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SYNTHESIS AND CHARACTERIZATION OF DILTIAZEM LOADED EUDRAGIT RS 100 "MICROSPONGES"

Nadezhda Ivanova*[1], Cinzia Di Franco[2], Adriana Trapani[3], Valentina Petkova[4], Milen Dimitrov[4]

[1]Faculty of Pharmacy, Medical University - Varna "Prof. Dr. Paraskev Stoyanov", 84 Tsar Osvoboditel Str., 9000, Varna, Bulgaria.

[2] CNR-IFN U.O.S. Bari, Bari Via Amendola 173 - 70126 Bari, Italy.

[3] Department of Pharmacy-Drug Sciences, University of Bari "Aldo Moro", via Orabona 4, 70125 Bari, Italy.

[4]Faculty of Pharmacy, Medical University of Sofia, 2, Dunav Str., 1000, Sofia, Bulgaria.

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***Corresponding Author**
Nadezhda Ivanova
Faculty of Pharmacy,
Medical University - Varna
"Prof. Dr. Paraskev
Stoyanov", 84 Tsar
Osvoboditel Str., 9000,
Varna, Bulgaria.

ABSTRACT

In this study, we report successful synthesis of porous polymeric microspheres with "microsponge" type morphology by quasi-emulsion solvent diffusion method. Over the past few years the "Microsponge Delivery Systems - MDS" started to stand out as promising carriers in solid and semi-solid drug dosage forms providing a number of benefits for the purposes of cutaneous, mucosal and stomach/colon-targeted drug delivery. In order to evaluate the potential for sustained/controlled drug release of our "microsponges", diltiazem was used as a model drug. Diltiazem-loaded microsponges were subjected to Scanning Electron Microscopy (SEM) for size and morphology assessment. Drug loading efficacy was evaluated after destruction of the particles in dichloromethane and spectrophotometric quantification. Compatibility study for drug and polymer was performed on FT-IR. In vitro dissolution test was carried out in PBS pH 7,4 and drug release kinetics was determined. The resulting particles showed desired spherical sponge-like morphology and mean size diameter of 59,09±13.00 µm. Satisfactory values of production yield and loading efficacy were observed – 82,78±0.34% and 90,66±1.65%, respectively. FT-IR revealed no chemical interaction between model drug diltiazem and polymer Eudragit RS 100. Drug release was found to follow zero-order kinetics and possessed drug release of 76,44% in 18 hours. These results

might be taken in consideration for future studies concerning development of microsponge-enriched dosage forms.

KEYWORDS: microsponges, diltiazem, polymeric microspheres, novel drug delivery systems.

INTRODUCTION

Among the variety of developed Novel Drug Delivery Systems (NDDS), Microsponge Delivery Systems (MDS) succeed to impress as promising chemically, physically and microbiologically stable drug carriers. Scientific interest in MDS continues to grow even 30 years after their first successful synthesis and patentable.[1] Microsponges are porous polymeric microspheres with non-collapsible structure, tailored to achieve controlled and sustained drug release when used as drug delivery systems.[2] Desorption mechanisms involve diffusion of the drug through the pores of the particles and the amorphous phase of the polymer. The microsponge particle remain intact through the entire process of drug delivery and applies control on it to its very end.[3] This defines the fundamental differences between MDS and other types of polymeric microspheres and microcapsules, which at a certain point of drug delivery or in contact with water or biological fluids, decompose, dissolve or undergo biodegradation.

MDS arouse particular interest in the field of targeted colon, stomach, dermal and mucosal drug delivery. As drug carriers they have the ability to incorporate a wide range of active ingredients, which is considered to be a result of their high degree of cross-linkage, relatively high chemical inertness and compatibility. Proper selection of a clinical case, in which their potential to be tested, could lead to beneficial results in the treatment of many diseases. In addition to the aforementioned advantages, MDS provide opportunity for dosage optimization, administration frequency and side effects reduction and bioavailability improvement by increasing the solubility and/or stability of certain drugs.[4-8]

Diltiazem (DTZ) is a non-dihydropyridines calcium channel blocker conventionally used in the treatment of hypertension, angina pectoris and some types of arrhythmia and was used as a model drug in our study. There are evidence in the literature testifying efficacy of the drug in prevention of migraine attacks and also in local treatment of anal fissures, when used as active ingredient in semi-solid rectal dosage forms.[9, 10, 11] Diltiazem base is very slightly soluble in water; therefor the freely water-soluble hydrochloride (HCL) salt of the drug is

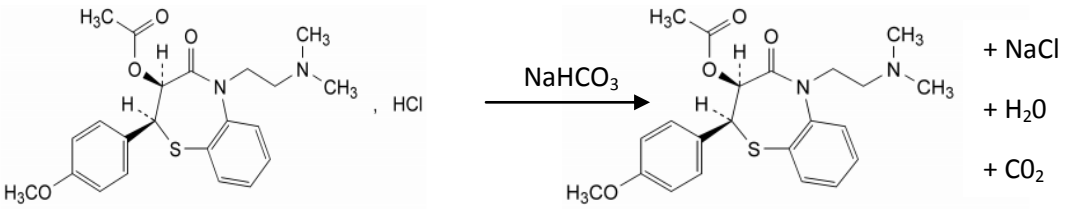
commercially used. Recent surveys have emphasized the need of new pharmaceutical alternatives (salts, complexes, esters) or drug delivery systems of DTZ which can achieve intermediate solubility (greater then DTZ, but lower then DTZ HCL) to be developed in order to compensate fast release and short elimination half-life (3,2±1,3 h) or low bioaccessibility in the case of DTZ base.[12] Diltiazem loaded microsponges (DTLM) could be useful and beneficial in oral dosage forms and semi-solid dosage forms for topical and mucosal administration as well.

MATERIALS AND METHODS

Diltiazem hydrochloride (DTZ HCL) was a gift from Sopharma Pharmaceuticals, Ammonio methacrylate copolymer (type B) - Eudragit RS 100 (ERS 100) was a gift from Evonik, Germany, other materials were purchased from (as follows): Dichloromethane (Himtex OOD), Ethanol anhydrous (Himtex OOD), Poly(vinyl alcohol) Mw 49 000 (Sigma Aldrich, USA), Sodium hydrogen carbonate (Valerus), Sodium hydroxide (Valerus), Potassium dihydrogen phosphate (Valerus), Disodium hydrogen phosphate (Valerus).

Conversion of diltiazem hydrochloride to diltiazem

DTZ HCL was converted to DTZ through the following reaction, using sodium hydrogen carbonate as soft alkalizing agent *(Figure 1).*



Diltiazem hydrochloride ***Diltiazem***

Figure 1: Conversion of DTZ HCL to DTZ pure base.

10,0 g of DTZ HCL (Mr 451,0) were dissolved in 100,0 ml distilled water under constant stirring on a magnet stirrer. Sodium hydrogen carbonate (Mr 84,0007) was then slowly added to the solution in slight excess. Precipitation of the very slightly water soluble DTZ base[12] was observed. The resulting slurry was stirred for another 15 minutes to allow complete reaction between the hydrochloride and sodium hydrogen carbonate. DTZ base was isolated through filtration and multiple washing with distilled water to remove residues of sodium hydrogen carbonate. The substance was then kept in desiccator for 24 hours and weighted to assess production yield.

The conversion of DTZ HCL to DTZ base was confirmed by FT-IR analysis of DTZ HCL and resulting compound in potassium bromide disks. FT-IR spectra were acquired using FT-IR spectrometer (PerkinElmer, Italy).

Calibration curves of DTZ

UV spectrophotometric quantification method was chosen for determination of DTZ during tests concerning microsponges' loading efficacy and in vitro dissolution tests because of its accessibility and feasibility in this particular study. For the purpose, calibration curves of DTZ HCL in distilled water, PBS pH 7,4 and ethanol anhydrous were built. Absorption maxima for diltiazem was found at wavelength of 240 nm.

Standard solutions of DTZ HCL in the three above mentioned media were prepared as follows: 15,0 mg of DTZ HCL was accurately weighed and transferred to a 100,0 ml volumetric flask containing sufficient quantity of media to dissolve the drug. This was further diluted up to the mark with dissolution media to obtain drug concentration of 150 mg /L. Proper dilutions were made to obtain concentrations of 1,5 mg/L, 4,5 mg/L, 7,5 mg/L and 15,0 mg/L.

Measurements were carried out on a UV/Vis Spectrophotometer, (Reileight UV-9200, China). Results were processed with the aid of UV/Vis Software program.

Where the analysis required determination of DTZ instead of DTZ HCL, the necessary recalculations based on molecular weight data for both compounds were made.

Microsponges preparation

Quasi emulsion solvent diffusion method was used for the synthesis of the microsponges. Drug DTZ and polymer ERS 100 (at drug-polymer ratio 1:3) were dissolved in 8,0 ml of dichloromethane (DCM) at room temperature under continuous stirring. The organic solution was then loaded to an analogue volumetric burette in order to facilitate and improve dropwise addition of the organic solution to an external aqueous phase. In addition, 25 G needle was attached to the burette orifice to reduce drops size and ensure drop uniformity. An external aqueous phase was prepared by dissolving polyvinyl alcohol (PVA) in 100,0 ml distilled water under stirring and heating up to 80 C^{o}. PVA was used as surfactant in concentration 0,15 % m/v. After cooling down to the room temperature, the aqueous solution was placed on a magnet stirrer at a constant stirring rate of 650 rpm and by releasing the stopcock of the

burette, dripping of the organic solution was initialized. The mixture was stirred for 3 hours in a laboratory hood. The flask was then attached to a vacuum system for another hour to eliminate residues of DCM. The microparticles were isolated by centrifugation of the suspension for 5 minutes at 4000 rpm, following filtration through Whatman filter paper №4 and multiple washing with distilled water. Blank sample without drug was also prepared. Samples were dried in desiccator at room temperature for 24 hours.

Determination of production yield (PY)

After 24 hours stay in desiccator each sample of microsponges was accurately weighted on analytical balance. Production yield (PY) was presented as percent, using the following equation (Eq. 1):

$$PY\% = \frac{\text{Actual wight of the microsponges}}{\text{Theoritical weight of the microsponges (drug+polymer)}}. 100 \quad \text{(Eq. 1)}$$

Reproducibility was assessed on the base of 3 replicates of DTLM synthesis.

Determination of drug loading efficacy (LE)

Test solutions were prepared as follows: 20,0 mg of microsponges, theoretically containing 5,0 mg DTZ, were accurately weighted on analytical balance and transferred to a 100,0 ml volumetric flask, containing 5,0 ml of DCM. After dissolution of the particles in DCM, further dilution with ethanol anhydrous up to the mark was made. 1,0 ml of the prepared solution was transferred in 10,0 ml volumetric flask and diluted to up the mark with ethanol anhydrous.

Reference solution was prepared as follows: 15,0 mg of ERS 100 were accurately weighed, transferred to a 100,0 ml volumetric flask, dissolved in 5,0 ml DCM and further diluted up to the mark with ethanol anhydrous. 1,0 ml of the prepared solution was transferred in 10,0 ml volumetric flask and diluted up to the mark with ethanol anhydrous.

DTZ concentration was measured by UV spectrophotometric analysis of test solution against reference solution at wavelength of 240 nm and with the aid of DTZ calibration curve in ethanol anhydrous.

Loading efficacy (LE) was presented as percent, using the following equation (Eq. 2).

$$LE\% = \frac{\text{Actual DTZ content in microsponges}}{\text{Theoritical drug content in microsponges}}.100 \quad \text{(Eq. 2)}$$

Repeatability was assessed by means of replicate analysis of each sample. Reproducibility was assessed by means of analysis of 3 batches of DTLM. Results are expressed as mean ± standard deviation (SD). Statistical analysis was performed on Excel 2013 and is presented in the supplementary material.

Morphology and particle size study

Morphology and surface characteristics of the microsponges were evaluated with SEM analysis. Samples were stuck on double-face carbon adhesive stubs and coated with a 10 nm palladium. The morphological reproducibility was assessed by means of replicate analyses belonged to each sample. The electron microscope images were taken by a Sigma Zeiss FE-SEM at 5-10 kV, 30 µm aperture. The statistical analysis of the microsponges was made by ImageJ Software.

Compatibility study

Compatibility study was carried out via FT-IR analysis of DTZ-loaded, blank microsponges and pure drug DTZ in potassium bromide disks. FT-IR spectra were acquired using FT-IR spectrometer (PerkinElmer, Italy).

In vitro drug release study

In vitro drug release test was carried out on European Pharmacopoeia Dissolution Apparatus 2 (Paddle apparatus), using 7-station Compact Dissolution Tester PT-DT 70 (Pharma test, Germany). Quantity of microsponges responding to 5,0 mg content of DTZ, was placed in a dialysis bag and dispersed in 1,0 ml buffer. The dialysis bag was then precisely attached to the paddle and immersed in to 400,0 ml dissolution medium. In vitro drug release behavior was investigated in PBS pH 7,4. All tests were carried out at 37 C^o ± 0,5 C^o and rotation speed of 30 rpm. Samples from dissolution medium were withdrawn at chosen times of the study and analyzed spectrophotometrically.

With the aid of the resulting data, drug release profile was obtained and extrapolated to four kinetic models – First order, Zero order, Highuchi model, Korsmayer-Peppas model - in order to determine and analyze drug release kinetics from the resulting microstructures.

RESULTS AND DISSCUSION

Microsponges characterization

SEM analysis revealed formation of highly porous, spherical microparticles - microsponges *(Figure 2).* Satisfactorily high values of yield and loading efficacy were observed. Size

analysis showed significant increase in the mean size diameter of DTLM compared to blank microsponges, which could be devoted to steric and other type of physical interaction between the polymer chains and DTZ.

PY%, LE%, morphology and size results for the resulting microsponge particles are presented on *Table 1*.

Drug-polymer compatibility study

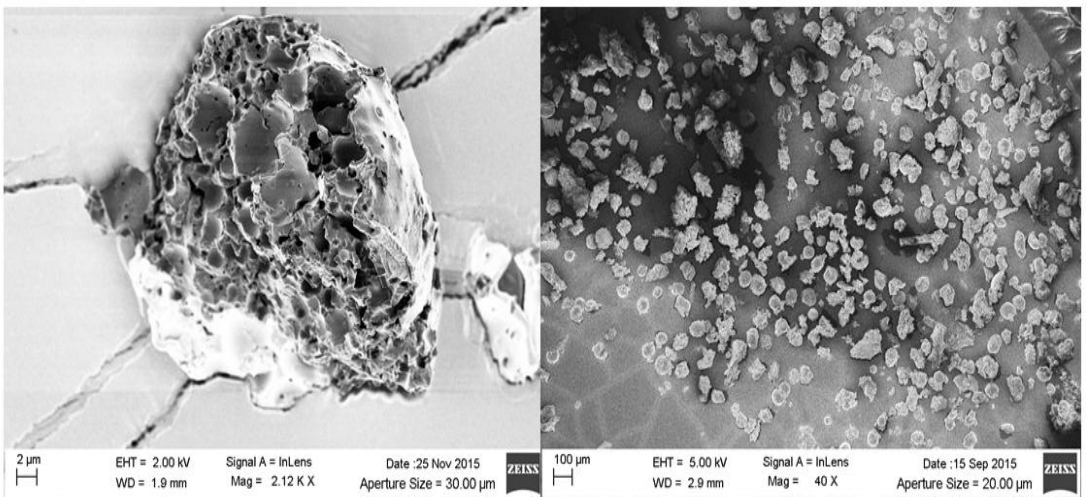
FT-IR spectroscopic study results of DTLM revealed no new peaks appearance or disappearance of existing peaks, when compared to reference spectra of blank microsponge formulation and pure drug DTZ. Thus, any chemical interaction among DTLM formulation was discarded. All characteristic peaks of DTZ (O-CH_3 2947 cm^{-1}, C=O acetate 1747 cm^{-1} C=O amide 1675 cm^{-1}) were observed in the microsponge formulation spectrum.[13] From the obtaining results, we can conclude that DTZ is compatible with polymer ERS 100 in the composition of MDS *(Figure 3).*

Table 1: PY%, LE%, morphology and size of microsponge formulations.

Formulation code	ERS 100, mg/ Drug-polymer ratio	PY%±SD	LE%±SD	Morphology	Mean size diameter, µm±SD
DTLM	600, 1:3	82,78±0.34	90.66±1.65	Spherical sponge-like particles	59,09±13,00
BLANK	600	83,33*	-	Spherical sponge-like particles	2,40±1,31

** No statistical analysis was performed*

a)



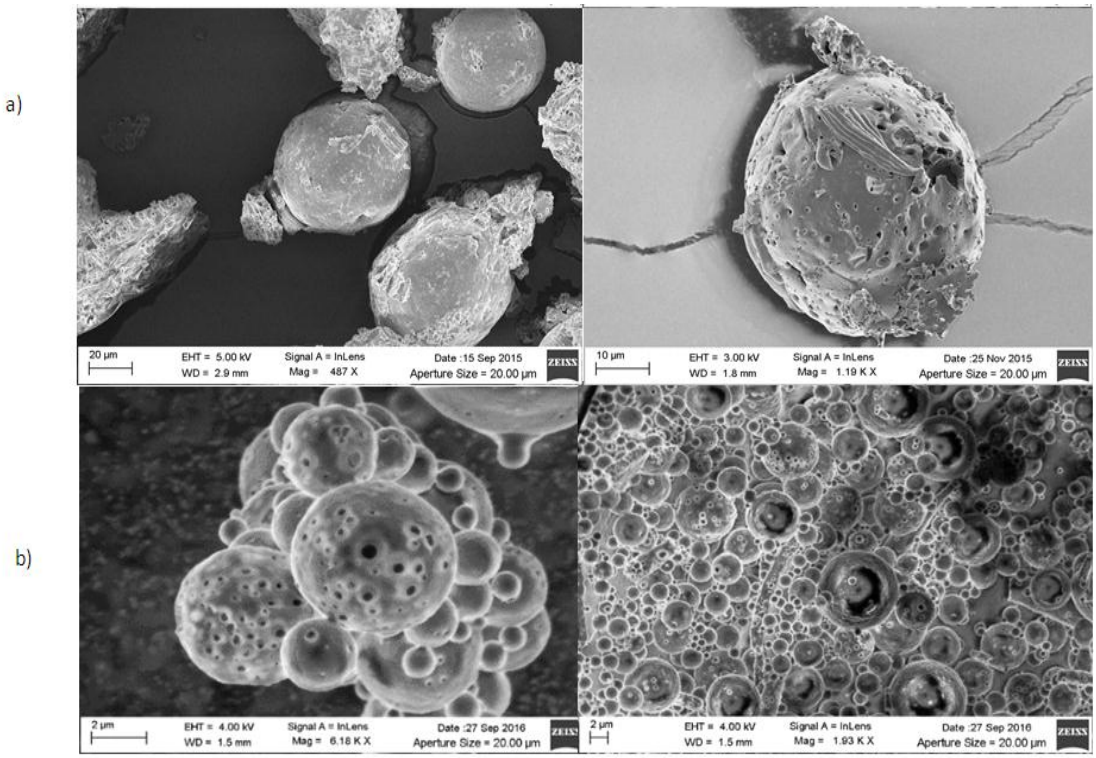


Figure 2: SEM micrographs of diltiazem-loaded (a) and blank (b) microsponges.

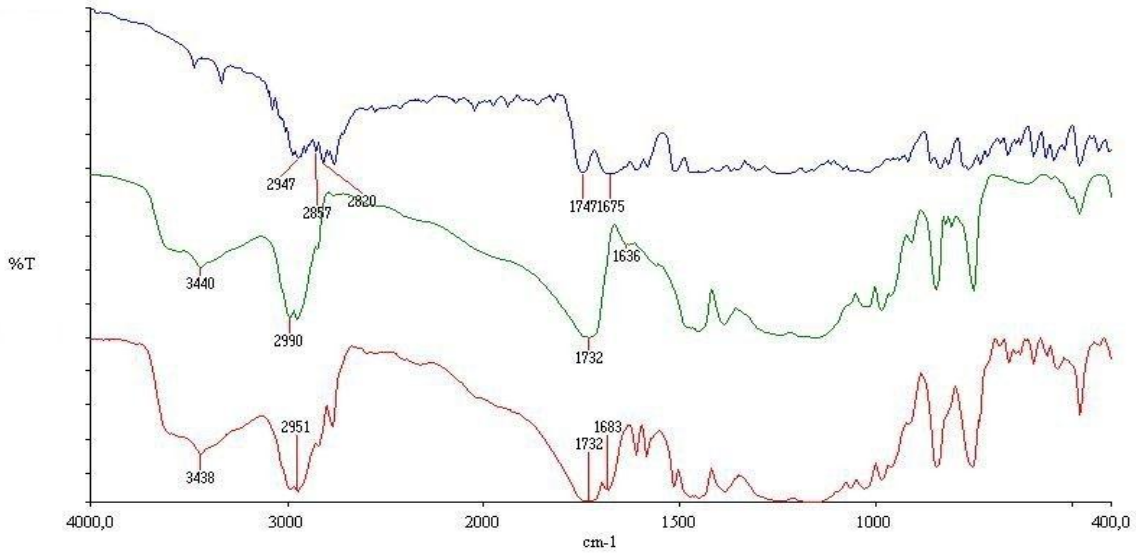


Figure 3: FT-IR spectra of DTZ (a), ERS 100 (b) and DTLM (c)

In vitro drug release

In vitro drug release profile of DTLM obtained in PBS pH 7,4 is presented on *Figure 4*. Drug release showed no initial burst effect, maximum cumulative drug release (CDR) in 18 hours – 76,44% and best fit to Zero order kinetic model (*Table 2*), which correlates with the drug and polymer nature and type of drug delivery system.[14]

Table 2: Drug release kinetics of DTZ from DTLM formulation

Formulation	Zero order, R^2	First order, R^2	Higuchi model, R^2	Korsmayer-Peppas model		
				R^2	K	n
DTLM	0,9950	0,9766	0,9651	0,9669	0,254	1,501

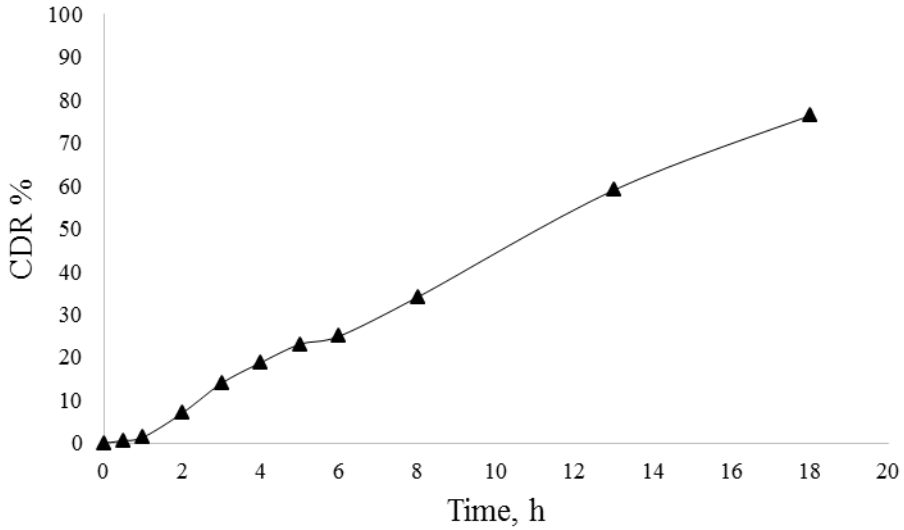


Figure 4: In vitro drug release profile of DTLM in PBS pH 7,4

CONCLUSIONS AND FUTURE PROSPECTS

Under the experimental conditions chosen for MDS synthesis, successful formation of desired spherical sponge-like structures was observed. Particles showed adequate PY%, LE% and drug release pattern, for which we consider the experiment a success. The need exist main formulation and process variables (such aa volume of the outer water phase and inner organic phase, PVA concentration, drug-polymer ratio, rotation speed and etc.) to be investigated in order to evaluate their significance for the particles' size, morphology, drug release, PY% and LE%. Diltiazem loaded microsponges showed potential to overcome some of the main disadvantages of conventional diltiazem therapy, such as short biological half-live of the drug, low bioavailability and frequent administration. In addition, rectal gels with DTZ suffer from low drug stability due to hydrolysis, fast release and high risk of unwanted for the purposes of anal fissure treatment, system absorption. Microsponges as eligible drug carriers in semi solid dosage forms have the ability to solve some of these problems as well and improve the efficacy of the local anal fissure treatment. Our future studies in the field will be focused in these directions.

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