Graphic abstract

**Clarithromycin (CAM)**
- Macrolide antibiotic
- Used for eradication of *Helicobacter pylori*
- Time dependent drug

**Three different hydrophobic binders**
- Glycerin monostearate (GM)
- Triglycerine full behenate (TR-FB)
- Lubriwax-101 (LW)

**Gastro-retentive DDS**

**High shear melt granulation**

**Heat Mix**

**CAM** + **Binder**

**75% CAM loaded granules with void space**

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**In vitro**

- Floated on solvent

- Sustained drug release

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**In vivo**

- Prolonged gastro-retentivity

- Enhanced eradication efficiency
Clarithromycin highly-loaded gastro-floating fine granules prepared by high-shear melt granulation can enhance the efficacy of *Helicobacter pylori* eradication

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**Abbreviations:** CAM, clarithromycin; GM, glycerin monostearate; GRDDS, gastro-retentive drug delivery system; HPLC, reversed phase high performance liquid chromatography; *H. pylori, Helicobacter pylori;* HSMG, high-shear melt granulation; LW, lubriwax-101; MB, meltable binder; SEM, scanning electron microscopy; SRCT, Synchrotron X-ray computed tomography; TR-FB, triglycerin full behenate; VVR, void voxel ratio

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Abstract

In an effort to develop a new gastro-retentive drug delivery system (GRDDS) without a large amount of additives, 75% clarithromycin (CAM) loaded fine granules were prepared with three different hydrophobic binders by high-shear melt granulation and their properties were evaluated. Granules containing the higher hydrophobic binder showed sustained drug release and were able to float over 24 h. The synchrotron X-ray CT measurement indicated that both the high hydrophobicity of the binder and the void space inside the granules might be involved in their buoyancy. In an in vivo experiment, the floating granules more effectively eradicated *H. pylori* than a CAM suspension by remaining in the stomach for a longer period. In short, CAM highly-loaded gastro-floating fine granules can enhance the eradication efficiency of *H. pylori* compared with CAM alone.

Highlights

- 75% clarithromycin loaded granules were prepared by high-shear melt granulation.
- The granules prepared with highly-hydrophobic binder had large void spaces inside.
- The hydrophobic granules showed sustained-drug release and buoyancy over 24 h.
- Strong water-repellency and low particle density were involved in their buoyancy.
- The floating granules improved recovery from *H. pylori* by retained in the stomach.
Keywords: Gastro-retentive drug delivery system; floating granules; clarithromycin;

Helicobacter pylori eradication; high-shear melt granulation; highly-drug loaded fine granules; synchrotron X-ray computed tomography
1. Introduction

A gastro-retentive drug delivery system (GRDDS) is an oral controlled drug delivery system in which a drug can remain in the gastric region for several hours to prolong its gastric residence time. GRDDS enhances the effects of drugs with a narrow absorption window in the upper part of the gastrointestinal tract, drugs with poor stability in the colon, or drugs which work in the stomach. Thus, GRDDS can improve the bioavailability of drugs and patient compliance. To date, various types of GRDDSs have been developed, including a gastro-floating system, a bioadhesive system, an expandable system, and a magnetic system among others [1]. However, these systems require a large amount of additives to exert not only gastro-retentivity but also sustained drug release. Consequently, incorporation of these additives may result in low drug loading, enlargement of the product size and complication of the manufacturing process. Therefore, a new GRDDS that can solve these concerns is desired.

Clarithromycin (CAM) is a macrolide antibiotic used for the treatment of a number of bacterial infections. A GRDDS is considered to be adequate for this drug because it is a time-dependent antibiotic used for the eradication of Helicobacter pylori (H. pylori) in the stomach. Recently, several GRDDSs for CAM have been developed [1, 2]; however, they still have many of the limitations mentioned above. In particular, for the eradication of H. pylori, the antibiotic amoxicillin and a proton pump inhibitor like omeprazole may be taken together,
such that low drug loading and enlargement of product size might be a serious problem for patient compliance.

In the present study, to solve the limitations of developing GRDDS for CAM, we focused on high-shear melt granulation (HSMG) for preparing gastro-floating fine granules with high CAM loading. Melt granulation uses materials with a low melting point as a meltable binder (MB). In HSMG, the granules are formed by agitation with heat, and cooling to ambient temperature congeals the MB and yields dried granules. The simplicity of the process is one of the advantages of HSMG. Additionally, as melt granulation is a solvent-free process, the drying step and toxic solvent are eliminated making it more economical and environmentally friendly. A further advantage is that selection of a MB can provide the sustained release properties [3] as well as buoyancy to the granules because of the strong water-repellent effect derived from the hydrophobicity of the MBs. Moreover, our synchrotron X-ray computed tomography (SRCT) measurements recently revealed that the granules prepared by HSMG had some void space in their structures—dependent on the operational and formulation conditions [4]—suggesting that granules with low particle density can be easily prepared. Therefore, HSMG incorporating higher hydrophobic MBs should be used to prepare the granules to ensure sustainability of drug release as well as buoyancy because of the water-repellent effect and low particle density.
Thus, we investigated the preparation and evaluation of gastro-floating fine granules with high CAM loading by HSMG. First, CAM highly-loaded granules were prepared with three different hydrophobic MBs and their powder properties, buoyancy, and drug release profile were investigated. Additionally, their internal structural changes while the drug release was observed by SRCT. Finally, in vivo evaluations such as bacterial recovery from \textit{H. pylori} and gastro-retentivity were examined.

\textbf{2. Materials and methods}

\textbf{2.1 Materials}

Clarithromycin (CAM) (stable form, form II) was purchased from Shiono Chemical Co., Ltd. (Tokyo, Japan). Glycerin monostearate (GM), which is often used as a hydrophobic MB, was purchased from Taiyo Chemical Industry Co., Ltd. (Tokyo, Japan). Triglycerin full behenate (TR-FB), which was recently reported as a highly-hydrophobic MB [4], was kindly provided by Riken Vitamin Co., Ltd. (Tokyo, Japan). Lubriwax®-101 (LW), which was reported as a base material to prepare microspheres with sustained-drug release, was kindly provided by Freund Corporation (Tokyo, Japan). Talc as a glidant was purchased from Gotoku Chemical Co., Ltd. (Tokyo, Japan).

\textbf{2.2 Contact angle of the water droplet}
The hydrophobicity of each MB was determined by measurement of the contact angle of the water droplet. MB was melted and casted into a mold to form a flat MB plate. A 10 µL water droplet was dropped on the MB plate and the contact angle was measured by the $\theta/2$ method.

2.3 Preparation of granules by HSMG

Granules were prepared by the HSMG method as reported previously [4]. Briefly, CAM 75 g, MB 20 g, and talc 5 g were placed into a high-shear mixer (MECHANOMiLL, Okada Seiko Co., Ltd., Tokyo, Japan) with a rubber heater and temperature sensor. The jacket temperature was fixed at 5°C above the melting point of each MB, as 78°C for GM, 77°C for TR-FB and 92°C for LW, and the impeller speed was set to 1000 rpm. After the product temperature reached 5°C above the melting point, the rubber heater was turned off, the lid was removed and the product was further mixed at 400 rpm for 2 min. To cool the obtained product, the mixture was spread on metal trays.

2.4 Particle size distribution

The particle size distribution of the granules was evaluated by sieve analysis using standard sieves (Tsutsui Scientific Instruments Co., Ltd., Tokyo, Japan) with aperture sizes
ranging from 54 to 1000 µm. Granules with diameters from 250–350 µm were used in all the 

studies except for flowability.


2.5 Characterization of powder property

The angle of repose was measured using the Powder Tester (Hosokawa Micron Co., 
Ltd., Japan) and evaluated by Carr’s flowability index [5]. As a standard, an angle of repose

“31–35°” represents “good” flowability, “36–40°” represents “fair”, “41–45°” represents

“passable” and “46–55°” represents “poor”. Measurement of roundness and scanning electron 

microscopy (SEM) were conducted as reported previously [4]. Granule strength was

measured with a particle hardness tester (GRANO, Okada Seiko Co. Ltd., Tokyo, Japan) and

calculated by Hiramatsu’s equation [6]. True density was measured with an air pycnometer

(type-1000, Tokyo Science Co. Ltd., Tokyo, Japan).


2.6 Buoyancy study

A total of 20 granules were placed in 50 mL of test solution (pH 4.0 or pH 6.5

phosphate buffer (0.05 M)) and shaken at 100 rpm with a Multi Shaker MMS and Incubator

FMS (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) [7]. At time points from 0 to 24 h, the number

of floating granules was counted and the rates of the floating granules were calculated.
2.7 Drug release test

The release behavior of CAM from the granules of each formulation was examined in accordance with the paddle method listed in JP 16th. The test medium was 900 mL of pH 6.5 phosphate buffer at 37±0.5°C and the paddle speed was 50 rpm. At each time point, 3 mL aliquots of the test solutions were withdrawn and replaced with an equal volume of buffer solution, and the samples were passed through a membrane filter (HLC-Disk 25, pore size: 0.20 µm, Kanto Chemical Co., Inc., Tokyo, Japan). The amount of CAM released into the medium was quantitatively determined by reversed phase high performance liquid chromatography (HPLC) (Shimadzu, Japan) in a binary mode (dual pump), with a UV detector at 210 nm. The HPLC column was reversed phase (TSKgel ODS-80T, 4.6 mm i.d. × 150 mm) maintained at 40°C. The mobile phase of 0.07 M potassium dihydrogen phosphate solution: acetonitrile at a ratio of 13:7 was delivered at a flow rate of 1.0 mL/min. The retention time of CAM was 12.5±0.5 min.

2.8 SRCT measurement

The SRCT measurement was conducted using a micro-CT instrument [8] installed at the undulator beamline BL37XU of SPring-8 (Hyogo, Japan). The samples were put into Lindemann glass capillaries with a diameter of 0.3 mm and then measured. Nine hundred transmission X-ray images, with parallel projection geometry, were recorded in 0.2° steps.
with continuous rotation of the sample. Tomographic reconstruction was performed by the convolution back-projection method using the software CBP and cross-sectional images were obtained. Voxel size was 0.526×0.526×0.526 µm. The sets of the cross-sectional images were analyzed using SLICE and ImageJ 1.48v. To evaluate the porosity of the granules, the void voxel ratio (VVR) was calculated with following equation.

\[
VVR = \frac{\text{(number of voxels regarded as void in the sample)}}{\text{(number of total voxels in the sample)}}
\]

Voxels with linear attenuation coefficient values less than 0.3 cm\(^{-1}\) were regarded as voids [4].

2.9 In vivo evaluation of gastro-retentivity of LW granules

The method used to evaluate the gastro-retentivity of the LW granules was based on a previous report by Nagahara et al. [9]. LW granules or CAM suspended in a 0.5% aqueous solution of methylcellulose at a concentration of 8.5 mg/mL (CAM suspension) was orally administered to 8-week-old male specific-pathogen-free Mongolian gerbils which were allowed ad libitum access to diet (weight: 54–61 g) obtained from Japan SLC (Shizuoka, Japan). The CAM dose was 30 mg/kg of body weight. At 2 or 4 h after administration, the stomach of each Mongolian gerbil was excised while the gerbil was under anesthesia, and the
amount of CAM remaining was evaluated; i.e., 10 mL of pH 6.5 phosphate buffer and 10 mL of methanol were added to each stomach and the amount of CAM extracted was determined by HPLC using the same condition as described in section 2.7. The percentage of CAM remaining was calculated according to the following equation: remaining percentage = \((R/T) \times 100\), where \(R\) represents the amount of CAM remaining in the stomach and \(T\) represents the amount of CAM administered.

2.10 In vivo bacterial recovery from H. pylori

The in vivo bacterial recovery was assessed using a method previously reported by Nagahara et al. [9]. A dose of 30 mg/kg clarithromycin in the form of either LW granules or suspension was orally administered to male Mongolian gerbils (weight: 56–68 g; age: 11 weeks) infected with H. pylori once a day for 3 consecutive days. One day after administration of the final dose, the Mongolian gerbils were killed and the stomachs were removed. Each stomach was homogenized with Brucella broth (3 mL/stomach), and serial dilutions were plated on modified Skirrow’s medium. The agar plates were incubated for 4 days at 37°C under microaerobic conditions in an Anaero Pack (Mitsubishi Gas Chemical Co., Inc., Japan). The viable cell counts for each stomach were calculated by counting the number of colonies on the agar plates. The number of colonies per plate was counted and expressed as the log CFU per gastric wall. All of the procedures used in the current study were conducted
in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of University of Shizuoka.
3. Results and discussion

The contact angle of GM, TR-FB and LW was 32±8°, 105±5° and 100±8°, respectively, and the values of TR-FB and LW were significantly higher than that of GM (Fig. 1A), suggesting that these two MBs have higher hydrophobicity than GM. Although the initial mean particle size of MBs were different (GM: 41 µm, TR-FB: 4 µm, LW: 22 µm), the obtained granules prepared with each MB had similar particle size distributions (Fig. 1B), angle of repose (34–36°), roundness (0.77±0.08–0.80±0.08), strength (203±104–218±96 mN/mm²) (Table 1) and morphology (Fig. 1C). In addition, before and after granulation, crystal transition of CAM from stable form (form II) to unstable forms was not observed (data not shown). In particular, as the angle of repose for the bulk CAM was 54°, the flowability of all the granules was improved compared with the bulk CAM. Moreover, fine globular particles in the range of 50–350 µm were obtained in high yield (GM granules: 58%, TR-FB granules: 62%, LW granules 60%), confirming that HSMG is an acceptable granulation method for the preparation of fine granules as we reported previously [4].

From the buoyancy study of the granules, TR-FB or LW granules floated in the test solutions at pH4.0 and pH6.5 over 24 h while the GM granules immediately sank (Fig. 2A and 2B). TR-FB and LW granules also showed more sustained drug release compared with GM granules (Fig. 2C). The improved sustained release of these granules can be explained by the higher hydrophobicity of the MBs [3] and the lower contact surface area of the granules.
when floated in the test solution. As CAM is a time-dependent antibiotic, use of sustained drug release should enhance its antibacterial effect.

As mentioned above, the strong water-repellency and low particle density arising from the large void space in the granules might be probably involved in the buoyancy of these granules. Therefore, to examine the buoyancy from a structural perspective, SRCT measurements were conducted. **Figure 3A–C** shows the inner structure of each granule before the drug release study. The black area inside the granule is the void space. From the value of VVR (**Table 2**), it was determined that the TR-FB granules and LW granules have larger void spaces than the GM granules. It is known that the density of the 0.05 M phosphate buffer and gastric content were 1.002 g/cm\(^3\)[10] and 1.004 g/cm\(^3\)[1], respectively, and the density of the product should be kept below these values for buoyancy. Thus, it was useful to calculate the particle densities of these granules. The VVR of the GM granules was 9.8±4.2% while its true density was 1.52±0.02 g/cm\(^3\), such that its particle density was calculated as 1.4±0.1 g/cm\(^3\). In the same way, the particle density of the TR-FB granules and LW granules were calculated as 1.3±0.1 g/cm\(^3\) and 1.1±0.1 g/cm\(^3\), respectively (**Table 2**). Although these floating granules have a lower particle density than the GM granules, they were still higher than 1.004 g/cm\(^3\). Therefore, the reason why the TR-FB and LW granules with the higher particle density floated might be explained by the stronger water-repellency that was derived from the high hydrophobic MB, such that the repellent force was stronger than the sinking force. Based on
these results, it can be concluded that buoyancy can be functioned into CAM highly-loaded
granules by using hydrophobic MBs with a contact angle larger than 100°.

Compared with the initial granules (Fig. 3A–C), the surface of granules from which
50% drug had been released became green in color and the sparse regions were recognized
(Fig. 3D–F), providing a tool by which CAM release from the surface of the granules could
be monitored. In contrast, the shape of the void space in the central part of the granules did
not change even after 50% drug release. This suggests that the void space retained air inside
the granules and the test solution may not penetrate the void space, indicating that the granule
density remained constant even when drug dissolution occurred. Therefore, although the
particle density of the granules was higher than 1.004 g/cm$^3$, the void space inside the
granules can also contribute to their buoyancy.

Finally, the bacterial recovery of Mongolian gerbils from $H. pylori$ infection was
conducted (Fig. 4). The pH of gerbils’ stomach was pH 4.1. LW granules provided
significantly higher bacterial recovery (2.27±0.53 logCFU) than the control (3.53±0.14
logCFU), whereas no significant effect was observed for the CAM suspension (3.38±0.08
logCFU). From the result of a gastro-retentivity test with Mongolian gerbils, the LW granules
showed over two-fold higher retentivity in the stomach at 2 or 4 h (75% or 34%) after
administration compared with the CAM suspension (32% or 14%), strongly suggesting that
the buoyancy of the LW granules was involved in this bacterial recovery. A further study
using polymerase chain reaction amplification of the 16s rRNA of *H. pylori* would be needed to precisely determine the efficacy of *H. pylori* eradication [2].

CAM highly-loaded gastro-floating fine granules were prepared by using hydrophobic MBs in HSMG. The LW granules improved the efficiency of bacterial elimination by prolonging the period of CAM exposure in the stomach. In the clinic, because amoxicillin and a proton pump inhibitor are normally prescribed for the eradication of *H. pylori*, this basic information in the present study can lead to develop new combination drug containing CAM and two other drugs for an improvement of patients’ compliance in the near future.

**Acknowledgements**

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References


850601.


[10] J. E. Schiel, D. S. Hage, Density measurements of potassium phosphate buffer from 4 to
**Table legends**

**Table 1. Powder properties of the granules**

<table>
<thead>
<tr>
<th></th>
<th>CAM bulk</th>
<th>GM granules</th>
<th>TR-FB granules</th>
<th>LW granules</th>
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<tbody>
<tr>
<td>Angle of repose (°)</td>
<td>54</td>
<td>36</td>
<td>34</td>
<td>35</td>
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<tr>
<td>Flowability</td>
<td>Poor</td>
<td>Fair</td>
<td>Good</td>
<td>Good</td>
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<tr>
<td>Roundness</td>
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<tr>
<td>Strength (mN/mm²)</td>
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<td>203±104</td>
<td>217±105</td>
<td>218±96</td>
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**Table 2. Densities and VVR of granules**

<table>
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<th>LW granules</th>
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<td>VVR (%)</td>
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<td>Particle density (g/cm³)</td>
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<td>1.4±0.1</td>
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**Figure legends**

**Figure 1. Powder property of MBs and the granules.**

A: Contact angle of a water droplet on the MBs. **: \( p < 0.001 \), B: Particle size distribution of each granule, and C: SEM image of (a) GM granules, (b) TR-FB granules and (c) LW granules, respectively.

**Figure 2. Characterization of the granules.**

A: Floating property of the granules in (a) pH 4.0 and (b) pH 6.5 test solution, B: LW granules float in the pH 4.0 test solution, and C: Drug release profiles of the granules.

**Figure 3. Inner structure of the initial, 50% drug released and 100% drug released granules.** GM granules (A, D, G), TR-FB granules (B, E, H), and LW granules (C, F, I). X-ray linear attenuation coefficients values less than 4 cm\(^{-1}\) are shown in black in the images of the granules, where the values from 4 cm\(^{-1}\) to 70 cm\(^{-1}\) are in rainbow colors.

**Figure 4. In vivo evaluation of LW granules.**

A: Bacterial recovery from \( H. \) pylori. and B: gastro-retentivity, *: \( p < 0.05 \) and **: \( p < 0.01 \)
Fig. 1

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A

Contact angle of water droplet (°)

GM  TR-FB  LW

B

Cumulative (%)

0-53  53-149  149-250  250-350  350-500  500-1000

Particle size (μm)

GM granules  TR-FB granules  LW granules

C

a) GM granule  

b) TR-FB granule  
c) LW granule

100 μm
Fig. 2

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A

a) pH4.0

Floating granules (%)

Time (h)

b) pH6.5

100

GM granules

TR-FB granules

LW granules

Time (h)

B

C

Cumulative drug release (%)

Time (h)
### Fig. 3

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<table>
<thead>
<tr>
<th>GM granules</th>
<th>TR-FB granules</th>
<th>LW granules</th>
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<td><img src="#" alt="Image H" /></td>
<td><img src="#" alt="Image I" /></td>
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<tr>
<td><strong>50% drug released</strong></td>
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<td></td>
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<tr>
<td><strong>100% drug released</strong></td>
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Fig. 4

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