THERMAL ANALYSIS STUDY OF FLAVONOID SOLID DISPERSIONS HAVING ENHANCED SOLUBILITY

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Purposes of this paper were to prepare and study new drug delivery systems for both flavanone glycosides and their aglycones based on solid-dispersion systems. These compounds are poor water soluble drugs, so an enhancement of their dissolution is a high priority. Solid-dispersion systems were prepared using PVP, PEG and mannitol as drug carrier matrices. Characterizations of these dispersions were done by differential scanning calorimeter (DSC) and X-ray diffraction (XRD). The glass transition (T_g) temperature of PVP was only recorded in the DSC thermograms of PVP solid-dispersions of both flavanone glycosides and their aglycones, while in case of PEG and mannitol solid-dispersions endotherms of both glycosides and aglycones were noticed with low peak intensity, indicating that high percent of drug is in amorphous state. The XRD patterns of all PVP solid-dispersions of aglycones show typical amorphous materials, but XRD patterns of their glycosides reveal the presence of crystalline material. However, in all solid dispersions shifts in T_g of PVP as well as T_m of PEG were observed, indicating the existence of some interactions between drugs and matrices. SEM and TEM microscopy revealed that PVP/aglycone flavanone compounds are nanodispersed systems while all the other solid dispersions are microcrystalline dispersions. The solubility of both flavanone glycosides and their aglycones was directly affected by the new physical state of solid dispersions. Due to the amorphous drug state or nano-dispersions in PVP matrices, the solubility was enhanced and found to be 100% at pH 6.8 in the nano-dispersion containing 20 mass% of aglycones. Also solubility enhancement was occurred in solid dispersions of PEG and mannitol, but it was lower than that of PVP nano-dispersions due to the presence of the drug compounds in crystalline state in both matrices.

Keywords: *enhanced solubility, flavonoids, solid dispersions, thermal analysis, XRD*

Introduction

Flavonoids are a group of naturally occurring polyphenolic compounds that are ubiquitous in all vascular plants and are widely used in the human diet [1]. They are usually present almost exclusively in the form of β-glycosides, and they can be divided on the basis of their molecular structure into four main groups, flavones, flavonols, flavanones, and isoflavones [2]. Naringenin and hesperetin, the aglycones of the flavanone glucosides naringin and hesperidin (Fig. 1) occur naturally in citrus fruits [3]. They exert a variety of pharmacological effects such as antioxidant [4, 5], blood lipid-lowering [6–9], anti-inflammatory activity through inhibition of the enzymes involved in arachidonate metabolism [10–12], anticarcinogenic [13, 14] and inhibit selected cytochrome P-450 enzymes resulting in drug interactions [15]. The physicochemical properties of naringin, hesperidin and their aglycones, naringenin and hesperetin, indicate that their crystalline phases are poorly soluble in water and show a slow dissolution rate from oral solid forms, restricting their use in therapy. The hydrophobic character of the molecule itself, the particles size distribution and the crystallinity of the substance, mainly affect the dissolution rate as well. The above properties indicate that the dissolution of either glycosides or their aglycones might be the most critical factor or rate limiting step in their bioavailability than the passage through intestinal barrier.

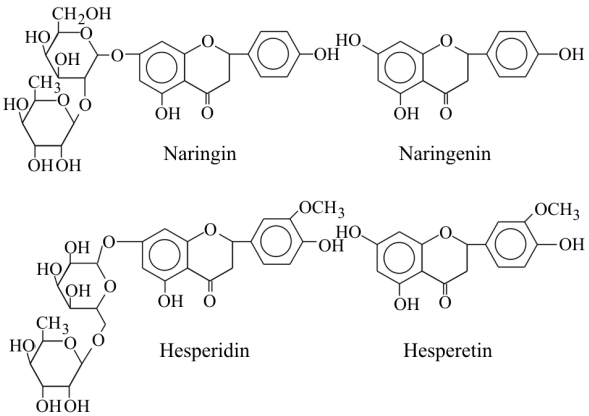

Fig. 1 Molecular structures of flavanone glycosides, naringin and hesperidin, and their aglycones, naringenin and hesperetin

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The most common and perhaps the oldest approach to improve the bioavailability of poorly water soluble drugs is to enhance their dissolution rate by the formation of a solid dispersion [16, 17]. Fine dispersion will increase the available surface so that wetting and dissolution can occur more rapidly. Furthermore in most cases the drug is not in the crystalline form but in amorphous state and such different solid forms can influence the dissolution, bioavailability, stability and other drug properties. According to Serajuddin 1999 [17], the advantage of solid dispersion, compared with capsule and tablet formulations, is that when the carrier is dissolved the drug is released as very fine colloidal particles with size less than 1 μm. Because of the large surface area, the dissolution rate is enhanced while in conventional formulations the dissolution rate is limited by the size of primary particle size, which is higher than 5 μm.

In the present study flavanone glycosides, naringin and hesperidin, and their aglycones, naringenin and hesperetin, were treated with the water-soluble carriers, polyvinylpyrrolidone (PVP) (PVP-K30), polyethylene glycol (PEG 4000) and mannitol, in order to select an appropriate carrier to destroy the space group (crystal structure), and to prepare amorphous dispersions of both glycosides and their aglycones. The aim was the preparation of a nanodispersion system by using the solvent method. These polymers are the most used drug carriers for solid dispersion preparations [18–23]. PVP is a synthetic polymer made up of linear groups of 1-vinyl-2-pyrrolidone monomers and PEG is a semi-crystalline polymer that results from polycondensation of ethylene glycol, they were selected due to their strong hydrophilic properties and their capability to form molecular adducts with many compounds, possibly due to their carbonyl or hydroxyl groups which are present in their repeated units with a result to increase water-solubility [18, 24–26], stability [25] and improve bioavailability of drugs [27].

Aims of this work are to prepare, to characterize the physicochemical properties of solid dispersion systems and to evaluate the effect of interactions between drugs and carriers on drug solubility.

Experimental

Materials

Hesperidin (3',5,7-trihydroxy-4'-methoxyflavanone 7-rhamnoglycoside), 97%, was purchased from Acros Organics (New Jersey, USA), naringin (4',5,7-trihydroxyflavanone 7-rhamnoglycoside), 97%, naringenin (4'-5,7-trihydroxyflavanone), 95% and hesperetin (3',5,7-trihydroxy-4'-methoxyflavanone), 95%, were supplied from Sigma (St. Louis, MO, USA). Poly(vinylpyrrolidone) type Kollidon K30 with a molecular mass of 50.000 supplied by BASF (Ludwigshafen, Germany), polyethylene glycol 4000 (PEG 4000) was obtained from BDH Chemical Ltd. (Poole, UK). Mannitol, acetic acid and absolute ethanol supplied by Merck (Germany). All the other materials and solvents which were used for the analytical methods were of analytical grade.

Preparation of solid dispersions

The solid dispersion systems (SD) were prepared using a solvent evaporation method for both flavanone glycosides and their aglycones in two different solvent systems. Acetic acid was used for glycosides and absolute ethanol for their aglycones. As carriers for solid dispersions two different water-soluble compounds PVP, PEG and mannitol were used. Solid dispersion systems of 80:20, 70:30, 60:40 and 50:50 mass/mass of each carrier/(naringin-hesperidin) glycosides, were prepared by dissolving the carrier and drug compounds in proper quantities of acetic acid. Naringin dissolves completely at room temperature after 15 min ultrasonication. Hesperidin dissolves completely only after heating in a mixture of acetic acid/water (80/20 V/V) until boiling for 20 min with continuous shaking and ultrasonication. After that the solutions were mixed and subsequently sonicated for 10 min. The solvent was fully evaporated under vacuum by rotary evaporator at 70°C for 30 min, then the created films were pulverized and stored in desiccators until studying.

In the same way, solid dispersion systems of 80:20, 70:30, 60:40 and 50:50 mass/mass of each carrier/(naringenin-hesperetin) aglycones, were prepared by dissolving the carrier and drug compounds in proper quantities of absolute ethanol at room temperature.

Characterization of solid dispersions

Thermal analysis

A PerkinElmer, Pyris 1 differential scanning calorimeter (DSC), calibrated with indium and zinc standards, was used. In solid dispersions with PEG and mannitol as carriers for each measurement a sample of about 10 mg was used, placed in an aluminium seal and heated up to 300°C with a heating rate of 20°C min^{-1}. From this scan the glass transition temperature (T_g), the melting temperature (T_m) and the heat of fusion (ΔH_m) were measured. In the case that PVP was used as carrier for each measurement a sample of about 6 mg was placed in aluminium seal and heated up to 130°C with a heating rate of 20°C min^{-1}. The sample remained at that temperature for 15 min in order to erase any thermal history and mainly to remove the moisture traces of PVP. Afterwards, the samples were quenched at 0°C and scanned again up to 300°C using the previous

heating rate. From this second scan the glass transition temperature (T_g), the melting temperature (T_m) and the heat of fusion (ΔH_m) were measured.

X-ray

The XRD analysis was performed on randomly oriented samples, scanned over the interval 5–50° 2θ, using a Philips PW1710 diffractometer, with Bragg–Brentano geometry (θ, 2θ) and Ni-filtered CuK_α radiation.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The morphology of the prepared solid dispersions as well as the initial materials was examined in an SEM, type Jeol (JMS–840). The films were covered with a carbon coating in order to have good conductivity of the electron beam. Operating conditions were: accelerating voltage 20 kV, probe current 45 nA and counting time 60 s.

Electron diffraction (ED) and TEM investigations were made on ultra thin films samples prepared by ultra-microtome deposited on copper grids. TEM micrographs were obtained using JEOL 120 CX microscope operating at 120 kV.

Release profile

A modified dissolution apparatus Pharma Test PT-DT7, with a stationary disk at 100 rpm and 1000 mL capacity was used. Samples corresponding to 180 mg of glycosides–naringin and hesperidin- and their aglycones, into hard gelatin capsules were placed in each vessel and were maintained at 37±0.5°C. Phosphate buffers, pH 6.8, containing 2 mass% Tween 20 was used. The disintegration time of the capsules was about 5 min. The samples were analyzed according to the RP HPLC methods developed by Kanaze *et al.* [3, 28].

Results and discussion

Differential scanning calorimetry (DSC)

Flavanone glycosides, naringin and hesperidin, and their aglycones, naringenin and hesperetin, are crystalline compounds with endotherm melting points of 163, 269, 255 and 231°C respectively (Fig. 2). In the DSC curves of solid dispersion systems of both flavanone glycosides and their aglycones with PVP (Fig. 3) the glass transition temperature (T_g) of PVP was only recorded.

The absence of the enthotherms corresponding to their crystal melting points (T_m) indicates the destruction of any crystalline phase during their dissolution into the PVP polymer matrix. The flavanone glycosides and their aglycones did not crystallized in these systems, possibly due to the optimum dispersion of their molecules in the polymer matrix. These systems can be referred as miscible, since only the glass transition of PVP is recorded, probably due to the molecular dispersion of drug compounds in polymer matrix. Furthermore, PVP is an amorphous polymer that has a T_g of 167°C, in all solid dispersions. It can be seen that its T_g was shifted to lower temperatures than that of pure PVP. The incorporation of low molecular mass compounds in all cases act as plasticizers reducing the T_g of PVP. This shift also indicates that may be some interaction involved between the polymer and the compounds, resulting in the formation of amorphous solid dispersion systems. However, these interactions may be very weak, resulting into T_g reduction. FTIR spectroscopy verified the existence of such interactions taken place between the carbonyl group of PVP and hydroxyl groups of aglycones, naringenin and hesperetin [29].

In the case of PEG solid dispersion systems with flavanone aglycones and their glycosides the DSC curves are different from that of PVP solid dispersions (Fig. 4). In all thermograms it can be seen clearly the melting point of PEG, near to the melting point of the pure polymer, which is 60°C. The endotherms of aglycones, naringenin and hesperetin, in their solid dispersion systems are shifted to lower values, compared to their pure crystals, also the peaks are broadened and the intensity is drastically reduced. In the systems of 50/50, 60/40 and 70/30 mass/mass of PEG/aglycones, there is a melting point that may correspond to hesperetin with a small shift at 229, 230 and 225°C, respectively. The melting point of naringenin is not observed, except in the mixture of 50/50 mass/mass, there is a small peak at 119°C it may be attributed to this compound. However, this peak is not strong evidence, because the melting point is about 44°C lower than that

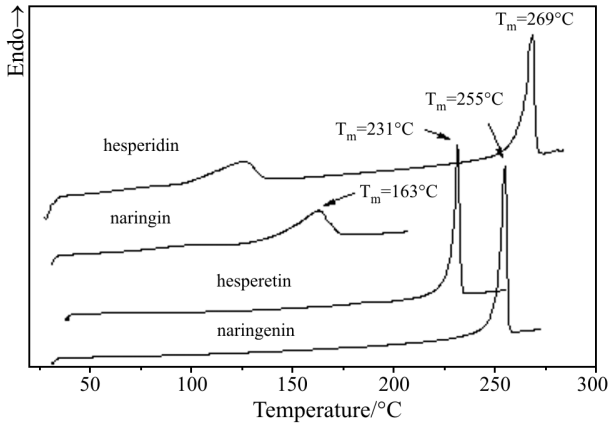

Fig. 2 DSC curves of flavanone glycosides (naringin and hesperidin) and their aglycone compounds (naringenin and hesperetin)

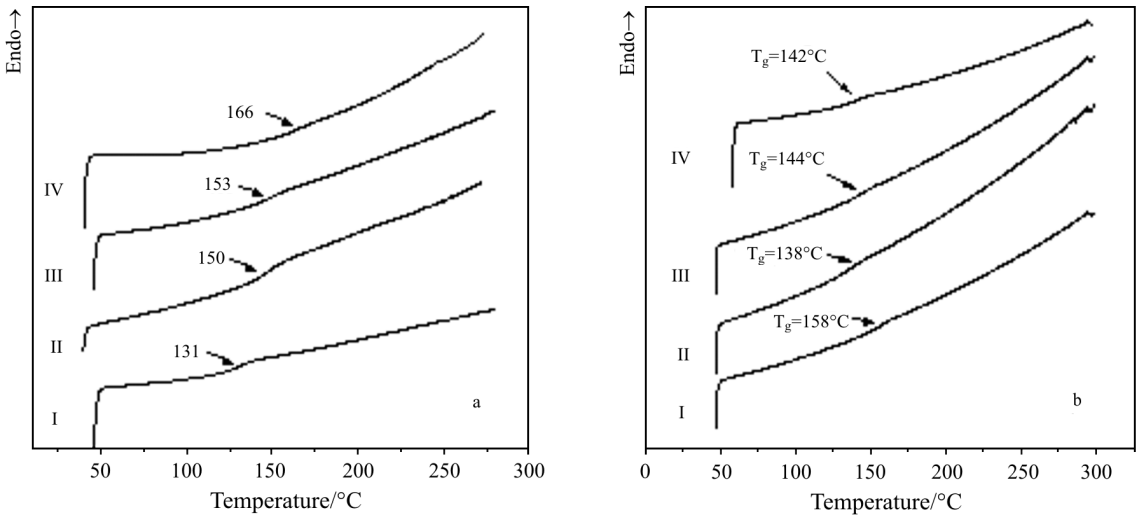

Fig. 3 DSC curves of a – PVP/flavanone glycosides (naringin and hesperidin) solid dispersions containing I=20/80, II=30/70, III=40/60, IV=50/50 mass/mass and b – PVP/flavanone aglycones (naringenin and hesperetin) solid dispersions containing I=20/80, II=30/70, III=40/60, IV=50/50 mass/mass

of the pure compound. Furthermore, the intensity of aglycone melting peaks are very small, it can be concluded that high percent of aglycone crystals are in amorphous state and only a small percentage is in crystalline state. This amorphization maybe the result of the interactions, which take place between PEG and the two aglycones. The shift of PEG melting point to lower temperatures by increasing the percentage of the aglycones in solid dispersions is a further evidence for the existence of such interactions. However, these interactions are not strong enough to ensure complete amorphization of the aglycones, naringenin and hesperetin. Since PEG melts at low temperature, there is a possibility that aglycone compounds may dissolve in the melt before reaching their melting points. Such behaviour in PEG solid dispersion was also mentioned [27]. The same behavior can be also observed in particular for the PEG/flavanone glycosides solid dispersions (Fig. 4b). In all samples there were small endothermic peaks appear close to 226–228°C and 160°C, they correspond to the flavanone glycosides, hesperidin and naringin, respectively. These peaks with small intensities support that small percentage of pure flavanone glycosides are in crystalline phase while the greater percentage is in the amorphous state. From these thermograms it is realized that the prepared solid dispersions containing PEG as carrier are not miscible, since two different phases are detected.

The DSC curves (Fig. 5) of mannitol solid dispersion systems of both aglycones at different ratios show that they were possibly present in crystalline phase due to the presence of melting points that can

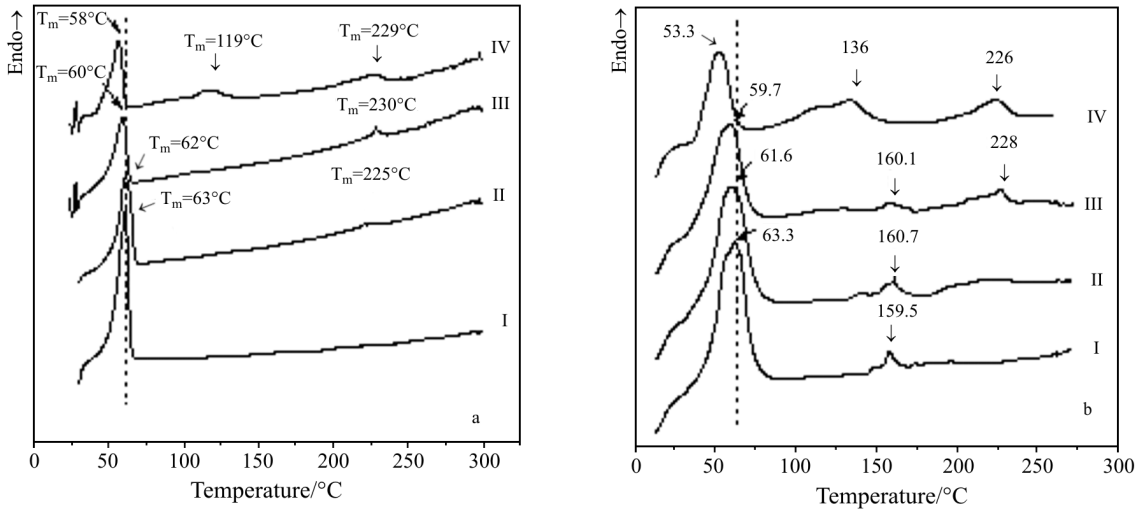

Fig. 4 DSC curves of a – PEG/flavanone aglycones (naringenin and hesperetin) solid dispersions containing I=20/80, II=30/70, III=40/60, IV=50/50 mass/mass and b – PEG/flavanone glycosides (naringin and hesperidin) solid dispersions containing I=20/80, II=30/70, III=40/60, IV=50/50 mass/mass

correspond to naringenin and hesperetin, but with large shifts to lower temperatures. The melting point temperatures of pure naringenin and hesperetin crystals were 255 and 231°C, while after their formulation in solid dispersion systems were 193.5 and 157.5°C, respectively, independently from their content ratio. Such behaviour is abnormal, similar results were also reported by others [30]. The reason that makes us to conclude that these endotherms attributes to pure aglycones is the increase of peak intensity by increasing their amounts in the solid dispersions. The melting point of mannitol in all solid dispersion systems remains the same around 170.7°C with a small shift (–2°C) at solid dispersions containing 40 and 50 mass% aglycone compounds. The presence of an endothermic peak around at 100°C, indicating the presence of residual moisture, because in the preparation step of mannitol solid dispersion systems, mannitol was dissolved in distilled water.

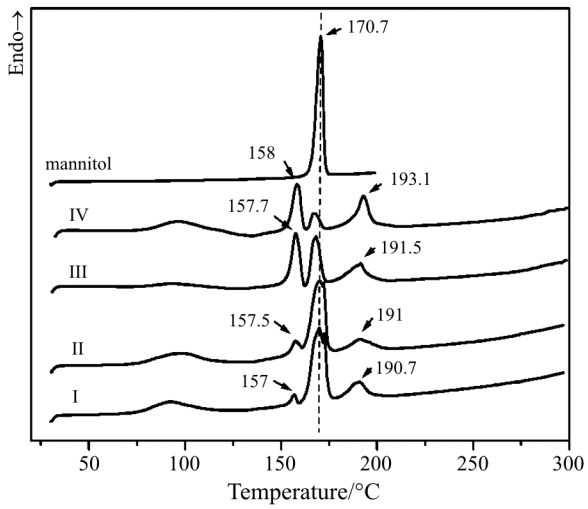

Fig. 5 DSC curves of mannitol solid dispersion systems with flavanone aglycones containing I=20/80, II=30/70, III=40/60, IV=50/50 mass/mass

Even though DSC can give very quick and safe results about the physical state of polymer/drug mixtures [31–35], it seems that some times lacks accuracy and sensitivity compared with other techniques. For example, in naproxen/PVP (30/70 mass/mass) physical mixtures, the DSC technique could not detect any crystalline structure, while the XRD technique was able to indicate the presence of residual naproxen crystals [36]. Another limiting reason to use DSC technique is the ability of low melting point polymers to act as solvent for a drug. In this case, by increasing the temperature, the polymer starts to melt and dissolves the drug crystals, with a result to form dispersion at lower temperatures than that of pure crystals. PEG has a low melting point with a result to melt at low temperatures before that of encountered drugs, so most of the used drugs can dissolve in the melt before reaching their melting points [37, 38]. For this reason XRD technique was used for further investigation, which seems to be more accurate and sensitive to study the crystalline state of the compounds.

X-ray diffractometry

XRD diffraction patterns of flavanone glycosides solid dispersions (Fig. 6a) revealed the presence of crystalline material, since several diffraction peaks, which are correspond to both glycosides, are recorded. This observation comes in disagreement with DSC curves, in which only amorphous PVP was detected without any endothermic peak that may correspond to both flavanone glycosides. One important difference between the two techniques is that the XRD measurements are done at room temperature while the DSC measurements are done at the melting

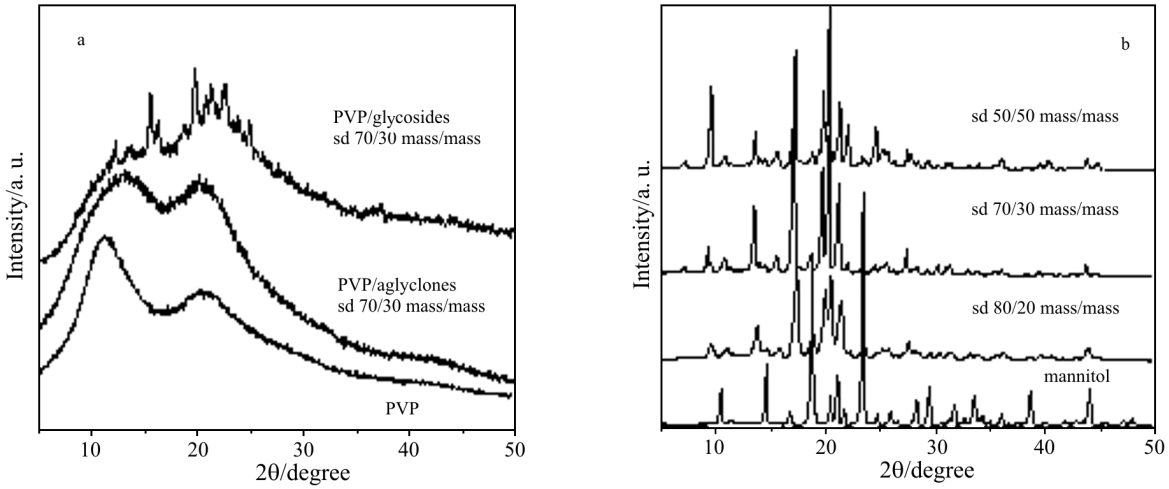

Fig. 6 XRD patterns of a – PVP solid dispersion systems with flavanone glycosides and their aglycones with concentrations 70/30 mass/mass and b – mannitol solid dispersion systems with aglycones at different concentrations

point of encountered drugs. On other hand, as can be seen from XRD patterns of PVP/aglycones (Fig. 6a), in all solid dispersions there are only two broad peaks that correspond to the diffraction pattern of pure PVP. The XRD patterns of these solid dispersions show typical profiles of amorphous material suggesting that the PVP macromolecules inhibited the drug crystallization. This finding comes in agreement with DSC analysis. From these data it realized that bulky molecules, such as flavanone glycosides can crystallize more easily than these of their respective aglycones in polymer matrices, this may be due to the difficulty of hydrogen bond formation.

In case of PEG and mannitol solid dispersions (Fig. 6b), in all XRD patterns, except of peaks that correspond to PEG or mannitol they have additional diffraction peaks with small intensities. These diffraction peaks are at the same angles (2θ), which characterize both aglycone and glycoside flavanones crystals, which suggest the existence of some crystalline structure. In a previous work concerning the preparation of inclusion complexes of flavanones with β-cyclodextrins, new diffraction patterns were appeared in XRD analysis, which indicates the formation of new crystal structure due to complexation [39]. In our study such new patterns were not detected. Furthermore, the existence of crystalline drug compounds enhances the hypothesis that the endothermic peaks with small intensities that recorded with DSC technique may attributed to these compounds.

SEM and TEM study

SEM is a technique which can give information about the morphology of the solid dispersions. Examining solid dispersion films of both flavanone aglycones with PVP, smooth surfaces can be seen in all samples and is no longer possible to find the original crystals of naringenin or hesperetin (Fig. 7). This means, in comparison with DSC and XRD data, that a solid dispersion or a molecular dispersion of naringenin and hesperetin was formed into PVP matrix. However SEM is not able to distinguish such a difference due to the low resolution. For this reason, solid dispersions were also examined by TEM. As can be seen from the micrographs taken in ultra thin films of PVP/aglycone dispersions (Fig. 10), aglycone compounds are in the form of nano-dispersions. In the solid dispersions containing 20 and 50 mass% aglycone compounds nanoparticles with sizes lower than 200 and 500 nm respectively were observed. From this study it was verified that aglycone compounds are in amorphous nanodispersions in PVP matrix. However, the possibility of the presence of small percent of aglycone compounds in molecular dispersion should not be excluded. This physical state can explain the drastic dissolution enhancement of particular compounds that will be discussed below.

In the case of solid dispersion systems of PEG and mannitol (Fig. 8), the film surfaces are very different from those of PVP, since roughness is dominated and many irregularities are detected. However, even at such surfaces it is possible to distinguish the crystals of particle size ranged from 1 to 5 μm, depending on the carrier percent. This comes in agreement with the above data revealing that flavanone aglycones remain in crystalline form.

Release profile

Flavanone glycosides, naringin and hesperidin, are partially crystals in solid dispersions of the three carriers, as can be seen from their XRD analysis. The release profiles of both glycosides from all their solid dispersion systems remain unchanged. It is known that crystalline compounds have always lower dissolution rate compared with amorphous due to the lower available surface area. Flavanone glycosides are bulky compounds. They might create hydrogen bond difficulty with drug carriers in order to form amorphous dispersions, with a result the particular compounds remain in crystalline state. However, the release profiles of aglycones, naringenin and hesperetin, from PVP solid dispersions in all ratios were

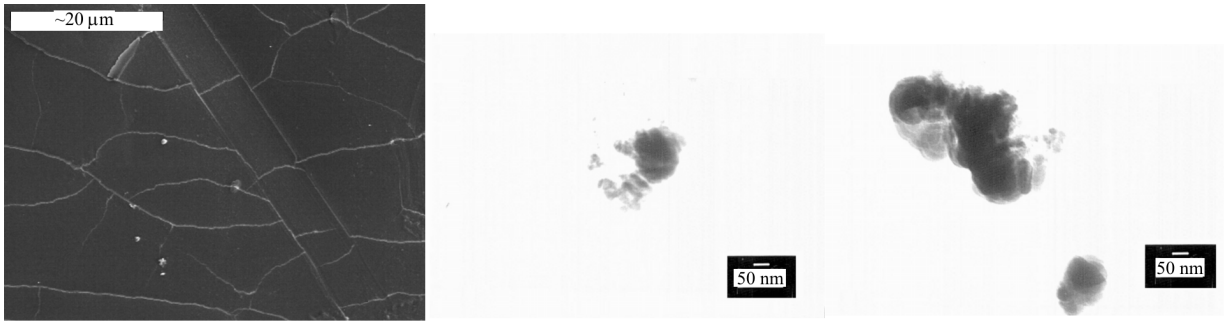

Fig. 7 a – SEM micrographs of PVP/aglycones solid dispersion film of 80/20 mass/mass, ratio, b – and c – TEM micrographs of PVP/aglycones solid dispersion films at of 80/20 and 50/50 mass/mass ratios, respectively

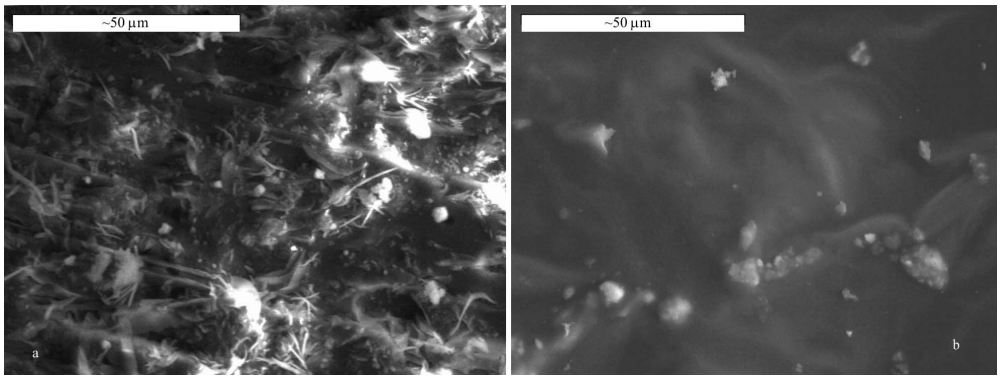

Fig. 8 SEM micrographs of a – PEG/aglycones and b – mannitol/aglycones solid dispersion films at 60/40 mass/mass ratio

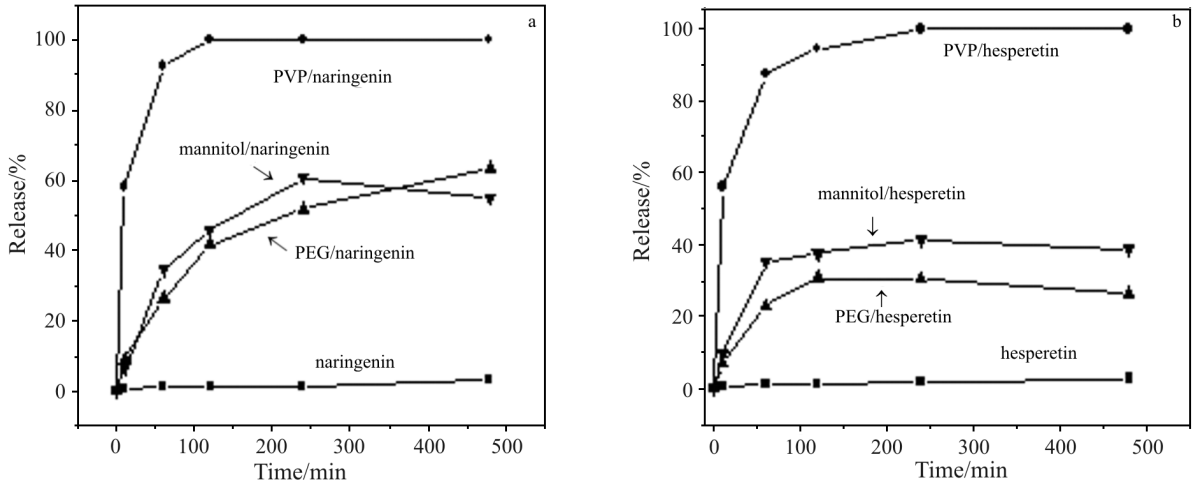

Fig. 9 The release profile of aglycone compounds from PVP, PEG and mannitol solid dispersion systems at pH 6.8 a – naringenin and b – hesperetin

dramatically improved relative to their pure aglycones alone (Fig. 9).

It was noticed that the highest release profile for both aglycones was at pH 6.8, this is obvious, especially in solid dispersion system of PVP: (naringenin/hesperetin) in the ratio of 80:20 mass/mass, since the release was 100% for both aglycones after almost 2 h, which means that there is a 51.4- and 64.3-fold increase in naringenin and hesperetin dissolution respectively. This enhancement in dissolution rate of both aglycones can be attributed to the crystal destruction of these compounds and the formation of nano-solution into PVP matrix. In the same way, the release profiles of flavanone aglycones from mannitol and PEG solid dispersion at different ratios 80:20, 70:30, 60:40 and 50:50 (carrier: naringenin/hesperetin, mass/mass) were studied at pH 6.8. Similar improvements in dissolution profiles of both aglycones from mannitol and PEG solid dispersions were also observed compared with pure compounds. However, the dissolved amounts of both drugs were lower compared with these of PVP solid dispersions (Fig. 9). The existence of these compounds as partially crystalline dispersions in mannitol or PEG matrix is a negative factor for their dissolution enhancement. The initial increase of dissolution rate was due to the dissolution of amorphous part of both drugs, a plateau was observed after 3–4 h due to the presence of residual crystalline drugs, which was not able to dissolve. From release profile, it could be estimated that only 50–60 mass% of flavanone aglycones were in amorphous state in solid dispersions of mannitol or PEG.

Conclusions

The combination of DSC and XRD techniques can be used successfully to characterize the physical state of solid dispersion systems of poorly water-soluble drugs. PVP is an effective polymer matrix that can form amorphous nano-dispersion systems with flavanone aglycones, naringenin and hesperetin, while can not form with their glycosides, naringin and hesperidin. This indicates than bulky molecules are very difficult to form amorphous dispersions.

When PEG and mannitol were used as drug carriers, both glycosides and their aglycones were suspended in the carrier matrix as crystalline dispersions. This indicates that these carriers are not appropriate particularly for these compounds.

The new physical state of the prepared solid dispersions was directly affecting the dissolution profile of the drugs. Solubility is dramatically improved in the case of PVP/aglycones due to the formation of amorphous nano-dispersions, while lower enhancement in the dissolution profiles of both aglycones were noticed in the case of PEG and mannitol solid dispersions, it was lower than 60% in all dispersion systems.

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