Research Article

Influence of Sodium Alginate on Hypoglycemic Activity of Metformin Hydrochloride in the Microspheres Obtained by the Spray Drying

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Received 23 March 2016; Accepted 27 April 2016

Academic Editor: Qiang Wei

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Alginate microspheres with metformin hydrochloride were prepared by the spray drying method in order to improve residence time of drug in the stomach. Nine formulations (F1–F9) with various drug:polymer ratio (1:2, 1:1, and 2:1) and different sodium alginate concentration (1%, 2%, and 3%) were evaluated for size, morphology, drug loading, Zeta potential, and swelling degree. In vitro drug release, mathematical release profile, and physical state of microspheres were also evaluated. Optimal formulation characterized by the highest drug loading was formulation F6 (drug:polymer ratio 2:1 and 2% alginate solution). Based on glucose uptake in Saccharomyces cerevisiae cells and α-amylase inhibition tests, it could be concluded that alginate microspheres enhance hypoglycemic activity of metformin hydrochloride evaluated in vitro. Designed microspheres are promising as alternative, multicompartment dosage form for metformin hydrochloride delivery.

1. Introduction

Sodium alginate (ALG) is nontoxic, biocompatible, and biodegradable polymer, which belongs to the group of polysaccharides naturally present in the seaweed [1, 2]. ALG is composed of monomers of β-D-mannuronic acid and α-L-guluronic acid residues joined together by (1–4) glycoside linkages. It is a biopolymer widely used for dietetic, biotechnology, cosmetic, and pharmaceutical industry [3]. Mucoadhesiveness and ability to gelate make ALG a promising excipient in the development of various dosage forms for modified drug delivery. In contact with acidic pH, ALG cross-links and forms swollen polymer matrix which acts as a reservoir enabling sustained drug release [4, 5]. Additionally, ALG is characterized by the ability to reduce body weight and to control glycemia in diabetic individuals by reduction after meal fluctuations of glucose concentrations, insulin secretion, decreasing of food intake, and delaying gastric emptying [6–8].

Metformin hydrochloride (MF) is an orally administered antidiabetic agent from biguanide group, which is the first line therapy to treat type 2 diabetes. Its hypoglycemic action includes decrease in the hepatic glucose production and in the intestinal glucose absorption and increase in glucose metabolism which, in consequence, lead to the reduction in plasma glucose concentration. In addition, MF decreases appetite and has effect on weight reduction and improvement of the lipid profile with no risk of hypoglycemia. MF is water soluble, but its bioavailability after oral administration in conventional dosage forms is only about 50% [9, 10]. Increase in the stomach residence time and the improvement in drug bioavailability might be achieved by mucoadhesive dosage forms. Mucoadhesive drug delivery systems through the intimate contact with the absorption surface enable prolonged residence time, better drug absorption, and enhanced bioavailability and they also permit decrease in the drug dosing frequency. Microspheres are multicompartment dosage forms which provide improved efficacy, reduced toxicity, and larger margin of safety in case of dosage form damage compared with traditional single unit formulations. Mucoadhesive polymer’s matrix enables them to adhere to the mucous membranes and to remain for longer time period in the gastrointestinal tract [11, 12].

One of the most widely reported methods for preparing ALG microspheres with MF is emulsion-cross-linking and nonaqueous evaporation [13–16]; therefore, the aim of this
research was to attempt to formulate for the first time ALG microspheres with MF by the new spray drying technique. Obtained microspheres were characterized for size, morphology, entrapment efficiency, drug loading, Zeta potential, and the in vitro MF release. The effects of the drug: polymer ratio and concentration of ALG solution on the properties of microspheres were also studied. Mucoadhesive properties of the microspheres were examined by using TA.XT.Plus Texture Analyzer and three different models of adhesive layer: gelatin disc, mucin gel, and porcine stomach mucosa. The physical state of microspheres was determined by differential scanning calorimetry (DSC). Additionally, the hypoglycemic properties of microspheres and the influence of ALG on MF activity were studied by determination of glucose uptake in Saccharomyces cerevisiae cells and by α-amylase inhibition test.

2. Experimental Section

2.1. Materials. Metformin hydrochloride (MF) was obtained from Debao Fine Chemical CO (Henan, China). Sodium alginate (ALG) low viscosity (2%, 100–300 cP), mucin type II, and gelatin type B from bovine skin were purchased from Sigma Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, methanol, propan-1,2-diol, acetonitrile, and starch pure soluble were obtained from Prolab (Nakło, Poland). Water was obtained from the veterinary service (Turośń Kościelna, Poland). Samples were stored at −20°C and before the experiment were defrosted and cut into 5 mm in diameter and 2 mm thick pieces. Saccharomyces cerevisiae was purchased from Lesaffre (Wolczyn, Poland), acarbose was obtained from Bayer Pharma AG (Berlin, Germany), and α-amylase was obtained from Polfa S. A. (Warsaw, Poland).

2.2. Formulation of Microspheres Containing MF. Microspheres were prepared using Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland). The flow rate was set to 4.5 mL/min, relative spray rate was fixed to 37 m³/h, and spray flow was fixed to 600 L/h. The inlet and outlet temperatures were established at 200°C and 96°C, respectively. The parameters of the spray drying were optimized in a number of preliminary tests. ALG of different concentrations (1%, 2%, and 3%) and different drug: polymer ratio (1 : 1, 1 : 2, and 2 : 1) was sprayed to obtain different formulations of microspheres (F1–F9).

2.3. Characteristics of Microspheres

2.3.1. Morphology and Size. Microspheres were analyzed by optical microscope (Motic BA400, Wetzlar, Germany) and by scanning electron microscope (SEM) (Hitachi S4200, Tokyo, Japan). Before imaging, samples were sputter-coated with gold in an argon atmosphere (Leica EM AC 2000, Wetzlar, Germany). The microspheres size distribution was studied using Zetasizer NanoZS90 (Malvern Instruments, Malvern, UK) by laser light-diffraction technique after suspending in propane-1,2-diol (propane-1,2-diol was used because in aqueous medium swelling and dissolving of microspheres were observed).

2.3.2. HPLC Analysis. The concentration of MF in the medium was determined by the HPLC system Agilent Technologies 1200 equipped with a G1312A binary pump, a G1316A thermostat, a G1379B degasser, and a G1315B diode array detector (Agilent, Waldbronn, Germany). Data collection and analysis were performed using Chemstation 6.0 software. Isocratic separation was achieved on Waters Spherisorb® 5.0 μM ODS 4.6 × 250 mm, 5 μm column (Waters Corporation, Milford, USA). Mobile phase was acetonitrile : methanol : phosphate buffer, pH 3.0 (20 : 20 : 60, v/v), the flow rate was 1.0 mL/min, and UV detection was performed at a wavelength of 240 nm [17]. The column temperature was maintained at 25°C. For injection into the HPLC system, 20 μL of sample was used. All reagents used for analysis were HPLC grade. The retention time of MF was 2.8 min. Standard calibration curve was linear over the range of 1–100 μg/mL with the correlation coefficient (R²) 0.999.

2.3.3. MF Loading and Percentage Yield. MF loading in the microspheres was determined by dissolving an accurately weighted amount of microspheres (20 mg) in 10 mL of distilled water and agitating it for 24 h at 150 rpm in a water bath [18]. The sample solution was further diluted and filtrated through 0.45 μm cellulose acetate Millipore filters (Billericia, USA). The percentage yield of MF in the ALG microspheres was determined by using the following formula:

\[ L = \frac{Q_m}{W_m} \times 100, \]  

where \( L \) is the percentage of drug loading, \( Q_m \) is the drug load in the microspheres, and \( W_m \) is the weight of the microspheres. The mean drug encapsulation efficiency was calculated by the following equation:

\[ EE = \frac{Q_s}{Q_t} \times 100, \]  

where \( EE \) is the percentage of encapsulation efficiency, \( Q_s \) is the actual drug content, and \( Q_t \) is the theoretical drug content. Percentage production yield was calculated as the relationship of the achieved weight of the microspheres related to the entire amount of the theoretical weight of drug and polymer:

\[ Y = \frac{W_m}{W'_i} \times 100, \]  

where \( Y \) is the percentage production yield, \( W_m \) is the weight of microspheres, and \( W'_i \) is the theoretical weight of drug and polymer.

2.3.4. Microspheres Porosity. Microspheres porosity was determined by using the solvent replacement method [19].
Dried microspheres were immersed overnight in absolute ethanol and weighed after excess ethanol on the surface was blotted. Then, porosity was calculated based on the following equation:

\[ P\% = \left( \frac{W_W - W_D}{\rho \times V} \right) \times 100, \]

where \( P\% \) is the porosity, \( W_W \) is the weight of microspheres before immersion in absolute ethanol, \( W_D \) is the weight of microspheres after immersion in absolute ethanol, \( \rho \) is the density of absolute ethanol, and \( V \) is the volume of microspheres.

2.3.5. Zeta Potential. Zeta potential measurements were performed using a Zetasizer NanoZS90 (Malvern Instruments, Malvern, UK). Before measurements, microspheres were suspended in propan-1,2-diol. Data was obtained from Zetasizer software 6.20.

2.3.6. Swelling Properties. Swelling properties were evaluated at \( 37 \pm 1^\circ C \) in beakers containing 25 mL of 0.1 M HCl (pH 1.2) and stirred at 100 rpm. The microspheres were periodically weighted at predetermined time intervals until a constant weight was obtained [20]. The swelling ratio was calculated by using the following formula:

\[ \text{SR} = \frac{W_S - W_0}{W_0} \times 100, \]

where \( \text{SR} \) is the swelling ratio, \( W_0 \) is the initial weight of microspheres, and \( W_S \) is the weight of microspheres after swelling.

2.3.7. Mucoadhesiveness. Evaluation of mucoadhesiveness was performed using TA.XT.Plus Texture Analyzer (Stable Micro Systems, Godalming, UK) and three different models of mucoadhesive material: gelatin disc, mucin gel, and porcine stomach mucosa. Experimental parameters of the process were chosen during preliminary tests and set as follows: pretest speed 0.5 mm/s, test speed 0.1 mm/s, contact time 180 s, posttest 0.1 mm/s, and applied force 1 N. Gelatin discs were prepared by pouring 30% (w/w) aqueous solution into a Petri dish. Layer of mucin was prepared by absorbing 10% mucin gel on discs with cellulose fiber (5 mm in diameter). The tests were conducted at \( 37 \pm 1^\circ C \). Adhesive layers were adhered to an upper probe and moisturized (excepted mucin) with 0.1 M HCl (pH 1.2) [21]. The mucoadhesive properties were determined as the maximum detachment force \( (F_{\text{max}}) \) and the work of mucoadhesion \( (W_{\mu}) \), calculated from the area under the force versus distance curve, expressed in J.

2.4. In Vitro MF Release. For the in vitro MF release test, apparatus type I (Erweka Dissolution tester type DT 600HH, Heusenstamm, Germany) was used [22]. Microspheres were placed in the basket, immersed in 500 mL of 0.1 M HCl (pH 1.2), and stirred at 50 rpm. In each study, the amount of microspheres equivalent to 500 mg of MF was analyzed. Samples were withdrawn and filtered through 0.45 μm cellulose acetate Millipore filters (Billerica, USA) at predetermined time intervals and replaced with fresh dissolution medium [23]. During the dissolution process, the temperature was maintained at \( 37 \pm 1^\circ C \). The amount of released MF was analyzed by HPLC method (as described in Section 2.3.2).

2.5. Mathematical Modeling of MF Release Profile. MF release data were analyzed according to zero-order kinetic, first-order kinetic, Higuchi model, Korsmeyer-Peppas equation, and Hixson-Crowell cube root law to characterize mechanism of the drug release. The constants of release kinetics and the regression coefficients \( (R^2) \) were calculated from the slope of plots by linear regression analysis.

Zero-order kinetic is as follows:

\[ F = k \times t. \]

First-order kinetic is as follows:

\[ \ln F = k \times t. \]

Higuchi model is as follows:

\[ F = k \times \sqrt{t}. \]

Korsmeyer-Peppas model is as follows:

\[ F = k \times t^n. \]

Hixson-Crowell model is as follows:

\[ 1 - (1 - F)^{1/3} = kt, \]

where \( F \) is the fraction of the drug release, \( k \) is the release constant, and \( t \) is the time. For the Korsmeyer-Peppas model, the fraction of drug remaining at time \( t \) was determined for every time interval \( \log (M_t/M_{\infty}) \) and plotted against the log of time \( t \). The slope of the line was taken as the value of \( n \), diffusional release exponent used for interpretation of release mechanism [24, 25].

2.6. Differential Scanning Calorimetry (DSC). DSC analysis of MF, ALG, and microspheres formulation F6 (with the highest drug loading) was performed using an automatic thermal analyzer system (DSC TEQ2000, TA Instruments, New Castle, USA). Each sample was precisely weighted (5 mg) and placed in sealed aluminium pan. An empty pan sealed was used as a reference. Temperature calibrations were performed using indium and zinc as standard. Samples were heated from 25°C to 200°C at scanning rate of 10°C/min under nitrogen flow of 20 mL/min [26].

2.7. Evaluation of ALG Influence on MF Hypoglycemic Activity

2.7.1. Glucose Uptake by Saccharomyces Cerevisiae Cells. Glucose uptake by Saccharomyces cerevisiae cells is often used model to in vitro study hypoglycemic activity [27–29]. Cells
were grown at 30 ± 1°C in a bottom flask containing 500 mL of sterilized modified minimal medium pH 5.3 for yeast growth (0.5 g KH₂PO₄, 0.5 g (NH₄)₂SO₄, 0.5 g MgSO₄, 1 g yeast extract, and 10 g glucose) [30]. Cells growth started from a stationary preculture of about 10⁷ cfu/mL. Then, cells were washed three times with distilled water and centrifugated (3,000 xg, 5 min). The cytocrsit was adjusted to 10% cells [31]. MF, microspheres placebo, and microspheres formulation F6 were added to 1 mL of various concentrations of glucose solution (5, 10, and 25 mM) and incubated for 10 min at 37 ± 1°C. Reaction was started by adding 100 μL of yeast suspension; then, mixture was vortexed and incubated at 37 ± 1°C for 60 min. After incubation, mixtures were centrifuged (2,500 xg, 5 min) and concentration of glucose remaining in the medium was estimated by using glucose assay kit (One Touch Select, Johnson & Johnson, New Brunswick, USA) [27].

\[ \text{% IA} = \left( \frac{C_S - C_C}{C_S} \right) \times 100, \]  

where IA is the percent of α-amylase inhibition, \( C_C \) is the concentration of starch in the control reaction, and \( C_S \) is the concentration of starch in the test sample [34–36].

### 2.8. Statistical Analysis.

Quantity variables were expressed as the mean and standard deviation. Statistical analysis was performed using nonparametric Kruskal-Wallis test and conducted by Statistica 10.0 software (StatSoft, Tulsa, USA). Differences between groups were considered to be significant at \( p < 0.05 \).

### 3. Results and Discussion

#### 3.1. Microspheres Morphology, Size, and Surface Charge Analysis

Spray drying is a relatively simple, one-step process which includes spray drying of drug solution or suspension in a stream of gas and depends on several parameters, for example, atomization devices, aspirator and feed rate, drying temperature, spray air flow, and properties of the drying material [37]. This method enables entrapment of both hydrophilic and hydrophobic drugs in polymer matrix and compared to other techniques (emulsification-precipitation, emulsification-cross-linking, ionotropic gela-

\[ \text{Porosity} = \left( 1 - \frac{\text{Volume of polymer}}{\text{Volume of dried material}} \right) \times 100 \]  

This formula is used to calculate the porosity of the microspheres. The results are presented in Table 1. The high porosity of the microspheres indicates that they can act as a reservoir for the drug, which can be released over time.

### 3.2. α-Amylase Inhibition

\[ \text{% IA} = \left( \frac{C_S - C_C}{C_S} \right) \times 100, \]  

\[ \% \text{ Inhibition} = \left( \frac{C_S - C_C}{C_S} \right) \times 100, \]  

where IA is the percent of α-amylase inhibition, \( C_C \) is the concentration of starch in the control reaction, and \( C_S \) is the concentration of starch in the test sample [34–36].

### 3.3. Encapsulation Efficiency

\[ \% \text{ Encapsulation Efficiency} = \left( \frac{C_S - C_C}{C_S} \right) \times 100, \]  

where \( C_C \) is the concentration of starch in the control reaction, \( C_S \) is the concentration of starch in the test sample, and IA is the percent of α-amylase inhibition.

### 3.4. Production Yield

\[ \% \text{ Production Yield} = \left( \frac{C_S - C_C}{C_S} \right) \times 100, \]  

where \( C_C \) is the concentration of starch in the control reaction, \( C_S \) is the concentration of starch in the test sample, and IA is the percent of α-amylase inhibition.

### Table 1: Characteristics of MF loaded ALG microspheres (formulations F1–F9).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug:polymer ratio</th>
<th>Zeta potential (mV)</th>
<th>Production yield (%)</th>
<th>Encapsulation efficiency (%)</th>
<th>Percent loading (%)</th>
<th>Mean diameter (μm)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:2</td>
<td>−5.3 ± 0.3</td>
<td>571 ± 1.6</td>
<td>119.8 ± 2.1</td>
<td>39.9 ± 1.7</td>
<td>3.5 ± 0.8</td>
<td>73.39 ± 2.3</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>−3.4 ± 0.4</td>
<td>861.1 ± 2.4</td>
<td>111.9 ± 2.7</td>
<td>55.6 ± 3.4</td>
<td>3.4 ± 1.1</td>
<td>67.02 ± 4.2</td>
</tr>
<tr>
<td>F3</td>
<td>2:1</td>
<td>−1.4 ± 0.9</td>
<td>85.9 ± 1.4</td>
<td>109.4 ± 3.2</td>
<td>72.9 ± 1.6</td>
<td>1.7 ± 0.9</td>
<td>60.71 ± 3.5</td>
</tr>
<tr>
<td>F4</td>
<td>1:2</td>
<td>−5.8 ± 2.3</td>
<td>61.8 ± 1.5</td>
<td>115.4 ± 1.9</td>
<td>38.4 ± 2.2</td>
<td>5.7 ± 1.1</td>
<td>75.04 ± 2.5</td>
</tr>
<tr>
<td>F5</td>
<td>1:1</td>
<td>−4.2 ± 0.9</td>
<td>68.0 ± 1.3</td>
<td>103.1 ± 2.1</td>
<td>51.5 ± 3.6</td>
<td>3.6 ± 0.5</td>
<td>65.53 ± 2.4</td>
</tr>
<tr>
<td>F6</td>
<td>2:1</td>
<td>−1.3 ± 0.7</td>
<td>61.7 ± 2.1</td>
<td>113.4 ± 2.3</td>
<td>75.6 ± 1.5</td>
<td>3.0 ± 1.6</td>
<td>61.81 ± 3.7</td>
</tr>
<tr>
<td>F7</td>
<td>1:2</td>
<td>−7.7 ± 3.8</td>
<td>68.9 ± 1.1</td>
<td>111.9 ± 2.6</td>
<td>37.3 ± 3.9</td>
<td>4.0 ± 0.8</td>
<td>72.97 ± 3.5</td>
</tr>
<tr>
<td>F8</td>
<td>1:1</td>
<td>−3.7 ± 1.5</td>
<td>58.9 ± 1.4</td>
<td>111.1 ± 2.9</td>
<td>55.7 ± 3.9</td>
<td>2.5 ± 1.3</td>
<td>60.88 ± 2.9</td>
</tr>
<tr>
<td>F9</td>
<td>2:1</td>
<td>−1.8 ± 1.1</td>
<td>45.0 ± 2.1</td>
<td>110.4 ± 3.1</td>
<td>73.6 ± 7.5</td>
<td>1.6 ± 0.4</td>
<td>59.70 ± 2.5</td>
</tr>
</tbody>
</table>
observed in formulations F7, F4, and F1 with drug : polymer ratio 1:2 (37.3±3.9%, 38.4±2.2%, and 39.9±1.7%, resp.). The maximum drug loading was in formulations F6, F9, and F3 (75.6±1.5%, 73.6±7.5%, and 72.9±1.6%) when drug : polymer ratio was 2:1. Interestingly, concentration of ALG solution had no significant impact on microspheres characteristics. Application of more concentrated solution of ALG (3%) did not result in higher production yield and diameter of obtained microspheres. It is worth noting that encapsulation efficiency in all microspheres was higher than 100%. It can be explained by a partial loss of the polymer during the preparation of microspheres, which decreased the theoretical polymer mass and changed the theoretical drug content to values higher than the previewed ones [41]. All formulations of microspheres had spherical shape. The morphology of microspheres F6 (with the highest MF loading) examined by optical and scanning electron microscopy is presented in Figure 1.

Zeta potential is an important parameter related to the stability of colloidal dispersions [42]. Zeta potential values of designed microspheres varied between −1.3 ± 0.7 and −7.7 ± 3.8 mV as shown in Table 1 (compared to −15.9 mV for ALG microspheres placebo). Although ALG is negatively charged, values of Zeta potential of microspheres with MF were close to zero. It is probably caused by complex formation between the polyanionic ALG and oppositely charged MF. Formulation F6 (with the highest drug loading) was characterized by the lowest Zeta potential value.

3.2. Swelling and Mucoadhesive Properties. Mechanism of mucoadhesion is directly connected with water uptake into the polymer matrix, swelling, and gel layer formation [43]. Swelling is mainly attributed to the hydration of the hydrophilic groups of ALG, where water penetrates inside the microspheres and fills the inert pores among polymer chains. ALG swelling and dissolution are pH dependent. At acidic pH, as a result of reduction of the electrical repulsion between the negatively charged ALG molecules and positively charged ions in the medium, polymer is protonated and creates insoluble form of alginic acid [44, 45]. After protonation of carboxylic acid groups, the polymer shrinks, absorption of water is decreased, and in consequence swelling degree decreases [43]. The swelling profiles, represented as the swelling ratio (SR) versus time, are reported in Figure 2.

Figure 2 illustrates the swelling properties of the designed microspheres in 0.1 M HCl (pH 1.2) at different time intervals. All the curves gave linear increase, indicating a loosening of the matrix with the creation of larger pores [46]. The results indicated that formulations of microspheres swelled.
gradually. Obtained curves showed an initial rapid increase within 30 min of the experiment due to the entry of water via metastable pores known as hysteresis of swelling mechanism and reached the highest value after 120 min for formulations F1–F9 and after 180 min for microspheres placebo. Formulation F7 (with the lowest MF content) showed the highest swelling ratio, while microspheres F6 (with the highest MF loading) attained lower value of SR. Swelling in acidic pH as a result of osmosis caused by unbound carboxyl groups enables forming a swollen gel and surface erosion, which results in disintegration of microspheres and sustained MF release.

The mucoadhesive drug delivery systems increase drug residence time and in consequence improve its bioavailability. ALG as polyanionic polymer is characterized by higher bioadhesive properties than polycationic (chitosan, poly(l-lysine)) or nonionic polymers (macrogol, hypromellose and polyvinyl alcohol) [47, 48]. Mechanism of ALG mucoadhesion is defined by interaction between carboxyl groups of polymer and mucin through electrostatic adsorption, van der Waals, and hydrogen bonds. The surface of mucin is positively charged and its flexible backbone chains enable interaction with ALG carboxyl groups. Initially, contact (wetting) between polymer and mucus and, subsequently, polymer swelling make the polymer strands relax [47, 48]. This is followed by the penetration of ALG into the mucus network and finally the formation of secondary chemical bonds between the mucus and the polymer. The influence of MF on the mucoadhesive properties of ALG microspheres is shown in Table 2.

The mucoadhesive properties were presented as maximum detachment force ($F_{\text{max}}$) and work of adhesion ($W_{\text{ad}}$) and as the adhesive layers gelatin discs, mucin gel, and porcine stomach mucosa were used. Porcine stomach mucosa model is often used in order to imitate in vivo conditions [49]. It was shown that ALG microspheres readily adhered to all tested mucoadhesive materials and that detachment force and work of adhesion increased when drug loading was decreased. When gelatin was used as adhesive layer, relatively large maximum detachment force and small values of the work of adhesion were observed. This fact might indicate that microspheres adhere to this layer for very short time, forming only weak bonds between gelatin and alginate. The lower mucoadhesiveness of microspheres F3, F6, and F9 is probably due to the high MF content causing disturbances in ALG gel structure. It is worth noting that significant ($p < 0.05$) differences of detachment force and work of adhesion values between gelatin and porcine stomach mucosa were observed; therefore, it might be suggested that mucin gel is a better substitute of mucous membrane.

Moreover, the obtained results indicate that there is direct correlation between mucoadhesive properties and SR. The highest values of $F_{\text{max}}$ ($0.9 \pm 0.1$) and $W_{\text{ad}}$ ($768.2 \pm 5.4$) were observed for formulation F7 with the highest SR value.
Table 2: Mucoadhesive properties of microspheres placebo (F0) and microspheres formulations F1–F9.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Type of adhesive layer</th>
<th>Gelatin</th>
<th>Mucin</th>
<th>Porcine stomach mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F_{\text{max}}$ [N]$^*$</td>
<td>$W_{\text{ad}}$ [$\mu$J]$^{**}$</td>
<td>$F_{\text{max}}$ [N]$^*$</td>
</tr>
<tr>
<td>F0</td>
<td></td>
<td>0.9 ± 0.3</td>
<td>362.9 ± 1.8</td>
<td>1.1 ± 3.7</td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>237.5 ± 1.0</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>F2</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>203.0 ± 0.6</td>
<td>0.5 ± 0.3</td>
</tr>
<tr>
<td>F3</td>
<td></td>
<td>0.4 ± 0.3</td>
<td>179.5 ± 0.4</td>
<td>0.5 ± 0.4</td>
</tr>
<tr>
<td>F4</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>392.8 ± 2.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>F5</td>
<td></td>
<td>0.4 ± 0.2</td>
<td>264.0 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>F6</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>238.3 ± 0.5</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>F7</td>
<td></td>
<td>0.5 ± 0.3</td>
<td>319.4 ± 1.5</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>F8</td>
<td></td>
<td>0.5 ± 0.2</td>
<td>303.7 ± 1.3</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>F9</td>
<td></td>
<td>0.4 ± 0.1</td>
<td>234.2 ± 0.6</td>
<td>0.7 ± 0.3</td>
</tr>
</tbody>
</table>

$^*$Maximum detachment force and $^{**}$work of adhesion.

Figure 3: MF release from microspheres formulations F1–F3 (a), F4–F6 (b), and F7–F9 (c), $n = 3$.

3.3. In Vitro MF Release. The release profiles of MF from microspheres formulations F1–F9 are shown in Figure 3. In all formulations, the release profile showed a burst effect, which is the first phase of the drug release and occurs due to the free MF binding at the microparticles surface. The highest burst effect was observed in formulation F6, with the highest drug loading; after 30 min, 41.7 ± 0.4% of MF was released. At acidic pH, ALG swells and creates gelling alginic acid matrix, which prevents disintegration of microspheres and controls water penetration inside the microsphere structure. Formulations F1, F4, and F7, with the lowest MF content, were characterized by the higher swelling ratio and ensured
Table 3: Models of MF release from microspheres formulations F1–F9.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero-order kinetics</th>
<th>First-order kinetics</th>
<th>Higuchi model</th>
<th>Korsmeyer-Peppas model</th>
<th>Hixson-Crowell model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>$K$</td>
<td>$R^2$</td>
<td>$K$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>F1</td>
<td>0.92</td>
<td>1.67</td>
<td>0.17</td>
<td>0.98</td>
<td>22.98</td>
</tr>
<tr>
<td>F2</td>
<td>0.86</td>
<td>1.68</td>
<td>0.55</td>
<td>0.95</td>
<td>29.37</td>
</tr>
<tr>
<td>F3</td>
<td>0.41</td>
<td>4.07</td>
<td>0.18</td>
<td>0.56</td>
<td>16.82</td>
</tr>
<tr>
<td>F4</td>
<td>0.87</td>
<td>6.69</td>
<td>0.22</td>
<td>0.96</td>
<td>25.03</td>
</tr>
<tr>
<td>F5</td>
<td>0.82</td>
<td>6.55</td>
<td>0.13</td>
<td>0.93</td>
<td>25.15</td>
</tr>
<tr>
<td>F6</td>
<td>0.52</td>
<td>4.62</td>
<td>0.21</td>
<td>0.66</td>
<td>18.76</td>
</tr>
<tr>
<td>F7</td>
<td>0.90</td>
<td>7.34</td>
<td>0.22</td>
<td>0.96</td>
<td>27.11</td>
</tr>
<tr>
<td>F8</td>
<td>0.70</td>
<td>6.01</td>
<td>0.37</td>
<td>0.84</td>
<td>23.64</td>
</tr>
<tr>
<td>F9</td>
<td>0.45</td>
<td>5.15</td>
<td>0.25</td>
<td>0.60</td>
<td>21.33</td>
</tr>
</tbody>
</table>

$R^2$: correlation coefficient, $K$: release constant, and $n$: the release exponent.

sustained drug release; after 8 h of the study, the amount of released MF was $82.6 \pm 1.6\%$, $88.9 \pm 2.2\%$, and $89.1 \pm 3.2\%$, respectively.

Afterwards, MF release profiles were fitted to zero-order and first-order equations and Higuchi, Korsmeyer-Peppas, and Hixson-Crowell models (Table 3).

In the Higuchi model, the best fit curve with $R^2$ (0.98) was observed for formulation F1. It was also noticed that mechanism of MF release from formulations F1, F2, F4, F5, and F7 was diffusion controlled first-order kinetics as the plots showed higher regression correlation coefficient. However, based on Korsmeyer-Peppas equation in case of sphere shape microspheres, the value of diffusional exponent $n \geq 0.85$ means that drug release is independent of time (case II transport), while value $n \leq 0.43$ means that release is controlled by Fickian diffusion. A value between 0.43 and 0.85 indicates combination of diffusion and swelling mechanism. The obtained values of diffusion exponent from 0.08 to 0.22 confirm diffusion as mechanism of MF release. The high values of $R^2$ in the Hixson-Crowell model indicate that this equation can also describe MF release and suggest that it was controlled also by disintegration process of the microspheres. The Hixson-Crowell cube root law describes the release from the systems, where drug release depends on the change in surface area and diameter of the particles with time and it is mainly applied to systems which are subjected to dissolution or erosion processes. In this case, MF release rate is limited by the microspheres erosion. The obtained data have shown that MF release from ALG microspheres is complex and includes simultaneously erosion and diffusion mechanism. MF release can be controlled by water penetration, responsible for polymer hydration, erosion of microspheres, and drug diffusion [50, 51].

3.4. Differential Scanning Calorimetry (DSC). DSC is an important tool to obtain information about possible interactions between drug and polymer, according to the appearance, shift, or disappearance of endothermic or exothermic peaks. Thermograms of MF, ALG, microspheres placebo, and microspheres formulation F6 (with the highest MF loading) are shown in Figure 4.

Under the experimental conditions, a sharp endothermic peak for pure MF was observed at 233.02°C corresponding to its melting point. Thermogram of microspheres formulation F6 has shown that peak of MF did not shift significantly (226.55°C). Lowering of MF melting point in microspheres might be due to its mixing with ALG, which lowered the purity of each component; MF crystallinity was reduced and the drug might convert into the amorphous form. In the DSC thermogram, a small endothermic peak of ALG at 129.55°C, attributed to the dehydration process, and strong exothermic peak at 247.80°C, corresponding to the decomposition of the polymer, were observed. Melting peak of ALG (129.27°C) was not detected in microspheres F6, which might suggest that ALG has dehydrated during the spray drying process [52].

3.5. Influence of ALG on MF Hypoglycemic Activity. Type 2 diabetes is a chronic metabolic disorder characterized by
 Obtained results indicate that MF significantly increased glucose uptake by *Saccharomyces cerevisiae* cells compared to the control sample. The uptake of glucose by *Saccharomyces cerevisiae* cells incubated with formulation F6 was lower than that by cells incubated with pure MF. This effect was observed at all examined glucose concentrations (5, 10, and 20 mM). In case of 10 mM glucose solution, concentration of glucose remaining in the medium was 3.13 ± 1.69 mM for microspheres F6, 1.50 ± 0.23 mM for pure MF, and 9.01 ± 1.61 mM for microspheres placebo. The highest glucose uptake inhibition was observed when *Saccharomyces cerevisiae* cells were incubated with microspheres placebo, which suggests that pure ALG demonstrates the highest intestinal glucose absorption inhibitory potential.

### 3.5.2. α-Amylase Inhibition

Therapeutic strategy of hyperglycemia in the treatment of type 2 diabetes involves decreasing the postprandial glucose concentration in the blood [53]. α-Amylase is an intestinal enzyme which plays an important role in the carbohydrate digestion. It hydrolyses α-bonds of polysaccharides (such as glycogen and starch) to glucose and maltose. Inhibitors of this enzyme delay carbohydrate digestion through binding to α-bonds and prevent decomposition of polysaccharides into mono- and disaccharides [62, 63]. This action prolongs the overall time for carbohydrate digestion and results in a reduction of glucose absorption. Consequently, inhibition of α-amylase reduces the postprandial fluctuations of blood glucose and decreases glycemic index of food [64–66]. It was shown that seaweeds can reduce activity of α-amylase; therefore, impact of ALG microspheres with MF on enzyme activity was studied. The results of α-amylase inhibition test are illustrated in Figure 5.

Inhibition of enzyme activity by microspheres F6 was associated with the amount of polymer; samples containing higher amount of ALG more effectively inhibited α-amylase. The values of enzyme inhibition were ranged from 5.35 ± 0.32% (for 7.56 mg of MF) to 63.82 ± 2.83% (for 10 mg of microspheres placebo) (Figure 5(a)). Microspheres formulation F6 was characterized by stronger α-amylase inhibition activity (36.34 ± 2.45%) compared to pure MF.

### 4. Conclusions

MF release and mucoadhesive properties of ALG microspheres obtained by the spray drying can be altered by varying the drug:polymer ratio. Optimal formulation characterized by the highest drug loading was formulation F6 (drug:polymer ratio 2:1 and 2% alginate solution). All microspheres possessed swelling and mucoadhesive properties depending on drug and polymer content. The release profile of MF from microspheres was prolonged and controlled by Fickian diffusion. Based on the *in vitro* hypoglycemic activity evaluation, it can be concluded that ALG microspheres enhance activity of MF. It was observed that ALG affects inhibition of glucose uptake in *Saccharomyces cerevisiae*.
cerevisiae cells and reduces α-amylase activity. As inhibition of intestinal glucose absorption is crucial in type 2 diabetes treatment, ALG might be valuable excipient in designing dosage forms with MF. Designed microspheres seem to be promising as alternative, multicompartement dosage form for metformin hydrochloride delivery. However, in vivo evaluation of ALG influence on hypoglycemic activity of MF in the microspheres is necessary and will be described in a due course.

Competing Interests

The authors declare no competing interests.

Acknowledgments

This research was conducted with the use of equipment purchased by the Medical University of Białystok as part of the OP DEP 2007–2013, Priority Axis I.3, Contract no. POPW.01.03.00–20–008/09, and supported by Medical University of Białystok Grant (no. N/ST/MN/16/001/2215).

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