj, 2000). Part of the magnesium stearate particles added tend to dissolve in the drug-derivative solution, an observation that was not seen with other detackifiers studied. The effect of the dissolved part on the detackifying effect, however,needs further investigation. Strong aggregation of particles was observed when talc or glycerol monostearate were used as detackifiers at all the concentrations studied

Effect of Speed of Rotation and Viscosity of Dispersion Media

Microspheres prepared with low propeller speed had larger particle size with greater entrapment efficiency; increment in the speed produce microspheres with smaller size but with less entrapment. Lowering the propeller speed reduced the shear forces acting upon the liquid droplets, resulting in increase in their particle sizes. These microspheres being larger in size entrapped higher amount of drug. Increase in the propeller speed provided additional shear forces on the liquid droplets so that particles with smaller sizes were obtained. These particles owing to their small diameters allowed migration of drug into the dispersion media thereby rendering the microspheres with reduced drug entrapment (Mateovic, 2002). Free acicular crystals of DCS were observed when the suspension was observed under a microscope.

The size and entrapment of microspheres initially decreased followed by an increase when prepared in liquid paraffin with increasing viscosities. The results clearly indicate that in the present system, at a particular viscosity the forces acting upon the liquid droplets are balanced by each other such that particles of uniform sizes are produced. Any alteration in the viscosity, however, shifts this balance so that the particle size increases. At low viscosity the shear forces acting upon the droplets are intense; centrifugal forces acting upon the particles outweigh influence of any other forces. Due to intense shear the droplets fail to attain definite size and spherical shape while drying. At higher viscosity of the dispersion media, the shear force generated due to stirrer speed is not enough to disperse the droplets in the media. Regions with least turbulence prove conducive to form droplet aggregation. The surface morphology of these microspheres, however, remained unchanged.

In Vitro Release and Release Kinetics

The release of DCS from microspheres of all formulations exhibited initial burst release followed by slow release. The initial burst release from the microspheres was due to the release of surface bound drug. The release of the drug present inside the microspheres was, however, restricted by the derivative matrix. The release of DCS retards proportionately with

the increase in the derivative concentration. This improved capsule formation around the drug molecules as a result of increase in derivative concentration provide barrier for it to diffuse out from the core into the dissolution media. Scanning electron photomicrograph of a batch of microspheres after dissolution is shown in Figure 3. Microspheres after the release of drug from them form porous structures. Although the derivatives were also partially soluble in the media (solubility < 32 mg/mL in 24 h), their solubility contributed little in the drug release. The pores seen in the microsphere structure are a result of drug diffusion that created the channels into the particle core. Increasing the derivatives concentration improves the entrapment of DCS as evidenced by the reduction in release rate from the microspheres, Figure 4.

The dissolution of DCS was proportional to the size of microspheres-smaller particles favored faster drug release. The release of drug from the microspheres was in linear agreement with the propeller speed. Increase in the propeller speed produced smaller sized microspheres with reduced encapsulation.

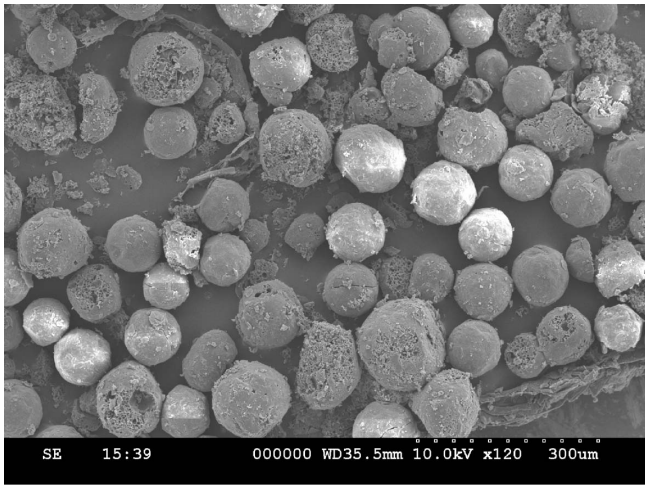

FIGURE 3. Scanning electron photomicrographs of DCS microspheres after dissolution study.

These small particles offered greater exposed surface area for drug release. Conversely a reduction in the propeller speed produced particles with greater drug encapsulation. Since the exposed surface area was lesser than that for small particles, a reduction in the release rate of the drug was observed, Figure 5. As seen earlier, an increase or decrease in viscosity of the media from 140 cps resulted in formation of large sized microspheres. The release of drug from these microspheres, however, was not proportional to their sizes, Figure 7. Presence of microspheres with varying shapes and sizes must have contributed to these results.

The release of drug from microspheres prepared from different solvent systems, however, resulted in microspheres with varying release profiles. When IPA or DCM was used as solvents, it resulted in microsparticles with reduced release rates. When acetone alone was used as solvent it resulted in microspheres with uniform distribution of drug across the derivative matrix. However when IPA was added in the derivative solution it reduced the solubility of drug in the derivative solution. This phenomenon caused precipitation of drug into large or small crystals that were absent when acetone was used as solvent. The process therefore produced a mixture of microspheres and microcapsules of varying sizes. The microcapsules due to complete entrapment of the drug crystals allowed slow release of drug from the core; the release is manifested as sharp reduction in the initial burst release. Increment in the IPA composition in the derivative solution produced more drug crystals in the derivative that further retarded the drug release. The drug release retarding effect therefore could be attributed to formation of crystals and presence of derivative coat around them. The crystals require large amount of energy to dissolve them and the coat provides additional shielding of drug from the media. Similar effect was observed when DCM was used as the other solvent. As discussed earlier, this decrease in drug release was attributed to the presence of microcapsules that offer greater resistance to drug release. It produced a system wherein derivative coat envelops a drug-derivative matrix. This system also retards the release of drug to a greater extent, Figure 6.

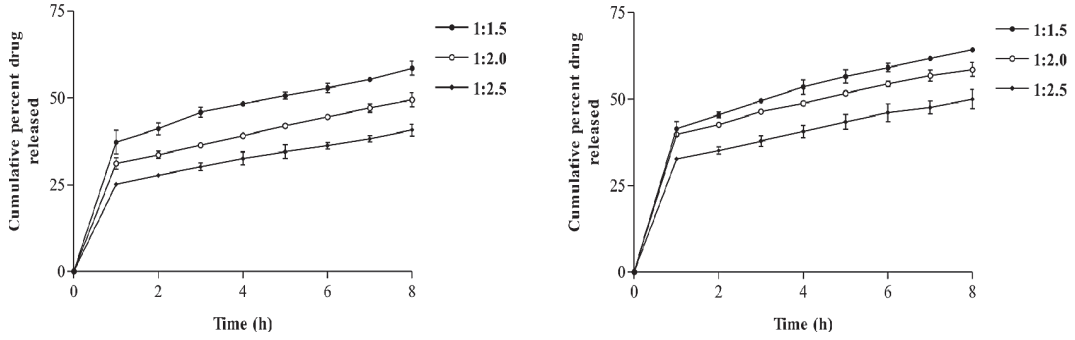

FIGURE 4. Dissolution profile of DCS from microspheres prepared from (a) PD-1 and (b) PD-2.

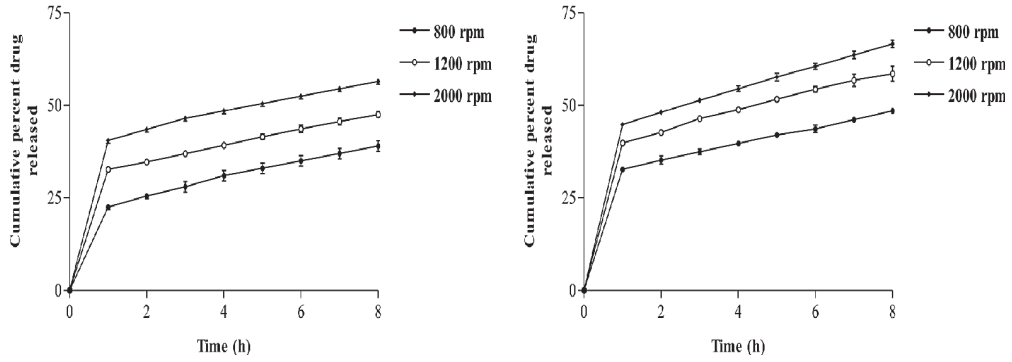

FIGURE 5. Effect of propeller speed on release of DCS from microspheres prepared from (a) PD-1 and (b) PD-2.

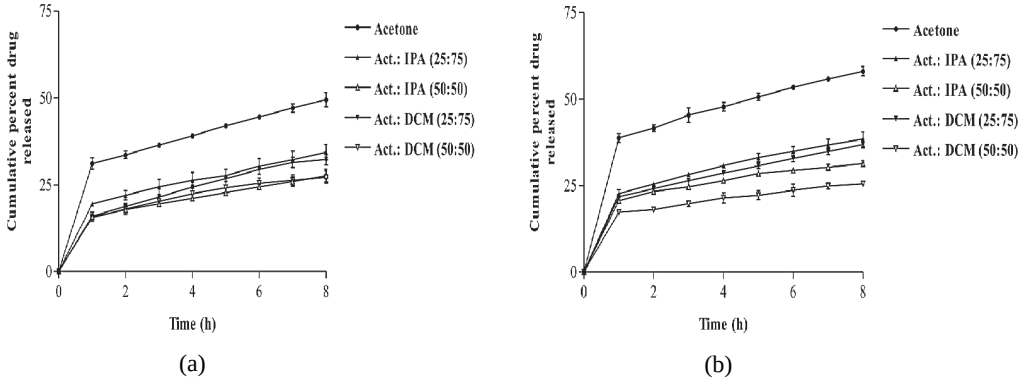

FIGURE 6. Effect of solvent system on dissolution of DCS from microspheres prepared from (a) PD-1 and (b) PD-2.

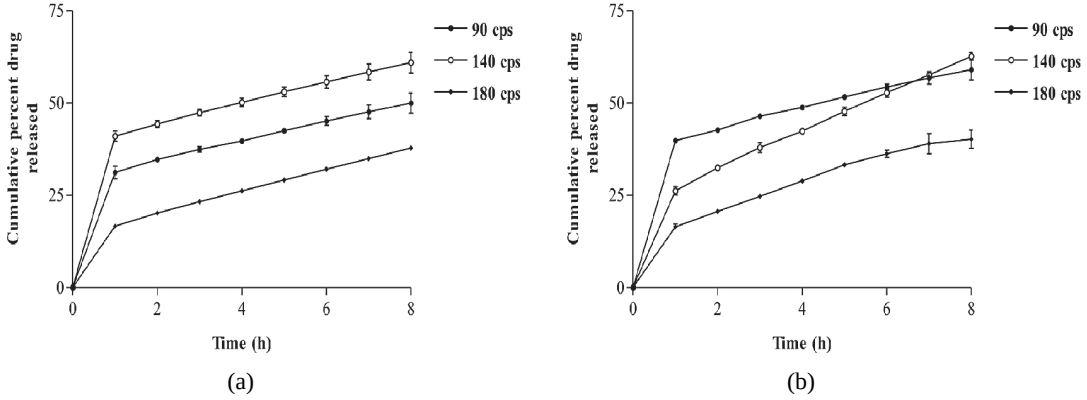

FIGURE 7. Effect of viscosity of dispersion media on release of DCS from microspheres prepared from (a) PD-1 and (b) PD-2.

The release of DCS from all the microsphere formulations occurred in two phases. The initial phase consisted of burst release (1st h) followed by sustained release for the next seven hours. The drug release in the second phase followed the Zero order model ($r > 0.999$). The combined data also complied with Higuchi equation of drug release kinetics ($r > 0.998$) indicating release from inert matrix by drug diffusion. When applied to the Peppas-Korsenmeyer equation, the exponent component, *n*, values were close to 0.5 with higher correlation coefficients, *r*, greater than 0.996, suggesting Fickian mechanism of drug diffusion. The transport or diffusion of media into the particles was through channels prepared by drug diffusion and through

hydrophilic segments of the particle; the drug diffused out through these water filled channels. The mechanism of drug release from the microspheres was similar to that observed from matrix tablets prepared by melt granulation (Communicated to Drug Development and Industrial Pharmacy, 2006).

In Vivo Study

Although none of the microsphere formulations resulted in complete drug release, microspheres prepared with drug: derivative ratio of 1:2.0 (either derivative), with 1200 rpm propeller speed prepared in liquid paraffin with 90 cps viscosity was taken for in vivo study. The reason for selecting these batches included their high correlation coefficients ($r > 0.9992$) and that these were the formulations upon which variations in process and formulation were carried out.

Carrageenan-induced rat paw edema test has been considered as a useful tool for studying anti-inflammatory effect of drug on rats (Arora, 2002; Winter, 1965). When carrageenan was administered into the rat paws it resulted in edema, manifested by an increase in the paw volume (increased to 110%); the inflammation remained constant throughout the experiment, Table 4. It must be noted that the circulation half-life of DCS is between 1–2 h; the anti-inflammatory effect of immediate release dosage form should therefore decrease after the 2nd hour. When DCS was administered as immediate release suspension the inhibition of edema was observed for initial two hours, thereafter the edema kept increasing. The drug was available at the site of edema at the concentration to inhibit inflammatory response for three hours from dosing (drug was administered one hour prior to carrageenan injection). Thereafter the concentration of DCS in circulation was not sufficient to produce the required effect.

PD-1 and PD-2 microspheres of DCS injected into the animals produced similar responses. The initial response to edema was weak, percent inhibition being lower than that of the DCS suspension. However from 2nd hour onward till the completion of experiment the percent inhibition of edema was very high as compared to the animals treated with DCS suspension. The inhibitory response in the microsphere-treated animals was observed throughout the experiment though complete inhibition was not observed in any case. This incomplete inhibitory response could be attributed to slow and incomplete release of DCS from the microspheres.

TABLE 4
Percent Inhibition of Paw Edema of Rats Administered with Different Formulations

Samples	Condition	Time (h)				
		1	2	4	6	10
Control group*	% Edema w.r.t. Initial paw volume	108.05 (2.58)	110.54 (1.65)	109.63 (2.65)	110.77 (1.32)	110.49 (1.16)
DCS suspension	% Edema w.r.t. Initial paw volume	84.45 (2.62)	63.25 (2.33)	69.21 (1.94)	77.98 (2.41)	86.14 (3.15)
	% inhibition w.r.t. control group	21.84 (1.47)	42.78 (1.65)	36.05 (2.65)	29.60 (2.16)	22.03 (3.05)
PD-1	% Edema w.r.t. Initial paw volume	90.14 (3.25)	56.24 (1.09)	23.61 (2.67)	15.44 (3.66)	15.65 (2.98)
	% inhibition w.r.t. control group	16.57 (2.64)	49.12 (1.77)	78.46 (2.51)	86.06 (2.73)	85.83 (3.38)
PD-2	% Edema w.r.t. Initial paw volume	93.12 (2.19)	63.27 (2.74)	31.25 (2.33)	17.25 (1.16)	15.44 (1.82)
	% inhibition w.r.t. control group	13.81 (2.88)	42.78 (2.45)	71.49 (1.94)	84.42 (1.54)	86.02 (3.40)

$n = 5$; *SD* in parenthesis; *without drug.

CONCLUSION

The synthesized PEGylated derivatives of rosin yielded microspheres with size range of 11–25 μm with drug entrapment being less than 80%. The system used in microsphere preparation was sensitive to choice of solvent system, viscosity of dispersion medium, propeller speed and drug: derivative concentration. The relative solubility of drug in the solvents used in microsphere preparation governed the fate of the process; microspheres or microcapsules resulted by changing the

solvent systems. These microspheres could sustain the release of diclofenac sodium for over 10 h period. The release of drug through these microspheres was by channel formation and drug diffusion. The in vivo study in rats also provided promising response by inhibiting the edema in the animals. The paw edema in rats was also satisfactorily inhibited for the time period tested. The derivatives therefore could be used successfully in preparing microspheres; entrapment of water-soluble drug, however, needs to be investigated.

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