A Novel Lactocin-Based Solid Lipid Nanoparticles for Smart Probiotic Nanofood: Rheological, Mucoadhesive and in vitro Release Properties

Dong-Myung Kim1*, Myungjune Chung2, Gee-Dong Lee3, Yong Kook Shin4 and Woo Kyu Jeon5
1*Nanofood Research Society of Seoul National University, Seoul, 151-742, Korea
2Cellbiotech Co., Ltd, Seoul, 157-030, Korea
3Daegu Gyeongbuk Institute for Oriental Medicine Industry, Gyeongsan, 704-230, Korea
4Chungbuk Health Industry Center, Chungbuk Technopark, Chungbukdo, 363-883, Korea
5Department of Gastroenterology, Kangbuk Samsung Hospital, Seoul, 110-746, Korea

(Received: July 5th, 2008; Accepted: July 23rd, 2008)

Abstract: The aim of the present work was to prepare and evaluate oral mucoadhesive sustained release nanoparticles of lactocin(CBT-LP2) these nanoparticles could then be used to improve patient compliance by simplifying the administration of lactocin, improving the therapeutic effect and reducing the dose related side effects. CBT-LP2 containing solid lipid nanoparticles(SLN) were prepared by ultrasonication using soybean lecithin as a stabilizing agent. The results showed that this method was reproducible, easily performed and led to efficient entrapment of CBT-LP2 as well as formation of spherical particles ranging from 80-200 nm. In addition, process variables, including the effect of gliadin concentration and the effect of surfactant, were also evaluated with respect to the percent CBT-LP2 entrapment and percent yields. The maximum percent CBT-LP2 entrapment and percent yield were approximately 73 and 88%, respectively. The sustained release behaviors of the gliadin nanoparticles and the smart probiotic nanofood(SPN) were evaluated in both phosphate buffered saline(pH 7.4) and simulated gastric fluid(pH 1.2) at 37±1°C. Their mucoadhesive properties were determined by in vitro and in vivo methods. The shelf life of prepared SPN was determined by storage at various temperatures in simulated gastric fluid(pH 1.2) with and without added enzymes.

Key words: lactocin(CBT-LP2), smart probiotic nanofood(SPN), solid lipid nanoparticles(SLN), mucoadhesive, release nanoparticles

1. Introduction

Antimicrobial chemotherapeutic agents have been widely used to control gastrointestinal infections. However, widespread use of antibiotics is now being discouraged due to problems including the emergence of drug resistant strains and chronic toxicity.1 Antibiotics are often responsible for acute diarrhea as they cause the loss of normal intestinal microbes in addition to the protection they provide against pathogenic organisms.2 As alternatives, probiotics such as lactobacilli and bifidobacterium, or their derivatives, have been administered. Indeed, some lactic acid bacteria(LAB) have health-promoting attributes including antimicrobial properties,3,4 immunomodulation,5-10 the ability to alleviate lactose intolerance,11 antitumor characteristics,10,12,13 and hypocholesterolemic effects.14,15 These findings have heightened the interest of nutrition, food and microbiology scientists in the production of functional foods.

We isolated L. plantarum CBT-LP2 and L. plantarum CBT-LP3 from kimchi, Pediococcus pentosaceus CBT-PP1 from goat’s milk, and L. lactis CBT-P7 from cow’s milk. These probiotics were cultured in the appropriate broth, condensed by vacuum evaporation and mixed with equal doses of each pathogenic strain. In this study, the antimicrobial effects of this probiotic culture condensate mixture(PCCM, LACTOCIN-W™) were evaluated using in vitro and in vivo models of food-borne pathogens.16,17

Lactocin(CBT-LP2) is well absorbed from the GI tract, but its systemic bioavailability(55%) is relatively low due to first pass metabolism. It undergoes rapid biodegradation to produce microbiologically active metabolites. Oral administration represents the most convenient and common route of nutrition delivery.18-21 However, the bioavailability of many nutrients after oral administration is very low for a variety of reasons, such as a short gastric residence time, instability in the GI tract or...
lack of intestinal permeation of nutrients. The gastrointestinal uptake of poor orally absorbed CBT-LP2 may be improved by binding them to colloidal solid lipid nanoparticles (CBT-LP2-SLN) as a smart probiotic nanofood (SPN) made with gliadin. SPN can protect labile molecules from degradation in the GI tract and might be able to transport non-absorbable molecules into the systemic circulation.

This SPN has the ability to both control the release of and protect the loaded CBT-LP2 against degradation. Moreover, the small particle size allows them to penetrate the mucus layer and thus bind to the underlying epithelium and/or adhere directly to the mucus network.22 The adhesive interactions of the SPN with the boundary layer may improve CBT-LP2 bioavailability through a number of different mechanisms. SPN may enhance the CBT-LP2 absorption rate by reducing the diffusion barriers between CBT-LP2 and the site of action or absorption.23 Similarly, they may prolong the residence time in the GI tract.

Gliadin SPN has a strong adhesive capacity for the GI mucosa, which may be due to the gliadin composition. The neutral amino acids of gliadin can promote hydrogen-bonding interactions with the mucosa whereas its lipophilic components can interact with biological tissues through hydrophobic interactions.24 The bioadhesive capacity of gliadin SPN has also been evaluated when orally administered to animals. Gliadin SPN shows great tropism for the upper gastrointestinal regions, but its presence in other intestinal regions is very low.25 Since DM Kim initially introduced the term 'nanofood', which means nanotechnology for food, and showed that encapsulated materials can be protected from moisture, heat or other extreme conditions, thus enhancing their stability, applications for this nanofood technique have increased.18-21,23-33

In the present report, CBT-LP2 loaded CBT-LP2-SLN for SPN was prepared by ultrasonication, and the sustained release behavior and the physicochemical characteristics of the SPN produced by this method were studied. The SPN produced high bioavailability, a targeting effect, and mucoadhesive properties, which suggest that oral administration would be possible.

2. Materials and Methods

2.1 Materials

CBT-LP2 was procured as a gift from Cellbiotech Co., Ltd., Korea. Stearic acid (obtained from Sigma, USA) was used as the lipid material for the CBT-LP2-SLN. Gliadin, purchased from Sigma-Aldrich (USA), was used as the coating material for the SPN. Soybean lecithin was obtained from Central Soya Co., LTD., USA. All other reagents were of analytical grade.

2.2 Preparation of CBT-LP2-SLN and Gliadin SPN

CBT-LP2, stearic acid and soybean lecithin were precisely weighed and dissolved in absolute ethanol in a water bath at 70°C. The resultant organic phase was then dissolved in absolute ethanol in a water bath at 70°C. An aqueous phase was prepared by dissolving glycerin in distilled water. The resulting solution was rapidly injected through an injection needle into the stirred aqueous phase (80°C). The CBT-LP2-SLN suspension was then ultrasonicated for 300 s using the Ultrahomogenizer (Heidolph Electro, Kelhaim Co., Ltd., Germany).

To obtain a sustained-release gliadin SPN, gliadin and CBT-LP2-SLN were dissolved in 20 ml of ethanol:water (7:3 v/v) and this solution was poured into stirred physiological saline (0.9% NaCl w/v in water) containing 0.5% soybean lecithin as a stabilizer. The ethanol was eliminated by evaporation under reduced pressure (Buchi R-140, Switzerland) and the resulting gliadin SPN was purified by centrifugation at 15,000 rpm for 1 h (Eltech Centrifuge, India). The supernatant was removed and the pellets were resuspended in water. The suspension was passed through a 0.45 mm pore size membrane filter and the filtrate was centrifuged again. Finally, the nanoparticles were freeze-dried using 5% glucose solution as a cryoprotector. Gliadin SPN was hardened by the addition of 2 mg glutaraldehyde per mg gliadin SPN and stirred for 2 h at room temperature before purification and freeze-drying (Table 1).

2.3 Particle Size and Morphology

The particle size, size distribution and zeta potential of CBT-LP2-SLN and gliadin SPN were determined using a Zetasizer 3000 HS (Malvern Instrument Ltd., UK). The surface morphology and internal structure of gliadin SPN were determined by scanning electron microscopy (SEM). A thin film of the aqueous dispersion of nanoparticles was applied on double-stick tape over an aluminum stub and air dried to obtain a uniform layer of particles. These particles were coated with gold to a thickness of about 450 Å using Sputter gold coater (Fig. 1).

2.4 Percentage CBT-LP2 Entrapment and Percentage Recovery

The percentage CBT-LP2 entrapment and the percentage recovery were determined using the following equations.23 The appropriate amount of freeze-dried gliadin SPN was digested with a minimum amount of ethanolic solution (water:ethanol,
Dong-Myung Kim, Myungjune Chung, Gee-Dong Lee, Yong Kook Shin and Woo Kyu Jeon

7:3 v/v). The digested homogenates were centrifuged at 15,000 rpm for 30 min and the supernatant was analyzed for CBT-LP2 entrapment. The entrapped CBT-LP2 was measured at 760 nm using the Shimadzu 1601 UV/Vis spectrophotometer (Table 2).

\[
\text{% CBT-LP2 entrapment} = \left( \frac{\text{Mass of CBT-LP2-SLN}}{\text{Gram of CBT-LP2 used in the formulation}} \right) \times 100
\]

\[
\text{% CBT-LP2-SLN recovery (% yield)} = \left( \frac{\text{Concentration of CBT-LP2-SLN}}{\text{Concentration of CBT LP2-SLN recovered}} \right) \times 100
\]

\[
\text{GNP1} \text{ is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:3).}
\]

### Table 1. Formulation for the preparation of gliadin SPN.

<table>
<thead>
<tr>
<th>CBT-LP2:gliadin (ratio)</th>
<th>Formulation code(s)</th>
<th>Volume (ml) of inner ethanol:water phase (7:3 ratio)</th>
<th>Volume of outer phase (ml, saline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:0.5</td>
<td>GNP1</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>1:1</td>
<td>GNP2</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>1:2</td>
<td>GNP3</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>1:3</td>
<td>GNP4</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

GNP1 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:3).

### Table 2. Percentage CBT-LP2 entrapment and percentage recovery.

<table>
<thead>
<tr>
<th>Formulation code(s)</th>
<th>% CBT-LP2 entrapment</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNP1</td>
<td>43.7±2.3</td>
<td>63.6±1.7</td>
</tr>
<tr>
<td>GNP2</td>
<td>55.6±3.4</td>
<td>65.2±2.3</td>
</tr>
<tr>
<td>GNP3</td>
<td>73.1±1.3</td>
<td>88.7±2.8</td>
</tr>
<tr>
<td>GNP4</td>
<td>68.6±2.1</td>
<td>76.1±1.9</td>
</tr>
</tbody>
</table>

GNP1 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN (CBT-LP2-SLN:gliadin ratio, 1:3).

### 2.5 In vitro Drug Release Studies

Various formulations of gliadin SPN were selected for in vitro CBT-LP2 release studies. In vitro drug release of CBT-LP2 from gliadin SPN was estimated by cell membrane dialysis in two different media, phosphate-buffered saline (PBS, pH 7.4) (Table 3) and simulated gastric fluid (SGF, pH 1.2) (Table 4) at 37±1°C for 24 h under sink conditions. The appropriate volume of gliadin SPN was added to the dialysis tube with 100 ml of media and continuously stirred with a magnetic stirrer at 37±1°C. After the appropriate time interval (1 h), a 1 ml of sample was withdrawn and analyzed for CBT-LP2 content at 760 nm in a Shimadzu 1601 UV/Vis. spectrophotometer. An equal volume of fresh media preheated to 37°C was added to replace the withdrawn sample.

### 2.6 In vitro Evaluation of Gastric Mucoadhesion of Gliadin SPN

Male Sprague-Dawley rats weighing 200-250 g were fasted overnight but allowed free access to water. Their stomachs were excised under anesthesia and then perfused with physiological saline to remove the stomach contents. The cleaned stomach was used immediately. A 100 mg gliadin SPN sample was suspended in SGF (pH 1.2) and added to the cleaned stomach, which was ligated and then incubated in physiological saline at 37°C for 30
min. The liquid contents of the stomach were then removed by injecting air and perfusing the stomach with SGF(pH 1.2) for 30 min at a flow rate of 1 ml per min. The stomach was cut open and the nanoparticles that remained in the stomach were recovered with SGF(pH 1.2). The final volume of washing solution was mixed with 10 ml of ethanolic solution and incubated for 2 h to allow complete digestion of the gliadin nanoparticles. After filtration through 0.45 mm filter paper, the absorbance was measured spectrophotometrically at 230.6 nm(gliadin) and gastric mucoadhesion was determined as the percentage of nanoparticles remaining in the stomach after perfusion.

### 2.7 In Vivo Evaluation of Gastric Mucoadhesion of Gliadin SPN

Sprague-Dawley rats(200-250 g) were fasted for 24 h but were allowed free access to water. Gliadin SPN(100 mg) in capsule form was administered to the rats using a gastric sonde. Four hr after administration, the rats were sacrificed and the stomach was removed and washed with SGF(pH 1.2) to recover the remaining CBT-LP2-SLN. The amount of CBT-LP2-SLN remaining in the stomach was determined as described above for the *in vitro* methods. For X-ray studies, gliadin SPN was prepared with barium sulfate as a contrast agent for the *in vivo* studies. Albino rats free of detectable gastrointestinal diseases or disorders were fasted overnight. Each subject then ingested the CBT-LP2-SLN formulation together with 2 ml of water. The intragastric behavior of the formulations was observed by taking a series of X-ray photographs at suitable time intervals (Fig. 2) beginning 30 min after dosing.

### 2.8 Stability studies

The stability of gliadin SPN was evaluated in PBS(pH 7.4)
Table 5. Percentage gastric retention of CBT-LP2-SLN.

<table>
<thead>
<tr>
<th>Formulation code(s)</th>
<th>Average% gastric retention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vitro</td>
</tr>
<tr>
<td>GNP1</td>
<td>74.9±2.7</td>
</tr>
<tr>
<td>GNP2</td>
<td>76.1±3.4</td>
</tr>
<tr>
<td>GNP3</td>
<td>81.5±2.3</td>
</tr>
<tr>
<td>GNP4</td>
<td>70.4±1.6</td>
</tr>
</tbody>
</table>

GNP1 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:3).

Table 6. Remaining percentage of gliadin content in different media.

<table>
<thead>
<tr>
<th>Media</th>
<th>% remaining of gliadin content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>PBS (pH 7.4)</td>
<td>98.3±1.4</td>
</tr>
<tr>
<td>SGF (pH 1.2)</td>
<td>98.4±1.8</td>
</tr>
<tr>
<td>SGF (pH 1.2)+enzyme(pepsin)</td>
<td>96.3±0.9</td>
</tr>
</tbody>
</table>

GNP1 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:3).

Table 7. Effect of storage on residual CBT-LP2 content.

<table>
<thead>
<tr>
<th>Formulation code(s)</th>
<th>Initial</th>
<th>% residual CBT-LP2 content</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 8o  RT</td>
<td>0 8o  RT</td>
<td>0 8o  RT</td>
<td>0 8o  RT</td>
<td>0 8o  RT</td>
</tr>
<tr>
<td>CGNP1</td>
<td>100 100 100</td>
<td>97.9±2.1</td>
<td>98.7±1.9</td>
<td>98.6±2.2</td>
<td>93.9±2.9</td>
</tr>
<tr>
<td>CGNP2</td>
<td>100 100 100</td>
<td>98.9±2.5</td>
<td>98.5±3.9</td>
<td>95.0±5.6</td>
<td>93.3±5.7</td>
</tr>
<tr>
<td>CGNP3</td>
<td>100 100 100</td>
<td>99.6±5.9</td>
<td>98.1±4.9</td>
<td>97.4±2.1</td>
<td>91.7±5.2</td>
</tr>
<tr>
<td>CGNP4</td>
<td>100 100 100</td>
<td>97.4±5.7</td>
<td>97.7±3.6</td>
<td>95.2±2.9</td>
<td>95.5±2.4</td>
</tr>
</tbody>
</table>

GNP1 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:0.5), GNP2 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:1), GNP3 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:2) and GNP4 is gliadin SPN(CBT-LP2-SLN:gliadin ratio, 1:3).

3. Results and Discussion

The protein and polysaccharide from the aqueous phase can be desolvated by pH or temperature change or by addition of appropriate counter ions or other desolvating agents such as ethanol and isopropanol. Desolvation deaggregates the protein and causes the suspension to become colloidal and milky in appearance. Gliadin SPN was prepared by the desolvation method for macromolecules in this method, a solvent phase of gliadin was added to a non-solvent phase, which has low viscosity and high mixing capacity in all proportions. Addition of the desolvating agent resulted in the desolvation of the macromolecules. In a process commonly known as coservation, a new phase, the coservate phase, was treated with a cross-linking agent(glutaraldehyde) to produce CBT-LP2-SLN macromolecules. The desolvation process can be regarded as the gradual removal of solvent molecules from around macromolecules in which the molecules “roll up” in the less friendly environment. The protein macromolecules thus become discrete, poorly solvated, rolled up molecules, which produce colloidal particles. Gliadin represents a group of polymorphic proteins extracted from gluten that are soluble in ethanolic solution and show remarkably low solubility in water except at extreme pH.20,23,28,30 Due to its physicochemical properties, gliadin SPN can be prepared by the desolvation...
method for macromolecules, using environmentally acceptable solvents such as ethanol and water. These macromolecules showed a high capacity for loading CBT-LP2 and were soluble without further chemical or physical cross-linking treatment. In the present system, the diffusion of ethanol (good solvent for gliadin) from the gliadin solution into the aqueous medium drastically reduced the solubility of gliadin solution, allowing formation of nanoparticles in the solution. The particle sizes of gliadin SPN ranged from 80-200 nm with a positive zeta potential of 22.8 mV. The gliadin SPN was spherical with a smooth surface (Fig. 1).

The percentage of CBT-LP2 entrapment in the CBT-LP2-SLN at different gliadin concentrations was 23.7±2.3, 41.6±3.4, 67.1±1.3, and 63.6±2.1. The release of CBT-LP2 mainly depended on the gliadin concentration. The burst release of CBT-LP2 from CBT-LP2-SLN at the initial stage resulted from the dissolution of CBT-LP2 crystals on the surface of CBT-LP2-SLN with increasing gliadin concentration, the release rate of CBT-LP2 from CBT-LP2-SLN decreased drastically. The smaller CBT-LP2-SLN particles released CBT-LP2 more rapidly than the larger ones in PBS and SGF at the initial stage since the smaller particles have a larger surface area.

Mucoadhesion involves various interactive forces between mucoadhesive materials and the mucus surface, including electrostatic attraction, hydrogen bonding, Van der Waals forces, mechanical interpenetration, and entanglement. The spectrophotometric method ($\lambda_{max}$ 230.6 nm) was used to measure the in vitro mucoadhesive capacity of the formulations developed. Table 5 shows the percentage of gastric retention of gliadin SPN in the rat gastrointestinal mucosa. The adhesion properties of CBT-LP2-SLN increased with increasing concentration of gliadin, and better mucoadhesion was observed for the GNP4 formulations (3%).

The disulfide bonds of GNP4 gliadin interact with the fucose and sialic acid groups that are present in the gastric mucosa. The mucolytic activity of thiols, such as N-acetyl cysteine, result in disulfide exchange reactions between the mucin glycoprotein in the mucus and the mucolytic agent. Due to the exchange reaction, intra- and intermolecular disulfide bridges within the glycoprotein structure are cleaved, leading to breakdown of the mucus. As the mucolytic agent is covalently bound to the mucin glycoprotein in mucus, other thiol-bearing compounds, in particular polymers with thiols, should also covalently bind to the mucus. Further, the in vivo bioadhesive behavior was confirmed by taking X-ray photographs of the CBT-LP2-SLN in the stomachs of rats (Figs. 2-7). To sharpen the radio-graphical contrast in vivo, sufficient CBT-LP2 must be enclosed in the CBT-LP2-SLN. To meet this opposing requirement, CBT-LP2-SLN was prepared with a particle density of 1.099/cm² sub.

In the early stages within 30 min after dosing, the CBT-LP2-SLN adhered to the stomach (Fig. 3). After 6 h, some CBT-LP2-SLN remained since the bioadhesive system delayed the arrival to the pylorus. The prolonged residence time of CBT-LP2-SLN in the stomach might be explained by its mucoadhesive properties as well as by a random emptying effect caused by the presence of a multiple unit system. Due to their spreading over the antrum, as the subunits approach the pylorus, they might pass through the stomach individually and release CBT-LP2 concurrently in the gut, leading to decreased variability of CBT-LP2 action among patients compared with that occurring in the single unit dosage form. The results of this study suggest that gliadin SPN could serve as a novel delivery device to improve the bioavailability of CBT-LP2 and possibly other compounds that are used to produce local and specific effects in the stomach and are observed in the upper region of the stomach.

In this study, we showed that lactocin resided in the stomach for a longer period of time when it was administered in the form of the mucoadhesive CBT-LP2-SLN than when administered as a suspension or in a conventional system. The CBT-LP2 containing gliadin SPN also provided greater anti-bacterial activity than the plain CBT-LP2 formulations.

Acknowledgements: This study was supported by a grant (ATC-10026108) from the Ministry of Knowledge Economy (MKE) and a grant (07052 KFDA 137) from the Korea Food & Drug Administration.

References

5. G Perdigon, MEN Marcias de, S Alvarez, et al., Prevention of gastrointestinal infection using immunobiological methods with milk fermented with Lactobacillus casei and Lactobacillus
17. D Kim, MJ Chung, A probiotics condensate mixture novel anti-
enteric-coated nanospheres containing the protease inhibitor camostat, Biomaterials, 28, 8 (2001).
18. DM Kim, Preparation and characterization of biodegradable or
selective oxidation of primary alcohol groups in chitin and chitosan,
19. DM Kim, Preparation and characterization of biodegradable or
enteric-coated nanospheres containing the protease inhibitor camostat, Biomaterials, 28, 8 (2001).
20. DM Kim, HS Kwak, Nanofood materials and approachable
development of nanofunctional dairy products, Kor Dairy Food Engin, 1, 1 (2004).
21. DM Kim, HS Kwak, Nanofood materials and approachable
development of nanofunctional dairy products, Kor Dairy Food Engin, 1, 1 (2004).
22. C Durrer, JM Irache, F Puisieux, et al., Mucoadhesion of latexes
23. DM Kim, GD Lee, YK Shin, Oral mucoadhesive sustained
24. MA Arangoa, G Ponchel, AM Orecchioni, et al., Bioadhesive
25. MA Arangoa, MA Companero, MJ Renedo, et al., Gliadin
nanoparticles as carriers for the oral administration of lipophilic
26. DM Kim, HS Kwak, Development of functional nanofood and
its future, Kor Dairy Food Engin, 2, 1 (2004).
27. DM Kim, EJ Cha, Clinical analysis of lutein in taking HPMC-
lutein nanoparticle (nanofood) and in taking raw or cooked
28. DM Kim, GS Cho, Nanofood and its materials as nutrient
29. DM Kim, GD Lee, Introduction to the technology, applications,
products, markets, R&D, and perspectives of nanofoods in
30. DM Kim, GD Lee, YK Shin, Oral mucoadhesive sustained
release nanoparticle coated probiotic nanofood, Tissue Engin
31. DM Kim, JE Kim, GS Lee, MJ Chung, Method of preparing
triple-coated lactic acid bacteria, triple-coated lactic acid bacteria
prepared thereby and articles comprising the same, PCT/
32. DM Kim, H Baek, JH Kim, MJ Chung, Anticancer composition
comprising a culture fluid of Lactobacillus casei as an effective
33. DM Kim, Target controlled nanofood using by smart probiotic
solid lipid nanoparticles(SLN), Kor Dairy Food Engin, 15, 31
(2007).