Preparation and Characterization of Genistein Containing Poly(ethylene glycol) Microparticles

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ABSTRACT: The purpose of this study was to prepare, characterize, and evaluate genistein-containing microparticles with enhanced dissolution profile using poly(ethylene glycol) (PEG) as polymer matrix. Genistein loaded microparticles were prepared by a solvent evaporation process and their surface, thermal, chemical, and dissolution properties were analyzed by microscopy, differential scanning calorimetry, ATR-FTIR spectroscopy, and USP dissolution apparatus II, respectively. The wettability index was also determined. Genistein exhibited an elongated crystal habit. However, the drug containing PEG microparticles were discrete and quasispherical. The ATR-FTIR studies performed on the formulation suggested hydrogen bonding between the drug and the polymer matrix. Thermal analysis indicated a conversion of the crystalline form of the drug to an amorphous form. Genistein, exhibiting low solubility and high permeability, is a Class II drug of the Biopharmaceutical Classification Scheme. However, there was a ~9-fold increase in the rate of dissolution of genistein in the case of all formulations as compared to native genistein. This study showed that genistein could be effectively encapsulated into PEG microparticles using an emulsion-solvent evaporation technique, therefore avoiding the potential disadvantages of other solid dispersion techniques. This approach provided a significant enhancement in the drug dissolution profile.
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Key words: amorphous; polyethylene; surfactants; thermal properties; water-soluble polymer

INTRODUCTION

Genistein [Fig. 1(A)], a phytoestrogen belonging to the isoflavone class of compounds, has a role in the treatment and prevention of various cancers and other diseases such as cardiovascular and osteoporosis among others.[1,2] It is a potent tyrosine kinase inhibitor and also possesses antioxidant activity.[3] There is a growing body of *in vitro* and animal studies suggesting that genistein can inhibit cancer cell growth.[4,5] A large number of products containing genistein are also being marketed and are available as nutritional supplements.

An understanding of the concept of biopharmaceutical classification scheme (BCS) gives insight into dealing with bioavailability problems of various orally administered drugs. The properties of drugs in BCS class II (low solubility, high permeability) can be conveniently modified to enhance the bioavailability when orally administered.[6] Oral absorption of any drug occurs in two steps; the drug first dissolves in the gastrointestinal fluid and the dissolved drug subsequently permeates across the gastrointestinal membrane. Out of these two processes, the slower kinetic process is the rate-limiting factor in the oral bioavailability of the drug. The oral bioavailability of poorly water-soluble drugs whose absorption is controlled by dissolution rate (Class II drugs in BCS) can be increased by various formulation approaches, which increase the solubility and dissolution rate. It is estimated that almost 41% of the new chemical entities are poorly soluble and most do not progress to preclinical evaluation because of formulation challenges.[7] The rate and extent of dissolution of the active ingredient from any solid dosage form determines the rate and extent of absorption of the drug. In the case of poorly water soluble drugs, dissolution is the rate limiting step in the process of drug absorption. Poorly soluble drugs have been shown to be unpredictably and slowly absorbed as compared to drugs with higher solubility.

Polyethylene glycol (PEG) polymer [Fig. 1(B)] has shown promise for the delivery of poorly soluble drugs.[8–12]This polymer is commercially available in a variety of molecular weights.

Several methods have been employed to improve the solubility of poorly water soluble drugs.[13,14] These

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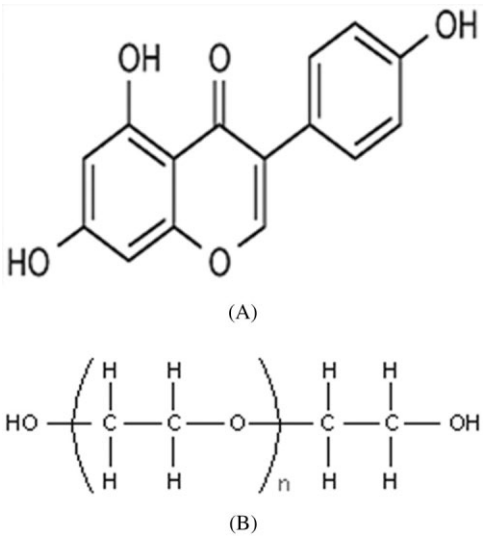


Figure 1 Chemical structure of (A) Genistein and (B) polyethylene glycol.

include approaches such as micronization, nanosuspension formulation, complexation, solubilization using surfactants, formation of micelles, and self-microemulsifying drug delivery systems.[15] There are some practical limitations to existing methods such as relatively higher surface area and inadequate solubility of drugs in carriers and the instability of drugs and carriers at elevated temperatures required in some cases for melting the carrier polymers and the drug. The goal of this study was to prepare solid dispersions by using emulsion-solvent evaporation technique to a model poorly soluble drug, genistein, to increase its dissolution.[16]

The use of nano- and microparticles of relatively insoluble drugs has enormously widened the window of achievable pharmacokinetic performance. For the successful development of such technology, it is essential to understand the characteristics of engineered particles.[17] There is a knowledge gap in the specific case of genistein-loaded PEG microparticles. The objective of this study was to apply emulsion-solvent evaporation technique to genistein, to increase its dissolution, and to evaluate the effect of process parameters on the percentage yield, encapsulation efficiency, and the physicochemical characteristics of the microparticles.

EXPERIMENTAL

Materials

Genistein was purchased from LC Labs (Woburn, MA). The solvents used were of analytical grade. The chemicals include ethanol, dichloromethane, corn oil, span 65 (sorbitan tristearate), poly(ethylene glycol) (PEG, MW 4000–10,000), and Tween 80 (polyoxyethylene sorbitan monooleate) purchased from Sigma Chemical (St. Louis, MO). The UV transparent microplates used for the analysis were purchased from BD Falcon (Franklin, NJ).

Estimation of genistein solubility and permeability

The value of genistein water-solubility was estimated using Yalkowsky's equation (eq. (1)). Yalkowsky and Valvani considered the Hildebrand–Scott equation to be limited in its ability to describe the solubility of nonelectrolytes in water, and proposed a semiempirical relationship (eq. (1)) based on the correlation of water solubility to physicochemical properties for weak electrolytes and nonelectrolytes.[18]

$$\text{Log } S_i \approx -1.0 \log \text{PC} - 1.11 \frac{\Delta S_f (T_m - T)}{2.303\, RT} + 0.54 \quad (1)$$

where S_i = solubility, PC = partition coefficient, $R = 8.3143\ \text{J K}^{-1}\ \text{mole}^{-1}$, $\Delta S_f = \Delta H_f / T_m$ (values obtained from Table I.)

The classification of drug solubility was based on the dimensionless dose number, D_0[19] which is given as follows:

$$D_0 = \frac{(M_0/V_0)}{S_i} \quad (2)$$

where D_0 is the ratio of drug concentration in the administered volume (250 mL) to the saturation solubility of the drug in water. M_0 = dose = 10 mg (based on average literature data[20]) , V_0 = 250 mL and S_i = solubility. Drugs with $D_0 < 1$ are classified as high-solubility drugs. Conversely, drugs with $D_0 > 1$ are poorly soluble drugs.[19]

The partition coefficient was estimated by Clog *P* values calculated using the software Pallas (version 2.0, CompuDrug, USA). Drugs with Clog *P* value ≥ 1.35 are classified as high-permeability drugs. Con-

TABLE I
Differential Scanning Calorimetry (DSC) Data of Genistein, PEG, and Physical Mixture of Genistein with PEG (1:1)

	T_1 (°C)	T_2 (°C)	Peak (°C)	ΔH (J/g)
Genistein	264	321.333	308.864	120.686
PEG	40	78.666	68.524	348.822
Physical mixture (1:1)	245.33	284.09	269.06	11.12
Microparticles	–	–	–	0

T_1 is Temperature at the onset of peak, T_2 is Temperature at the end of peak. The microparticles were prepared using a speed of homogenization of 1000 rpm; drug to polymer ratio is 1:40; and MW of PEG is 10,000 Da.

versely, drugs with Clog *P* values lower than 1.35 are classified as low-permeability drugs.[19]

Preparation of genistein containing PEG microparticles

Microparticles were prepared by solvent evaporation using alcohol-in-oil method.[21] Typically, 16 mg of genistein and between 100 and 1500 mg of polyethylene glycol were accurately weighed and dissolved in a 1:10 mixture of dichloromethane (DCM) with alcohol. Span 65 at a concentration of 1% was dissolved with stirring in 150 mL of corn oil which is heated to 70°C. The stirring was continued for a period of 45 min. The oil phase was allowed to cool to room temperature. The organic phase was added slowly by means of a pipette to the oil phase and immediately homogenized at the specified speed for a period of 5 min. The resultant emulsion was kept in a fume hood to allow for evaporation of the organic phase for a period of 12 h with continuous magnetic stirring. The emulsion was further subjected to centrifugation at a speed of 3000 rpm for 10 min. The oily phase was separated and the resultant particles were washed three times with hexane to remove the excess oil. The mixture was subjected to centrifugation and the hexane was decanted. The washings with hexane were repeated three times to ensure complete removal of the oil. The microparticles were allowed to dry in a fume hood for a period of 12 h and the resultant particles were stored at room temperature (~20°C). PEGs of various molecular weights were used as carriers in this study as these are hydrophilic polymers. Effect of drug to carrier ratios and speed of homogenization on the dissolution of genistein was also studied.

Microparticle characterization

Microplate reader based assay for analysis of genistein

A stock solution of 50 μg/mL was used for serial dilutions to obtain genistein concentrations from 1 to 30 μg/mL. The latter was used to obtain the standard curve. The samples were assayed using a spectrophotometer and microplate reader (Tecan Spectra Flour Plus Tecan systems, San Jose, CA) and their UV absorbance recorded at 260 nm. Special UV transparent plates, which do not absorb in the UV range, were used in the microplate reader.

Percent yield of formulations

The percent yield was calculated using the following formula:

$$\text{Percent yield} = [(\text{Practical yield})/(\text{Theoretical yield})] \times 100 \quad (3)$$

where, practical yield is the weight of the microparticles or simple dispersions collected after preparation, and the theoretical yield is the total weight of the ingredients used in the preparation.

Encapsulation efficiency and drug loading

The concentration of genistein in the formulation was calculated and expressed as percent drug loading. The percent encapsulation efficiency was defined as:

$$\text{Percent encapsulation efficiency} = [(\text{Practical drug loading})/(\text{Theoretical drug loading})] \times 100 \quad (4)$$

The percent actual drug loading was defined as:

$$\text{Percent actual drug loading} = [(\text{Weight of drug})/(\text{Weight of drug} + \text{polymer})] \quad (5)$$

Morphological analysis

The shape and surface characteristics of the preparations both with and without drug were analyzed both by optical and scanning electron microscopy (SEM).[22] SEM was performed after coating the preparations with gold in a Polaron E5150 film thickness controlled Sputter Coater, under vacuum of <0.1 Torr at a temperature of 6°C to a thickness of 20 nm. The accelerating voltage was 20 kV and the working distance was 10 mm. The instrument used was a Hitachi S500 SEM at 500× and 2000× magnification. The particle size was measured from the micrographs obtained.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed to characterize the formulations and for excipient compatibility testing with genistein. Thermal analysis was performed on genistein, PEG, the physical mixture, and microparticles according to a modification of previously described method.[16] The equipment used was a PerkinElmer DSC 7 attached with a thermal analysis data station (TADS), interface, and a printer. The instrument was calibrated using an indium standard. Samples corresponding to 5 mg of genistein were accurately weighed in aluminum pans. The pans were covered with lids and sealed. Thermograms were obtained at a scanning range of 10°C/min conducted over a temperature range of 40–340°C.

Melting point determination

Melting points were determined using a SMP 10 melting point apparatus. Small amounts (~50 mg) of the preparations under examination were placed in capillary tubes sealed at one end. The temperature at which the drug completely melted was then observed and recorded.

Attenuated total reflectance–fourier transform infrared spectroscopy

Attenuated total reflectance (ATR) fourier transform infrared (FTIR) spectra for the excipients and formulations were recorded on a Nicolet Nexus 470 spectrometer (Thermo Nicolet, Madison, WI) using the Smart Miracle ATR accessory. Approximately 100 μg of sample was loaded onto the sample holder and scanned in the range of 4000–400 cm^{-1}. Interferograms were processed using Happ-Genzel apodization, followed by automatic baseline correction.[23]

Wettability index

The change in wettability of genistein upon incorporation of the drug with polymer, either as microparticles or by forming solid dispersions was evaluated using a technique similar to that employed to screen suspending agents.[24] The end of a glass Pasteur pipette was plugged with glass wool and the pipette was filled with the various powdered formulations (pure drug or drug-carrier formulations). The powder (~2 g) was tap-packed to a height of 5 cm. One milliliter of distilled water was then placed on top of the powder bed and the time required for the solvent to wet or soak through a distance of 3 cm was noted. This value was termed the wettability index.

In vitro dissolution studies

Dissolution experiments were performed using the paddle method as described in USP dissolution apparatus 2. The samples previously filled in capsules (which dissolve in 5 min) were placed in phosphate buffer media (pH 6.8) containing 1% Tween 80. Formulations containing equivalent amounts of genistein were taken in all the dissolution studies. The capsules were held to the bottom of the vessel using aluminum sinkers. Hundred milliliters conversion kits were used as the dissolution vessel. Dissolution was carried out for a period of 2 h. The dissolution medium was maintained at a temperature of 37°C ± 0.5°C by means of a constant temperature water bath. The medium was stirred at 50 rpm by means of an adjustable constant speed motor. Two milliliter samples were withdrawn at predetermined time intervals at 0, 10, 20, 30, 40, 60, 80, 90, 100, and 120 min. After each sampling, an equivalent amount of fresh buffer was added to the dissolution vessels to maintain constant volume. The samples were immediately assayed using a microplate reader at 260 nm. From the absorbance values, the cumulative percent of genistein released was calculated and expressed as cumulative percent released. Dissolution experiments for each formulation were performed in triplicate.

Effects of various process parameters such as polymer concentration or drug to polymer ratio, molecular weight of PEG, were studied. For polymer concentration, PEG in increasing concentration was used to study the effect on dissolution characteristics. In terms of drug to polymer ratio; 1:10, 1:20, 1:40, 1:80; ratios were used to characterize the effect of polymer concentration on dissolution of genistein. Different molecular weight (4000, 6000, and 10,000 Da) of PEG was also used to evaluate the effect of molecular weight on dissolution of genistein.

Statistical analysis

Most experiments were performed in triplicate. The significance of the differences between groups was tested using one-way analysis of variance (ANOVA), and each group was compared using Tukey's test. A probability level (P value) of <0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Genistein solubility and permeability

Since no reports on solubility of genistein were available in the literature, the solubility was estimated using eq. (1) and thermal data. The estimated solubility of native genistein S_i was 3.04 ng/mL. This was consistent with preliminary HPLC analysis. Since the initial HPLC method that we previously reported[25] using a C18 column had a limit of detection (~5 ng/mL) relatively higher than the solubility of genistein, this method was not appropriate for the current study. To address this limitation, a microplate reader approach was developed and used. HPLC analysis with different conditions (e.g., column and mobile phase) would be an alternative future approach for this study. The obtained S_i value was used to compute the dose number D_0 which was 13,157.9 (practically water-insoluble drug).[19] Clog P was 1.79 in the case of native genistein. Since the calculated D_0 was found to be >1 and the Clog P was >1.35, genistein clearly falls in the BCS class II of compounds, which includes compounds having low solubility and high permeability. Being a poorly water soluble molecule, the delivery of genistein is challenging.

Process variables affecting genistein encapsulation into PEG microparticles

Studies were conducted to determine the ratio of solvents, drug to polymer ratio (1:80–1:10), and speed of homogenization (500–4000 rpm) to obtain free flowing particles. In this method of preparation, the choice of solvent can be of crucial importance. A mixture of 1:10 DCM to ethanol was chosen as the solvent for the dispersed phase based on its ability to dissolve both the drug and polymer, and its immiscibility with the continuous phase solvent. Also, DCM has low enthalpy of vaporization at 25°C (28.52 kJ/mol) and high volatility (vapor pressure 39.3°C at 100 kPa) that facilitates easy removal by evaporation.[26] Corn oil was chosen as the solvent for the continuous phase based on the criteria of immiscibility with dispersed phase solvent, inability to dissolve PEG, low solubility toward drug, higher boiling point than dispersed phase solvent, ease of recovery of microparticles, and low toxicity.[27] A speed of homogenization at 1000 rpm was initially selected from preliminary experimentation, as discrete particles were obtained at this speed. In the case of microparticles, its final structure and composition was expected to result from a complex interaction between polymer, drug, solvent, continuous phase, and emulsifier.

The role of the emulsifier in microparticle production by solvent evaporation is the short-term stabilization of the suspended polymer solution droplets.[28] In microencapsulation, stabilization, to prevent aggregation and coalescence, is only a short-term requirement; once adequate solvent evaporation has taken place to produce some hardening of the drug–polymer droplets, coalescence and aggregation does not occur. Addition of span 65 in these experiments helps to overcome this coalescence and it is essential to ensure droplet stability during the emulsification and subsequent particle hardening process.

There were no differences between the percentage yield values for microparticles. The slightly higher yield with total solids content for 1:80 might possibly be due to the greater viscosity; hence retention of the fraction adhered to glass beaker and homogenizer tip surfaces. Percent yield for the microparticle preparations was found to be between 57 and 78%. Some of the product loss was unavoidable during washings with hexane.

The encapsulation efficiency determined was about 60–70% in the case of microparticles. The loss of genistein may be due to washings with hexane. Additionally, genistein may be partially soluble in corn oil which may lead to a reduction in the encapsulation efficiency.

Particle characteristics

The physicochemical properties of the solid formulations were analyzed using spectroscopy, thermal analysis, microscopy, and by dissolution data. These techniques are essential to fully understand the increase in dissolution rates of solid dispersions. Knowledge of the interactions on a molecular level between a polymer, drug, and other excipients is of interest for the elucidation of physical and chemical properties of solid dispersion systems.

Morphological analysis

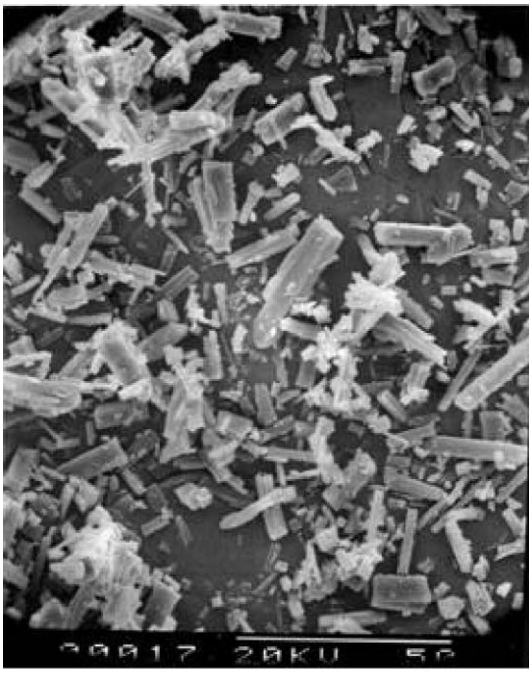
When observed under a light microscope, native genistein showed elongated needle-shaped crystal habits. However, the genistein containing microparticles were typically quasispherical. This was confirmed by SEM studies. The micrographs obtained are shown in Figure 2. SEM also showed some porous structures with the presence of few drug crystals on particle surface, which probably occurred by small ring deposit. Ring deposits are common wherever drops containing dispersed solids evaporate on a surface and occur by capillary flow.[29]

Wettability index

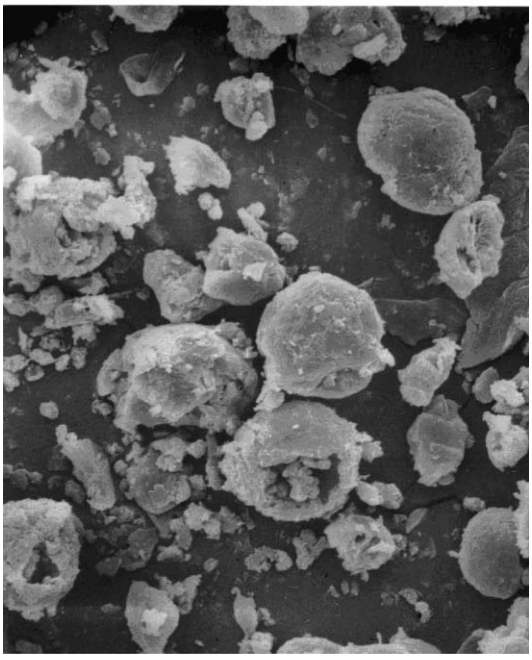
The change in wettability of genistein upon incorporation of the drug with water-soluble polymer has been observed by the wettability index determination. The wettability indices were found to be 222 $\pm$ 1.44 s in case of native genistein and 116 $\pm$ 1.5 s in the case of PEG–genistein microparticles prepared by emulsion-solvent evaporation method. Higher the wettability index, the longer is the time taken for the substance to get wet and hence lower the solubility. Microparticles used were prepared using speed of homogenization 1000 rpm, drug to polymer ratio 1:40, and PEG MW 10,000. The wettability index was markedly decreased in case of the formulation. This difference may be attributed to the surface properties and porosity of powders. The close packing of the microparticles with minimum of voids in addition to the residual oil layer detected by ATR-FTIR studies may decrease wettability index.

Attenuated total reflectance–fourier transform infrared spectroscopy

Genistein compatibility with the excipients used in the formulation was tested with ATR-FTIR. Figure 3(A) and (B) shows the absorbance spectra of pure PEG and pure genistein, respectively. Genistein showed several sharp characteristic peaks; the most prominent being at 3400 and 3100 cm^{-1} representing OH and CH stretching vibrations, respectively. The changes in bands assigned to OH deformation in genistein were observed in PEG–genistein microparticles [Fig. 3(F)] but not in their physical mixture [Fig. 3(E)] suggesting hydrogen bonding between PEG and the polymer.[30]



(A)



(B)

Figure 2 SEM micrograph of (A) Genistein and (B) PEG–genistein microparticles. Bar shown for micrographs at ×500 represents 50 μm.

The characteristic peak for PEG is observed at 3000 cm^{-1} with another sharp peak at 1150 cm^{-1}. The spectrum of the 1:1 physical mixture containing PEG [Fig. 3(E)] and genistein had features of each of the components. PEG did not change the infrared spectrum of genistein indicating no chemical interaction in the binary mixture with the molecular structure of genistein remaining completely intact. Characteristic bands are seen at 3500 cm^{-1} for genistein in addition to the peaks at 2900 cm^{-1}, which is characteristic of the olefins, and the peaks at 1150 cm^{-1} for ester groups, which are present in the span 65 [Fig. 3(C)] and corn oil spectra [Fig. 3(D)]. The increase in absorption peaks at 2900 cm^{-1} for aliphatic acetate esters and the aliphatic hydrocarbons at 1000 cm^{-1}, which are the functional groups in corn oil, can be seen in Figure 3D and also in the spectrum for the microparticle preparation [Fig. 3(F)]. These spectra indicated peaks for corn oil as well as span 65. This suggests that there is a residual film of oil and span 65 on the surface of the microparticles. The particles could not be made absolutely free of the corn oil by multiple washings with hexane. Also, the presence of span 65 on the surface confirms that the emulsifier helps in the stabilization of the particles at the oil–alcohol interface and may explain difference in wettability index observed as indicated earlier.

Thermal analysis

Data obtained from DSC of genistein, PEG, physical mixture, and microparticles are shown in Table I. Genistein showed a sharp melting peak with a melting onset at 300°C, which corresponds to its melting point and the melting peak itself was at 305°C. These observations were consistent with the data in the literature. A DSC scan of PEG yielded a peak at 63°C, which was consistent with the reported melting temperature range of this polymer.[31] The enthalpy of fusion values are shown in Table I.

The disappearance of endothermic peaks of genistein for physical mixtures, compared to the pure drug was noteworthy (data not shown). This may be due to a decrease in crystallinity of the drug or partial change in crystal form. The drug probably consists of predominantly amorphous material, since no drug melting peaks were observed. These observations suggest that the crystalline genistein in native form was converted to an amorphous form, which explains the absence of drug peak in the DSC thermograms obtained. This was supported by melting point determination, which is discussed below, as well as by the higher rates of dissolution as seen in the case of the preparation. This was due to conversion to amorphous form which is known to have higher solubility. Any strong interaction between the drug and polymer used can be ruled out based on the supporting data obtained by ATR-FTIR. Further, X-ray diffraction studies should be carried out to confirm and compute the proportion of amorphous to crystalline conversion of drug in the formulations.

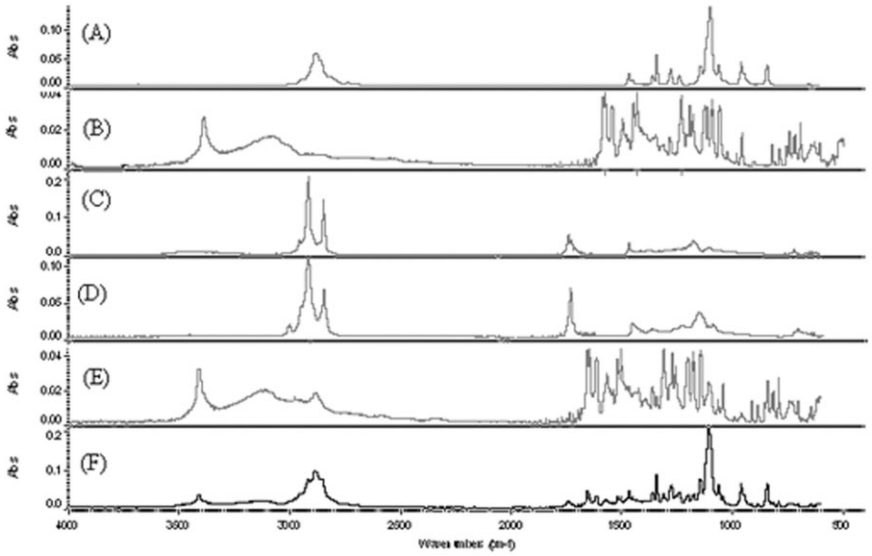


Figure 3 ATR-FTIR spectra of (A) PEG (MW 10,000), (B) genistein, (C) span 65, (D) corn oil, (E) 1:1 physical mixture of genistein and PEG (MW 10,000), and (F) microparticles of PEG–genistein.

Determination of melting point

Melting point determination may be a useful indication to the polymer–drug interaction. Genistein shows a melting point of 300°C, whereas PEG shows a melting point at 60°C as given in the literature. Therefore, the formulations may have melting points of variable nature depending on their physical structure. The melting points were found to be 302, 65, and 277–300°C, and for genistein, PEG, and physical mixture (1:1, genistein:PEG), respectively, using melting point apparatus. Melting point of genistein was found to be 300°C, which is very close to the value of 302°C obtained using DSC. The melting point of genistein from the formulations was found to be over a range of 277–300°C. Based on Van't-Hoff equation, the presence of impurities tends to make a crystalline peak broader, and therefore, in the present case PEG may be responsible for the wide range of genistein peak in the mixture.[32]

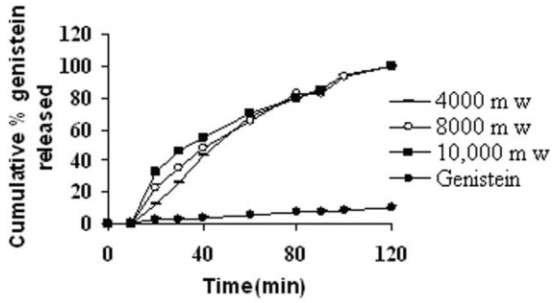


Figure 4 Effect of PEG molecular weight (MW) on the dissolution of genistein in genistein containing PEG microparticles.

Effect of molecular weight of PEG on particle characteristics

Dissolution for all the dispersions using PEG MW 4000, 8000, and 10,000 Da were significantly greater than those for genistein alone ($P = 0.0079$) (Fig. 4). The dissolution profiles of all the PEG microparticles prepared exhibited significant increase in the rate of dissolution in the phosphate buffer system as compared to native genistein.

In case of the genistein–PEG microparticles, it was seen that there was a slight increase in the rate of dissolution with an increase in the molecular weight of PEG. During the later stage of the dissolution, no difference in the rates of dissolution could be discerned as seen in Figure 3. Table II shows effect of molecular weight of PEG on percent drug loading, encapsulation efficiency, and yield of genistein microparticles. This may be attributed to the fact that higher molecular weight PEG form more viscous solutions, which further reduces molecular mobility and the rate of crystallization of drug. In fact, viscosity is related to molecular weight by Mark-Howink equation:

TABLE II
Effect of PEG Molecular Weight (MW) on Percent Encapsulation Efficiency and Percent Yield of Genistein Microparticles

PEG MW used in microparticles	Actual drug loading (%)	Encapsulation efficiency (%)	Yield (%)
4000	0.69 ± 0.01	30 ± 0.40	60 ± 2.39
8000	1.16 ± 0.01	50 ± 0.73	62 ± 1.24
10,000	1.63 ± 0.02	70 ± 0.42	75 ± 0.79

Values given are mean ± SD for $n = 3$.

TABLE III
Effect of Speed of Homogenization on Percent Encapsulation Efficiency and Percent Yield of Genistein Microparticles Prepared Using PEG of MW 10,000.

Speed of homogenization (rpm) used in microparticles	Actual drug loading (%)	Encapsulation efficiency (%)	Yield (%)
500	1.32 ± 0.09	57.53 ± 3.19	72 ± 5.87
1000	1.18 ± 0.03	51.92 ± 1.08	65 ± 2.46
2000	0.97 ± 0.11	42.87 ± 4.94	57 ± 1.44
4000	1.07 ± 0.08	46.01 ± 3.06	69 ± 3.71

Values given are mean ± SD for $n = 3$.

$$[\eta] = KM^{\alpha} \quad (6)$$

where K and α are constants for a given polymer–solvent combination at a given temperature and M is the polymer molecular weight. The higher molecular weight polymer increasingly favors the incorporation of drug as solid solutions or merely flakes more readily during dissolution. The lack of a considerable difference in dissolution profile ($P = 0.076$) at later time points because of differences in molecular weight might be due to the fact that all the PEGs in the present study are soluble in the dissolution medium at a similar rate. However, the dissolution of genistein from microparticles was ~9-fold higher than that for pure genistein at the end of 2 h (from 9.35% to 98.79% at 2 h).

Effect of speed of homogenization on particle characteristics

Table III shows the effect of speed of homogenization on encapsulation efficiency and yield. It was observed in the dissolution profiles given in Figure 5 that the higher the speed of homogenization, the greater is the rate of dissolution of genistein from the produced microparticles. This may be due to a reduction in the particle size of the microparticles as observed by SEM and optical microscopy.

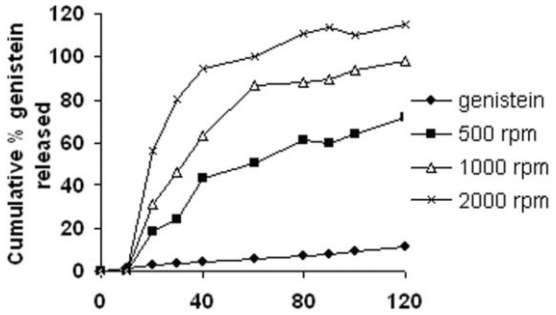


Figure 5 Effect of speed of homogenization on the dissolution of genistein in PEG–genistein microparticles.

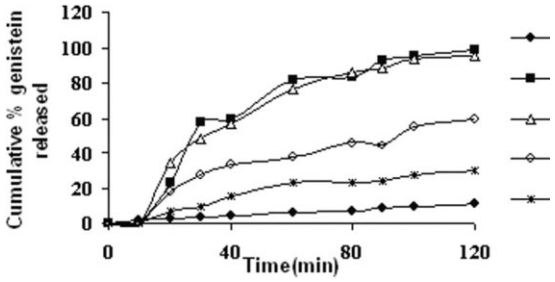


Figure 6 Effect of drug to polymer ratio on dissolution of genistein containing PEG microparticles. (Speed of homogenization was 1000 rpm, while the molecular weight of PEG was 10,000.)

A decrease in particle size increases the specific surface area and, consequently, the dissolution rate and sometimes the solubility, according to the combined Noyes–Whitney and Nernst equation,

$$dC/dt = AD(C_s - C)/hV \quad (7)$$

where, dC/dt is the rate of dissolution, A is the surface area presented by drug for dissolution, h is the diffusion layer thickness, D is the diffusion coefficient of the drug in this layer, C_S is the saturation solubility of the drug in the dissolution medium, C is the concentration of the drug in the dissolution medium at time t, and V is the volume of dissolution medium. Both the reduction in particle size and increase in solubility form the rationale for the use of solid microparticles.

Effect of drug to polymer ratio on particle characteristics

The microparticles prepared with 40 parts of PEG had the highest ($P = 0.0038$) cumulative dissolution at the end of 2 h of 95%, which was greater than the other preparations using 1:10 and 1:20 of drug to polymer ratio (Fig. 6). Table IV shows the effect of drug to polymer ratio on encapsulation efficiency and yield. The microparticles with 10 and 20 parts of PEG

TABLE IV
Effect of Drug: Polymer Ratio on Percent Encapsulation Efficiency and Percent Yield

Drug–polymer ratio used in microparticles	Actual drug loading (%)	Encapsulation efficiency (%)	Yield (%)
1:10	5.92 ± 0.21	68 ± 2.87	78 ± 3.6
1:20	2.54 ± 0.09	56 ± 2.04	64 ± 2.0
1:40	1.14 ± 0.03	49 ± 1.60	59 ± 2.8
1:80	0.37 ± 0.01	32 ± 1.91	58 ± 1.1

The speed of homogenization was 1000 rpm and the MW of PEG was 10,000 Da. Values given are mean ± SD for $n = 3$.

showed a slightly lower dissolution than that of 40 parts. The percent genistein released at the end of 2 h increased with an increase in the amount of PEG contained in microparticles.

An exception to this can be seen in the case of microparticles containing 80 parts of PEG. Microparticles containing 40 parts of PEG appear to have an advantage over the others in terms of dissolution rate as they provide ~9-fold increase over genistein alone.

Depending on the solubility of the drug, the drug-to-carrier ratio has to be optimized to achieve an optimum dissolution rate. In our case, 1:40 was found to be the optimum ratio. Microparticles containing higher (e.g., 80) parts of PEG did not show significant enhancement of drug dissolution in the release probably due to the limiting factor of the drug solubility in this release medium. When the solubility of a drug is very low, a higher fraction of carrier has to be used to deliver the drug in higher soluble state. Supersaturation of the drug in the carrier system might lead to stability problems.

CONCLUSIONS

Genistein falls in the biopharmaceutical classification system class II of compounds based on its low solubility and high permeability. This drug was incorporated into polymeric microparticles prepared using PEG by emulsion-solvent evaporation without altering its molecular structure. This formulation increased the dissolution profile of genistein significantly. DSC measurements indicated a conversion from crystalline to amorphous form of genistein consistent with greater dissolution of formulations as compared to the native form. ATR-FTIR studies provided data suggested hydrogen bonding interactions between the drug and formulation ingredients. Among the process variables studied, the speed of homogenization had maximum effect on the rate and extent of dissolution of genistein. Overall, solid dispersion systems of genistein–PEG result in increased dissolution of genistein by virtue of reduction in particle size and presence of noncrystalline genistein dispersed in the systems. The results of this study suggest that solid dispersion technology using microencapsulation may be used for formulating poorly water soluble drugs with the benefit of circumventing some of the disadvantages of methods such as extrusion method. However, further *in vivo* bioavailability studies and *in vitro* cytotoxicity studies are needed to verify whether the results obtained in this study can be translated to the *in vivo* situation.

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