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Effect of Superdisintegrant on Antigen Release from Enteric-Coated Antigen Microspheres

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ABSTRACT

The effect of incorporation of a superdisintegrant into an oral enteric-coated antigen microsphere (ECAM) delivery system was studied. The mixture of antigen and sodium starch glycolate (SSG, Explotab®) was applied onto 25- to 30-mesh nonpareil beads followed by coating with polymethacrylic acid–ethylacrylate. (PMA–EA, Eudragit® L-30D). Lipopolysaccharide (LPS) was used as a model antigen. The amount of antigen released from the ECAMs in dissolution medium as a function of time was determined by a size-exclusion HPLC method with UV detection. The level of superdisintegrants incorporated was optimized by adding different amount of SSG onto the beads. Study results indicated 12% (w/w) SSG provided the significantly faster release of LPS than other formulations. The time of the beads with SSG incorporated began to break apart the enteric film was 1.24, 1.26, 1.34, 1.58 and 1.86 min for 12%, 9%, 5%, 3%, and 1% superdisintegrant added formulation, respectively. The disintegrating time of SSG incorporated beads was significantly less than that of non-SSG incorporated beads (2.97 min, $p < 0.05$). Beads incorporated with 9% and 12% SSG always yielded significantly larger swelling and liquid uptake capacity. The significantly faster antigen release rate and the reduced time needed for breaking the Eudragit film may due to the swelling forces generated by incorporation of the superdisintegrant in the beads. The 12% (w/w) SSG formulation has the fastest release rate and the shortest film breaking time because 12% SSG microspheres take up a larger amount of water and provide higher swelling forces.

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INTRODUCTION

Oral administration of antigen containing proteins, glycoproteins, polysaccharides, lipopolysaccharides, or haptens with carriers can result in the induction of humoral and cellular immune responses; however, the required doses of antigens are much higher when compared to the doses used for systemic immunization (1–3). This fact has been a major reason why the oral immunization route has not been widely used. Denaturation of highly purified antigens such as viral glycoproteins and bacterial enzymes by gastric hydrochloric acid, digestion of antigens by proteolytic enzymes, and limited antigen absorption (4,5) are other factors causing low efficacy of oral vaccination.

Enteric-coated antigen microspheres (ECAMs) can protect antigens from gastric secretions and increase their presentation to the immune system throughout the gastrointestinal tract, thereby inducing more effective antibody responses (6,7). Further modification of the antigen delivery system by incorporation of a superdisintegrant in the formulation may increase the antigen's availability to quickly interact with the immune system in the GI tract and therefore decrease the amount of antigen needed to induce a desired immune response, increasing the efficacy of oral vaccination.

The properties of disintegrants have been evaluated in terms of water uptake (9-13), swelling rate (9,10), and disintegrating force (10,12,14,15) to interpret the drug release behavior of tablets. The liquid uptake and swelling capacity of enteric-coated microspheres have not been reported. The purpose of present study was to determine the effect of sodium starch glycolate (SSG) incorporation on the swelling capacity and liquid uptake of ECAMs.

MATERIALS AND METHODS

Sodium starch glycolate (Explotab®, Edward Mendell, Patternson, NY), 25- to 30-mesh size of NU-PARELL®, PG, Sugar Spheres NF (Ingredient Technology Corporation, Pennsauken, NJ), lipopolysaccharide (LPS, Sigma Chemical Co., St. Louis, MO, serotype: 055:B5), Eudragit® L-30D (Röhm Pharma, Weiterstadt, Germany), triethyl citrate (TEC, Aldrich Chemical Company, Inc., Milwaukee, WI), dibutyl sebacate (DBS, Sigma Chemical Co., St. Louis, MO), and talc (USP powder, Matheson Coleman & Bell, East Rutherford, NJ) were used as obtained from the supplier without further purification or modification.

Formulation Preparation

Nonpareil sugar beads, 100 g, were loaded into a preheated modified fluid bed bottom spray coater (Labline/PRL, Melrose Park, IL) at 60°C. when the sugar bead temperature equilibrated with the coating unit, either 0.03% LPS (based on the weight of sugar beads loaded in the coating chamber), which was dissolved with 5% (w/v) gelatin solution, or the mixture of the LPS and SSG was applied onto the beads. Each formulation contained 0% or 1%, 3%, 5%, 9%, 12% (w/w) SSG, respectively. A 0.8-mm bottom spray nozzle was used and the nozzle pressure was maintained at 18 ± 2 psi, blower speed was set at 40–50% of full capacity to cause the beads to move freely. LPS or the mixture of LPS and SSG was constantly delivered by a peristaltic pump (Gilson Medical Electronics, Middleton, WI) at a rate of 2 to 3 ml/min. After applying LPS and SSG, the pellets were dried in the coating chamber for another 5 min at the same temperature and air flow. The antigen-coated beads were removed from the coating chamber and placed in a 37°C oven overnight to remove residual moisture before application of PMA–EA enteric-coating. A mixture of Eudragit L-30D, 15% DBS, and 15% TEC (based on enteric coating solid contents) and 5% (w/w) talc was applied in the same manner as antigen coating to form an enteric-protected film. Eudragit L-30D, 20% (w/w), was applied based on the final dry weight of antigen loaded beads.

Superdisintegrant Study

The disintegrant was tested to measure its capacity to influence liquid uptake, swelling, time to rupture, and dissolution rate of LPS when incorporated into enteric-coated and non-enteric-coated antigen microspheres.

Liquid Uptake Measurement

A laboratory liquid penetration apparatus (13,16–19) (Fig. 1) was employed to determine liquid uptake and swelling of enteric-coated and non-enteric-coated beads. The apparatus consisted of a buchner funnel (15 ml) fitted with a sintered glass filter connected to a horizontally positioned 2-ml graduated pipette via tubing. The penetration liquid was simulated intestinal fluid. One-gram beads of each formulation were placed into the up section of the funnel. The prevent loss of water due to evaporation, the glass filter was covered with parafilm during the entire course of measurement. Ten replicate measurements were carried out for each group of for-

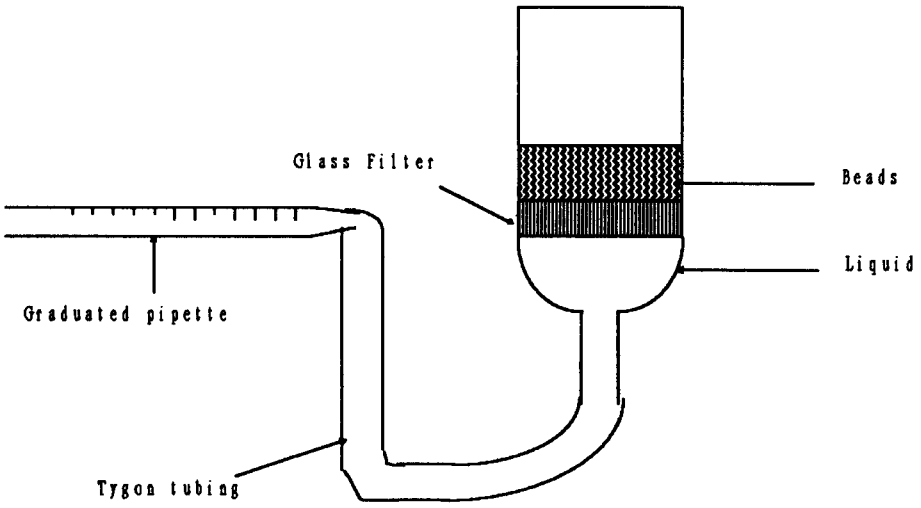

Figure 1. Apparatus for measurement of liquid uptake and swelling capacity.

mulated beads. In each measurement, the timing was started as soon as the bead surface touched the moist glass filter. The volumetric changes of simulated intestinal fluid in the pipette as a function of time were recorded. The water uptake rate profiles were generated by plotting the volume of liquid penetrated into the beads versus time.

Swelling Percentage of Antigen-Loaded Microspheres

The diameters of enteric-coated and non-enteric-coated beads were measured under a microscope before and after being exposed to 300 µl of liquid. The swelling percentage increase from the original volume was determined by Eq. (1).

$$\% \text{ swelling} = \left[\frac{V_{\text{after}} - V_{\text{before}}}{V_{\text{before}}}\right] \times 100 \quad (1)$$

where V indicates the volume of the sphere-shaped beads.

Enteric Film Coating Rupture Time

Ten enteric-coated antigen beads as a group from each formulation, with and without SSG, were tested. Each bead of a test group was placed under the microscope. The time after adding 400 µl simulated intestinal fluid to the point when the coating film began to rupture was noted.

Dissolution

The dissolution of LPS from enteric-coated microspheres with different percentages of SSG incorporated were studied by using a modified USP rotating basket method on a six-spindle dissolution tester (Vanderkamp® 6000, Van-Kel Industries, Inc., Chatham, NJ) at a speed of 100 rpm. Dissolution tests were performed in triplicate. Each replicate contained 3 g of non-SSG-incorporated ECAMs or SSG-incorporated ECAMs. The first hour of dissolution testing was conducted in enzyme-free pH 1.5 simulated gastric fluid, followed by transferring the coated beads into simulated intestinal fluid (pH 7.2) for the remainder of the test procedure. The amount of LPS released from ECAMs in the dissolution medium as a function of time, which was normalized each time with a batch antigen coating efficiency, was determined by a modified size-exclusion HPLC method with UV detection (20).

Antigen Coating Efficiency

From each batch of ECAMs product (with or without SSG), 5 g of antigen beads were randomly taken and ground into a fine powder and then transferred to a 50-ml volumetric flask with 30 ml of intestinal fluid. The powder sample was agitated for 4 hr. An aliquot of this solution was filtered through a 20-gauge 5-µm filter needle and assayed by the HPLC method aforemen-

tioned. These triplicate determinations were obtained and used as the batch antigen coating efficiency.

Statistical Analysis

The statistical significance of the differences in total volume of liquid uptake and swelling percentage was evaluated by an analysis of variance (ANOVA), and Turkey's Studentized range (HSD) test was used for multiple range analysis. The analysis was performed using SAS (SAS Institute, Cary, NC) with $p < 0.05$ being considered statistically significantly different for all tests.

RESULTS AND DISCUSSION

Determination of Volumetric Liquid Uptake of Antigen Microspheres

An increased amount of SSG disintegrant in the microspheres from 1% to 12% (w/w) resulted in an increase in the total volume of liquid taken up by the beads, as well as faster rates of liquid uptake (Figs. 2 and 3). At 60 min, the total volume of liquid taken up by 5%, 9%, and 12% SSG incorporated beads were significantly greater than that of 0%, 1%, and 3% SSG incorporated formulations (Fig. 2). However, among the 5%, 9%, and 12% SSG incorporated formulations, the total volume of liquid uptake was not significantly different ($p > 0.05$). The total volume of liquid uptake among 0%, 1%, and 3% SSG formulations was also not different. In non-enteric-coated beads, only 12% and 9% SSG incorporated beads absorbed more liquid than the non-SSG bead formulations. The total volume of liquid uptake of enteric-coated microspheres was significantly larger than that of non-enteric-coated beads (Figs. 2 and 3). This indicates that the enteric film of PMA-EA also absorbs liquid when it is exposed to the simulated intestinal fluid.

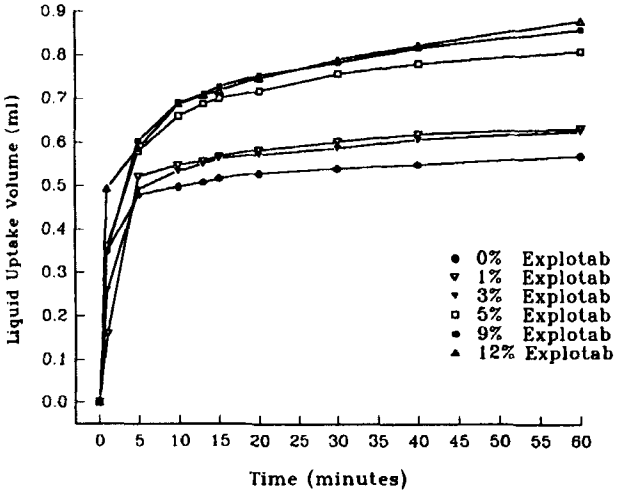

Figure 2. Volumetric liquid uptake rate profiles for enteric-coated antigen microspheres containing different levels of superdisintegrant. Each data point represents the mean value of 10 replicates.

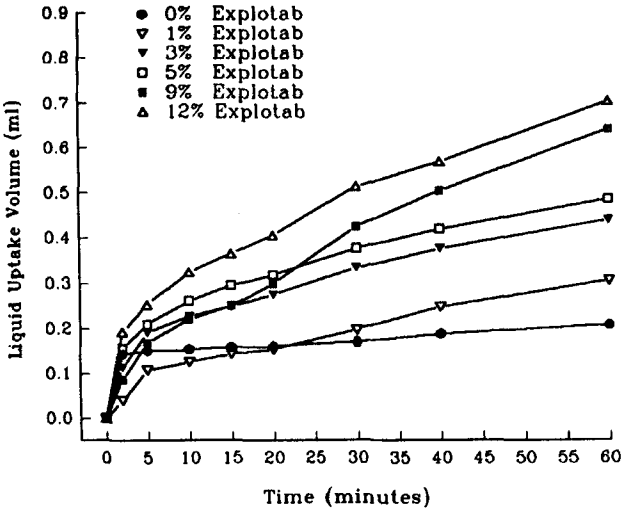

Figure 3. Volumetric liquid uptake rate profiles for non-enteric-coated antigen microspheres containing different levels of superdisintegrant. Each data point represents the mean value of 10 replicates.

Swelling Capacity

The percentage increase in swelling from the original volume of 5%, 9%, and 12% SSG incorporated enteric-coated beads was significantly greater than the 0% SSG formulation. Microspheres incorporated with 12% SSG have significantly larger swelling capacities than other formulations. The same results were observed in non-enteric-coated antigen microspheres (Figs. 4 and 5).

Enteric Film Coating Rupture Time and Dissolution of LPS

The time at which the enteric-coated film started to break up was influenced by the amount of the SSG in the formulation. The time at which the film began to break apart decreased as the amount of SSG in the formulation increased. It took 2.97, 1.86, 1.58, 1.34, 1.26

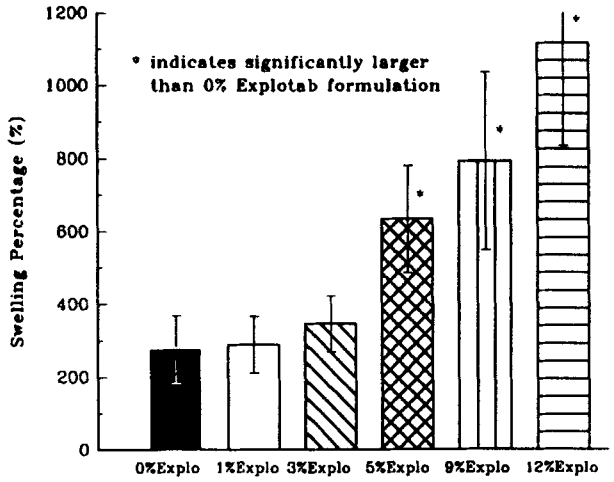

Figure 4. Effect of different levels of superdisintegrant on swelling capacity of enteric-coated antigen microspheres.

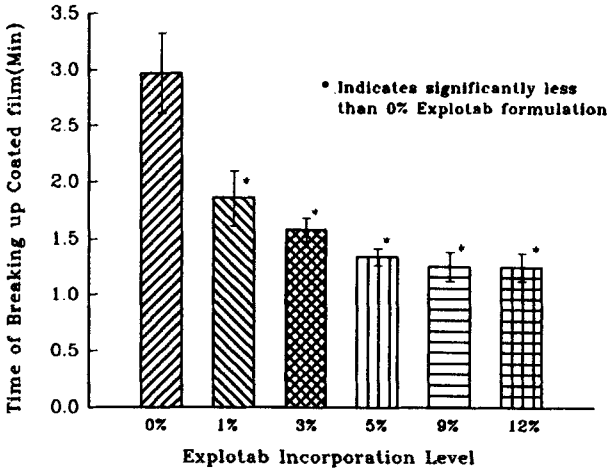

Figure 6. Effect of different levels of superdisintegrant on the rupture time of enteric film coat.

and 1.24 min for 0%, 1%, 3%, 5%, 9%, and 12% SSG incorporated beads to break apart the enteric film, respectively (Fig. 6). Beads with disintegrant incorporated require significantly less time to rupture the enteric film coat than non-disintegrant-incorporated beads. In vitro dissolution results indicate that with increasing amounts of SSG in the formulation, the antigen release rate increased. However, the initial release rate of LPS from ECAMs in simulated intestinal fluid was not significantly different when 1% or 3%, 5%, and 9% of SSG was incorporated in the beads (Fig. 7). When the amount of SSG was increased to 12%, the LPS release rate increased significantly.

There was no detectable LPS released from SSG incorporated ECAM up to 1 hr in pH 1.5 simulated gastric fluid. During the exposure of the microspheres to the acidic environment, the film coating showed minimum swelling. Lehmann et al. (21) indicated that the anionic polymethacrylates with coating thickness 20–40 μm have minimum swelling in the acid milieu of the

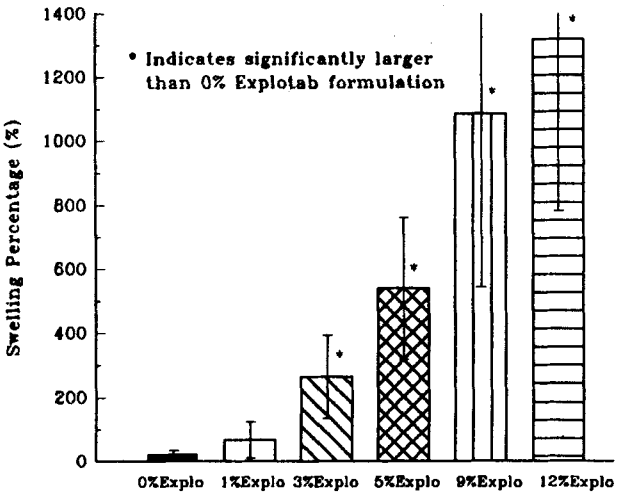

Figure 5. Effect of different levels of superdisintegrant on swelling capacity of non-enteric-coated antigen microspheres.

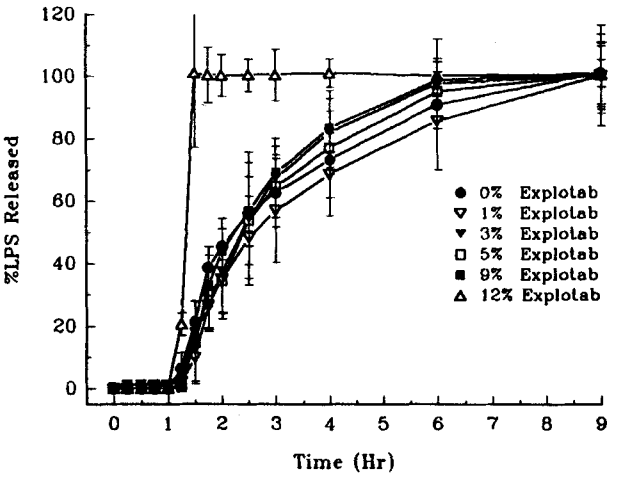

Figure 7. LPS dissolution rate profiles from enteric-coated antigen microspheres with different levels of superdisintegrant incorporated. Dissolution was conducted in pH 7.2 simulated intestinal fluid with 1 hr pH 1.5 simulated gastric fluid pretreatment.

stomach at pH 1–4 and remain largely impermeable within a period 2–4 hr and are free from technical imperfections. The present results confirm their observation. The average observed film thickness of all formulations in this study was about 26 μm, which is similar to Lehmann et al.'s findings (21).

Drug dissolution from tablet matrix or encapsulated dosage forms (8) can be improved significantly by the addition of disintegrating agents in the formulation. A superdisintegrant, such as sodium starch glycolate, is now frequently used in pharmaceutical formulations to improve the rate and extent of dissolution, and thereby increase the rate of drug absorption. When a superdisintegrant is incorporated into enteric-coated antigen microspheres, it may aid swelling processes, helping to release antigen upon the dissolving of the enteric-protected film when ECAMs reach the small intestine. Fully releasing antigens from the enteric-coated antigen delivery system in small intestine allows for better antigen absorption or uptake and, therefore may increase the amount of antigen available to the immune system. The faster antigen release rate and the significantly less time needed for breaking the film in SSG-incorporated formulations may be due to the swelling forces generated by a superdisintegrant. When the pH is increased to 7.2 in simulated intestinal fluid, the film coat swells and may create large pores on the surface. Because of the pores, water can easily penetrate into the film. As the water contacts the sodium starch glycolate, the disintegrant swells and creates sufficient swelling forces to break the film barrier, causing rapid release of LPS. The rapid antigen release rate of LPS from the ECAMs may be caused by a rapid absorption of water into beads plus the enormous swelling of the SSG particles. The swelling of enteric-coated beads is an important factor in controlling the release of a bioactive agent as it affects PMA–EA film permeability, the available surface area for contact with the surrounding solution, and the intrabead concentration of active agent.

It was observed that 12% SSG formulation has the fastest antigen release rate and the shortest time for rupturing the enteric film coat. This may be due to the fact that 12% SSG beads can take up more liquid and generate larger swelling forces. Incorporation of SSG in oral enteric-coated antigen microspheres provides rapid release of antigens at a high pH environment, which may improve the antigen absorption and/or uptake in the intestinal tract of the host, and thereby increase both its systemic and secretory immunities.

CONCLUSIONS

Water wicking (uptake) and disintegrant swelling represent two means commonly used in a determining disintegrant performance. Sodium starch glycolate has outstanding water wicking capacities and good swelling properties. This dual functionality translates into superior disintegrating characteristics in enteric-coated antigen microspheres. SSG also maintains good disintegrating properties after enteric film coating. SSG aids in breaking up an enteric-coated film of ECAM delivery systems and increases LPS dissolutions; it therefore may improve the system's subsequent availability and absorption. Incorporation of a superdisintegrant into an enteric-coated antigen delivery system can increase the release rate of a bioactive agent. Fluid-bed spray coating of the disintegrant onto the beads with the antigen provides a quick and effective way of speeding up the antigen release rate, which may be useful in improving availability of oral delivery of enteric-coated drugs, proteins, or other bioactive molecules, and may potentiate the effectiveness of oral vaccination.

ACKNOWLEDGMENTS

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