ORIGINAL ARTICLE

CHITOSAN MICROPARTICLES LOADED WITH ANTIDIABETIC DRUGS – PREPARATION AND CHARACTERIZATION

IUSTINA AVRAM[1], FLORENTINA GIANINA LUPAŞCU[1], LUMINIŢA CONFEDERAT[1], SANDRA MĂDĂLINA CONSTANTIN[1], CRISTINEL IONEL STAN[2]*, LENUŢA PROFIRE[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universităţii Street, 700115, Iaşi, România*
[2]*Department of Morphofunctional Science, Faculty of Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universităţii Street, 700115, Iaşi, România*

**corresponding author: cristi_stan00@yahoo.com*

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Abstract

The objective of this study was to develop new binary polymeric systems based on chitosan in order to achieve an improvement of the pharmacokinetic and pharmacological profile of the two most used oral antidiabetic drugs - metformin and glibenclamide. The presence of the antidiabetic drugs in the polymer matrix was proved using IR spectroscopy. The optimized formulations were studied in terms of morphology, particle size, swelling degree and loading efficiency. The binary polymeric formulations (chitosan-metformin-glibenclamide) were characterized by the swelling degree and loading efficiency, higher than the unitary polymeric systems (chitosan-metformin and chitosan-glibenclamide). The highest loading efficiency was shown by the chitosan-metformin-glibenclamide formulation in 1:0.5:0.5 ratio (w/w/w).

Rezumat

Obiectivul acestui studiu a fost dezvoltarea de noi sisteme polimerice binare pe bază de chitosan cu scopul de a obţine o îmbunătăţire a profilului farmacocinetic şi farmacologic a două dintre cele mai utilizate antidiabetice orale - metformin şi glibenclamid. Prezenţa antidiabeticelor orale în matricea polimerică a fost evidenţiată prin spectrospie IR. Formulările optimizate au fost studiate din punct de vedere morfologic, al mărimii microparticulelor, al gradului de umflare şi al eficienţei încapsulării substanţei active. Sistemele polimerice binare (chitosan-metformin-glibenclamid) se caracterizează printr-un grad de umflare şi o eficienţă a încapsulării mai mare, comparativ cu sistemele polimerice unitare (chitosan-metformin şi chitosan-glibenclamid). Cel mai mare grad de încapsulare s-a obţinut pentru formularea chitosan-metformin-glibenclamid în raport de 1:0,5:0,5 (m/m/m).

Keywords: chitosan, metformin, glibenclamide, microparticles, polymer matrices

Introduction

Diabetes mellitus is a chronic metabolic disorder resulting from a defect in the insulin secretion, the insulin action or both. This disorder claims four million lives every year and it is a leading cause of blindness, kidney failure, heart attack, stroke and amputation [1, 2, 4]. The current oral treatment options for type 2 diabetes mellitus (T2DM) include sulfonylureas, glinides, biguanides, thiazolidinediones and alpha-glucosidase inhibitors, drugs which are often associated with serious side effects [2, 7, 15]. Metformin is a biguanide drug used as first-line therapy in type 2 diabetes mellitus treatment. However, metformin has a low degree of bio-availability (50-60%) and short and variable half life time (0.9 - 2.6 h) which requires repeated administration of high doses in order to maintain effective plasma concentration [9, 12]. Unfortunately, at higher doses, metformin is often associated with several side-effects such as lactic acidosis, diarrhoea, nausea, vomiting and flatulence [14]. Glibenclamide is a drug which belongs to the third-generation sulfonylureas with enhanced potency and increased duration of action [8, 13] but with reduced bio-availability (45%) attributed to its poor dissolution properties [5]. It is administrated in doses of 2.5 - 5 mg/day as monotherapy or in combination with metformin (500 mg/day) as Glucovance®, Bidiab®, Glibomet® and Gliformin®. Metformin/glibenclamide fixed-dose combination should be avoided in the elderly and those with renal or hepatic impairment [10]. This combination can also cause gastro-intestinal side effects, hypoglycaemia and weight gain and it increases the risk of severe and prolonged hypo-glycaemia [10]. In order to increase the pharmaco-kinetic and safety profile of metformin and gliben-clamide, new binary drug microparticles based on chitosan were developed. Chitosan is a biopolymer very suitable for biomedical and pharmaceutical applications based on its properties such as bio-degradability, low

toxicity, low immunogenicity and good bio-compatibility [6]. The objective of this study was to develop new binary polymeric systems based on chitosan in order to achieve an improvement of the pharmacokinetic and pharmacological profile of the association between metformin and glibenclamide.

Materials and Methods

Materials. Chitosan medium molecular weight (CS), metformin hydrochloride, glybenclamide, acetic acid, sodium tripolyphosphate (TPP), dimethylsulfoxide (DMSO) were purchased from Sigma Aldrich Company.

Preparation of chitosan microparticles loaded with antidiabetic drugs

The antidiabetic drugs (metformin, glibenclamide) were loaded into chitosan microparticles using ionic gelation method [11]. Briefly, the drugs were dissolved in the minimum volume (0.5 mL) of proper solvent (distilled water for metformin hydrochloride and DMSO for glibenclamide). The drug solutions were added into 3 mL of 1% chitosan solution in acetic acid. The mixture was stirred at room temperature for 3 h and then was dropped through a syringe needle (26 G) into 20 mL of 2% TPP solution in distilled water under stirring (325 rpm). The mixture was stirred again (200 rpm) at room temperature for 24 h. The formed beads: chitosan-metformin (CS-M), chitosan-glibenclamide (CS-G) and chitosan-metfomin-glibenclamide (CS-MG) were separated from the TPP solution and washed three times with distilled water and then dried at room temperature. In order to obtain high loading efficiency and stable microparticles, three concentrations for each drug have been used (30 mg, 22.5 mg and 15 mg), which means that the ratio between antidiabetic drug and chitosan was 1:1, 0.75:1 and 0.5:1 (w/w).

Chracterization of chitosan microparticles loaded with antidiabetic drugs

Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectra of chitosan and chitosan microparticles loaded with antidiabetic drugs were recorded using a Biorad FT-IR spectrometer FTS 575C in the range between 4000 cm^{-1} and 500 cm^{-1}, after 32 scans at a resolution of 4 cm^{-1}. The spectra processing was carried out with the Horizon MB^{TM} FTIR Software.

Particle size measurements and morphology. The size of the microparticles (in wet and dry state) was measured using a Zeiss (Axiotech) optical microscope (5 times magnification). The scanning electron microscopy technique (SEM) using a Desktop SEM (Phenom, The Netherlands) was chosen to study the morphology of the microparticles.

Swelling degree. The swelling degree (SD) of the polymeric systems was performed in distilled water and simulated gastric fluid (SGF) at pH 1.6 and 37°C by measuring the microparticles weight as a function of time [11]. A sample of dried micropaticles (W_1) was place in distilled water and simulated gastric fluid respectively (SGF). At different times microparticles were removed from water and SGF respectively, filtered and weighed (W_2). The experiments were performed in triplicate and average values were calculated. The swelling degree at different times was calculated using the following formula:

$$SD\ (\%) = (W_2 - W_1)/W_1 \times 100 \qquad (1)$$

where: W_1 = the weight of the dried microparticles; W_2 = the weight of the swollen microparticles at different times.

Loading efficiency. The loading efficiency (LE %) of the antidiabetic drugs into chitosan microparticles was evaluated using a UV spectrophotometric method (UVIKNO XL, BIOTECH Instruments) [8, 12]. The content of drug (metformin, glibenclamide) in the TPP solution after removing the beads was calculated by measuring the absorbance of the solution at 233 nm (for metformin) and 300 nm (for glibenclamide) respectively, using the standard curve for each drug. The loading efficiency (%) was calculated using the following formula:

$$LE\ \% = C_1/C_0 \times 100 \qquad (2)$$

where: C_0 = the initial concentration of the anti-diabetic drug; C_1 = the antidiabetic drug concentration in the TPP solution.

Results and Discussion

Fourier transform infrared (FT-IR) spectroscopy

The presence of the antidiabetic drugs in the polymer matrices has been proven by the FT-IR spectral data (Figure 1). The spectra of chitosan micropaticles revealed the following characteristic bands: 1636 cm^{-1} (-CO-NH-NH_2), 1456 cm^{-1} (CH_2), 1040 cm^{-1} (C-O-C) and 1375 cm^{-1} (CH_3). The presence of metformin in the polymer matrix was proved by the following spectral bands (cm^{-1}): 3367, 3294, 3150 (N-H); 1558, 1541 (-NH_2); 1684 (-C=N); 1080, 1063, 1040 (C-N) and 2974, 2943 (-CH_3). The spectral bands at 3365, 3292, 3153 (N-H), 1624, 1558 (C=O); 1165 (SO_2) and 737 (C-Cl) cm^{-1} were attributed to glibenclamide and the cross linking agent (TPP) was identified by spectral bands at 1219 cm^{-1} (P=O) and 895 cm^{-1} (P-O-P).

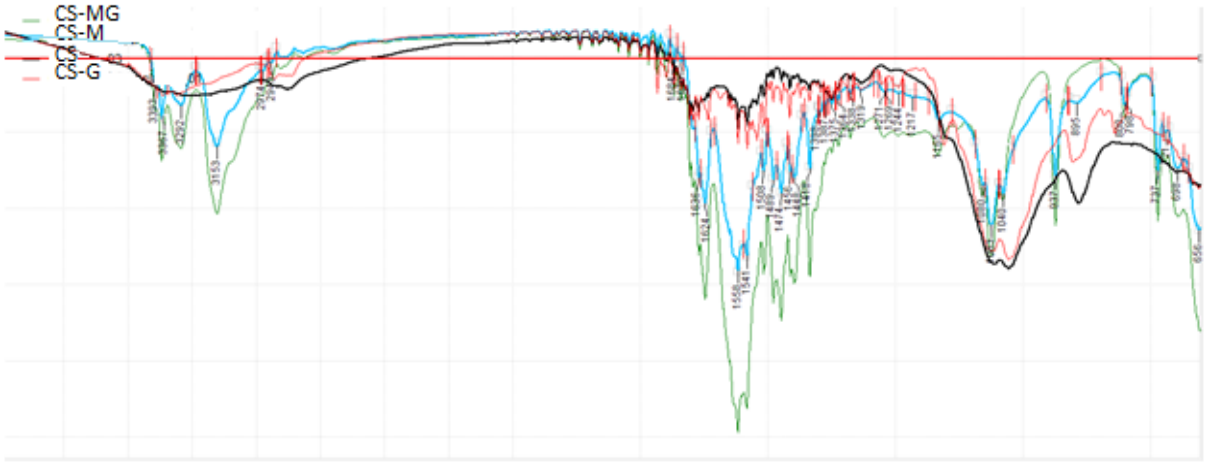

Figure 1.
The FT-IR spectra of the chitosan (CS) and of the loaded chitosan microparticle (CS-M, CS-G, CS-MG)

Particle size measurements and morphology

The size of the chitosan loaded microparticles in wet and dry state and the data regarding their stability are presented in Table I. Stable microparticles were successfully formed at all chitosan-drug ratio used. This parameter is very important because it influences other characteristics of the beads such as swelling degree and loading efficiency. The size of the micro-particles ranged between 500 - 710 µm in wet state and between 300 - 480 µm in dry state. It was also observed that chitosan-metformin-glibenclamide micro-particles are larger than chitosan-metformin and chitosan-glibenclamide microparticles respectively. The scanning electron microscopy (SEM) revealed that chitosan microparticles (CS) have a regular, spherical shape with smooth surface, whereas upon loading the compounds, the shape becomes irregular with rough surface (Figure 2).

Table I
The characteristics of the chitosan-drug microparticles

Antidiabetic drug(s)	Ratio Drug:CS (w/w)	Particle size (µm)	
		Wet	Dry
Metformin	1:1	506.85 ± 12.2	306.4 ± 8.9
	0.75:1	686.92 ± 9.30	319.2 ± 10.5
	0.5:1	647.18 ± 23.4	348.1 ± 6.4
Glibenclamide	1:1	651.82 ± 15.8	368.3 ± 10.9
	0.75:1	644.67 ± 10.4	357.3 ± 15.7
	0.5:1	639.87 ± 14.9	379.4 ± 8.6
Metformin/Glibenclamide	1:1	715.91 ± 18.7	482.4 ± 13.5
	0.75:1	709.45 ± 16.2	479.1 ± 11.4
	0.5:1	710.13 ± 19.6	482.5 ± 6.3

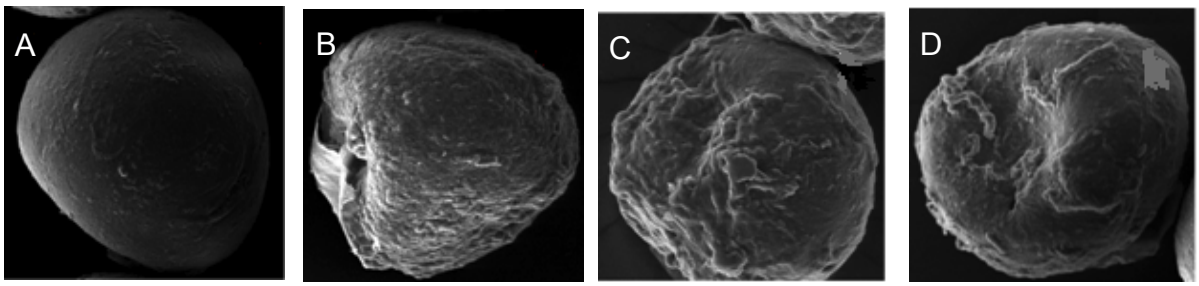

Figure 2.
SEM micrographs for CS (A) and CS-M (B), CS-G (C) and CS-MG (D) systems

In distilled water, the chitosan-metformin (CS-M) microparticles showed a higher swelling degree (304 - 380%) compared to chitosan (179%), while for chitosan - glibenclamide (CS-G) systems the swelling degree was lower (53-106%) (Figure 3 A-C).

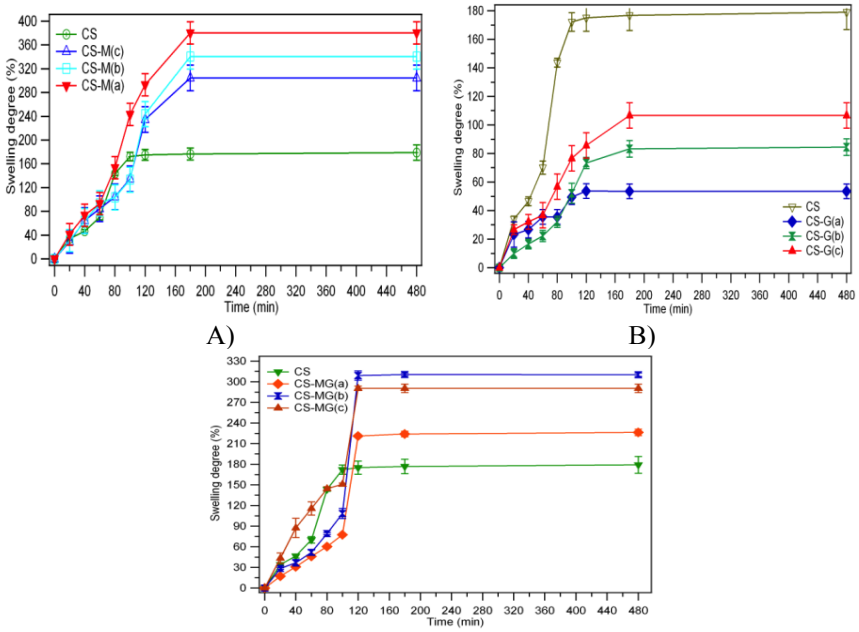

Figure 3.

The swelling degree of CS and CS-drugs: CS-M (A), CS-G (B), CS-MG (C) at different concentrations: 30 mg (a), 22.5 mg (b) 15 mg (c) in distilled water

The binary systems (chitosan-metformin-glibenclamide) showed the highest swelling degree, which ranged between 226% (30 mg:30 mg) to 310% (22.5 mg:22.5 mg) compared to chitosan (179%). The dynamic equilibrium was reached after 3 h and it remained at a constant value for about 8 h.

Also it was observed that the swelling degree of the unitary and binary systems was higher in SGF compared to the values recorded in distilled water (Figure 4 A-C).

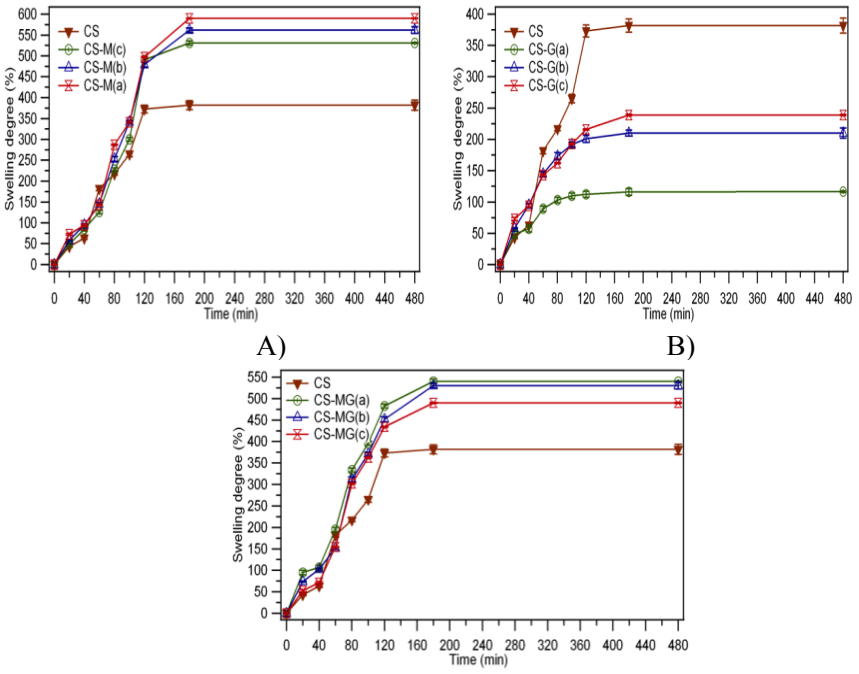

Figure 4.

The swelling degree of CS and CS-drugs: CS-M (A), CS-G (B), CS-MG (C) at different concentrations: 30 mg (a), 22.5 mg (b) 15 mg (c) in distilled water

For CS-M the swelling degree ranged between 530% (CS-Mc) and 590% (CS-Ma) while the values recorded for CS-G ranged between 116% (CS-Ga) and 239% (CS-Gc). For binary systems (CS-MG) the swelling degree ranged between 490% (CS-MGc) and 540% (CS-MGa).

Loading efficiency

The loading efficiency (LE) of the antidiabetic drugs in the chitosan microparticles, at different concentrations is shown in Figure 5. As it can be observed, glibenclamide is efficient if loaded into the matrix of chitosan, regardless of the used concentration (30 mg, 22.5 mg, 15 mg). For CS-G, the loading efficiency ranged between 96% (15 mg) and 98% (30 mg). For metformin the loading efficiency is inversely proportional to the used concentration, the highest value being obtained at a concentration of 15 mg (40%). This can be explained by the hydro-philic properties of metformin, which facilitate the release of the drug from the polymer matrix to the aqueous medium in which the cross-linking process performs. For the binary systems (chitosan-metformin-glibenclamide), the loading efficiency was higher than the unitary systems (chitosan-metformin and chitosan-glibenclamide respectively) and inversely proportional to the used concentration. The highest percentage of the loading efficiency for metformin (51%) and glibenclamide (98%) was recorded at a concentration of 15 mg of each drug.

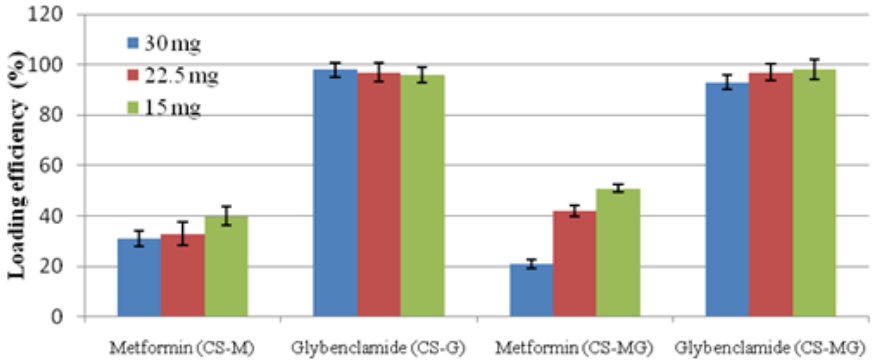

Figure 5.
The loading efficiency (%) of metformin, glibenclamide, metformin-glibenclamide in chitosan polymer matrix at different concentrations

Conclusions

New binary polymeric systems based on chitosan-metformin-glibenclamide have been developed and they were physico-chemical characterized in order to improve the pharmacokinetic and pharmacological profile of the used antidiabetic drugs. These polymeric systems showed improved swelling degree compared to chitosan and unitary polymeric systems, chitosan-metfomin and chitosan-glibenclamide. Also the loading efficiency of the antidiabetic drugs was higher for binary systems compared to unitary systems, which means that these formulations can be a good therapeutic alternative for the management of diabetes mellitus treatment.

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