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ORIGINAL ARTICLE

Effect of cyclodextrins on physico-chemical and release properties of Eudragit RS 100 microparticles containing glutathione

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Abstract The objective of this work was to develop a novel microparticulate system based on the mucoadhesive polymer Eudragit-RS 100 and cyclodextrins (CDs), potentially useful for the oral administration of Glutathione (γ–glutamylcysteinylglycine, GSH). For this purpose, an oil-in-oil (O/O) emulsion-solvent evaporation method was used for the preparation of microparticles (MPs) containing GSH alone or together with one of the following CDs: α-, β-, γ-, methyl-β-(Me-β-), hydroxypropyl-β-(HP-β-) or sulfobutylether-β-cyclodextrin (SBE_{7m}-β-CD). MPs were obtained by emulsifying a mixture of Eudragit RS 100, GSH, CD and magnesium stearate in acetone or acetonitrile with a mixture of liquid paraffin and Span 80. Size, encapsulation efficiency, and drug release of the prepared MPs were evaluated. The results clearly indicated that all the examined properties were dependent on the water-miscible solvents and CD used. In particular, MPs prepared by using acetone or acetonitrile showed different size distributions with mean diameters in the ranges 82–350 and 15–22 μm, respectively. Moreover, encapsulation efficiency values were found to be high in all cases (71–99%) and was significantly affected by the CD type. The GSH release rates were evaluated employing dissolution media with different pH values (1.2, 6.8 and 7.4) and the following rank order was obtained for MPs prepared using acetone: MPs incorporating Me-β-CD > MPs without CD > MPs incorporating the remaining CDs. On the other hand, MPs prepared using acetonitrile gave the highest GSH release rate. Finally, stability of GSH encapsulated in MPs containing HP-β-CD to enzymatic attack by pepsin A, α-chymotrypsin, and γ-glutamyltranspeptidase was also investigated.

Keywords Cyclodextrin · Glutathione · Microparticles · Morphology · Release · Stability

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Introduction

The tripeptide glutathione (γ–glutamylcysteinylglycine, GSH) is one of the main detoxification factors present in the human body. It plays an important role in the protection against both free radicals and reactive oxygen compounds. Furthermore, it is clinically used for approved therapeutic indications including treatment of alcohol and drug poisoning, protection against toxicity induced by cytotoxic chemotherapy, radiation trauma and AIDS-associated cachexia [1–4]. However, following intravenous administration of GSH, rapid enzymatic degradation by γ-glutamyl-transpeptidases and γ-glutamyl-cyclotransferases occur leading to the generation of the constituent aminoacids; thus, the plasma half-life of the tripeptide is very short. In an attempt to develop a dosage form enabling oral GSH administration, in a previous work [5] it was demonstrated that encapsulation of GSH in Eudragit RS 100 microparticles (MPs) in the presence of hydroxypropyl-β-cyclodextrin (HP-β-CD) represents a new and convenient delivery system. For the preparation of

these MPs containing GSH alone or together with HP-β-CD, an oil-in-oil (O/O) emulsion-solvent evaporation method [6] was used. In particular, MPs were obtained by emulsifying a mixture of Eudragit RS 100, GSH, CD and magnesium stearate in acetone with a mixture of liquid paraffin and Span 80.

In the present study, new MPs with different physico-chemical and release properties are described. They were prepared by replacing HP-β-CD with α-, β-, γ-, Me-β- or SBE_{7m}-β-CD and moreover, the use of acetonitrile instead of acetone was also investigated.

Experimental

Materials

Reduced GSH, glutathione disulfide (GSSG), Span 80, perchloric acid (70%), Pepsin A (from porcine stomach mucosa), α-chymotrypsin (TLCK treated from bovine pancreas Type VII), and γ-glutamyltranspeptidase, (from equine kidney Type VI, 11 U/mg) were purchased from Sigma-Aldrich (Milan, Italy). Eudragit RS 100 (Röhm GmbH & Co) was a kind donation from Rofarma (Gaggiano, Milan, Italy). Liquid paraffin (density measured at 20°C 0.840 kg/dm^3; viscosity measured at 20°C 31.31 cPs) and magnesium stearate (Italian Pharmacopoeia, having a magnesium content of 4.7% w/w) were obtained from Polichimica S.r.l. (Bologna, Italy). Acetone (HPLC grade), Acetonitrile (HPLC grade), and *n*-hexane (HPLC grade) were purchased from Baker (Deventer, Holland). 2-hydroxypropyl-β-cyclodextrin with a degree of substitution, 5.88, (calculated by means of ^{1}H-NMR) was obtained from Roquette (Cassano Spinola, Italy). Sulfobutylether-β-cyclodextrin (SBE_{7m}-β-CD, denoted as SBE-β-CD in this work, Captisol®) was purchased from CyDex Inc (Lenexa, KS), (average degree substitution 6.4, MW 2163). α-, β-, γ-CD and mono methyl-β-cyclodextrin (Me-β-CD) were kind gifts from Waker–Chemie (Peschiera Borromeo, Italy). All these cyclodextrins were kept in a desiccator until their use.

Preparation of microparticles

MPs were prepared according to an O/O emulsion-solvent evaporation method [6]: (a) *MPs without CDs*: Eudragit RS 100 (1.25 g) was dissolved in 5 ml of acetone or acetonitrile at room temperature and then GSH (50 or 100 mg) and magnesium stearate (75 mg) were added. The suspension was homogenized using an Ultraturrax homogenizer (Janke and Kunkel, Germany, model T25) equipped with an S25N dispersing tool at 13,000 rpm for 30 s. The resulting suspension was dispersed in 15 ml of a similarly prepared 1% v/v mixture of Span 80 in liquid paraffin by employing the above mentioned homogenizer at 13,000 rpm for 2 min. The resulting emulsion was maintained overnight at room temperature and under stirring using a paddle stirrer at 900 rpm. The MPs were harvested by centrifugation, washed three times with *n*-hexane, dried overnight under vacuum and then stored at 4°C until their further use; (b) *CDs containing MPs*: Eudragit RS 100 (1.25 g) was dissolved in 5 ml of acetone or acetonitrile at room temperature. To this solution GSH (50 or 100 mg), CD (50 or 150 mg) and magnesium stearate (75 mg) were added. By working-up the resulting suspension as above, MPs incorporating GSH and CD were obtained.

Quantitative analysis of GSH

High-performance liquid chromatography (HPLC) analyses were performed with a Waters (Waters Corp., Milford, MA) Model 600 pump equipped with a Waters 2996 photodiode array detector (set at the wavelength of 220 nm), a 20 µl loop injection autosampler (Waters 717 plus) and Empower software. For analysis, a reversed phase Hydro Synergy C_{18} (25 cm × 4.6 mm; 4 µm particles; Phenomenex, Torrance, CA) column in conjunction with a precolumn C18 insert was eluted with acetonitrile: 0.025 M phosphate buffer pH 2.7 (1:99 v:v) in isocratic mode. The flow rate of 0.7 ml/min was maintained and the column effluent was monitored continuously at 220 nm. The retention times of GSH and GSSG were 7.2 and 18 min, respectively. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions. Standard calibration curves of GSH in 0.025 M phosphate buffer pH 2.7 were prepared at 220 nm wavelength using 0.025 M phosphate buffer pH 2.7 as solvent and were linear ($r^2 > 0.999$) over the range of tested concentrations (3.32×10^{-3} M–5.46×10^{-6} M). Under these conditions, the quantification limit (LOQ), defined as the analyte concentration that could be analysed with acceptable precision and accuracy, was determined to be 2 µg/ml for both GSH and GSSG. The linear range for this assay was checked from 2 µg/ml to 1000 µg/ml.

GSH content of the microparticles

About 10 mg of MPs were poured into 4 ml of a mixture containing 0.025 M phosphate buffer pH 2.7/CH_3CN (95/5 v/v) and the resulting suspension shaken

overnight in a thermostatically controlled water bath (25°C, 150 rpm). The supernatant was filtered through 0.22 μm membrane filter (Millipore® cellulose acetate) and then analyzed by HPLC. The results are expressed as percentage of GSH encapsulation efficiency (EE%) calculated as 100× (μmol GSH loaded per g microspheres/μmol GSH employed per g polymer).

Size and morphology of microparticles

The surface morphology and shape of MPs (with or without CDs) were examined by scanning electron microscopy (SEM). The samples were sputtered with a carbon film 30 nm thick. The electron microscope utilized for this study was a EVO50XVP of LEO Instruments operating with 15 kV accelerating potential, 300 pA probe current and working distance 10 mm.

The size of MPs was determined by direct observation, using a light stereomicroscope (Leica Galen III) equipped with a Panasonic (WV CP 230) camera and Leica Qwin v. 2.4 software. The arithmetic mean diameter of MPs was determined by averaging the individual values of 150 MPs taken from each batch.

Solid state characterization

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer. Samples of pure GSH powder or Eudragit RS 100 GSH containing MPs (with or without CD) were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 450–4000 cm^{-1} and the resolution was 1 cm^{-1}.

X-ray analysis

Powder X-ray diffraction (PXRD) patterns were recorded on a Philips X'PERT PRO X-ray diffractometer using Cu $K\alpha$ radiation, a voltage of 40 kV, a current of 40 mA, and a scanning rate of 1/2°/min.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms were obtained using a Mettler Toledo DSC 822 e Star^{e} 202 System (Mettler Toledo, Switzerland) equipped with a automatic thermal analysis program. Aliquots of about 5 mg of each sample were placed in an aluminium pan of 40 μl capacity and 0.1 mm thickness, press-sealed with a perforated aluminium cover of 0.1 mm thickness. An empty pan sealed in the same way was used as reference. DSC curves of Eudragit RS 100 and GSH powder and MPs including GSH alone or together with CDs were recorded. Conventional DSC measurements were performed by heating the sample up to 230°C at the rate of 5°C/min, under a nitrogen flow of 50 cm^3/min. The starting temperature was 25°C. Indium (99.99% of purity) was used as standard for calibrating the temperature. Reproducibility was checked by running the sample in triplicate.

In vitro drug release studies

In vitro release of GSH from each formulation was determined at pHs 1.2, 6.8, 7.4 and at 37°C in an USP III (BioDis, VanKel, Cary North Carolina, USA) dissolution apparatus. Three dissolution media (HCl pH 1.2, 0.05 M phosphate buffer pH 6.8, and 0.05 M phosphate buffer pH 7.4) were used in order to mimic the main pH conditions of the gastrointestinal tract. The glass vessels of the dissolution apparatus were filled with 230 ml of dissolution medium and apparatus settings were as follows: 25 dips/min, drain time of 1 min. For each sample, analysis was performed in triplicate. Samples from each MP formulation (corresponding to at least 15 mg of pure GSH) were weighted on a polypropylene stainless steel screen (125 mesh) and put on the base of the glass vessels. At predetermined time intervals, 2 ml of the mixture were withdrawn in the manifold and filtered through a 0.22 μm membrane filter (Millipore® cellulose acetate) in thermostated test tubes. The initial volume of dissolution medium was maintained by adding 2 ml of the same medium after each withdrawal. The clear filtrate was allowed to stand in the test tube at 37°C until analyzed by HPLC. Similarly, 15 mg of GSH powder were submitted to the above reported dissolution test.

Stability to enzymatic degradation of GSH encapsulated in Eudragit MPs

Eudragit MPs containing GSH together with HP-β-CD prepared in acetonitrile or in acetone were selected as representative formulations for stability studies.

Pepsin A

About 5 mg of pepsin was dissolved in 5 ml of HCl buffer pH 1.2 and the resulting solution was further diluted (1:1000) with HCl pH 1.2 to give a 370 mU/ml solution.

Pure GSH (6.3 mg) was added to 10 ml of 370 mU/ml pepsin A solution. The resulting mixture was kept

in screw-capped test tubes in a thermostatically controlled water bath at 37°C shaking at 150 rpm. At given time intervals, aliquots of 400 μl were withdrawn from the incubated solutions and centrifuged at 13,200 rpm for 15 min. The supernatant was diluted 1:1 with 0.025 M phosphate buffer pH 2.7 and the remaining intact peptide was determined by HPLC.

About 132 and 240 mg of MPs (from acetone and acetonitrile, respectively, both containing 6.1 mg of pure GSH) were suspended in 10 ml of pepsin A solution 370 mU/ml. This dispersion was kept in screw-capped test tubes in a thermostatically controlled water bath at 37°C shaking at 150 rpm. By working-up as above the MPs stability in the presence of pepsin A was determined.

α-Chymotrypsin

The α-chymotrypsin solution of 7.3 U/ml was prepared by dissolving 2.6 mg of α-chymotrypsin in 20 ml of 0.05 M phosphate buffer pH 6.8.

Pure GSH (5.38 mg) were solubilized in 20 ml α-chymotrypsin solution (7.3 U/ml). The resulting solution was put in a screw-capped test tube in a thermostatically controlled water bath at 37°C shaking at 150 rpm. At given time intervals, aliquots of 500 μl were withdrawn from the incubated solutions and centrifuged at 13,200 rpm for 15 min. The supernatant was diluted 1:1 with 0.025 M phosphate buffer pH 2.7 and the remaining intact peptide was determined by HPLC.

About 110 and 200 mg of MPs (from acetone and acetonitrile, respectively, both containing 5 mg of pure GSH) were suspended in 20 ml α-chymotrypsin solution (7.3 U/ml). This mixture was put in a screw-capped test tube in a thermostatically controlled water bath at 37°C shaking at 150 rpm. By working-up as above the MPs enzymatic stability in the presence of α-chymotrypsin was determined.

γ-Glutamyltranspeptidase

The*e* γ-glutamyltranspeptidase solution was prepared by dissolving 1.2 mg of γ-glutamyltranspeptidase in distilled water (2 ml) and then, 0.8 ml of this solution was mixed with 5.2 ml of phosphate buffer pH 6.8.

Pure GSH (3.8 mg) were solubilized in the γ-glutamyltranspeptidase solution prepared as above in a screw-capped test tube and incubated at 37°C in a shaking (150 rpm) and thermostatically controlled water bath. At given time intervals, aliquots of 400 μl were withdrawn, and the enzymatic reaction was stopped by addition of perchloric acid 10% (0.25 ml). Each sample was centrifuged at 13,200 rpm for 15 min and the supernatant was diluted 1:1 with 0.025 M phosphate buffer pH 2.7 and the remaining intact peptide was determined by HPLC.

About 80 and 152 mg of MPs (from acetone and acetonitrile, respectively, both containing 3.8 mg of pure GSH) were suspended in the γ-glutamyltranspeptidase solution prepared as above in a screw-capped test tube in a thermostatically controlled water bath at 37°C shaking at 150 rpm. By working-up as above the MPs stability in the presence of γ-glutamyltranspeptidase was determined.

Results and discussion

In a previous work [5], we demonstrated that Eudragit RS 100 MPs containing GSH and HP-β-CD represent a new delivery system which may be useful for oral administration of the considered tripeptide. These MPs were prepared by using an O/O emulsion evaporation method [6] involving acetone as dispersed phase. In the present work we report the investigation carried out with the aim of obtaining MPs with different physicochemical and release properties by using new solvents instead of acetone and CDs. Firstly, acetone was replaced by an analogous volume of a new solvent. When ethyl acetate was used, no MPs formation was observed. On the other hand, the use of methanol or isopropanol led to MPs of size greater than the previously prepared ones by using acetone. On employing acetonitrile, MPs of much reduced size were obtained (15–22 μm). Therefore, this last solvent was employed to encapsulate GSH alone or together with a different CD in Eudragit RS 100 MPs. Furthermore, the effect of cyclodextrin (CD) on the mean diameter and encapsulation efficiency (EE%) was also investigated. The mean diameters of all prepared MPs in acetonitrile are shown in Table 1. This table also depicts the mean diameters of the corresponding formulations prepared in acetone for an immediate comparison. By using acetone, the lowest mean diameter (82 μm) was observed when Me-β-CD and GSH were used at the highest level (i.e., 150 mg and 100 mg, respectively). The notable reduction in size observed when acetone was replaced by acetonitrile may be ascribed to the difference of interfacial tension between the involved solvent and liquid paraffin. This interpretation seems reasonable considering the result obtained by optical microscopy analysis of two analogously prepared O/O emulsions. Droplets having a very different mean size were observed, the largest being those formed in acetone/paraffin emulsion.

Table 1 Size, encapsulation efficiency, and yield of Eudragit MPs from selected formulations containing GSH (50 mg) and CDs (150 mg)

Formulations	Mean diameter (μm)	Encapsulation efficiency (%)	Yield (%)
Prepared by using acetone[a]			
Whitout CD	265.1	75.4	
α CD	350.0	87.4	83.9
β CD	182.0	91.6	82.8
γ CD	349.0	84.5	87.2
HP-β-CD	178.5	77.1	
Me-β-CD	140.0	90.7	84.0
SBE-β-CD	243.0	86.6	78.1
Prepared by using acetonitrile[a]			
Without CD	22.5	99.0	83.5
α CD	15.0	81.0	84.0
β CD	14.6	75.0	87.0
γ CD	15.3	76.5	89.6
HP-β-CD	16.0	89.7	83.3
Me-β-CD	22.5	70.7	91.9
SBE-β-CD	15.3	93.0	95.0

[a] Data are means of three determinations with coefficients of variation (CV) less than 10%

SEM micrographs (Fig. 1) revealed that most of the prepared MPs had a spherical shape and a quite smooth surface.

EE% values were found to be high in all cases (71–99%) and was significantly affected by the CD type (Table 1). For MPs prepared in acetonitrile the following rank order of E.E.% was observed: MPs without CD > SBE-β-CD > HP-β-CD > α-CD > γ-CD > β-CD > Me-β-CD. The yields of the recovered and dried MPs were calculated by comparing their amount multiplied by 100 with the total weight of polymer and CD used. These yields were found high enough and ranging from 78% to 90% (Table 1).

To assess whether the GSH was incorporated in the MPs in its crystalline or amorphous form, FT-IR-, PXRD- and DSC-studies were performed. FT-IR and PXRD spectra and DSC profiles of pure GSH and of all the loaded MPs were recorded. In Fig. 2, for the sake of concision, only the PXRD spectra are shown. These results, taken together, suggest that the microencapsulation process produces a marked decrease in crystallinity of GSH and/or confers to this drug a nearly amorphous state.

The in vitro drug release at pH 1.2 from the MPs prepared both in the presence of acetone and acetonitrile is shown in Fig. 3. In this study, the possible release of the CD from MPs was not investigated. Comparing the data shown in Fig. 3, it is apparent that, with the exception of Me-β-CD containing MPs, those prepared using acetonitrile gave the highest GSH release rate. Furthermore, Me-β-CD containing MPs prepared in acetone and in acetonitrile showed the highest and the lowest, respectively, percentage of drug released. This behavior may be related to the fact that, as shown by SEM analysis, MPs prepared in acetone were essentially broken, together with some almost intact and of spherical shape (Fig. 4). On the other hand, Me-β-CD containing MPs prepared in acetonitrile were of greater size and hence characterized by a reduced specific surface.

It is also apparent from the data reported in Fig. 3 that the release rate of GSH from MPs prepared using acetonitrile is independent on the size of the CD cavity. In fact, the following rank order of release rate was observed for natural CDs: β-CD > γ-CD > α-CD. The

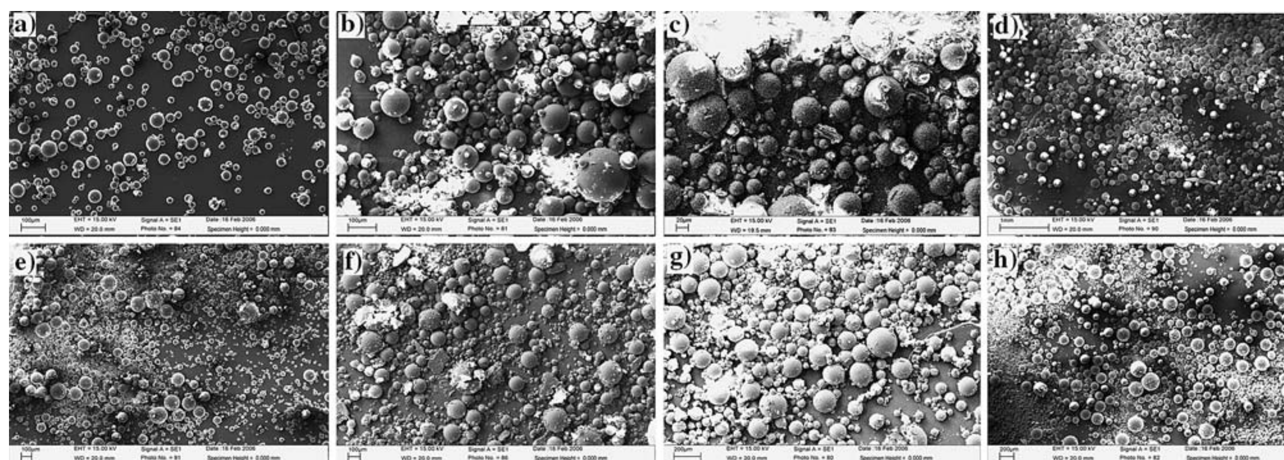
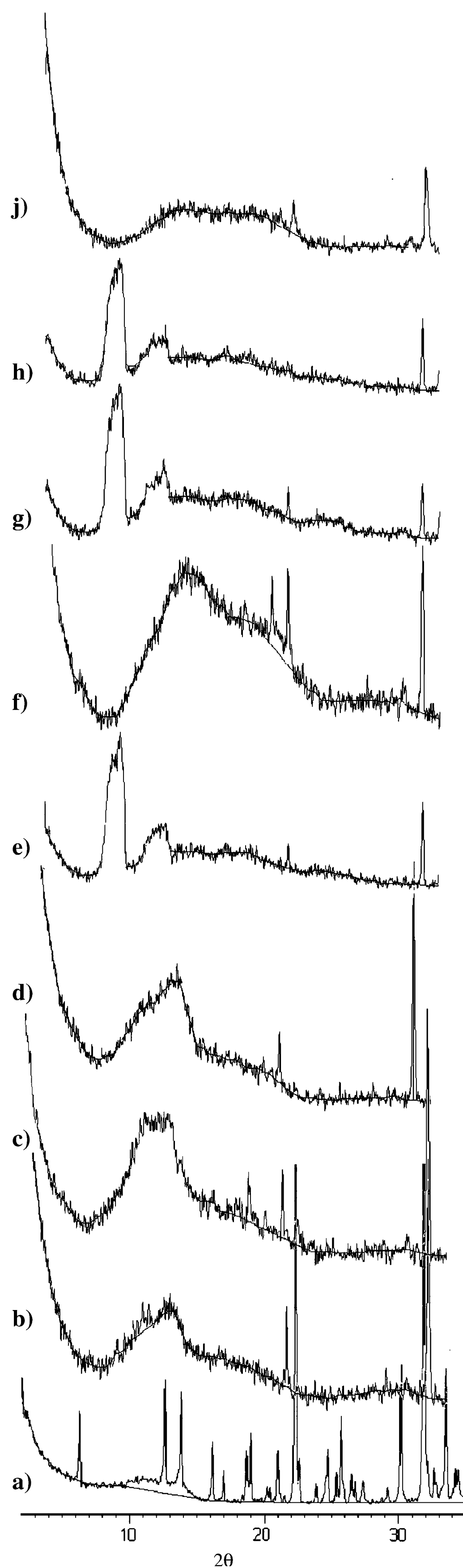


Fig. 1 SEM micrographs of MPs prepared in the presence of acetonitrile (**a**) without GSH and CD, and SEM micrographs of MPs containing (**b**) GSH alone, (**c**) GSH and α-CD, (**d**) GSH and β-CD, (**e**) GSH and γ-CD, (**f**) GSH and HP-β-CD, (**g**) GSH and SBE-β-CD, (**h**) GSH and Me-β-CD



◀ **Fig. 2** PXRD spectra of (**a**) pure GSH; (**b**) Eudragit RS 100 alone. PXRD spectra of MPs prepared in the presence of acetonitrile and (**c**) without CD, (**d**) containing GSH and α-CD, (**e**) containing GSH and β-CD, (**f**) containing GSH and γ-CD, (**g**) containing GSH and Me-β-CD, (**h**) containing GSH and HP-β-CD, (**i**) containing GSH and SBE-β-CD

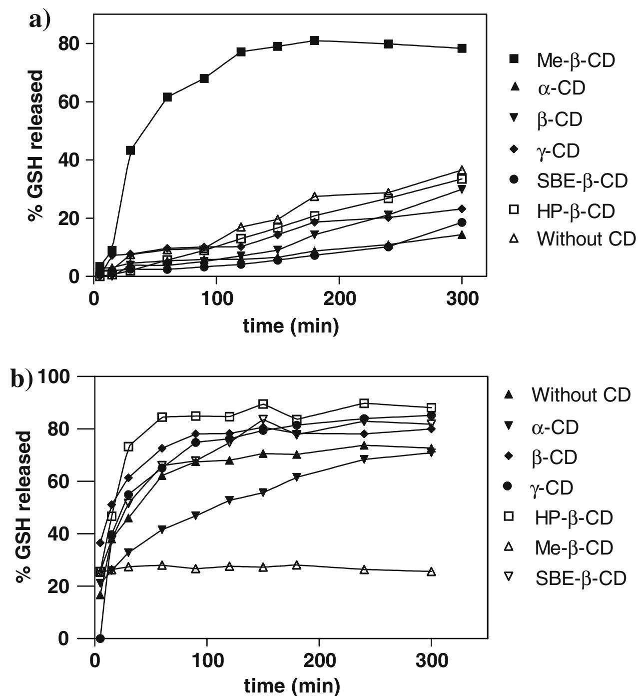


Fig. 3 Release of GSH at pH 1.2 from MPs prepared in acetone (**a**) and acetonitrile (**b**)

influence of the solvent used for the preparation of MPs on their release properties was also noted for other CDs. Thus, SBE-β-CD containing MPs prepared in acetone showed the lowest percentage of drug released, while the corresponding ones prepared in acetonitrile displayed a high percentage of GSH released after 5 h.

The in vitro GSH released from the MP formulations prepared in acetonitrile at pH 6.8 and 7.4 (data not shown) was in general slower than that observed at pH 1.2. Moreover, under neutral or basic conditions, α-CD containing MPs generally gave rise to the slowest GSH release rate. It was also apparent that at pH 6.8 or 7.4, the amount of GSH released after 5 h was lower than that observed at pH 1.2 and progressively increasing amounts of glutathione disulfide were detected by HPLC analysis. This is consistent with the fact that higher GSH oxidation rate is observed under neutral or basic conditions [5].

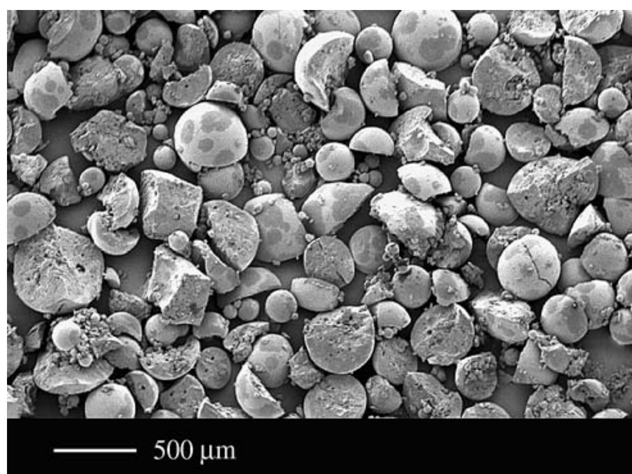


Fig. 4 SEM micrograph of Me-β-CD containing MPs prepared in acetone

The stability of GSH encapsulated in Eudragit MPs containing HP-β-CD and prepared using acetonitrile was investigated in the presence of pepsin A, α-chymotrypsin, and γ-glutamyl-transpeptidase. The results were compared with those obtained under the same conditions using GSH alone or MPs containing GSH together with HP-β-CD and prepared employing acetone. Results indicate that MPs protect GSH against proteolytic degradation. As shown in Fig. 5, for the peptide alone, after 5 h incubation with the enzymes examined, a clear disappearance of GSH resulted. In contrast, in the case of GSH containing MPs together with HP-β-CD no degradation occurred but a progressive increase in GSH level was observed. However, in the presence of pepsin this effect was more intense for MPs prepared in acetonitrile than the corresponding ones arising from acetone (Fig. 5a). In α-chymotrypsin no differences were noted between the two types of MPs (Fig. 5b). In the presence of γ-glutamyl-transpeptidase, the peptide alone showed a very marked degradation, while MP formulations again showed a progressive increase in GSH released (Fig. 5c). These results indicate that the encapsulation prevents to some extent the enzymatic degradation of the peptide.

In conclusion, GSH was encapsulated in Eudragit RS-100-based MPs containing different CDs. The obtained results suggest that (i) by using acetonitrile instead of acetone in the O/O emulsion-solvent evaporation a marked reduction in size of the resulting MPs together with satisfactory entrapment efficiencies can be obtained; (ii) the physico-chemical and release properties of MPs are dependent on the experimental protocol and CD used. Stability studies showed a stabilizing effect of encapsulation in Eudragit RS-100 since peptide degradation was reduced to some extent. Therefore, Eudragit RS 100 MPs prepared using acetonitrile may represent a new GSH delivery systems potentially useful for oral administration of this tripeptide.

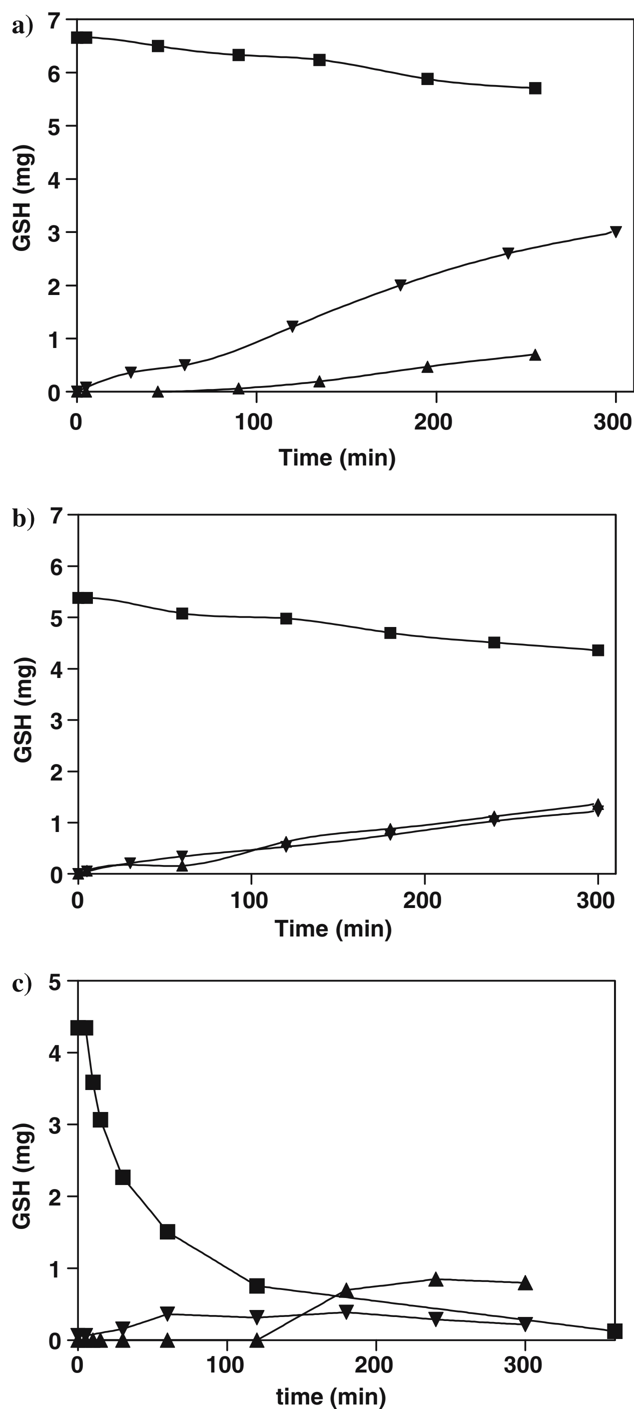


Fig. 5 Enzymatic degradation of pure GSH (■) and GSH released from MPs prepared in acetone (▲) and in acetonitrile (▼) in the presence of (**a**) pepsin A, (**b**) α-chymotrypsin, and (**c**) glutamyltranspeptidase

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References

1. Sweetman, S.C. (ed.): Martindale. The Complete Drug Reference, 33rd edn. The Pharmaceutical Press, London (2002)
2. Shigesawa, T., Sato, C., Marumo, F.: Significance of plasma glutathione determination in patients with alcoholic and non-alcoholic liver disease. Gastroenterol. Hepatol. **7**, 7–11 (1992)
3. Townsend, D.M., Tew, K.D., Tapiero, H.: The importance of glutathione in human disease. Biomed. Pharmacother. **57**, 145–155 (2003)
4. Deleve, L.D., Kaplowitz, N.: Glutathione metabolism and its role in hepatotoxicity. Pharmacol. Ther. **52**, 287–305 (1991)
5. Trapani, A., Laquintana, V., Denora, N., Lopedota, A., Cutrignelli, A., Franco, M., Trapani, G., Liso, G.: Eudragit RS 100 microparticles containing 2-hydroxypropyl-β-cyclodextrin and glutathione: Physicochemical characterization, drug release and transport studies. Eur. J. Pharm. Sci. **30**, 64–74 (2007)
6. de Brito Amorim, M.J.L.G., Ferreira, J.P.M.: Microparticles for delivering therapeutic peptides and proteins to the lumen of the small intestine. Eur. J. Pharm. Biopharm **52**, 39–44 (2001)