Preparation and Determination of Drug-Polymer Interaction and In-vitro Release of Didanosine Microspheres made of Cellulose Acetate Phthalate or Ethyl cellulose Polymers

Sethi R. K.*, Barik B. B., Sahoo S. K.
University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Odisha, India

Abstract
The objective of this study was to formulate and evaluate the drug-polymer interaction of Didanosine using two polymers with different characteristics as Ethyl cellulose or Cellulose acetate phthalate. Microspheres were prepared by the emulsion solvent evaporation. The effect of drug-polymer interaction was studied for each of microspheres. Important parameters in the evaluation of a microencapsulation technique are encapsulation efficiency, yield production, particle size, surface characteristics of microspheres, scanning electronic microscopy (SEM), powder X-ray diffraction analysis (XRD) and differential scanning calorimetry (DSC). The in-vitro release studies are performed in buffer (pH 7.4). Microspheres containing cellulose acetate phthalate and Ethyl cellulose showed 80-87% and 75-79% of entrapment efficiency, respectively. The thermogram X-ray and DSC showed stable character of Didanosine in the microspheres and revealed an absence of drug polymer interaction. The prepared microspheres were spherical in shape and had a size range of 355 µm for Cellulose acetate phthalate microspheres and 345-383 µm for Ethyl cellulose microspheres. The results suggest that Didanosine was successfully and efficiently encapsulated; the release rates of matrix microspheres are related to the type of polymer, only when formulation (FDEC3) used to get prolonged drug release with increasing the polymers content in the microspheres. Data obtained from in-vitro release for microspheres were fitted to various kinetic models and the high correlation was obtained in the First order model.

Key words:
Didanosine, dispersing agent, modified emulsion-solvent evaporation.

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INTRODUCTION
Didanosine (ddI) is a drug commonly used in acquired immune deficiency syndrome (AIDS) therapy [1, 2]. Although it may suffer hydrolysis in acid pH, which consequently produces hypoxanthine,
concomitant administration of ddI with food may still compromise the stability of the drug due to the acid pH generated by the digestion process. In general, the free drug is administered in buffered tablets to prevent its deactivation when exposed to the low pH of the stomach. The tablet formulation requires the addition of 50% of carbonate or magnesium hydroxide to obtain the buffering effect. However, some collateral effects are referred, such as diarrhea and renal problems, and may also show wide inter-individual bioavailability [3, 4]. Furthermore, the buffered tablets are large; being therefore, difficult to be administered orally.

Great benefits in ddI administration were obtained in 2001 with the commercial formulation Videx EC composed of gastro-resistant granules. However, Videx EC still requires frequent administration and lacks in controlled release profile. Furthermore, the lower bioavailability makes ddI a good candidate for extended release formulations.

In recent years, a continuous interest has been focused on the development of controlled drug release formulations using multiparticulate systems (e.g., pellets), offering various advantages over single dosage forms, namely, an improved bioavailability [5,6] and easy administration for elderly people and for children [7]. These include a low risk of dose dumping, flexibility of blending units with different release patterns, reproducible gastric residence time, and prevention of high local drug concentration in the gastrointestinal tract [8, 9]. Because of instability of ddI in acid pH, sustained release pellets have been frequently obtained using different types of coatings [10].

However, no commercially long-acting product exits in the market. The short biological half-life of 1.5 h following oral dosing necessitates frequent administration of the drug in order to maintain the desired steady state levels[11]. The formulation of ddI as a modified release dosage form of ethyl cellulose (EC) and cellulose acetate phthalate (CAT) microspheres seems to be an alternative approach in overcoming the potential problems in the gastrointestinal tract. Microencapsulation is a well-known method that is used to modify and delay drug release from pharmaceutical dosage forms. A great number of microencapsulation techniques are available for the formation of sustained release of microparticulate systems. One of the popular methods for the encapsulation of drugs within water-insoluble polymers is the emulsion solvent evaporation method [12]. The emulsion solvent evaporation technique was fully developed at the end of the 1970s and has been used successfully in the preparation of microspheres made from several biocompatible polymers such as poly (D, L-lactide-co-glycolide) [13-17], poly (ε-caprolactone) [18-23] and Eudragit [24-26].

The technique of emulsion solvent evaporation offers several advantages and is preferred over other preparation methods such as spray drying, sonication and homogenization etc, as it requires only mild conditions such as ambient temperature and constant stirring [12]. CAT has been widely used as an enteric coating for tablets and capsules. Lately, several workers have described investigations using CAT as a polymer employing either aqueous [27]. The microencapsulation of drugs with CAT has been carried out successfully in either an aqueous or an organic vehicle. There are several methods available which may be employed in the microencapsulation with CAT and EC. They include coacervation-phase separation method, spray-drying method and extrusion method [28].

The physicochemical properties of a drug are usually the main concern in the selection of a suitable method for use. While studies evaluating drug release from microspheres prepared with individual cellulose esters have been conducted in the past [29], a comparative evaluation of drug release from microspheres prepared using a range of cellulose esters of similar molecular weights has not been available.
The purpose of this paper is to study sustained release microspheres prepared through modified emulsion solvent evaporation method (O1/O2 emulsion) and the effects of variations of drug/polymer ratio on the preparation of microspheres (using CAT/EC polymers separately to prepare different microspheres). The micromeritics properties (incorporation efficiency, yield value, particle size and distribution, surface characteristics of microspheres), powder X-ray diffraction analysis (XRD), differential scanning calorimetry (DSC) and dissolution tests were evaluated afterwards.

**MATERIALS AND METHODS**

**Materials**

Didanosine, Ethyl cellulose and Cellulose acetate phthalate and was obtained as a gift sample from Cipla Ltd., Mumbai. All the solvents are procured from Merck. All other chemicals and reagents used in the study were of analytical grade.

**Method**

**Preparation of ddI microsphere with CAT or EC polymers**

Microspheres were prepared through oil-in-oil (O1/O2 emulsion solvent evaporation method) using different ratios of ddI to CAT or EC ratios (as shown in Tables 1). Liquid paraffin is preferred as an appropriate dispersing medium to methanol and acetone, because when a solvent with a dielectric constant about 10 or above is used, non-polar liquid paraffin is preferred [30,31]. Acetone is a unique organic solvent which is polar, water-miscible and oil-immiscible. All other organic solvents like methanol, ethyl alcohol, ethyl acetate, acetone, dimethyl sulfoxide and tetrahydrofuran are oil-miscible and do not form emulsions of the polymer solution in oil [12,15]. ddI and Aluminium stearate was dispersed in 14 ml of the mixed solvent system consisting of acetone and methanol in a 10 ml and 4 ml. The drug suspension was then emulsified in a liquid paraffin solution under stirring at 500-700 rpm (Cuprit Electrical Co. India) for 30 min. Then, 30 ml of n-hexane (non-solvent, for CAT or EC, respectively) was added to harden the microspheres and stirring was continued for a further 3 h. Next, the hardened microspheres were collected by filtration and washed with three portions of 30 ml of non-solvent to remove any remained oily phase, and then was air dried for 12 h.

**Evaluation of microspheres**

**Determination of drug content, encapsulation efficacy and production yield (%)**

50 mg of formulations was dissolved in 50 ml of phosphate buffer pH 7.4 and filtered. The samples were assayed for drug content by UV-spectrophotometer (Shimadzu UV-1700) at λmax 249.5nm and the drug content was calculated using the Eq (1).

\[
\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \text{ Eq (1)}
\]

All experiments were done in triplicate. The production yield of the microsphere was determined through accurately calculating the initial weight of the raw materials and the last weight of the polymeric particles obtained. All of the experiments were performed in triplicate.

**Particle size analysis** [32]

Microspheres were separated into different size fractions by sieving for 10 minutes using a mechanical shaker (Cuprit Electrical Co. India) containing standard sieves # 16, # 24, # 30, # 44 and # 60 and mean particle sizes of microspheres were calculated. Each sample was measured in triplicate.

**Microsphere morphology:**

Morphological characterization of the microspheres was carried using scanning electron microscopy (Joel, SEM Model JSM - 6400, TOKYO, Japan). For SEM the double sided sticking tape coated with gold film (thickness 200nm) was used under the reduced pressure (0.001torr).

**Fourier Transform Infrared Spectroscope [FTIR]**

The IR Spectra of DDI was recorded by using (PerkinElmer Spectrum Version 10.03.02). Drug sample was prepared in KBr disks [2mg sample in
200mg KBr. The scanning range was 400-4000cm⁻¹ and the resolution was 2cm⁻¹.

**Differential scanning calorimetry (DSC)**

The DSC analysis of pure drug, drug-loaded microspheres were carried out using Perkin Elmer, USA (Diamond DSC) to evaluate any possible drug-polymer interaction. 5mg drug loaded microspheres were triturated to get finely divided powder. The powder was passed through sieve No.100. In a similar way, pure drug was also passed through sieve No.100. Sample [2-4mg] were accurately weighed and heated in sealed aluminium pans at a rate 5.00°C/min from 50°C to 200°C temperature range under nitrogen flow of 25ml/min.

**X-ray diffractometry (XRD)**

X-ray diffractometry was used for diffraction studies. XRD studies were performed on the samples by exposing them to copper (Cu kα) radiation (40kV,30mA) and scanned from 2°C to 80°C, 2θ at a step size of 0.045° and step time of 0.5 sec. XRD analysis was performed on the pure drug and for the prepared formulation of microspheres with various polymers.

**Dissolution studies**

*In vitro* dissolution studies were performed using USP type II dissolution apparatus (VEEGO, VDA-6D). The rotating basket method specified in USP-XXI at 75 rpm. The microspheres were weighed and tied in the muslin bag and placed in the basket. The dissolution medium (500ml) consisted of phosphate buffer pH 7.4. The temperature was maintained at 37°C ± 0.5°C. An aliquot of (5ml) sample was withdrawn at specified time interval and replaced with an equivalent volume of dissolution fluid. Drug content was determined by UV-Visible spectrophotometer (Schimazdu UV 1700 E 23) at 249.5nm. The release studies were conducted in triplicate.

**Determination of stability of the microspheres**

The microspheres prepared in the present study were filled in the hard gelatin capsules (No.1) and stored in HDPE container at RT 37°C, and 45°C for 6 months as per ICH guidelines. The samples were then characterized for % drug content.

**RESULTS AND DISCUSSION**

**The effect of drug: polymer ratio on the physical properties microsphere**

Microspheres were formed after a series of steps like solvent evaporation and addition of non-solvent. Microspheres (cellulose acetate phthalate or ethylcellulose) were prepared using different drug-polymer ratios as shown in Tables 1. The drug-polymer ratio was varied through maintaining the amounts of drug, dispersing agent and solvent constant in all preparations and changing the amount of polymer. The results of the effect of drug-polymer ratio (microspheres containing cellulose acetate phthalate/ethylcellulose) on production yield, drug loading efficiency and mean particle size are shown in Table 2 figure 1. The pore formation is induced by diffusion of solvent from surface of the microsphere. In all of the formulations, the mean amount of drug entrapped in prepared microspheres was different from the theoretical value, since the drug loading efficiency is the range of 87.20-95.49% (microspheres containing CAT) and 85.60-90.15% (microspheres containing EC). The highest and the lowest encapsulation efficiency were obtained with cellulose acetate phthalate polymer (95.49%) and ethylcellulose polymer (85.60%), respectively. The encapsulation efficiency of the drug depended on the solubility of the drug in the solvent and continuous phase. According to Table 2, raising the polymer-drug ratio increased the production yield (when the ratio of polymer–drug increased from 1:1 to 1:2 (microspheres containing CAT) and 1:1 to 1:2 (microspheres containing EC), the production yield was 64-85%. The reason for decreased production yield at low polymer: drug ratios could be due to the increased diffusion rate of solvents (acetone and methanol) from concentrated solutions into emulsion, since through increasing the polymer...
amounts, the viscosity of solution was increased as well. The size of microspheres containing CAT was found constant (Table 2) and the size of microspheres EC was found to be decreased by means of decreasing in the concentration of polymer (Table 2). It can be attributed to the fact that with the higher diffusion rate of non-solvent to polymer solution, the smaller size of microcapsules is easily obtained [33]. A volume-based size distribution of drug, polymer and drug loaded microspheres, indicated a log-probability distribution. Mean particle size of CAT and EC was 355 and 359.09 ± 21.37 µm, respectively.

SEM of microspheres (FDCAT and FDEC) is demonstrated in Figure 2. When the viscosity of internal phase of these formulations was investigated, it was found that the particle sizes of microsphere were proportional with the viscosity of the dispersed phase. The results showed that the apparent viscosities of different drug: polymer ratios of microspheres containing CAT (1: 1, 1: 1.5 and 1: 2), were 13, 23 and 32 mPa.S, respectively. The results indicated that the apparent viscosities of different drug: polymer ratios of microspheres containing EC (1: 1, 1: 1.5, and 1: 2) were 9, 13 and 16 mPa.S, respectively. When the dispersed phase with higher viscosity was poured into the continuous phase (external phase), due to the higher viscosity of the internal phase, the globules of the formed emulsion might need more energy to divide into smaller particles and the bigger droplets were formed and the mean particle sizes were increased. In other studies, it was showed that the particle size depends on the solvent volume and the drug/polymer ratio, when solvent diffusion method is utilized for preparing microspheres [34-36].

Fourier Transform Infrared Spectroscope [FTIR]
The FTIR spectra of the pure drug and microspheres with polymers were compared and the characteristic peak for microspheres in spectra was found to be super imposable to that of the pure drug (Figure 3). There were no extra peaks, which gave evidence that there was no drug polymer interaction. The FTIR spectrum of the physical mixture of drug and polymer showed no significant shift or reduction in intensity of peaks. 3307(OH stretching vibration), 1721(cyclic ketone, C=O), 1704(C=N stretching), 1343 (OH bending vibration), 1104(CO stretching vibration), and 1214 (CN stretching vibration). If the drug and polymer would interact, then functional groups in the FTIR spectra would show band shift and broadening compared to the pure drug and polymers.

Differential scanning calorimetry (DSC)
The drug may have been dispersed in crystalline or amorphous form or dissolved in the polymeric matrix during the formation of microspheres. Any abrupt or drastic change in the thermal behavior of rather the drug or polymer may indicate a possible drug-polymer interaction [37]. The endothermic peak of pure drug was observed at about 194.78°C (Figure 4). However, in the thermogram of the microsphere, (containing CAT or EC) there was an endothermic peak of the drug melting with a lower intensity than the pure drug peak, suggesting the crystalline state of the drug in the microsphere. The DSC shows the stable character of ddI in the drug loaded microspheres and revealed crystallinity form.

X-ray powder diffractometry
The X-ray diffraction patterns of pure drug, shows that the pure drug is crystalline in nature (Figure 5). However, when it was incorporated into the polymer matrix, the principal peaks of the drug appeared with lower intensity. This could be ascribed to the crystalline state of the drug in the microsphere. It confirms the results obtained from DSC experiments.

In-vitro release studies
Figure 6 shows the release profile of the drug from microsphere. The in-vitro release of ddI from microspheres containing CAT exhibited an initial burst effect, which may be due to the presence of some drug particles on the surface of the microspheres. The initial burst effect may be attributed as a desired effect to ensure the initial
therapeutic plasma concentrations of drug. For microsphere containing EC, dissolution of ddI at pH 7.4 was strongly reduced, resulting in an overall slower drug in most cases. The initial slow drug leakage generally ended very timely; in the remaining time, nearly linear behavior was observed. Two phenomena can combine in enhancing in the diffusion of the remaining dispersed drug into the bulk phase as well as the formation of pores within the matrix due to the initial drug dissolution; particle wetting and swelling which enhances the permeability of the polymer to the drug [33]. The results indicated that some factors such as polymer-drug ratio governed the drug release from these microspheres. In order to keep the total surface area of the microspheres constant and thus, to get comparable results, the release studies were carried out using the same size fractions of microspheres containing equivalent amount of ddI from different batches. Drug release rates increased with decreasing the amounts of ddI in the formulation (containing CAT or EC polymer). Higher level of polymer corresponding to lower level of the drug in the formulation resulted in a decrease in the drug release rate (Figures 6). As more drugs are released from the microspheres, more channels are probably produced, contributing to faster drug release rates. However, Figure 6 demonstrates that the burst effect is higher when the ddI is loaded to CAT polymer. Moreover, nearly the same amount is released at 7 h from the FDCAT3 (drug: polymer 1: 2 ratio). Therefore, formulations containing CAT could not prolong the release of ddI. Only formulations containing EC are prolonged release, which could be due to the thicker polymer membrane that controls the release rate (Figure 6). One of the goals in drug microencapsulation systems development is to have an initial burst release and achieve a constant release rate thereafter. The degree of initial burst from the microsphere depends on the drug encapsulate ability of the polymer matrix, which thereby, making it unavailable for immediate diffusion [36]. For this reason, efforts to reduce the initial burst have followed in the same track as those, increasing encapsulation efficiency, so, understanding the previous effort to maximize the encapsulation efficiency will thus be useful in controlling the release profile. Pristine ddI had a higher release in comparison with microspheres containing CAT or EC, (Figure 6).

The in-vitro release profiles were fitted on various kinetic models in order to find out the mechanism of drug release [38,39]. The fit parameters of first-order, Higuchi, Hixon Crowell and zero-order equations are given in Table 3. A high correlation was observed for the First order model release plots stated non-fickian and diffusion controlled (Table 3). The release mainly depended on the ratio of the polymer.

Stability studies
The accelerated stability studies showed the stable nature of the drug and showed a good correlation between the original and the aged samples.

CONCLUSION
ddI microspheres were prepared using the modified solvent evaporation method. ddI microspheres (containing of CAT or EC) could be prepared with high drug encapsulation efficiency. Polymer: drug ratio influenced the sphericity of the microspheres. It was observed that increasing the polymer concentration leads to an increase in the mean particle size of the microspheres. The drug release from CAT or EC microspheres exhibited a lower initial burst effect and the mechanisms of the drug release non-fickian and diffusion controlled. The controlled release without the initial peak level that is achieved with these formulations may reduce dose frequency and side effects as well as improving the patient’s compliance.

ACKNOWLEDGMENT
The authors greatly acknowledge Cipla Ltd., Mumbai, India for providing Didanosine as gift
samples respectively. The authors are also thankful to Bhubaneswar, Odisha, India for providing research facility.

**Table 1**: Didanosine microsphere containing cellulose acetate phthalate and ethyl cellulose formulations prepared by modified solvent evaporation method (O1/O2).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Drug : Polymer ratio</th>
<th>Emulsion (O1/O2)</th>
<th>Internal organic phase (O1)</th>
<th>External oily phase (O2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Didanosine (mg)</td>
<td>Cellulose acetate phthalate (mg)</td>
</tr>
<tr>
<td>FDCAT1</td>
<td>1:1</td>
<td>500</td>
<td>500</td>
<td>10</td>
</tr>
<tr>
<td>FDCAT2</td>
<td>1:1.5</td>
<td>500</td>
<td>750</td>
<td>10</td>
</tr>
<tr>
<td>FDCAT3</td>
<td>1:2</td>
<td>500</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>FDEC1</td>
<td>1:1</td>
<td>500</td>
<td>500</td>
<td>10</td>
</tr>
<tr>
<td>FDEC2</td>
<td>1:1.5</td>
<td>500</td>
<td>750</td>
<td>10</td>
</tr>
<tr>
<td>FDEC3</td>
<td>1:2</td>
<td>500</td>
<td>1000</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2**: Effect of formulations on yield, drug entrapment, % drug entrapment efficiency and particle size of didanosine microspheres.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Yield (%± SD)</th>
<th>Mean amount of drug entraped (%± SD)</th>
<th>% Drug entrapment efficiency</th>
<th>Mean particle size (µm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDCAT1</td>
<td>78.2±2.31</td>
<td>21.80±4.11</td>
<td>87.20</td>
<td>355±1.52</td>
</tr>
<tr>
<td>FDCAT2</td>
<td>83.7±2.68</td>
<td>18.07±3.25</td>
<td>90.35</td>
<td>355±1.72</td>
</tr>
<tr>
<td>FDCAT3</td>
<td>85.1±3.79</td>
<td>15.91±4.56</td>
<td>95.49</td>
<td>355±1.34</td>
</tr>
<tr>
<td>FDEC1</td>
<td>64.5±3.56</td>
<td>21.40±2.33</td>
<td>85.60</td>
<td>345.30±2.31</td>
</tr>
<tr>
<td>FDEC2</td>
<td>60.0±4.71</td>
<td>17.59±4.52</td>
<td>87.95</td>
<td>348.26±4.75</td>
</tr>
<tr>
<td>FDEC3</td>
<td>69.3±3.41</td>
<td>15.02±5.21</td>
<td>90.15</td>
<td>353.72±5.94</td>
</tr>
</tbody>
</table>

**Table 3**: R² values of mathematical models for dissolution profiles of Didanosine microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>R² values of mathematical models for dissolution profiles of Didanosine microspheres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order</td>
</tr>
<tr>
<td>FDCAT1</td>
<td>0.937</td>
</tr>
<tr>
<td>FDCAT2</td>
<td>0.939</td>
</tr>
<tr>
<td>FDCAT3</td>
<td>0.923</td>
</tr>
<tr>
<td>FDEC1</td>
<td>0.954</td>
</tr>
<tr>
<td>FDEC2</td>
<td>0.957</td>
</tr>
<tr>
<td>FDEC3</td>
<td>0.876</td>
</tr>
</tbody>
</table>

**Figure 1**: % Yield, Mean Amount of Drug Entrapped (MADE), Drug Encapsulation Efficiency (DEE) and Mean Particle Size (MPS)
**Figure 2:** SEM Photographs indicate Ethyl Cellulose loaded ddI Microspheres (A, B & C) and Cellulose acetate phthalate loaded ddI Microspheres (A*, B* & C*).

**Figure 3:** FTIR Spectra of CAT (A), CAT loaded microspheres (B), Pristine ddI(C), EC(D) and EC loaded microsphere(E)
Figure 4: DSC thermogram of pristine ddI (A), CAT loaded microsphere (B) and EC loaded microsphere(C)
Figure 5: X-ray diffraction of Didanosine (ddl), formulation Didanosine and cellulose acetate phthalate (ddl & CAT) and formulation Didanosine and Ethyl Cellulose (ddl & EC)
**Figure 6:** Cumulative % drug release of Didanosine microspheres

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