The requirement for an oral colonic drug delivery system is to reduce the drug release to a minimum prior to the cecum (1). Colon as a site offers distinct advantages on account of a near neutral pH, a much longer transit time, reduced digestive enzymatic activity and a much greater responsiveness to absorption enhancers (2). These criteria favor this distal part of the gastrointestinal tract (GIT) as a site for the delivery of vermicides, colonic diagnostic agents and sustained release of drugs in treatment of nectural asthma, angina and arthritis (3). A colon-specific drug delivery system should prevent drug release in the stomach and small intestine and affect an abrupt onset of drug release upon entry into the colon (4).

Various approaches have been used for delivery of drugs to the colon via oral route, which include coating with pH-dependent polymers, design of time-release dosage forms and the utilization of carriers that are degraded exclusively by the colonic bacteria (5). Every system has advantages as well as disadvantages. The poor site-specificity of pH-dependent systems, because of large variations in the pH of the gastrointestinal tract, is very well documented. The site-specificity of timed-release dosage forms is considered poor because of large variations in gastric emptying times and passage across the ileo-cecal junction (6). However, microflora-activated systems formulated making use of non-starch polysaccharides are highly promising because the polysaccharides remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic microflora of the colon. Furthermore, this strategy exploiting the abrupt increase of the bacteria population and corresponding enzyme activities will also accomplish greater site specificity of initial drug release (7). The polysaccharides for colonic drug delivery are also inexpensive, naturally occurring and abundantly available (8).

The requirement for an oral colonic drug delivery system is to reduce the drug release to a minimum prior to the cecum (1). Colon as a site offers distinct advantages on account of a near neutral pH, a much longer transit time, reduced digestive enzymatic activity and a much greater responsiveness to absorption enhancers (2). These criteria favor this distal part of the gastrointestinal tract (GIT) as a site for the delivery of vermicides, colonic diagnostic agents and sustained release of drugs in treatment of nectural asthma, angina and arthritis (3). A colon-specific drug delivery system should prevent drug release in the stomach and small intestine and affect an abrupt onset of drug release upon entry into the colon (4).

Various approaches have been used for delivery of drugs to the colon via oral route, which include coating with pH-dependent polymers, design of time-release dosage forms and the utilization of carriers that are degraded exclusively by the colonic bacteria (5). Every system has advantages as well as disadvantages. The poor site-specificity of pH-dependent systems, because of large variations in the pH of the gastrointestinal tract, is very well documented. The site-specificity of timed-release dosage forms is considered poor because of large variations in gastric emptying times and passage across the ileo-cecal junction (6). However, microflora-activated systems formulated making use of non-starch polysaccharides are highly promising because the polysaccharides remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic microflora of the colon. Furthermore, this strategy exploiting the abrupt increase of the bacteria population and corresponding enzyme activities will also accomplish greater site specificity of initial drug release (7). The polysaccharides for colonic drug delivery are also inexpensive, naturally occurring and abundantly available (8).

Single unit colon targeted drug delivery systems may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology.

**Abstract:** In the present work, paracetamol loaded eudragit based microsponges were prepared using quasi-emulsion solvent diffusion method. The compatibility of the drug with various formulation components was established. Process parameters were analyzed in order to optimize the formulation. Shape and surface morphology of the microsponges were examined using scanning electron microscopy. The formulations were subjected to in vitro release studies and the results were evaluated kinetically and statically. The in vitro release data showed a bi-phasic pattern with an initial burst effect. In the first hour drug release from microsponges was found to be between 17–30%. The cumulative percent release at the end of 8th hour was noted to be between 54-83%. The release kinetics showed that the data followed Higuchi model and the main mechanism of drug release was diffusion. The colon specific tablets were prepared by compressing the microsponges followed by coating with pectin:hydroxypropylmethylcellulose (HPMC) mixture. In vitro release studies exhibited that compression coated colon specific tablet formulations started releasing the drug at 6th hour corresponding to the arrival time at proximal colon. The study presents a new approach for colon specific drug delivery.

**Keywords:** microsponge, colonic delivery, pectin, diffusion

* Corresponding author: phone: +919219610427, fax: +911212575724 e-mail: vikasjain11118059@rediffmail.com, vikasjain111180@gmail.com
that may lead to drastically compromised systemic drug bioavailability or loss of local therapeutic action in the colon. Recently, much emphasis is being laid on the development of multiparticulate dosage forms in comparison to single unit systems because of their potential benefits like increased bioavailability, reduced risk of local irritation and predictable gastric emptying (9).

Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge like spherical particles that consist of myriad of interconnecting voids within a non-collapsible structure with large porous surface (10). Moreover, they may enhance stability, reduce side effect and modify drug release favorably (7).

Paracetamol (PCM), an antipyretic and analgesic drug, which has been widely used in clinical practice, was selected as a model drug. It has a short half life in plasma about 1–4 hours.

The present study is aimed at developing microsponge based novel colon specific drug delivery system containing PCM. The microsponges of PCM were prepared and characterized. They were formulated as colon specific tablets and subjected to in vitro characterization for various attributes.

**EXPERIMENTAL**

**Materials**

Paracetamol was purchased from Jackson Laboratories Pvt. Ltd. Amritsar (India). Eudragit RS-100 was kindly gifted by Evonic India Pvt. Ltd. Mumbai (India), Polyvinyl alcohol 30,000–70,000 (PVA), triethylcitrate, and HPMC (100,000 cps) were purchased from Sigma-Aldrich (USA). Pectinex Ultra SP-L (26,000 FDU/mL), pectin (from citrus fruits, methoxy content 9.4%) and sodium carboxymethylcellulose (Na-CMC) were procured from Sigma (USA). All chemicals used for analysis were of analytical grade.

**Methods**

**Paracetamol loaded microsponge preparation**

Paracetamol microsponges were prepared by quasi-emulsion solvent diffusion method. The internal phase consisted of Eudragit RS-100 (200 mg) and triethylcitrate (1% w/v) dissolved in 5 mL of dichloromethane. Triethylcitrate (TEC) was used as plasticizer. This was followed by addition of drug with gradual stirring (500 rpm). The internal phase was then poured into polyvinyl alcohol 30,000–70,000 (PVA) solution in water, the external phase. After 8 h of stirring, the microsponges were formed due to removal of dichloromethane from the system. The microsponges were filtered and dried at 40°C for 12 h. The composition of microsponge formulations are given in Table 1.

**Fourier transform infrared (FTIR) analysis**

FTIR spectra of the drug, physical mixture of the drug and Eudragit RS-100, and formulations FPRS1–FPRS4 were recorded in potassium bromide disc using a Shimadzu Model 8400 FTIR spectrometer to ascertain compatibility.

**Differential scanning calorimetric (DSC) analysis**

Thermal analysis using DSC was carried out on drug, physical mixture of the drug and Eudragit RS-100, and formulations FPRS1–FPRS4 (Shimadzu DSC-60 Thermal Analyzer). Accurately weighed samples were loaded into aluminum pans and sealed. All samples were run at a heating rate of 20°C/min. over a temperature range 40–430°C.

**Morphology**

The morphology and surface characteristics of the microsponges were studied using scanning electron microscopy (SEM). All the samples were coated with gold–palladium alloy under vacuum. Coated samples were then examined using LEO 430 SEM analyzer.
Actual drug content and encapsulation efficiency

The weighed amount of drug loaded microsponges (100 mg) was kept in 100 mL of phosphate buffer pH 6.8 for 12 h with continuous stirring. The samples were filtered using 0.45 µm membrane filter and the samples were analyzed at 256 nm against blank using UV spectrophotometer (UV 1700, Shimadzu, Japan). The drug content and encapsulation efficiency were calculated using the following formulas (7):

Actual drug content (%) = Mact/Mms × 100
Encapsulation efficiency (%) = Mact/Mthe × 100

where Mact is the actual drug content in microsponges, Mms is the total amount of the microsponges and Mthe is the amount of drug added to the microsponges. All analyses were carried out in triplicate.

In-vitro drug release studies of microsponge formulations

The microsponges containing 250 mg of paracetamol were subjected to in vitro drug release studies. In vitro release studies were carried out in USP basket apparatus with stirring rate of 50 rpm at 37 ± 0.5°C. Initial drug release was carried out in 900 mL of 0.1 M HCl for 2 h, followed by phosphate buffer pH 6.8 for next 6 h. Samples were withdrawn at regular intervals of time. The sink conditions were maintained by adding equal amount of dissolution medium. The samples were analyzed spectrophotometrically (Shimadzu UV-1700) at a wavelength of 256 nm. Dissolution tests were performed in triplicate for each sample.

Preparation of colon specific tablet formulations

The core tablets consisting of microsponges containing 250 mg drug, Na-CMC and magnesium stearate were prepared by direct compression method. All tablet constituents were weighed and mixed in motor passel for 15 min. Final powder mixture was compressed using 10 mm round flat punches on an eight station tablet punch machine (Cambart, D-8) using 1500 kgf/cm² compression pressure. Core tablet formulations are given in Table 2.

Pectin:HPMC (80 : 20) mixture was used as outer shell for compression coating. The coating material used was 400 mg. Fifty percent of coating material was placed in the die cavity and the core tablet was placed in centre followed by addition of the remainder of the coating material. The coating material was compressed around the core tablet at an applied pressure of 2500 kgf/cm² using round flat punches (14 mm) on the same tabletting machine.

RESULTS AND DISCUSSION

Quasi-emulsion solvent diffusion method was used for preparation of microsponges because of its simplicity and reproducibility. Moreover, it has advantage of avoiding solvent toxicity. The drug and polymer in the ratios 3 : 1, 6 : 1, 9 : 1, and 12 : 1 were taken to prepare different microsponge formulations namely FPRS1, FPRS2, FPRS3, and FPRS4, respectively. In each formulation, the amounts of polymer (200 mg), dichloromethane (5 mL), PVA (0.5% w/v) were kept constant. The microsponge formulations were prepared using mechanical stirrer (Remi RQ1217-D) at a stirring speed of 150 rpm for 20 min. The core tablets were coated by wet granulation method. The coating material (Pectin:HPMC) was prepared by mixing 80% of the coating material with 20% low melting waxes. The coating material was compressed around the core tablet at an applied pressure of 2500 kgf/cm² using round flat punches (14 mm) on the same tabletting machine.

The drug release studies were done with the same method used for microsponges and core tablets. Additionally, Pectinex Ultra SP-L was added to the dissolution medium at 6th hour in order to simulate the enzymatic action of the colonic bacteria. Samples were withdrawn periodically and compensated with an equal amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance at 256 nm using UV spectrophotometer.

![Table 2. Core tablet formulations of PCM microsponges.](image)
rate of 500 rpm for 8 h. The composition of various microsponge formulations are presented in Table 1.

The effect of various variables like drug/polymer ratio, stirring rate, volume of internal phase, amount of emulsifying agent on the nature of microsponges was studied.

Effect of drug-polymer ratio on microsponges

The morphology of the microsponges was studied by scanning electron microscopy (SEM). The representative photographs of the microsponges are shown in Figure 1. The microsponges were observed to be spherical and uniform with no drug crystals on the surface. Figure 1 shows that drug-polymer ratio has considerable effect on the morphology and size of microsponges. It was observed that as the ratio of drug to polymer was increased, the particle size decreased. This could probably be due to the fact that in high drug to polymer ratios, the amount of polymer available per microsponge was comparatively lower. Probably in high drug-polymer ratios less polymer amount surrounds the drug and microsponges with smaller size were obtained (11).

Production yield, actual drug content, encapsulation efficiency, and mean particle size of formula-

Figure 1(a-h). SEM photograph of microsponge formulations (drug: Eudragit RS-100). The photograph coded ‘A’ represents whole image. ‘B’ represents surface photograps.
The production yield, actual drug content, encapsulation efficiency, and mean particle size of various microsponges formulations (n = 3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug:Polymer ratio</th>
<th>Production yield (% ± S.D.)</th>
<th>Theoretical drug content (%)</th>
<th>Actual drug content (% ± SD)</th>
<th>Encapsulation efficiency (% ± SD)</th>
<th>Mean particle size (µm ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPRS1</td>
<td>3 : 1</td>
<td>72 ± 0.43</td>
<td>75.00</td>
<td>73.56 ± 0.09</td>
<td>98.08 ± 0.89</td>
<td>62.34 ± 6.89</td>
</tr>
<tr>
<td>FPRS2</td>
<td>6 : 1</td>
<td>74.12 ± 0.34</td>
<td>85.71</td>
<td>84.32 ± 0.04</td>
<td>98.37 ± 0.56</td>
<td>54.67 ± 5.39</td>
</tr>
<tr>
<td>FPRS3</td>
<td>9 : 1</td>
<td>76.23 ± 0.36</td>
<td>90.00</td>
<td>88.32 ± 0.45</td>
<td>98.13 ± 0.09</td>
<td>48.23 ± 7.24</td>
</tr>
<tr>
<td>FPRS4</td>
<td>12 : 1</td>
<td>75.02 ± 0.60</td>
<td>92.30</td>
<td>90.81 ± 0.34</td>
<td>98.38 ± 0.67</td>
<td>41.45 ± 5.34</td>
</tr>
</tbody>
</table>

Effect of stirring rate on the morphology and yield of microsponges

The effect of stirring rate on the morphology of microsponges is shown in Figure 2. The formulation with the lower drug to polymer ratio (i.e., 3:1) was chosen to investigate the effect of stirring rate on the morphology of microsponges. The stirring rate was varied in the range of 300 to 500 rpm. The dispersion of the drug and polymer into the aqueous phase was found to be dependant on the agitation speed. As the speed was increased, the size of microsponges was reduced and the microsponges were found to be spherical and uniform (12). When the rate of stirring was increased up to 500 rpm the spherical microsponges were formed with mean particle size of 62.34 ± 6.89 µm.

It was noted that at higher stirring rate the production yield was decreased. Possibly, at the higher stirring rates the polymer adhered to paddle due to the turbulence created within the external phase, and hence production yield decreased (13).

Effect of volume of internal phase on the production of microsponges

It was observed that on increasing the volume of internal phase from 5 to 10 mL microsponges were not formed. This may be due to the decrease in viscosity of internal phase (14). As the amount of dichloromethane was increased, the finely dispersed
spherical quasi-emulsion droplets were seen in solvent under the agitation, but as the stirring was discontinued emulsion droplets adhered together and coalesced. Consequently, no microsponges could be formed. The result suggests that the amount of dichloromethane need to be controlled within an appropriate range to effect not only the formation of quasi-emulsion droplets at the initial stage but also the solidification of drug and polymer in the droplets. The good microsponges were produced when 3 to 5 mL of dichloromethane were used.

Effect of amount of emulsifying agent on the production yield and particle size of microsponges

An increase in amount of emulsifying agent resulted in decreased production yield and increased mean particle size, as the emulsifier was non-ionic in nature and possibly formed some hydrophobic region which dissolved some of the drug and polymer. The molecules might have associated away from the oil-water interface at higher concentrations resulting in alternative hydrophobic region, which dissolved some portion of drug resulting in a reduction in production yield of microsponges (13). On the other hand, an increase in the amount of emulsifying agent resulted in increased larger microsponges. This could be due to the increased viscosity wherein larger emulsion droplets formed resulted in larger microsponges. The dispersion of the solution of the drug and polymer into droplets was effected by the concentration of polyvinyl alcohol in the external phase. When the concentration of PVA was increased in dispersion phase, the size of microsponges was found to be decreased (18). The results of the significant effect of emulsifying agent on production yield and mean particle size are shown in Table 4. An increased amount of emulsifying agent decreased the production yield from 72 to 67% and increased the mean particle size from 62 to 66 µm.

Characterization of microsponges

DSC studies were carried out to confirm compatibility (15). The thermal behavior of drugs, physical mixture of drug and polymer, and formulations FPRS1–FPRS4 were studied. In the thermogram, the drug showed a sharp endothermic peak (at 174.23°C) which corresponds to the melting point of drug in the crystalline form. In the DSC curve of physical mixture of drug and polymer, and formulations FPRS1–FPRS4, the characteristic peaks of drug(s) were observed. The result showed that there is no incompatibility between the drug and polymers. Microsponge production process did not change the nature of drug in microsponges. The thermal behavior of the drug, physical mixture of...
FTIR spectra were recorded to assess the compatibility of the drug and excipients (16). FTIR spectra of paracetamol powder, characteristic N-H stretching band at 3325 cm⁻¹, O-H stretching band at 3161.11 cm⁻¹, and carbonyl stretching band at 1654 cm⁻¹ were seen. Eudragit RS 100 showed an ester C=O stretching peak around 1726.17 cm⁻¹. All characteristic peaks of paracetamol were observed in the FTIR spectra of FPRS1–FPRS4 formulations. The results showed that no chemical interaction or changes took place during preparation of the formulations and the drug was found to be stable in all the formulations. The FTIR spectra of the drug, physical mixture of drug

Table 4. The effect of emulsifying agent on microsponges formulation.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Internal Phase</th>
<th>External phase</th>
<th>Yield (%)</th>
<th>Mean diameter µm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCM (mg)</td>
<td>Polymer (mg)</td>
<td>Dichloromethane (mL)</td>
<td>Water (mL)</td>
</tr>
<tr>
<td>FPRS1 (a)</td>
<td>600</td>
<td>200</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>FPRS1 (b)</td>
<td>600</td>
<td>200</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5. In vitro drug release models for different microsponges formulations.

<table>
<thead>
<tr>
<th>Code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Korsmeyer-Peppas model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>K (mg/h)</td>
<td>R</td>
<td>K (h⁻¹)</td>
</tr>
<tr>
<td>FPRS1</td>
<td>0.9729</td>
<td>7.7948</td>
<td>0.9846</td>
<td>0.1255</td>
</tr>
<tr>
<td>FPRS2</td>
<td>0.9710</td>
<td>8.3785</td>
<td>0.9869</td>
<td>0.1497</td>
</tr>
<tr>
<td>FPRS3</td>
<td>0.9805</td>
<td>9.4463</td>
<td>0.9941</td>
<td>0.2057</td>
</tr>
<tr>
<td>FPRS4</td>
<td>0.9865</td>
<td>11.296</td>
<td>0.9934</td>
<td>0.3473</td>
</tr>
</tbody>
</table>

Figure 4. FTIR spectra of paracetamol physical mixture of drug & Eudragit RS-100 and FPRS1-FPRS4 microsponges formulations
and Eudragit RS-100 and formulations FPRS1–FPRS4 are presented in Figure 4.

**In vitro release studies of the microsponge formulations**

The microsponge formulations were subjected to *in vitro* release studies using USP XXIV dissolution assembly at the stirring rate at 50 rpm and temperature at 37 ± 0.5°C. Initially, drug release was carried out in 0.1 M hydrochloric acid for 2 h followed by phosphate buffer pH 6.8 for the next 6 h. The release profiles obtained for the formulations FPRS1–FPRS4 are presented in Figure 5. It was observed that the drug release decreased with an increase in the amount of polymer for each formulation. This may be due to the fact that the release of drug from the polymer matrix takes place after complete swelling of the polymer and as the amount of polymer in the formulation increases the time required to swell also increases. The release showed a bi-phasic pattern with an initial burst effect. In the first hour drug release was found to be 17–30%. This may be attributed to the drug present in the pores of the microsponges or improper entrapment of drug (17). The cumulative percent release for FPRS1–FPRS4 at the end of 8 h was found to be 54–83%. The microsponge formulations were subjected to *in vitro* dissolution studies and the data were analyzed using various mathematical models (Table 5). Based on highest regression value, the best fit was observed for Higuchi matrix. The n value for Peppas model was found to be between 0.5–1 indicative of non-fickian diffusion.

The *in vitro* dissolution data were subjected to statistical analysis using ANOVA. The p value was found to be 0.5207 indicating no significant difference in the release behavior (p > 0.05).

**In vitro dissolution studies of the colon specific tablet formulations**

In order to prepare the compression coated tablet formulations, core tablets were prepared as the first step. The homogenous granular characteristic of microsponges is due to their highly porous structure and in these means, microsponges have the compressibility to produce strong tablets and 1000–2000 kgf/cm² pressure did not cause the structure deformation of microsponges (19). *In vitro* drug release studies of the colon specific tablet formulations were carried out using USP basket apparatus with stirring rate 50 rpm at 37 ± 0.5°C. The release profiles obtained for the formulations CPRS1–CPRS4 are presented in Figure 6. No drug was released in the first 6 h. After this lag time, the drug release started at the beginning of 7th hour due to the addition of the Pectinex Ultra SP-L and continued up to 14th hour for CPRS1 (68.65%), 14th hour for CPRS2 (88.23%), 13th hour for CPRS3 (92.45%) and 12th hour for CPRS4 (95.76%).

The results of *in vitro* drug release showed that the ratio of Pectin: HPMC (80 : 20) protected the cores up to 6th hour corresponding to the time to
reach the colon and after that under the influence of the enzyme, the system could be degraded faster and deliver the drug to the proximal colon that forms the main site of bacterial carbohydrate metabolism. So, the results were in accordance with the triggering mechanism due to the very active metabolism in the proximal part compared with the distal part of colon and pectin could find the appropriate environment to be degraded.

CONCLUSION

This study presents new approach for the preparation of modified microsponges. The prepared microsponges exhibited characteristics of an ideal delivery system for colon targeting. The unique compressibility of microsponges offers a new alternative for producing mechanically strong tablets. Further colon specific tablets based on microsponges could provide effective local action as microsponges may selectively be taken up by the macrophages present in colon.

Acknowledgment

The authors are thankful to the Director, School of Pharmaceutical Sciences, Shobhit University, Meerut for providing necessary facilities.

REFERENCES


Received: 11. 10. 2009
Dicyclomine-loaded Eudragit®-based Microspponge with Potential for Colonic Delivery: Preparation and Characterization

Vikas Jain* and Ranjit Singh
School of Pharmaceutical Sciences, Shobhit University, Meerut, Uttar Pradesh, 250110, India

Abstract

Purpose: The purpose of this work was to develop a prolonged microspponge drug delivery system containing dicyclomine.

Methods: Dicyclomine-loaded, Eudragit-based microsponges were prepared using a quasi-emulsion solvent diffusion method. The compatibility of the drug with formulation components was established by differential scanning calorimetry (DSC) and Fourier transform infra-red (FTIR). Process parameters were modulated to optimise the formulation. Shape and surface morphology of the microsponges were examined using scanning electron microscopy.

Results: The results of compatibility tests showed that no chemical interaction or changes took place during preparation of the formulations; furthermore, the drug was stable in all the formulations. In increase in drug:polymer ratio resulted in a reduction in the release rate of the drug from the microsponges. Kinetic analysis showed that the main mechanism of drug release was by Higuchi matrix-controlled diffusion. Drug release was bi-phasic with an initial burst effect with 16 – 30 % of the drug was released in the first hour. Cumulative release for the microsponges over 8 hours ranged from 59 - 86 %.

Conclusion: This study presents an approach for the modification of microsponges for prolonged drug release of dicyclomine. The unique compressibility of microsponges can be applied to achieve effective local action since microsponges may be taken up by macrophages present in colon.

Keywords: Microspponge; Dicyclomine; Quasi-emulsion solvent diffusion; Eudragit RS-100; Colonic drug release

Received: 26 April 2009        Revised accepted: 21 December 2009

*Corresponding author: E-mail: vikasjain1118059@rediffmail.com; Tel: +91-1212575724, 9219610427; Fax: +91-1212575724
INTRODUCTION

Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles that consist of a myriad of interconnecting voids within a non-collapsible structure with a large porous surface. Moreover, they may enhance stability, reduce side effect and modify drug release favourably [1,2].

Dicyclomine, an anticholinergic drug, has direct smooth muscle relaxant action, and in addition to being a weak anticholinergic, it exerts antispasmodic action. Its plasma half life is 4 - 6 h. Dicyclomine causes gastrointestinal (GI) side effects like other antispasmodic drugs. The present study was designed to formulate a delivery system based on microsponges that would reduce the GI side effects of the drug.

EXPERIMENTAL

Materials

Dicyclomine was purchased from Jackson Laboratories Pvt Ltd, Amritsar, India. Eudragit RS 100 was a gift from Evonic India Pvt Ltd, Mumbai, India while polyvinyl alcohol (PVA, mol weight 30,000 – 70,000) and triethylcitrate were purchased from Sigma-Aldrich, USA. All the chemicals used for analysis were of analytical grade.

Preparation of microsponges

The composition of the microsponge formulations is outlined in Table 1. The microsponges were prepared by quasi-emulsion solvent diffusion method [2,3] using an internal phase that consisted of Eudragit RS-100 (200 mg) and triethylcitrate (1 %v/v) dissolved in 5 ml of dichloromethane. Triethylcitrate (TEC) was then added to enhance the plasticity of the polymer. This was, followed by the addition of dicyclomine with stirring (500 rpm). The mixture was then poured into 0.5 % w/v aqueous solution of polyvinyl alcohol (PVA) which served as the external phase. After 8 h of stirring, microsponges were formed due to the removal of dichloromethane from the system by evaporation. The microsponges were washed with water, filtered and dried at 40 °C for 12 h.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>FDRS1</th>
<th>FDRS2</th>
<th>FDRS3</th>
<th>FDRS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicyclomine (mg)</td>
<td>600</td>
<td>1200</td>
<td>1800</td>
<td>2400</td>
</tr>
<tr>
<td>Eudragit RS-100 (mg)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Triethylcitrate (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dichloromethane (ml)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>PVA (0.5 % w/v)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of dicyclomine, Eudragit RS 100, physical mixture(s) of dicyclomine and Eudragit RS-100, and microsponge formulations having drug:polymer ratios of 3:1, 6:1, 9:1 and 12:1 (FDRS1 – FDRS4) were incorporated in potassium bromide discs and evaluated with a Shimadzu Model 8400 FTIR spectrometer.

Differential scanning calorimetry (DSC)

DSC analysis was carried out on the drug, Eudragit RS 100, physical mixtures of the drug and polymer, and formulations FDRS1-FDRS4, using a Shimadzu DSC-60 Thermal Analyzer. Samples (approximately 2 mg) were placed in aluminum pans and sealed and run at a heating rate of 20 °C/min over a temperature range 40 - 430 °C.

Morphology and particle size studies

The morphology and surface characterization of the microsponge formulations were
evaluated by SEM analysis using LEO 430 SEM analyzer after coating the microsponges with gold–palladium under vacuum.

**In-vitro dissolution studies**

**In-vitro** dissolution studies were carried out using USP XX1V dissolution assembly (basket type, Electrolab TDT-08L) in 900 ml of 0.1N HCl at a stirring rate of 50 rpm and temperature of 37±0.5 °C. Initial drug release was monitored for 2 h; thereafter, the dissolution medium was replaced with 900 ml of phosphate buffer (pH 6.8) and drug release monitored for another 6 h. Samples (5 ml) were withdrawn at regular time intervals and sink conditions were maintained by replacing an equal amount of fresh dissolution medium. The samples were analyzed spectrophotometrically (Shimadzu UV-1700) at a wavelength of 420 nm. Dissolution tests were performed in triplicate.

The dissolution data were subjected to various release models, namely, Zero order, First order, Higuchi and Korsmeyer-Peppas

**Statistical analysis**

The data obtained from each experiment were subjected to statistical analysis by Student t-test and one-way analysis of variance (ANOVA) using Graph Pad Instat software. $P < 0.05$ was considered to be indicative of significance.

**RESULTS**

**Effect of stirring rate on microsponge**

The effect of stirring rate on the size of microsponges was studied. As the stirring speed was increased, microsponges of smaller size were obtained, as shown in Table 2. When the rate of stirring was increased from 300 to 500 rpm, the mean particle size decreased from 67.23±4.45 to 60.25±5.67 µm but this was not statistically significant.

**Effect of volume of internal phase on microsponges**

It was observed that when the volume of internal phase (dichloromethane) was increased from 5 to 10 ml, microsponges were not formed. Good microsponges were produced only when 3 to 5 ml of internal phase was used.

**Effect of amount of emulsifying agent on microsponge**

Increase in the amount of PVA (emulsifying agent) incorporated in the microsponges decreased production yield and increased the particle size.

**Effect of drug/polymer ratio on microsponges**

The microsponges were spherical and uniform with no drug crystal on the surface, as shown in Figure 1. Drug/polymer ratio had an effect on the morphology and size of microsponges with increase in drug:polymer ratio leading to a decrease in particle size. The mean particle diameters for the drug/polymer ratios of 3:1 and 12:1 were 60.25±5.67 µm and 43.66±6.20 µm, respectively (see Table 2).

The production yield, actual drug content, encapsulation efficiency, and mean particle size of the microsponge formulations were 70 – 77 %, 62 – 80 %, 81 – 88 %, and 44 - 60 µm, respectively, as given in Table 2. On subjecting the data obtained for production yield, actual drug content, and encapsulation efficiency to statistical analysis, no significant difference was observed amongst the formulations ($p < 0.05$).

**Characterization of microsponges**

Analysis of the FTIR spectra (see Fig 2) of the drug (dicyclomine), physical mixture of drug and Eudragit RS-100, and formulations FDRS1–FDRS4 indicate a characteristic C=O stretching band at 1716.53 cm⁻¹ for the drug,
Jain & Singh

Figure 1: SEM of microsponge formulations. (Note: Photographs in the left column represent whole images of microsponge while those in the right column show their surface morphology; FDRS1, FDRS2, FDRS3 and FDRS4 denote formulations with drug to polymer ratios of 3:1, 6:1, 9:1, and 12:1, respectively).

and an ester C=O stretching peak around 1726.17 cm\(^{-1}\) for Eudragit RS 100, as has also been reported in the literature for this acrylate polymer [2]. All the characteristic peaks of dicyclomine were observed in the spectra of all the microsponges (FDRS1 – FDRS 4), thus indicating that no chemical interaction or changes took place during the preparation of the formulations and that the drug was stable in all the formulations.

In the DSC studies (see Fig 2), the thermogram of the drug alone showed a sharp endothermic peak at 175.97 °C which corresponds to the melting point of drug in the crystalline form. In each of the DSC thermograms of the physical mixture of drug and polymer, and microsponge formulations FDRS1 – FDRS4, respectively, the characteristic endothermic peak of the drug was also observed, thus indicating that there was compatibility between the drug and polymers used.

**In vitro drug release**

The release profiles obtained for the microsponge formulations are presented in Figure 4. The profiles showed a bi-phasic release with an initial burst effect. In the first hour, about 16 – 30 % of the drug was released. Cumulative release for the microsponges after 8 h ranged from 59 - 86 %. Drug release from the formulations decreased with increase in the amount of polymer in the microsponges.

| Table 2: Production yield, drug content, encapsulation efficiency, and mean particle size of the microsponge formulations (±SD, n=3) |
|---|---|---|---|---|---|
| Formulation | Drug: polymer ratio | Production yield (%) ± S.D | Theoretical Drug Content (%) | Actual drug content (%) ± S.D | Encapsulation efficiency (%) ± S.D. | Mean particle size (µm) ± S.D. |
| FDRS1 | 3:1 | 79.01±0.57 | 75.0 | 62.05±0.01 | 82.73±0.45 | 60.25±5.67 |
| FDRS2 | 6:1 | 70.65±0.28 | 85.7 | 70.12±0.01 | 81.91±0.04 | 53.62±7.11 |
| FDRS3 | 9:1 | 76.60±0.56 | 90.0 | 75.32±0.08 | 83.68±0.23 | 49.34±6.45 |
| FDRS4 | 12:1 | 70.48±0.78 | 92.3 | 80.69±0.03 | 87.42±0.56 | 43.66±6.20 |

* FDRS1, FDRS2, FDRS3 and FDRS4 denote microsponge formulations with drug to polymer ratios of 3:1, 6:1, 9:1, and 12:1, respectively.
DISCUSSION

The quasi-emulsion solvent diffusion method used for the preparation of the microsponges was simple, reproducible, and rapid. Furthermore, it was observed that as drug:polymer ratio increased, particle size decreased. This is probably due to the fact that at higher relative drug content, the amount of polymer available per microsponge to encapsulate the drug becomes less, thus reducing the thickness of the polymer wall and hence, smaller microsponges [4]. The smaller size of microsponges obtained at higher stirring rate may be attributed to better dispersion at higher stirring rates [2].

Failure to form microsponges on increasing the volume of internal phase from 5 to 10 ml may be due to incomplete removal of internal phase solvent with the result that the droplets could not solidify as most of the internal phase remained in the droplets [5]. This warrants the use of internal phase solvent in an appropriate amount to ensure the formation of quasi emulsion droplets, and solidification of the drug and polymer thereafter. Increase in the amount of emulsifying agent resulted in decreased

Table 3: Kinetic data from in vitro drug release models for the microsponge formulations

<table>
<thead>
<tr>
<th>Code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Korsmeyer-Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>K (mg/h)</td>
<td>R</td>
<td>K (h⁻¹)</td>
</tr>
<tr>
<td>FDRS1</td>
<td>0.9538</td>
<td>7.4595</td>
<td>0.9748</td>
<td>0.1181</td>
</tr>
<tr>
<td>FDRS2</td>
<td>0.9529</td>
<td>8.587</td>
<td>0.9745</td>
<td>0.1506</td>
</tr>
<tr>
<td>FDRS3</td>
<td>0.9520</td>
<td>9.1038</td>
<td>0.9835</td>
<td>0.1787</td>
</tr>
<tr>
<td>FDRS4</td>
<td>0.9733</td>
<td>10.351</td>
<td>0.9854</td>
<td>0.2575</td>
</tr>
</tbody>
</table>

Figure 4: In vitro drug release profile of microsponge formulations.

[▲ = FDRS1 (3:1); ○ = FDRS2 (6:1); ■ = FDRS3 (9:1); □ = FDRS4 (12:1)]
production yield. This may be due to the development of some hydrophobic regions which probably dissolved some of the drug and polymer. Furthermore, the increase in microsponge particle size as the amount of emulsifying agent increased could be due to increased viscosity leading to larger droplets which, in turn, resulted in larger microsponges [1].

On analysing the in vitro dissolution data with various release models, the highest regression coefficient showing the best fit was found for the Higuchi model. The n value for Peppas model was between 0.5 and 1.0 which is indicative of non-Fickian diffusion. Statistical analysis using ANOVA yielded a p-value of 0.5930 for all the formulations, thus indicating that there was no significant difference among them.

CONCLUSION

This study presents an approach for the production of dicyclomine microsponges with prolonged release characteristics. The unique compressibility of microsponges offers an alternative way for producing mechanically strong tablets. This is significant since colon-specific tablets based on microsponges could provide effective local action, since microsponges can be taken up by macrophages present in colon.

ACKNOWLEDGMENT

The authors are thankful to the Director, School of Pharmaceutical Sciences, Shobhit University, Meerut for making available the facilities used in this work.

REFERENCES

From: vikas jain <vikasjain11118059@rediffmail.com>
To: <vikasjain111180@gmail.com>, <vikasjain11118059@rediffmail.com>
Subject: Fw: ARPR: Your manuscript entitled Development and Characterization of Colon-Specific Drug Delivery System Containing Paracetamol Microsponges
Date: Thu, 17 Sep 2009 13:32:51 IST

Note: Forwarded message attached

-- Original Message --

From: "Mi-Ock Lee" eic.pskor@gmail.com
To: vikasjain11118059@rediffmail.com
Subject: ARPR: Your manuscript entitled Development and Characterization of Colon-Specific Drug Delivery System Containing Paracetamol Microsponges
Ref.: Ms. No. ARPR-D-09-00086R2
Development and Characterization of Colon-Specific Drug Delivery System Containing Paracetamol Microsponges
Archives of Pharmacal Research

Dear Mr. Jain,

I am pleased to tell you that your work has now been accepted for publication in Archives of Pharmacal Research.

It was accepted on 16-09-2009.

Thank you for submitting your work to this journal.

With kind regards

Soojeong Lim, Associate Editor
Sang-Geon Kim, Editor in Chief
Mi-Ock Lee, Editor

Archives of Pharmacal Research
Journal of Pharmaceutical Sciences - Message from Ron Borchardt - Acceptance Letter

1 message

tdunning@ku.edu <tdunning@ku.edu> Tue, Sep 7, 2010 at 2:45 PM
To: vikasjain111180@gmail.com
Cc: deepika.ranka08@gmail.com, ranjithngbu@rediffmail.com

07-Sep-2010

Dear Mr. Jain:

I would like to take this opportunity to thank you and your co-authors for submitting your manuscript entitled “Factors effecting the morphology of natural polysaccharide based microsponges bearing dicyclomine for colonic delivery” (#10-517) to the Journal of Pharmaceutical Sciences (JPharmSciTM). I was pleased to hear that your manuscript was recently found to be acceptable for publication in JPharmSciTM by Associate Editor Marcus Brewster.

Now that you have submitted to Becky Whaley a completed Copyright Transfer Form and your manuscript (clean, production-ready), Tables and Figures in the proper format for publication by Wiley Online Library, I am pleased to inform you that your manuscript has been accepted for publication in JPharmSci™.

Upon publication of your manuscript in J. Pharm. Sci, it will appear in the Table of Contents under the subject area: Pharmaceutical Technology. If this is the incorrect subject area, please contact Tammy Dunning (tdunning@ku.edu) and she will help you select a more appropriate subject area.

Critical to the publication of your manuscript in both the electronic and hard copy formats of JPharmSciTM is your timely review of the galley proofs of your manuscript. You can expect to receive correspondence via e-mail from Wiley InterScience concerning your galley proofs in 2-3 weeks. The publisher will ask you to proof read and return your galley proofs in 48 hours. PLEASE ABIDE BY THEIR TIMELINES! If you don't receive your galley proofs in 2-3 weeks, please contact Becky Whaley bwhaley@ku.edu in the JPharmSciTM editorial office.

Once your manuscript gets published online in JPharmSciTM, readers of the journal will have the opportunity to download your paper. As I described in two recent editorials in JPharmSciTM (October 2006, September 2007), “usage metrics” (i.e. article downloads) are rapidly becoming an important measure of a manuscript's impact. Since author access to “usage metrics” is becoming as important as author access to a journal's impact factor, I am pleased to inform you that Wiley InterScience has agreed to make this download information available to authors of papers published in JPharmSciTM. Requests for this information should be sent to Tammy Dunning (tdunning@ku.edu).

Please note that you can view the ten most accessed (i.e., downloads) papers published in JPharmSciTM in the past six months, as well as the ten most cited papers in 2006-2007 by going to http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1520-6017 and click on the “Most Accessed” link. In addition, it is my intention to write an editorial on an annual basis that recognizes the “The Ten Most Highly Cited Articles” and “The Ten Most Frequently Downloaded Articles” published in JPharmSciTM (See Editorial in September 2007 issue of JPharmSciTM ).

Thank you again for submitting your most significant scientific finding for publication in JPharmSciTM.

Best regards,

Ronald T. Borchardt
Editor