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Enhancement of the dissolution profile of the diuretic hydrochlorothiazide by elaboration of microspheres

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Abstract: Hydrochlorothiazide (HCTZ), which was developed and introduced in the late 1950s, is still one of the most frequently employed drugs in antihypertensive treatments. Its poor aqueous solubility is one of the reasons for its limited bioavailability after oral administration. The present paper provides details of the preparation of HCTZ-loaded microspheres by the solvent evaporation technique. A total of seven formulations were prepared using ethyl cellulose, poly(ε-caprolactone) (PCL), β-cyclodextrin (β-CD) and synthesized poly(methyl methacrylate) (PMMA) of different molecular weights in different drug-to-carrier ratios in order to investigate their effect on the encapsulation efficiency and drug release kinetics. The prepared formulations were characterized by Fourier transform-infrared (FTIR) spectroscopy, powder X-ray diffractometry, differential scanning calorimetry, yield, drug loading, optical microscopy, surface morphology by scanning electron microscopy (SEM), and *in vitro* release studies in simulated gastrointestinal tract fluid. The loading efficiency was found in the range from 18±0.34 to 39±0.95 %. The microspheres were spherical, and the mean Sauter diameter (d_{32}) of the obtained microparticles ranged from 26±0.16 to 107±0.58 μm. The presence of the drug and polymer carriers in the microparticles was confirmed by FTIR spectroscopy and XRD analysis. *In vitro* dissolution studies showed that the release rate was largely affected by the characteristics of the microparticles, namely the particle size and the nature of the matrix. The release data are best fitted to the Higuchi model with high correlation coefficients (r^2).

Keywords: microparticles; polymer carriers; solvent evaporation method; kinetic study; in *vitro* release.

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INTRODUCTION

The technique of microencapsulation by solvent evaporation is widely applied in pharmaceutical industries to obtain formulations for the controlled release of drug. The obtained polymer microspheres with drug trapped inside can degrade and release the encapsulated drug slowly with a specific release profile.[1] Controlled drug release has outstanding clinical benefits, such as, reduction of dosing frequency, more convenience and acceptance for patients, and drug targeting to specific locations resulting in higher efficiency.[2,3]

In the solvent evaporation process, a solution or dispersion of a drug in a polymer solution is emulsified into an aqueous medium to form the microspheres. Various polymers have been used to develop drug delivery systems for entrapping and delivering drugs orally. In recent years, the use of hydrophilic polymers, in particular cellulose derivatives, such as: ethyl cellulose (EC),[4–10] hydroxypropyl cellulose (HPC),[11,12] carboxymethyl-ethyl cellulose CMEC,[4,5] hydroxypropyl-methyl cellulose HPMC[5,8,13] and cellulose acetate,[14] has attracted considerable attention for the development of controlled release technology in the formulation of pharmaceutical products.

Thus, a range of microspheres prepared using both synthetic biocompatible and biodegradable polymer matrices have been extensively studied and are available on the market for long-term treatment in various applications. The polymer types used most frequently in such studies include PLGA,[15,16] poly(ε-caprolactone) PCL[17,18] and PMMA.[19,20] The final one contains ionisable groups (carboxylic acid) and has been the focus of intensive research, since its pH-dictated ionization can be utilized for the development of systems with reversible water solubility controlled by pH.[21,22] This polymer is typically water-soluble when charged, but becomes water-insoluble when neutral. These materials also find application in areas such as controlled release of drugs in the form of hollow microspheres for the protection of sensitive species in acidic media.[23]

Cyclodextrins (CDs) are doughnut-shaped cyclic oligosaccharides with an interior cavity and they form specific inclusion complexes with many organic compounds. They are widely used in foods, pharmaceuticals, agricultural, analytical and cosmetics industry encapsulations.[24] In food related applications, flavour compounds have been encapsulated into CDs for better retention and protection from various possible means of deterioration, as well as for controlled delivery.[25] CDs may also act as drug carriers and provide optimized efficacy, safety and convenience. Moreover, CDs may prolong the release characteristics of particular compounds entrapped within the cavities.[9,26]

Hydrochlorothiazide (HCTZ, 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadia-zine-7-sulphonamide, a poorly water soluble drug (0.7 mg mL^{-1})), is diuretic belonging to the benzothiadiazines. It has been proved in the management of hypertension in combination with cardiovascular drugs. According to the

Biopharmaceutics Classification System (BCS), hydrochlorothiazide is considered as a class IV drug.[27]

It inhibits sodium re-absorption in the distal tubules, causing increased excretion of sodium and water as well as potassium and hydrogen ions. It has low and variable oral bioavailability, which is attributed to its poor solubility, slow dissolution and poor membrane permeability.[24] Hydrochlorothiazide is absorbed from the gastrointestinal tract (GI) and apparently not metabolized and excreted unchanged in urine. At least 61 % of the drug is reportedly eliminated from the body when the excretion is essentially completed within 24 h post administration. The oral bioavailability of the drug is reported to be 60–80 % of the administered dose.[28]

The main objectives of this research work were the preparation and evaluation of monolithic systems known as microspheres of HCTZ prepared by the solvent evaporation technique using ethyl cellulose as a polymeric biomaterial, PCL, β-cyclodextrin (β-CD) and synthesized poly(methyl methacrylate) (PMMA) of different molecular weights. The HCTZ microspheres were prepared, using different matrices with different compositions in order to assess the effect of polymers on the encapsulation efficiency and the kinetics of the drug release in the gastrointestinal tract.

EXPERIMENTAL

Chemicals

Hydrochlorothiazide HCTZ (M = 297.739±0.02 g mol^{-1}) was obtained as a gift sample from the Laboratory of Therapeutic Chemistry, Faculty of Medicine and Pharmacy of Rabat, Mohamed V University, Morocco, ethyl cellulose (viscosity: 22 mPa s of 5 mass % in toluene/ethanol solution extent of labelling: 48 %) was purchased from Sigma–Aldrich, poly-ε-caprolactone (PCL, $\bar{M}_w$: 70000–90000 g mol^{-1}) was purchased from Sigma–Aldrich, β-cyclodextrin (β-CD) from Sigma–Aldrich (USA), poly(vinyl alcohol) (PVA; 87–90 % hydrolyzed, $\bar{M}_w$: 30000–70000 g mol^{-1}) from Sigma–Aldrich. Dichloromethane (DCM, purity >98 %) and absolute methanol (99 % purity) were purchased from Riedel–de Hâen (USA). Methyl methacrylate was purchased from Sigma–Aldrich (USA), tetrahydrofuran (THF) anhydrous (≥ 99.9 %), inhibitor-free from Sigma–Aldrich. The corresponding structures are shown in Shceme S-1 of the Supplementary material to this paper. A solution of simulated gastric fluid (pH 1.2) was prepared by dissolving 2 g of NaCl and 60 mL of HCl solution (1 M) in 1 L of deionised water. A solution of simulated intestinal fluid (pH 7.4) was prepared by mixing 50 mL of a solution (0.2 M) of potassium phosphate monobasic (KH_2PO_4) and 39.1 mL of a solution (0.1 M) of sodium hydroxide (NaOH) in 1 L of deionised water.

Synthesis and characterization of PMMA

Poly(methyl methacrylate) (PMMA) was obtained by radical polymerization under a nitrogen atmosphere, in anhydrous tetrahydrofuran (THF) as solvent, at 90 °C, and in the presence of 0.5 % benzoyl peroxide as initiator for 4 h.

The polymer was fractionated by precipitation of the polymer by the addition of a polymer solution to a non-solvent (precipitant).[29] The polymer was dissolved in 20 mL of acetone. This solution was poured gradually (drop-by-drop) under vigorous stirring into the meth-

anol/water as a non-solvent until the appearance of turbidity. After a few hours of hardening, the turbidity was dissolved by temperature variation. The solution was allowed to stand several hours. The concentrated phase was separated by settling; then dissolved in a small amount of solvent and finally isolated by pouring the solution into (methanol/water). The first fraction obtained after vacuum filtration was dried at 40 °C to constant weight constant. The volume of the supernatant (reduced by evaporation) is treated again with an additional amount of precipitant in such a manner as to obtain a new fraction. This process was repeated until a large amount of precipitant was ineffective. The first three fractions were recovered as drug coating matrices.

PMMA characterization

The first three fractions of PMMA were characterized by IR spectroscopy using an Alpha Brucker spectrometer in the spectral wavelength range from 400 to 4000 cm^{-1}, carbon magnetic resonance (^{13}C-NMR) spectroscopy on a Brucker instrument at 300 MHz, and viscosimetric measurements at 20±0.1 °C using a Cannon–Fenske capillary viscometer type KPG. The average mass $\bar{M}_v$ of the polymers were determined by application of the Mark–Houwink equation. The characterisation data for the prepared PMMA are given in the Supplementary material.

Preparation of microspheres

Microspheres were prepared by the solvent evaporation process. Briefly, one or more polymers (depending on the requirement) and HCTZ in the mass ratio (polymer(s):drug) 2:1 (content of HCTZ/polymer = 50 mass %) were co-dissolved in a water-immiscible organic solvent, *i.e.*, in dichloromethane (DCM), 66.5 g (polymer/DCM = 1.5 mass %). The organic solution was poured into de-ionized water (250 g) as an external phase containing 1 % of PVA (content of PVA/water = 0.5 mass %). The resulting O/W emulsion was continuously agitated in a glass reactor (600 mL, $\varnothing$ = 80 mm) using a six-blade turbine impeller stirrer (blade length = 50 mm, blade width = 8 mm, IKA, RW20 digital, UK) at a constant stirring speed (800 rpm) at room temperature for 3 h. The solidified microspheres were collected by filtration, washed several times with de-ionized water, and dried under vacuum in a desiccator containing $CaCl_2$ for at least 48 h. The starting compositions of the different microspheres prepared along with formulations are summarized in Table I. The details of microspheres characterization are given in Supplementary material.

TABLE I. Processing conditions for the formulation of the prepared microspheres

Lot	Composition	Matrix/drug ratio
L1	EC–HCTZ	2:1
L2	PCL–HCTZ	2:1
L3	EC/PCL–HCTZ	1:1:1
L4	EC/PCL/PMMA(F1)–HCTZ	2 :1:1:2
L5	EC/PCL/PMMA(F2)–HCTZ	2:1:1:2
L6	EC/PCL/PMMA(F3)–HCTZ	2:1:1:2
L7	EC/*β*-CD–HCTZ	1:1:1

In vitro *hydrochlorothiazide (HCTZ) release measurements*

The HCTZ release kinetics from the obtained formulations was followed using an appropriate glass dissolution reactor plunged in a bath regulated at 37±0.5 °C. The reactor was equipped with a filter tube to enable solution withdrawal without microparticles. Appropriate

amounts of formulations containing 25 mg HCTZ were placed in 1000 mL glass flasks, filled with 900 mL of simulated liquid at pH 1.2 or 7.4 at 37 °C. A stirring speed of 500 rpm was used. Aliquots of the medium (3 mL) were withdrawn periodically at predetermined time intervals, and analyzed by UV spectroscopy using a Jenway 7305 UV–Vis mono beam spectrophotometer at the appropriate wavelength for the study medium: λ_{max} = 264 nm (15100 L mol^{-1} cm^{-1}) in the gastric medium and λ_{max} = 271 nm (16000 L mol^{-1} cm^{-1}) in the intestinal medium. The withdrawn volume was replaced with an equal volume of fresh medium. The amount of HCTZ present in each sample was determined and the corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release (calculated from the total amount of HCTZ contained in each formulation) *versus* time. Each experiment was performed in duplicate.

Several mathematical models, *i.e.*, zero order, first order, the Higuchi equation and the Korsmeyer–Peppar equations, have been developed for elucidation of the drug transport processes and to predict the resulting drug release kinetics.[30-33]

RESULTS AND DISCUSSION

Characterization of the microspheres

The seven formulations loaded with hydrochlorothiazide prepared with various polymers by the solvent evaporation method using various proportions of polymer were characterized by studies such as drug loading (*DL*), percentage yield, size and size distribution (Table II).

TABLE II. Drug loading, percentage yield, and size and size distribution of the prepared microspheres

Lot	*DL* / %	Yield, %	d_{10} / μm	d_{32} / μm	d_{43}/ μm	δ
L1	18±0.34	94	38±0.37	40±0.65	42±0.30	1.09±0.01
L2	33±2.12	60	28±0.34	33±0.35	37±0.46	1.33±0.00
L3	33±1.62	71	45±0.17	48±0.32	51±0.43	1.13±0.00
L4	30±2.27	58	36±0.07	46±0.22	51±0.73	1.41±0.01
L5	23±2.71	60	22±0.29	26±0.16	30±0.49	1.34±0.03
L6	23±2.66	69	77±0.47	107±0.58	125±0.26	1.61±0.00
L7	39±0.95	46	52±0.62	68±1.04	76±1.41	1.46±0.01

The content of HCTZ in each solid dispersion formula is found to be between 18±0.34 and 39±0.95 % and the yield varied from 46 to 94 %. The drug loading and yield were found to be dependent on the nature of the polymer used in the formulation. Thus, the CD-containing microspheres (L7) showed a desirably high drug content. β-CDs are fairly water soluble and can form water-soluble complexes with lipophilic guests in the cavity of CDs.[34,35] Hence, the use of the β-CD in physical mixture of the formulation (L7) improved drug entrapment.

The drug loading decreased in the microspheres composed only of EC as the matrix. Whereas HCTZ loaded values increase when the microspheres were PCL-based or based on a combination of EC and PCL. The hydrophobic nature of PCL and its high mass promotes the encapsulation of drug and consequently a

higher *DL* value. Concerning the microspheres containing PMMA with different viscosimetric masses, there was a real increase in the drug content with the growth of the mass of the different fractions but the yield decreased. However, the hydrophobic property of PMMA and the entangled structure of the first fraction F1 (L4 with an $\bar{M}_v$ of 31903 g mol^{-1}) prevents solubilisation and the transfer of HCTZ to the aqueous phase, consequently increases the *DL* value to 30±2.27 %.

L1 produces a good yield (94 %), which indicates minimum loss of microspheres during the preparation and recovery, while the yield of L7 did not exceed 46 %, which was not excellent because an oil-in-water (O/W) emulsion was used to prepare the microparticles and the low yield could be due to the water solubility of β-CD and its possible transfer to the external phase (water).

An optical microscopic analysis of various samples was performed. The study indicated that the microparticles were spherical with different sizes. Taking the microparticles forms into account, the microparticle mean diameter was measured and finally the number mean diameter, the weight mean diameter and the surface mean diameter were calculated by examining 500 microparticles. Depending on the microparticles composition, the mean Sauter diameter (d_{32}) of batches of microparticles ranged between 26±0.16 to 107±0.58 μm, the number mean diameter (d_{10}) was between 22±0.29 and 77±0.47 μm and the weight mean diameter (d_{43}) between 30±0.49 and 125±0.26 μm. It was observed that under the same operative conditions, the stirring speed of 800 rpm gives small sizes microparticles. However, small microspheres sizes are obtained with L5 (d_{32} = = 26±0.16 μm) and larger particles developed in L6 (d_{32} = 107±0.58 μm).

The surface and morphology of the microspheres were studied using SEM; the photographs of different formulations are presented in Fig. 1. The microparticles composed only of ethyl cellulose were perfectly individualized and spherical in shape with rough and very porous surface and a number mean diameter d_{10} of 38±0.37 μm. It shows that the drug leach out through these channels. Microspheres based on PCL alone (Fig. 1) were also spherical but with a smooth surface and without any visible pores. It was noted that the number mean diameter (d_{10} = 28±0.34 μm) of the PCL/HCTZ microspheres (Fig. 1) was lower than that of EC–HCTZ microspheres (L1). Moreover, the combination of EC and PCL increased the size of microparticles (d_{32} = 48±0.32 μm). They were always spherical with a rough and very porous surface and a large dispersion was obtained. A slightly deflated microsphere appearance was observed.

On the other hand, microparticles containing EC, PCL and PMMA at different fractions (L4, L5 and L6, examples in Fig. 1) were irregular in shape and heaped up (formation of aggregates), the majority spherical and porous in the surface. There were also rod-forms in three batches; a similar finding was reported by Poovi *et al.*,[8] which were due to undissolved polymer that produces irregular particles in the shape of rods. DCM effectively forms an emulsion with an

aqueous phase containing PVA but its rapid evaporation rate does not allow the formation of stable microparticles and the development of this form, thus causing the subsequent aggregation of the microparticles and consequently, the undissolved polymer produced irregular and rod-shaped particles. However, the microparticles containing EC and β-CD (Fig. 1) appear spherical with a porous surface.

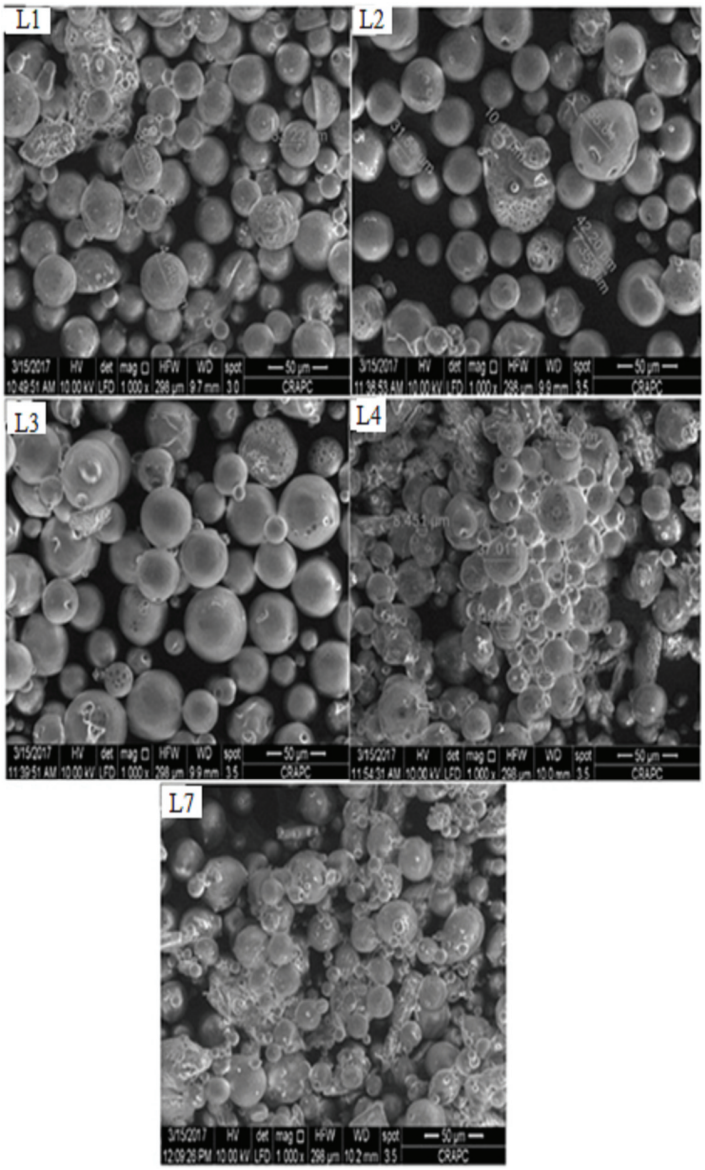

Fig. 1. SEM micrographs of the surface and the morphology of the prepared HCTZ-loaded microspheres.

The XRD patterns of HCTZ, the carriers, and the corresponding solid dispersion are presented in Fig. 2. It is well described in the literature that the differences in the solid phases are responsible for the differences in the solubility drugs.[36] The crystalline nature of HCTZ was clearly demonstrated by its characteristic XRD pattern containing well-defined peaks within the 2θ range from 5 to 60°. The drug microspheres exhibited a characteristic diffraction pattern that was less intense compared to that of pure HCTZ. These changes indicate that the crystallinity was reduced.

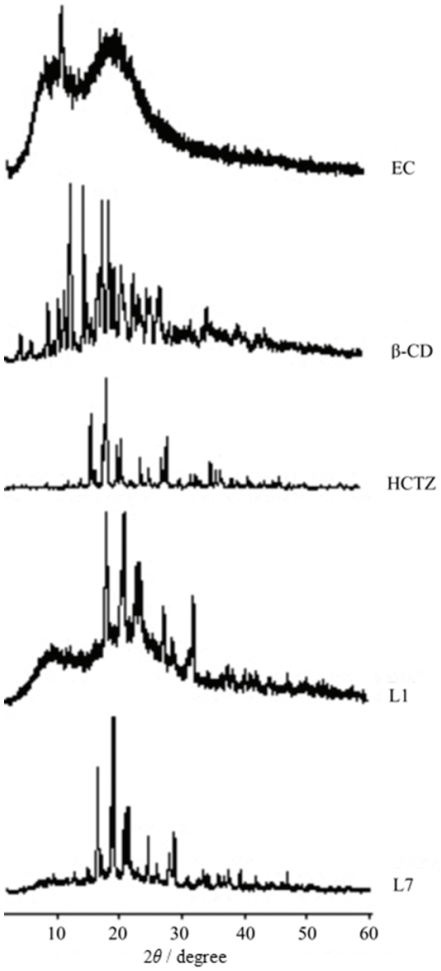

Fig. 2. XRD patterns of ethyl cellulose, β-CD, pure HCTZ, L1 and L7.

The XRD pattern of the microspheres indicate the existence of major peaks of the drug HCTZ and the polymers, *e.g.*, the spectrum of formulation L1 indicates peaks at 2θ 16.64, 19.17 and 39.29 that correspond to peaks from HCTZ. The X-ray diffraction data indicate a slight reduction in the extent of crystallinity.[37]

In the XRD pattern of EC/*β*-CD/HCTZ (L7, Fig. 2), some diffraction peaks were displaced. These changes are indicative of the diminution of the crystalline form of HCTZ and *β*-CD.[38]

The DSC curves for EC, *β*-CD, HCTZ, L1 and L7 are displayed in Fig. 3. The DSC curve for HCTZ presents two thermal events, one at about 273 °C corresponding to the melting point of HCTZ and the second at 311 °C indicates its thermal decomposition.[39] Analyzing the DSC curve of *β*-CD, two endothermic peaks could be observed, one at 106 °C and the second at around 311 °C. These peaks could be related to the loss of water from *β*-cyclodextrin and the melting of *β*-CD with decomposition, respectively.[37] On the DSC curve of EC, the thermal transition of the polymer was observed at 112 °C, which corresponds

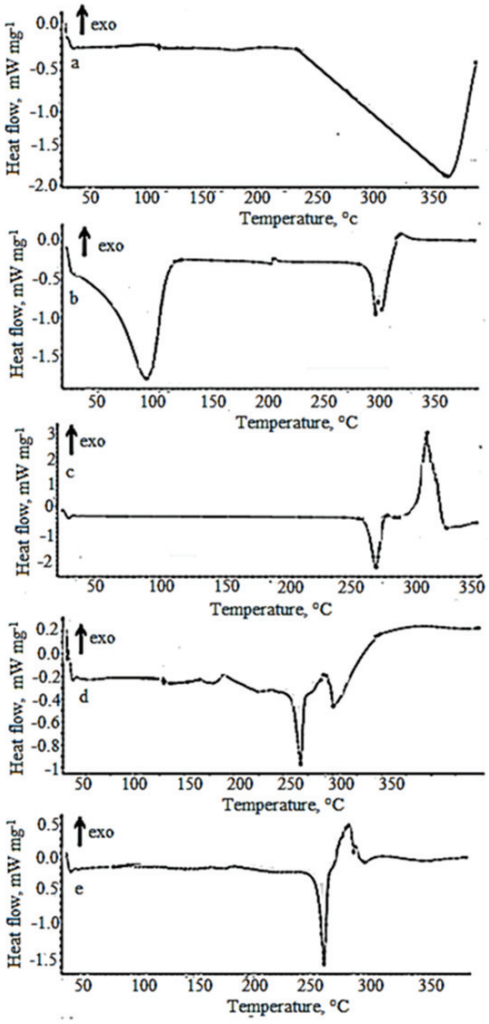

Fig. 3. DSC curves of: a) ethyl cellulose, b) *β*-CD, c) pure HCTZ, d) L1 and e) L7.

to the glass transition temperature (T_g) of EC and its melting point appeared at 374 °C.

Analyzing the microspheres DSC curves (Fig. 3d and e), the peak of melting point of HCTZ appeared and it is clear that there was a slight reduction in the melting point value of HCTZ for L1 and L7 (267 and 269 °C, respectively). This change is due to the presence of the matrixes and it indicates that there is no interaction between the drug and matrixes.[13] Moreover, there is a small variation in HCTZ crystallinity (crystalline form of HCTZ).

The FTIR spectra for HCTZ pure, physical mixtures and solid dispersions with different carriers (selected formulations) are shown in Fig. 4. The FTIR spectrum of pure HCTZ is characterized by an absorption band at 3357.15 cm^{-1}, most probably attributable to the N–H stretching band of the primary amine group, and at 3262.04 and 3163.08 cm^{-1} for the N–H stretching bands of the secondary amine group. The peak at 1594.95 cm^{-1} arises from the stretching of the C=C aromatic ring, the peak at 1273.24 cm^{-1} denotes the C–N stretching vibration of aromatic amines and the peak at 1241.20 cm^{-1} arises from SO_2 stretching.

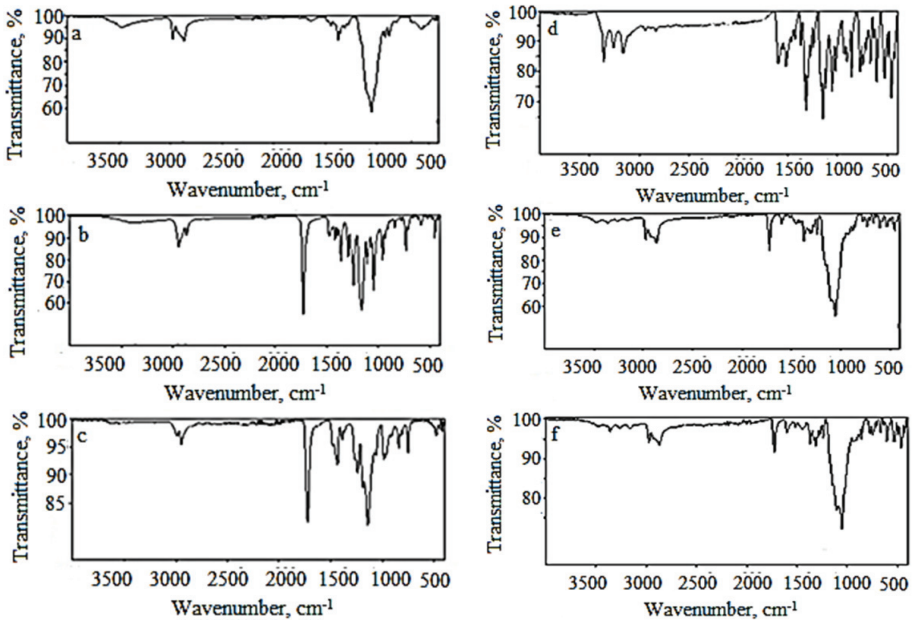

Fig. 4. IR spectra of: a) ethyl cellulose, b) PCL, c) PMMA, d) pure HCTZ, e) L3 and f) L5.

The infrared spectra of the microspheres are compared in Fig. 4 with those of HCTZ and the polymer matrix. An analysis showed the presence of similar characteristic bands of the polymers and HCTZ in the HCTZ-loaded microspheres. The spectra of HCTZ delayed release formulations showed that most of

the peaks of the drug were present and broad peak at the same place as the peak observed in the spectrum of pure drug, which indicates that the spectra of the HCTZ loaded microspheres appear as the sum of the spectra of pure HCTZ and the polymers. This confirms the absence of any chemical interaction between drugs and polymers. The differences in transmittance values are due to varying drug and polymer concentrations.

In vitro *dissolution of hydrochlorothiazide from the microspheres formulations*

The drug release study was realized in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). The *in vitro* dissolution study was performed for various microspheres formulations using EC, PCL, PMMA and β-CD as polymers for the formulations. The drug release profiles from the microspheres after 500 min in the gastrointestinal tract are shown in Figs. 5 and 6, from which it could be seen that all formulations underwent an initial linear release phase followed by equilibration. The release rate was largely affected by the characteristics of the microparticles, namely the particle size and nature of the matrix. The results of the *in vitro* dissolution studies of the formulations L1–L7, presented in Table S-I of the Supplementary material, show that the microencapsulation technique improved the dissolution rate of HCTZ.

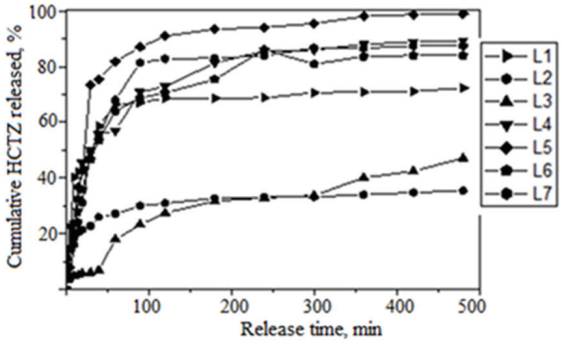

Fig. 5. Cumulative percent HCTZ released *vs.* time in the simulated gastric fluid (pH 1.2).

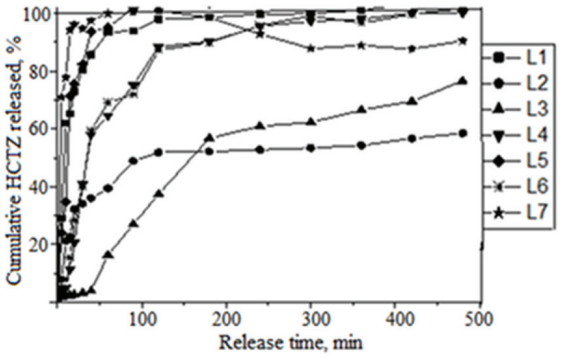

Fig. 6. Cumulative percent HCTZ release *vs.* time in simulated intestinal fluid (pH 7.4).

First, the presence of polymeric carriers, such as EC and β-CD, improved the solubility of the microspheres, which could reduce the interfacial tension between a poorly water-soluble drug and the dissolution medium.[8,40] Loyd *et al.*[41] and Pokharkar *et al.*[42] demonstrated that the improvement in the dissolution rate of a drug may be due to a reduction in its crystallinity. During the dissolution process of a drug in its crystal state, a certain amount of energy is required to break the crystal lattice.[43] However, amorphous drugs do not need such energy,[44] although this enhancement might also be attributed to the increase in the wetability and solubility of the drug.[45] This improvement in drug release promotes the bioavailability of the drug, which makes the solid dispersions ideal for the delivery of oral hydrophobic drugs.[46]

Secondly, the drug/polymer ratio is an important factor affecting the rate of release of drugs from matrices. The results reveal that the release rate decreased as the concentration of PCL at high molar mass ($\bar{M}_v$ = 70000–90000 g mol^{-1}) increased in formulations (L2 and L3). At higher polymer loading, the viscosity of the matrix was increased, resulting in a decrease in the effective diffusion coefficient of the drug.[8]

Thirdly, the type of the polymer had a significant effect on the sizes of the microspheres and the drug release rate from the obtained microspheres.[47,48] Thus, factors that may also contribute to drug dissolution profile are the porosity and the surface morphology of the microspheres.

In vitro *release of HCTZ in simulated gastric fluid (pH 1.2)*

The percentages of drug released at 60 min in acidic medium at pH 1.2 were 82, 68, 66, 64, 57, 27 and 18 % for L5, L7, L1, L6, L4, L2 and L3, respectively. Whereas, after 2 h (the time corresponding to the drug retention in the human stomach), the percentages of the drug released from L1–L7 were 67, 31, 27, 73, 91, 71 and 83 %, respectively. First, the effect of the matrix and the size of the microspheres are noticeable. By comparing the three formulations L1–L3, the EC-based microspheres (L1) show good release of the drug. This percentage cumulative drug release was lower in L2 and L3. These results could be explained by the competition of the effects of the nature and the molar mass of the polymer and the size and porosity of microparticles on the rate of HCTZ release.

It was also observed that the drug release rate was higher for the EC microspheres compared to the PCL-based microspheres. The low release of HCTZ from the PCL-based microspheres could be explained by the effect the hydrophobic nature of PCL and of its $\bar{M}_v$ value of 70000–90000 g mol^{-1}. However, a significant effect of the polymer type on the size of the microspheres and the drug release rate from the obtained microspheres was noted. The cumulative release was faster from the smaller and porous microspheres prepared with EC (d_{32} = 40±0.65μm) than the EC+PCL mixture (d_{32} = 48±0.32 μm). It was rep-

orted previously that for smaller microspheres, the larger effective area results in a greater number of drug molecules in the surface of the microspheres, leading to faster drug release.[47,48]

On the other hand, an effect of the PMMA molar mass on drug release was not registered. On the contrary, the low mass of PMMA relative to the EC and PCL favours the penetration of water into the microparticles and consequently increases the release of the drug. L5 released 100 % of HCTZ in 8 h with an initial burst of nearly 74 % of the drug being released within 30 min.

The combination of EC with β-CD was previously found to increase the release of HCTZ during the first hour with a release burst of about 68 %. CDs are hydrophilic cyclic oligosaccharides with an outer surface and a somewhat lipophilic central cavity. In aqueous solutions, CDs are able to solubilise hydrophobic drugs by taking up a lipophilic fraction of the drug molecule into the central cavity, *i.e.* formation of hydrophilic inclusion complexes.[49]

These properties were confirmed by the present results demonstrated in Fig. 5 and Table S-I. The results have also proven the improvement of the solubility of HCTZ and allowing its diffusion from the polymer matrix.

In vitro *release of HCTZ in simulated intestinal fluid (pH 7.4)*

The effect of the pH of the release medium was studied for the prepared microspheres. The obtained release profiles are presented in Fig. 6. Thus, hydrochlorothiazide was rapidly released at pH 7.4. For example, the percentages of drug dissolved at 2 h were 100, 100, 98, 89, 87, 52 and 37 % for L5, L7, L1, L4, L6, L2 and L3, respectively. The solubility of HCTZ in the alkaline medium favoured largely its diffusion. These results could be explained by the presence of cations in the simulated intestinal fluid (Na^+ and K^+) altering the solubility of hydrochlorothiazide leading to the enhanced release of the drug in this medium.

Release mechanisms and mathematical analysis

The *in vitro* release data of all formulations were subjected to model fitting analysis to determine the mechanism of drug release from the formulations by treating the data according to the zero order, first order, Higuchi and Korsmeyer––Peppas equation (the details are presented in Supplementary material). The coefficients of correlation (r^2) and dissolution rate constants of HCTZ from microspheres according to the studied models are given in Tables III and IV for the gastric and intestinal medium, respectively.

The values of the Higuchi's dissolution constant in the intestinal milieu are relatively high (see Table IV). These values are in agreement with the experimental results already reported.

From the results of the Korsmeyer–Peppas equation, the values of n for the microspheres prepared ranged from 0.22 to 0.65 in the acidic medium and from

0.23 to 0.55 in intestinal medium (Tables III and IV), indicating a shift of Fickian transport.

TABLE III. Coefficients of correlation and dissolution rate constants of HCTZ from the microspheres in simulated gastric fluid (pH 1.2)

Lot	Zero Order		First Order		Higuchi		Korsmeyer–Peppas		
	K_0	r^2	K_1	r^2	K_H	r^2	ln K_{KP}	n	r^2
L1	0.00010	0.980	0.0046	0.968	6.588	0.990	–1.830	0.35	0.986
L2	0.00003	0.968	0.0025	0.941	1.795	0.994	–2.205	0.22	0.993
L3	0.00001	0.992	0.0044	0.986	0.544	0.991	–3.522	0.22	0.984
L4	0.00010	0.994	0.0060	0.970	7.480	0.999	–2.614	0.50	0.999
L5	0.00020	0.965	0.0094	0.994	9.523	0.987	–2.594	0.55	0.962
L6	0.00010	0.900	0.0047	0.947	6.878	0.956	–2.158	0.45	0.973
L7	0.00020	0.953	0.0067	0.969	9.021	0.981	–3.108	0.65	0.982

TABLE IV. Coefficients of correlation and dissolution rate constants of HCTZ from the microspheres in simulated intestinal fluid (pH 7.4)

Lot	Zero Order		First Order		Higuchi		Korsmeyer–Peppas		
	K_0	r^2	K_1	r^2	K_H	r^2	ln K_{KP}	n	r^2
L1	0.00020	0.975	0.0148	0.982	8.232	0.974	–1.056	0.24	0.962
L2	0.00003	0.987	0.0012	0.969	2.260	0.999	–1.835	0.23	0.989
L3	0.00002	0.997	0.0093	0.983	0.585	0.993	–5.019	0.47	0.992
L4	0.00010	0.938	0.0072	0.960	7.8049	0.953	–2.493	0.49	0.932
L5	0.00020	0.933	0.0221	0.998	11.185	0.976	–2.335	0.55	0.999
L6	0.00010	0.903	0.0065	0.926	7.3863	0.915	–2.432	0.47	0.890
L7	0.00050	0.926	0.0100	0.927	12.445	0.931	–0.744	0.24	0.924

In fact, from the kinetic results, for formulations that have an exponent n less than 0.5, the mechanism of drug release could be related to the quasi-Fickian model. Furthermore, the n = 0.5 obtained from formulation L4 at pH 1.2 followed the Fickian diffusion model. Then, the n values between 0.5 and 1.0 obtained from the Korsmeyer–Peppas model showed that the formulations followed non-Fickian (Anomalous) release, which indicated that drug release from microspheres was produced by diffusion and dissolution controlled release.

Finally, it could be concluded that with the development of the microencapsulation technique, solid dispersions have enormous potential in the design of the controlled release dosage form, such as microspheres, due to the high availability of a variety of carriers to solve problems associated with the delivery of poorly soluble drugs.

CONCLUSIONS

The present study was conducted to improve and compare the dissolution of the water-insoluble drug HCTZ using the microencapsulation technique with microspheres prepared by the solvent evaporation method with different carriers

such as ethyl cellulose, PCL, β-cyclodextrin (β-CD) and synthetic poly(methyl methacrylate) (PMMA) of different molecular weights. The solvent evaporation method was used to prepare several formulations with the selected polymers. The microspheres prepared were characterized by Fourier transform infrared spectroscopy (FTIR), X-Ray diffraction (XRD) and DSC studies indicating the presence of the drug in the microspheres and a slight decrease in the crystalline form of the latter and there was no interaction between drug and polymers. The structure and porosity of the microsphere surface were investigated by scanning electron microscopy. Various rates of drug release from microspheres of different matrices were experimentally determined. The drug dissolution from all formulations was significantly influenced by the nature of matrix and the characteristics of the microparticles. The highest release of HCTZ was obtained with the formulation L5 EC/PCL/PMMA (F2)/HCTZ (2:1:1:2), which gave complete liberation after 2 h in the intestinal medium. It was also noted that β-CD is able to solubilise hydrophobic drugs by taking up the lipophilic part of the drug molecule into its central cavity, leading to good release of HCTZ. The nature of medium greatly affects the release of HCTZ as a poorly soluble drug. The dissolution of HCTZ in the simulated intestinal medium is important for various formulations tested. Finally, the release kinetics using the Higuchi and Korsmeyer–Peppas equations showed a Fickian diffusion mechanism and the release rate could be controlled by adjusting the selected polymers and different ratios of carriers.

SUPPLEMENTARY MATERIAL

Additional experimental results and considerations are available electronically on the pages of the journal's website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

ПОБОЉШАЊЕ ПРОФИЛА БРЗИНЕ РАСТВАРАЊА ДИУРЕТИКА ХИДРОХЛОРОТИАЗИДА ИЗРАДОМ МИКРОСФЕРА

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Хидрохлоротиазид (HCTZ), мада је развијен и уведен крајем педесетих година перошлог века и даље је један од најчешће коришћених лекова у лечењу повишеног крвног притиска. Мала растворљивост у води је један од разлога за његову ограничену биорасположивост након оралног узимања. У овом раду је приказана израда микросфере на бази HCTZ користећи поступак отпаравања лако испарљивог растварача из емулзије. Укупно седам различитих формулација је припремљено на бази етил-целулозе, поли(ε-капролактона) (PCL), бета-циклодекстрина (β-CF) и синтетисаних поли(метил-метакрилата) (PMMA), различитих моларних маса. Коришћени су различити

масени односи лека и полимера ради испитивања њиховог утицаја на ефикасност инкапсулације и кинетику ослобађања лека. Припремљене микросфере су карактерисане помоћу FTIR спектроскопије, рентгенске дифракције, диференцијалне скенирајуће калориметрије, приносом и садржајем лека, оптичком микроскопијом, а морфологија површине на основу скенирајуће електронске микроскопије (СЕМ). Такође је приказана студија кинетике отпуштања лека *in vitro*, који су симулирали услове у гастроинтестиналном тракту. Међутим, ефикасност инкапсулације лека је била мала у опсегу од 18±0,34 до 39±0,95 %. Микросфере су биле сферичне, а средњи пречник Саутер (d_{32}) добијених микрочестица је био у опсегу од 26±0,16 до 107±0,58 m. Присуство лека и полимерних носача у микрочестима је потврђено FTIR и XRD спектрима. Резултати *in vitro* испитивања растворљивости лека су показали да брзина отпуштања лека из полимерних матрица у великој мери зависи од карактеристика микрочестица, тј. од величине честица и природе матрице. Анализа кинетичких параметара отпуштању лека показује најбоље слагање са Higuchi моделом са високим коефицијентима корелације (r^2).

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