USP Dissolution Apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations

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Abstract

The reciprocating cylinder dissolution apparatus (USP Dissolution Apparatus III) was used to evaluate performance of several colonic delivery systems. Its dissolution tubes move between successive rows of vessels, allowing delivery systems to release drug in different media sequentially, i.e. simulated gastric fluids, simulated intestinal fluids, and simulated colonic fluids (SCF). The objective of this study was to assess USP dissolution apparatus III using formulations based on guar gum, a galactomannan polysaccharide. Drug (dexamethasone or budesonide) release in SCF was markedly increased at galactomannanase concentrations >0.01 mg/ml. The galactomannanase concentration and dipping speeds in the SCF were adjusted to produce dexamethasone release profiles similar to those estimated from absorption data in vivo. Thus, this dissolution setup may have predictive value for in vivo colonic delivery using guar gum-based colonic dosage forms.

Keywords: Dexamethasone; Budesonide; Guar gum; Hydroxypropyl methylcellulose; USP Dissolution Apparatus III; Colon-specific drug delivery

1. Introduction

Ulcerative colitis is currently treated with antiinflammatory medications, such as sulfasalazine, mesalamine, hydrocortisone, prednisone and ACTH [1–3]. Enemas and suppositories are not effective for extensive proximal ulcerative colitis; in addition, traditional oral dosage forms do not deliver drug locally to the large intestine. To reduce their adverse effects, these agents should be targeted to the colon for the treatment of inflammatory bowel disease by modified release formulations [4–13]. Oral delivery of drugs to the colon can be achieved in several ways, such as pro-drugs which rely on enzymatic reduction of azo-bonds or hydrolysis of β-glycoside bond, coatings (pH-dependent dissolution, enzymatic hydrolysis of polysaccharides, enzymatic reduction of azo bond) and hydrogel matrix (pH-dependent swelling+reduction of azo crosslinks). Ideally, as much drug as possible should be delivered in a controlled manner into the colon, including the distal regions, to topically treat ulcerative colitis.

Guar gum, a naturally occurring galactomannan polysaccharide, can be used to deliver drugs to the colon due to its susceptibility to microbial degradation in the large intestine [14–16]. For instance, the viscosity of a guar gum solution incubated with a homogenate of feces has been reduced by 75% over 40 min [17]. Guar gum contains a polysaccharide built by β-(1→4) linkages with a side-branching...
unit of a single D-galactopyranose unit joined to
every other mannose unit by α-(1→6) linkages [18–
20]. On average, most guar gum contains about 80%
galactomannan, 12% water, 5% protein, 2% acid
insoluble residue, 0.7% ash and 0.7% fat. Guar gum
hydrates and swells in cold water forming viscous
colloidal dispersions or sols [21–23]. It is thus used
as a gelling agent to retard drug release from tablets
[24–26]. Guar gum has been proposed for use in
colonic delivery when complexed with borax [27].
The dosage forms described herein do not require the
use of borax, the safety of which, when complexed
to guar gum, is unknown in humans.

During the development of guar gum-based
formulations for colonic delivery, human stool sus-
pensions were used as colonic fluid to evaluate drug
released from potential candidates in a 3 part-dissolu-
tion setup (i.e. simulated gastric fluid (USP, SGF),
simulated intestinal fluid (USP, SIF) and stool sus-
pensions). However, this system presented mixing
problems due to the viscosity of stool suspensions.
Though total disintegration of the ‘colonic release’
tablets was observed at 24 h in stool suspensions,
total dissolution was not detected. In addition, there
was substantial variability in drug dissolution among
samples from the same formulation, possibly due to
incomplete recovery of dexamethasone during ex-
traction, low drug solubility, drug instability in the
stool suspensions, or variable enzyme content be-
tween stool samples.

Based on these problems we investigated the
reciprocating cylinder dissolution apparatus (USP
Dissolution Apparatus III). Its design makes it
applicable for drug release testing of colonic delivery
systems. The dissolution tubes move between suc-
cessive rows of vessels, allowing colonic delivery
systems to automatically move and to assess drug
dissolution in various media sequentially, i.e. SGF,
SIF, and simulated colonic fluids (SCF), which
contains one or more enzymes capable of degrading
a guar gum-based dosage form. Thus, drug release
from potential colonic delivery formulations in each
medium can be determined and the potential for
colonic drug delivery assessed. Finally, the replace-
ment of stool samples with a defined enzyme prepa-
ration (SCF) eliminates the variability and difficulty
in using human stools. The objective of this study
was to examine the reciprocating cylinder dissolution
apparatus using colonic delivery formulations based
on guar gum.

2. Materials and methods

2.1. Materials

Ethyl alcohol (reagent, denatured, HPLC grade),
monobasic potassium phosphate, pancreatin, pepsin
and triamcinolone acetonide were purchased from
Sigma (St. Louis, MO); sodium chloride, sodium
acetate, sodium hydroxide, hydrochloric acid and
acetic acid from Mallinckrodt (Paris, KY); methyl-t-
butylether, acetonitrile and pentane from Baxter-
Burdick and Jackson (Muskegon, MI); guar gum
(G3 and U grades) from Aqualon (Birmingham, AL);
hydroxypropyl methylcellulose (Methocel E3 and
E50 LV) from The Dow Chemical Company (Mich-
igan); dibasic calcium phosphate (Emcompress, USP,
FCC, BP) from Mendell (Patterson, NY); dexam-
ethasone (USP, micronized) from Upjohn Com-
pany (Midland, MI); budesonide (micronized) from
Vinchem (Chatham, NJ); galactomannanase
(Gamanase) from Novo Nordisk (Danbury, CT). The
specific activity of the galactomannanase preparation
as supplied was 300 nmol of mannose liberated in 1
h per mg of protein at 37°C (pH 6.5).

2.2. Tableting

Table 1 lists the formulations used for this study.
Dexamethasone or budesonide were pre-mixed with
a small amount of guar gum G3 by spatulation,
followed by mixing in a V-blender for 10 min with
the remaining guar gum and all other ingredients.
Powder mixtures were compressed manually into
tablets by a rotatory press (concave, monoradius,
diameter of 13/32 in; 330 mg (dexamethasone), 500
mg (budesonide)).

2.3. Dissolution

The following dissolution conditions were estab-
lished: 2 h in 250 ml of SGF (3.2 mg/ml of pepsin
and 2 mg/ml of sodium chloride in 0.05 M hydro-
chloric acid, pH 1.2), 4 h in 250 ml of SIF (10
mg/ml of pancreatin in 0.05 M phosphate buffer, pH
Table 1
CD formulations prepared and evaluated

<table>
<thead>
<tr>
<th>Formulation</th>
<th>CD₁</th>
<th>CD₂</th>
<th>CD₃</th>
<th>CD₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>Dex*, 3%</td>
<td>Dex, 3%</td>
<td>Dex, 3%</td>
<td>Bud†, 1.5%</td>
</tr>
<tr>
<td>Guar gum</td>
<td>Coarse, 60.5%</td>
<td>Coarse, 60.5%</td>
<td>Fine, 60.5%</td>
<td>Coarse, 60.5%</td>
</tr>
<tr>
<td>Additives</td>
<td>Methocel E3, 36%</td>
<td>Methocel E50LV, 36%</td>
<td>Emcompress, 36%</td>
<td>Methocel E50LV, 37.5%</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
</tbody>
</table>

*Dex, dexamethasone; †Bud, budesonide; tablets weighed 330 mg (dexamethasone), 500 mg (budesonide).

7.5) and the last 18 h in 250 ml of SCF (0.1 mg protein/ml of galactomannanase in 0.05 M phosphate buffer, pH 6.5) or control (0.05 M phosphate buffer only, pH 6.5). Because of the low water solubility of budesonide, the dissolution medium was also replaced at 12 and 18 h to maintain sink conditions. Dissolution medium (6 ml) was extracted at each time point, without replacement. The dip speed in SGF/SIF was 20 cycles/min; that for SCF was 3 cycles/min.

2.4. Sample preparation

Samples (dexamethasone: 4 ml; budesonide: 2 ml) were combined with 2.0 ml of saturated sodium chloride and triamcinolone acetonide (250 µg/ml; dexamethasone: 4 µl and budesonide: 100 µl) as an internal standard. Then, 5.0 ml of methyl-α-butyl-ether:pentane (6:4, v/v) were added and the samples were vortexed for 1 s. Samples were centrifuged to separate the layers, and the organic layer was transferred to a separate test tube. The extraction was repeated, and the organic solvent was evaporated to dryness under nitrogen. The residue was dissolved in 50% ethanol (dexamethasone: 0.5 ml and budesonide: 1.0 ml). Standards (50 µg/ml) were diluted in the simulated fluids and then extracted in the same manner [28,29].

2.5. Chromatography

For the chromatographic analysis, a Hitachi L-7100 pump, L-7200 autosampler, and L-7420 UV detector were used. A 30 cm×3.9 mm (i.d.) column packed with 10 µm ODS (Waters model no. 27324) was used. The compositions of mobile phase were as follows: 20 mM sodium acetate/acetonitrile (57:43) for dexamethasone in simulated fluids; ethanol/water (48:52) for budesonide. The mobile phase flow rate was 1.0 ml/min, the injection volume was 50 µl for dexamethasone and 100 µl for budesonide. The effluent stream was monitored at 239 nm (dexamethasone) or 240 nm (budesonide). Drug concentrations were calculated by comparing the peak area ratio of drug to internal standard given by the unknown with the ratio given by the standard [30]. Both epimers of budesonide were separated and measured; data are expressed as total budesonide released.

3. Results

The effect of several variables (dip speeds of reciprocating cylinders in SGF, SIF and SCF and the concentrations of galactomannanase) on dissolution profiles of formulations CD₁, CD₂, and CD₃ was assessed. The data indicated that drug dissolution and tablet disintegration vary as a function of formulation and dissolution variables.

3.1. Dip speed in SGF and SIF

Dexamethasone dissolution from formulations CD₁ and CD₂ was evaluated in SGF for 2 h followed by 4 h in SIF at various dip speeds: 10, 20 and 30 cycles/min (Fig. 1). The drug release for CD₃ (with higher viscosity grade HPMC, Methocel E50 LV) was relatively independent of dip speed. On the other hand, dip speed affected dissolution and tablet disintegration of CD₃ markedly. At a dip speed of 30 cycles/min, CD₁ disintegrated completely in SIF (over the first 2 h). Thus, dip speed of 20 cycles/min was selected for the dissolution of colonic delivery systems.
3.2. Dip speed and galactomannanase concentrations in SCF

Because transit and other movements of colon are relatively slow compared with those of the small intestine, dip speeds >5 cycles/min may be unrealistic for dissolution in SCF. It was found that the drug release was sensitive to dip speed in SCF, even when the difference in dip speed was only 2 cycles/min (Fig. 2).

The effect of varying the concentration of galactomannanase in the SCF on dexamethasone dissolution from formulation CD₂ is shown in Fig. 3. Galactomannanase concentrations >0.01 mg/ml accelerated dissolution of dexamethasone from formulation CD₂. The magnitude of increased dissolution was dependent on the concentration of galactomannanase. Data presented in Fig. 3 also demonstrate the independence of the onset of increased drug release rate on the range of concentrations of galactomannanase investigated. Slower drug release at 0 mg/ml of galactomannanase in SCF compared to that in SGF/SIF was due to a reduced dip speed (5 cycles/min) used for dissolution in SCF compared with that used in the SGF/SIF (20 cycles/min).

3.3. Dissolution from dexamethasone formulations

Using all variables selected from the experiments described above, dexamethasone dissolution profiles of the CD₁, CD₂, and CD₃ were evaluated (Fig. 4). Dissolution from each formulation was studied in a manner similar to that using stool suspensions, i.e. SGF, SIF followed by SCF all at 37°C. The observed drug release and tablet disintegration in the simulated fluids was that generally expected based on the previous experiments with the stool suspensions. Though the dip speed was decreased from 20 (first 6 h; SGF/SIF) to 3 cycles/min (last 18 h; SCF), drug release rate did not decrease in SCF. These data indicated that release of dexamethasone from these three formulations can be enzymatically triggered in SCF. Dissolution of dexamethasone from formul-
Fig. 4. Dissolution of dexamethasone from formulations CD$_1$, CD$_2$, and CD$_3$ using USP Dissolution Apparatus III; $n=3$. Conditions were as follows: 2 h in 250 ml of SGF (USP; 37°C; dip speed=20 cycles/min), next 4 h in 250 ml of SIF (USP; 37°C; dip speed=20 cycles/min), and 18 h in 250 ml of phosphate buffer (galactomannanase concentrations=0.1 mg/ml; pH 6.5; dip speed=3 cycles/min; 37°C).

Like dexamethasone, dissolution of budesonide was dependent on the galactomannanase present in the SCF. Because of the low water solubility of budesonide, the SCF was replaced by fresh dissolution medium (SCF) at 12 and 18 h to determine if apparent dissolution was limited by factors other than disintegration of the guar matrix. It was found that the drug release was unchanged by such replacement (see Fig. 6).

4. Discussion

Bacteria residing in the colon produce significant amounts of galactomannanases which can hydrolyze guar gum [14–17,31]. In this study, we demonstrated that galactomannanases accelerated dissolution of dexamethasone and budesonide from guar gum-based matrix tablets. The more rapid dissolution of dexamethasone from CD$_1$ was probably due to the use of the hydrophilic low viscosity grade HPMC (Methocel E3). The gel formed on the tablet surface eroded faster under higher shearing force (i.e. at higher dip speeds). Consequently, this caused fast tablet disintegration and drug release of CD$_1$. The higher viscosity grade HPMC (Methocel E50 LV; formulation CD$_3$) slowed dexamethasone dissolution somewhat relative to that from formulation CD$_1$. 

Fig. 5. Dissolution of budesonide from formulation CD$_2$ using USP Dissolution Apparatus III; $n=3$. Conditions were as follows: 2 h in 250 ml of SGF (USP; 37°C; dip speed=20 cycles/min), next 4 h in 250 ml of SIF (USP; 37°C; dip speed=20 cycles/min) and 18 h in 250 ml of phosphate buffer (galactomannanase concentrations=0.1 mg/ml; pH 6.5; dip speed=3 cycles/min; 37°C).

Fig. 6. Dissolution of budesonide from formulation CD$_4$ using two dissolution conditions; $n=3$. The dissolution conditions (setup 1) were as follows: 2 h in 250 ml of SGF (USP; 37°C; dip speed=20 cycles/min), next 4 h in 250 ml of SIF (USP; 37°C; dip speed=20 cycles/min), and 18 h in 250 ml of phosphate buffer (galactomannanase concentrations=0.1 mg/ml; pH 6.5; dip speed=3 cycles/min; 37°C). For dissolution setup 2, the SCF was replaced with fresh SCF at 12 and 18 h.

3.4. Dissolution from budesonide formulations

Budesonide tablets (formulation CD$_4$) were also evaluated by this dissolution system. Fig. 5 shows the dissolution of budesonide in SGF, SIF, and SCF; about 10% of budesonide released in the upper gastrointestinal fluids and the remaining 90% release in SCF. Compared to dexamethasone, the lower amount of budesonide released in SGF/SIF was probably due to its lower relative water solubility.
Finally, the slower dissolution observed from formulation CD$_1$ was probably due to the use of a relatively water insoluble additive (i.e. Emcompress) and smaller particle size of guar gum (fine grade).

The different release rates demonstrated under the in vitro dissolution conditions developed, coupled with clinical data collected with these formulations [32], suggest that guar-based colonic delivery systems may be useful in treating either Crohn’s disease or ulcerative colitis. For instance, formulation CD$_1$ released drug more rapidly in the SCF than did formulations CD$_2$ and CD$_3$; in vivo data collected from CD$_1$ indicated that relatively large amounts of dexamethasone were delivered and released in the distal ileum and cecum [32]. Thus, formulation CD$_1$ may be useful in the local treatment of Crohn’s disease or proximal ulcerative colitis. On the other hand, formulations CD$_2$ and CD$_3$ release drug in a more delayed and prolonged manner compared with CD$_1$. Therefore, these formulations may be beneficial in treating distal disease (viz. Crohn’s colitis and ulcerative colitis).

An in vitro dissolution setup should ideally correlate to data collected in vivo (preferably in humans). The three dexamethasone formulations evaluated in this study were tested in a human clinical study [32]. The dissolution conditions (i.e. dip speeds) for SGF and SIF were found to release about 20% dexamethasone in 6 h. This percentage of drug release was nearly identical to the total percent dexamethasone absorbed after the 6 h following oral administration to fasted human volunteers [32]. Absorption of dexamethasone was complete on average within 14–18 h in vivo following administration. This finding is also consistent with the dissolution data collected under specific conditions using USP Apparatus III.

Dissolution characteristics of the budesonide colonic delivery system, i.e. CD$_4$, suggest that this formulation could deliver relatively large amounts of budesonide into the large intestine over a prolonged period of time. Other drugs, such as prednisolone and triamcinolone acetonide, are also suitable using this technology. However, disease could alter the delivery characteristics due to changes in transit through various segments of the colon and variations in gut microflora levels.

5. Conclusions

In this study, problems of mixing and drug solubility in stool suspensions were addressed by using USP Dissolution Apparatus III. The results suggest that guar gum-based tablets may be a useful biodegradable polymeric matrix material for delivery and release in the distal ileum, cecum, and colon. Dip speed of the reciprocating cylinders affected dissolution from tablets composed of low viscosity grade HPMC. Galactomannanase accelerated dissolution of both dexamethasone and budesonide from guar gum-based tablets in SCF at galactomannanase concentrations >0.01 mg/ml. The extent of acceleration in drug dissolution depended on the concentration of galactomannanase. Finally, this dissolution system is simpler and easier to use than that recently proposed for the evaluation of pectin-based colonic delivery systems [33].

References


