JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1465–1472

Received April 28, 2009
Accepted August 19, 2009
DOI 10.1211/jpp/61.11.0005
ISSN 0022-3573

Research Paper

Evaluation of molecular pharmaceutical and in-vivo properties of spray-dried isolated andrographolide–PVP

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Abstract

Objectives Andrographolide, a natural lipophilic molecule, has a wide range of pharmacological actions. However, due to low aqueous solubility, it has low oral bioavailability. The purpose of the study was to increase the solubility and dissolution rate of isolated andrographolide by formulating its solid dispersion.

Method Solid dispersions were obtained by a spray-drying technique using different ratios of drug to polyvinylpyrrolidine (PVP K-30). Solid dispersions in compression with isolated drug and corresponding physical mixtures were characterized for various molecular pharmaceutical properties and subjected to stability study for up to 3 months.

Key findings A five-fold increase in saturation solubility of andrographolide with higher values of Q_{5min} (cumulative percentage release in 5 min) and lower values of $t_{75\%}$ (time required for 75% w/w drug release) for solid dispersion was observed in different dissolution mediums. This was attributed to the formation of amorphous nature and intermolecular hydrogen bonding between drug and PVP K-30. The stability study showed there to be no significant change in molecular pharmaceutical properties and dissolution profile over the period of 3 months. Moreover, the in-vivo study in Wistar albino rats also justified improvement in the therapeutic efficacy of andrographolide after solid dispersion.

Conclusions This study demonstrates the utility of solid dispersion to improve primary and secondary pharmaceutical properties of andrographolide using PVP K-30 as a carrier.

Keywords andrographolide; amorphous; PVP K-30; solid dispersion; spray drying

Introduction

Andrographolide is a diterpene lactone present in *Andrographis paniculata* Nees (Acanthaceae family). It is also known as 'king of bitters'. Andrographolide has several pharmacological actions, including analgesic, antipyretic, anti-inflammatory, hepatoprotectant, antiviral, antithrombotic, anti-cancer and hypoglycaemic activity.[1–6] It has an experimental log P value of 2.632 ± 0.135 and an aqueous solubility of 3.29 ± 0.73 μg/ml at 25°C. Due to its low aqueous solubility, andrographolide has a low bioavailability after oral administration, leading to poor therapeutic application.[7] The enhancement of oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development.

An array of methods have been attempted in recent years to improve solubility of pharmaceuticals, including use of pro-drugs, addition of surfactants, salt formation, complexation, particle size reduction, etc.[8–10] Solid dispersion of water-insoluble drugs using water-soluble surface-active carriers enhances drug dissolution and bioavailability by the reduction of particle size to the microcrystalline or molecular level. Polyvinylpyrrolidone (PVP K-30), a high-molecular-weight water-soluble carrier, has been demonstrated to retard and inhibit the crystallization of drugs, producing amorphous solid dispersions with increased drug dissolution rates, solubility, stability and minimal enthalpy relaxation, thus offering stabilization of the higher-energy amorphous systems.[11–13] An attempt has also been made to improve solubility of andrographolide by chemical complexation with cyclodextrin.[14] However, the slow process of complexation, high molecular weight of cyclodextrin and specific pH requirement for processing limit their practical utility.

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The preparation and molecular properties of solid dispersion of andrographolide have not been reported previously. Therefore, the purpose of the study was to prepare solid dispersions of isolated andrographolide by a spray-drying technique using different ratios of PVP K-30 (PVP). The solid dispersions were characterized for various molecular pharmaceutical properties in comparison with isolated andrographolide and corresponding physical mixtures using drug content, residual solvent content determination, saturation solubility, scanning electron microscopy (SEM), particle size, flow property, X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), Fourier transform infrared (FT-IR) spectroscopy and in-vitro drug release. Solid dispersions were further subjected to stability study at 40 ± 2°C/75 ± 5% relative humidity (RH) over a period of 3 months and evaluated for in-vitro drug release and crystalline characteristics. An optimized batch was also subjected to in-vivo testing in Wistar albino rats for pharmacodynamic studies.

Materials and Methods

Chemicals

Andrographolide was isolated from powdered leaves of *Andrographis paniculata* by a previously reported method and it was compared with standard andrographolide (Research Organic, Chennai, India) for its purity.[15] PVP K-30 (PVP) and carboxymethyl cellulose (CMC) were gifts from Cipla Pharmaceuticals (Mumbai, India). Methanol GR grade was purchased from Merck Chemicals (Mumbai, India). Carrageenan was a gift from Scitech Centre (Mumbai, India). All other solvents and chemicals used were of analytical grade.

Isolation of andrographolide

Isolation of andrographolide was carried out by a cold maceration method.[15] The powdered leaves (100 g) of *Andrographis paniculata* were extracted with a 1 : 1 mixture of dichloromethane (75 ml) and methanol (75 ml). The filtrate was subjected to evaporation to remove the organic solvent. The dark green crystalline mass obtained was washed several times with toluene to remove colouring matter from the residue. The obtained crystalline material was dissolved in hot methanol and cooled rapidly in a refrigerator for recrystallization of andrographolide. The structure and purity were checked by various spectral studies and compared with standard andrographolide.

Preparation of solid dispersions and physical mixtures

Homogeneous solutions containing andrographolide–PVP were obtained by adding different amounts of PVP to a solution of isolated andrographolide (1 g) in methanol (50 ml). The resultant solutions were spray dried using a laboratory scale spray dryer (Jay Instruments & Systems Pvt. Ltd, Mumbai, India) under the following set of conditions: inlet temperature, 60°C; outlet temperature, 45°C; feed rate, 6–8 ml/min; atomization air pressure, 2 kg/cm^2 and aspiration, 280 mm WC. Physical mixtures of andrographolide and PVP in the same ratio of andrographolide : PVP were prepared by dry blending and passing through a fine mesh (150 μm).

Yield and drug content

The dried weight of spray-dried product was recorded as practical yield. The solid dispersions were dissolved in a suitable quantity of methanol by use of a cyclomixer and ultrasonicator. The drug content was determined at 227 nm using a spectrophotometer (V-530; JASCO, Japan) after suitable dilutions.

Residual solvent content determination

The amount of total residual solvent in solid dispersions was determined by using a thermal gravimetric analyser (TGA-60WS0; Shimadzu Corporation, Japan). Thermal gravimetric analysis was performed by heating a weighed amount of sample in nitrogen atmosphere from 25°C to 80°C at the rate of 2°C/min and the loss of weight as a function of temperature was recorded.

Evaluation of molecular pharmaceutical properties

Saturation solubility

Saturation solubility was determined by dispersing a known excess amount of andrographolide, physical mixtures and solid dispersions into 10 ml of 0.1 M HCl, phosphate buffer pH 6.8 and distilled water. The suspensions were magnetically stirred (20 rev/min) at 37 ± 0.5°C for 48 h, at the end of which samples were withdrawn and filtered through 0.45-μm membrane filters. The filtrates were suitably diluted and analysed using a UV spectrophotometer (Jasco V-500) at 227 nm. The results of triplicate measurements and their means were reported.

Scanning electron microscopy

The external morphology of isolated andrographolide and solid dispersions was determined by scanning electron microscopy (SEM) (Stereoscan S120; Cambridge, UK). Samples were mounted on double-faced adhesive tape and coated with a thin gold–palladium layer by sputter-coated unit (VG-Microtech, Uckfield, UK) and surface topography was analysed at ×1000 and ×3000.

Particle size analysis

Particle size analysis was performed by using a laser diffraction technique (Malvern 2000 SM; Malvern Instruments, Malvern, UK), which allows sample measurement in the range of 0.05–20 000 μm. The particle size measurements were carried out at a 90° scattering angle. The powder sample was dispersed in distilled water and the average particle size was determined. The data presented are mean values of three independent samples produced under identical production conditions.

Flow property

The flow properties were characterized in terms of angle of repose (θ), Carr's index and Hausner ratio. For determination of angle of repose (θ), the powder sample was poured through the walls of a funnel, which was fixed at a position

such that its lower tip was at a height of exactly 2.0 cm above a hard surface. The powders were poured until a time when the upper tip of the pile surface touched the lower tip of the funnel. The angle of repose (θ) was calculated using the following equation:

$$\mathrm{Tan}^{-1}\theta = \text{Height of the pile/radius of its base} \quad (1)$$

The powders were poured gently through a glass funnel into a graduated cylinder cut exactly to the 10-ml mark. Excess powders were removed using a spatula and the weight of the cylinder with powder required for filling the cylinder volume was calculated. The cylinder was tapped from a height of 2.0 cm until the time when there was no more decrease in the volume. Bulk density (σb) and tapped density (σt) were calculated. Carr's index and Hausner ratio were calculated according to the following equation:

$$\text{Carr's index} = [(\sigma t - \sigma b)/\sigma t] \times 100 \quad (2)$$

$$\text{Hausner ratio} = \sigma t/\sigma b \quad (3)$$

X-ray powder diffraction

The crystalline properties of isolated andrographolide, physical mixtures and solid dispersions were studied by X-ray powder diffraction (XRPD) using an X-ray diffractometer (PW 1729; Philips, Almelo, Netherlands). The samples were irradiated with monochromatized Cu Kα radiation (1.542 Å) and analysed at 2θ between 5° and 50°. The voltage and current used were 30 kV and 30 mA, respectively. The range and the chart speed were 2×10^3 CPS and 10 mm/degree 2θ, respectively.

Differential scanning calorimetry

Thermal properties were studied using differential scanning calorimetry (DSC) in a calorimeter equipped with an intracooler (DSC 821e; Mettler-Toledo, Greifensee, Switzerland). Indium standards were used to calibrate the temperature and enthalpy scale. Approximately 5 mg of sample was hermetically sealed in an aluminium pan with a hole and heated at a constant rate of 10°C/min over a temperature range of 0–250°C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded after appropriate background subtraction using an FTIR spectrometer (FTIR-8400; Shimadzu Corporation, Kyoto, Japan) equipped with a diffuse reflectance accessory (DRS-8000; Shimadzu Corporation, Japan) and a data station. About 2–3 mg of the sample was mixed with dry potassium bromide and the samples were scanned from 4000 to 400 cm^{-1} wave numbers at a resolution of 2 cm^{-1}.

Dissolution studies

The dissolution studies were performed using a USP 24 type II dissolution test apparatus (Electrolab TDT-08L, Mumbai, India). The samples, equivalent to 50 mg of isolated andrographolide, were placed in the different dissolution vessel containing 900 ml of 0.1 M HCl, phosphate buffer pH 6.8 and distilled water at 37 ± 0.2°C and stirred at 100 rev/min. Periodically, samples were collected and replaced with fresh dissolution medium. After filtration through Whatman filter paper No. 41 (Whatman, Middlesex, UK), the concentration of andrographolide was determined UV-spectrophotometrically at 227 nm. A standard calibration curve for andrographolide was measured over the range of 5–30 μg/ml and shown to be linear. Analysis of data was performed using PCP-Disso software (V3; Poona College of Pharmacy, Pune, India). Q_{5min} (cumulative percentage release in 5 min) and $t_{75\%}$ (time required for 75% w/w drug release) were calculated using the Korsmeyer–Peppass equation:

$$Q = kt^n \quad (4)$$

where Q is the cumulative percentage release, t is the time required for Q release, and k and n are constants.

Stability studies

The solid dispersions were stored at 40 ± 2°C/75 ± 5% RH for three months and the effect of temperature and humidity on the solid dispersions was studied by measuring in-vitro drug release and the presence of crystallinity was assessed using XRPD and DSC studies.

Statistical analysis

Statistical analysis of the changes in solubility and dissolution rate of andrographolide and solid dispersions in various dissolution media was performed using the Kruskal–Wallis test followed by Dunnett's post-test. Parameters of andrographolide versus solid dispersions in various media were analysed using unpaired *t*-test with a two-tailed *P*-value test at the 95% confidence level. $P < 0.05$ denoted significance in all cases.

In-vivo study

The anti-inflammatory activity of solid dispersion was determined in comparison with isolated andrographolide in Wistar albino rats, 150–200 g, obtained from Yash farm (Pune, India). The rats were housed in polypropylene cages with free access to standard laboratory diet and water. They were kept at 25 ± 1°C and 45–55% RH with a 12-h light–dark cycle. Animal handling routines were performed according to Good Laboratory Practice. The research protocol of the animal experimentation was approved by the 'Institutional Animal Ethics Committee' of Poona College of Pharmacy (Pune, India). The total number of rats used in this study was 18. According to OECD guidelines the acute toxicity study was performed for andrographolide solid dispersions in both sexes of Wistar albino rats.

Carrageenan-induced paw oedema test

After overnight fasting, rats were divided into three groups of six rats each: group I received distilled water served as a control; group II received an oral dose of solid dispersion as a fine aqueous suspension equivalent to 30 mg/kg andrographolide; and group III received an oral dose of isolated andrographolide (30 mg/kg) suspended in 0.5% (w/v) carboxymethyl cellulose.

The effective dose of andrographolide was based on earlier reports and clinical dosages.[16] Thirty minutes after drug administration, 0.1 ml of freshly prepared carrageenan suspension (1%) in normal saline was injected

subcutaneously into the plantar region of the right hind paw to induce acute inflammation. The paw oedema volume was recorded at 0.5, 1, 2, 3, 4 and 6 h after carrageenan injection using a plethysmometer (UGO Basile 7140; Ugo Basile, Milan, Italy). The percentage inhibition of inflammation was calculated for each rat of all the groups using the following formula:

$$\text{P.I.} = [(V_1 - V_0)\text{Control} - (V_1 - V_0)\text{Treated}]/(V_1 - V_0)\text{Control} \quad (5)$$

where P.I. = percentage of inhibition, V_1 = paw volume after drug treatment and V_0 = paw volume before drug treatment.

The percentage inhibition of paw volume in the solid dispersion-treated group was compared with the control group and isolated andrographolide-treated group. Data were expressed as mean ± SD and statistical analysis was carried out using Kruskal–Wallis following by Dunnett's test and $P < 0.01$ was taken as significant.

Results

The spectral data of the isolated andrographolide and standard andrographolide confirmed the identity of the isolated compound as andrographolide.[15] By this method, 100 g of leaves yielded 1.5 g of 95.9% pure andrographolide. Purity was assessed using HPTLC (Figure 1). Solid dispersion of isolated andrographolide by spray drying in the presence of PVP K-30 was attempted to overcome the problem of low aqueous solubility. The preliminary batches of spray-dried andrographolide were prepared using various proportions of PVP (data not shown). At constant drug concentration, when the amount of PVP exceeded 4 parts the product was sticky and at below 2 parts the product was crystalline with poor flow properties. Based on the powder properties, drug content and saturation solubility, batches containing andrographolide–PVP in a ratio of 1 : 2, 1 : 3 and 1 : 4 parts by weight were used for further studies. The batches of solid dispersions were denoted as SD A (1 : 2), SD B (1 : 3) and SD C (1 : 4) and physical mixtures containing the same ratios were denoted, respectively, as PM A, PM B, PM C.

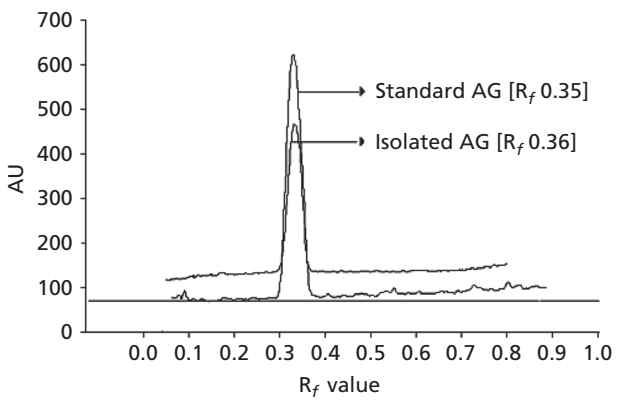

Figure 1 HPTLC spectra of standard andrographolide and isolated andrographolide. The standard andrographolide (AG) and isolated andrographolide (1 mg/ml of methanol) were applied on HPTLC silica gel plates using a CAMAG LINOMAT IV automatic spotter. The plates were developed using chloroform–methanol (9 : 1) as the mobile phase. The plates were scanned on a CAMAG TLC Scanner 3, which records the UV spectrum. The data was processed with *Win*CATS software for purity assessment. The retardation factor, R_f, of isolated andrographolide was 0.36, which was also the R_f region of standard andrographolide. UV absorption spectra recorded at start, middle and end position of the band completely overlapped, exhibiting an absorption maxima at 230 nm.

The selection of solvent and inlet and outlet temperature conditions for spray drying were set considering the thermal properties of PVP. The outlet temperature was kept below the melting point of PVP where methanol also gets preferentially evaporated.

The yield and drug content of the product obtained by spray drying were 60–70% (w/w) and 90–98% (w/w), respectively. The total residual solvent in all the solid dispersion batches was below 0.22% (w/w).

Molecular pharmaceutical properties

The saturation solubility of the isolated andrographolide, physical mixtures and spray-dried product were studied in 0.1 M HCl, phosphate buffer IP pH 6.8 and distilled water. The isolated andrographolide was most soluble in acidic medium (7.76 ± 2.01 μg/ml) and least soluble in distilled water (3.29 ± 0.73 μg/ml) (Table 1). The saturation solubility of solid dispersions was 5–6 times greater than isolated andrographolide and the physical mixtures showed moderate increase in saturation solubility.

The microphotographs showed that isolated andrographolide consisted of plate-shaped oblong crystals with fully developed corners; a few were cylindrical rods (Figure 2). The spray-dried powder consisted of spherical-shaped microparticles with a narrow particle size distribution and smooth surface. The particle size of isolated andrographolide was 4961.00 ± 6.6 μm, whereas spray-dried product was in the range of 2.8–3.6 μm (Table 1). As compared with the size of fully developed crystals of isolated andrographolide, the physical mixture obtained by trituration showed a 10-fold reduction in particle size.

The powder obtained by spray drying, the physical mixture and isolated andrographolide were evaluated for their flow properties. The angle of repose was in the range of 26.01 ± 0.14 to 27.50 ± 0.16° for solid dispersions, 40.42 ± 0.26 to 41.15 ± 0.19° for physical mixtures and 42.37 ± 0.23° for andrographolide. Carr's index was found to be 16.96–9.38% for solid dispersions, 40.04–41.07% for physical mixtures and 42.69% for andrographolide. The Hausner ratio ranged from 1.19 to 1.24 for solid dispersions, 1.66 to 1.69 for physical mixtures and 1.74 for andrographolide. These values indicate that the microparticles obtained by spray drying exhibited good flow properties as compared with andrographolide and physical mixtures.

The powder X-ray diffractogram of isolated andrographolide drug powder from 5 to 50° 2θ showed numerous distinctive peaks that indicated a highly crystalline nature (Figure 3). Due to its amorphous nature PVP did not display diffraction peaks. The XRPD peaks of crystalline andrographolide in all physical mixtures were similar, having low intensity compared with those in isolated andrographolide, indicating that the crystallinity of andrographolide did not

Table 1 Pharmaceutical properties of isolated andrographolide and physical mixtures and solid dispersions of andrographolide–PVP K-30

Formulation	Saturation solubility (μg/ml)			Particle size (μm)	Angle of repose (θ)
	Water	0.1 M HCl	PB pH 6.8		
IAG	3.29 ± 0.73	7.76 ± 2.01	5.32 ± 1.27	4961.0 ± 6.6	42.37 ± 0.23
PM A	4.89 ± 0.96	11.34 ± 1.14	8.45 ± 1.41	422.6 ± 5.2	41.15 ± 0.19
PM B	5.16 ± 1.05	15.38 ± 1.40	11.17 ± 1.24	413.4 ± 3.7	40.91 ± 0.31
PM C	5.66 ± 1.25	18.32 ± 1.05	12.38 ± 1.52	404.1 ± 3.2	40.42 ± 0.26
SD A	8.73 ± 1.54	26.42 ± 2.19	16.19 ± 1.64	3.6 ± 0.3	27.50 ± 0.16
SD B	11.37 ± 1.67	32.38 ± 2.17	20.11 ± 1.68	3.1 ± 0.6	26.26 ± 0.13
SD C	14.34 ± 2.08	37.75 ± 1.38	23.18 ± 1.82	2.8 ± 0.4	26.01 ± 0.14

PB: phosphate buffer; IAG, isolated andrographolide; PM, physical mixture; SD, solid dispersion. Data are means ± SD, $n = 3$.

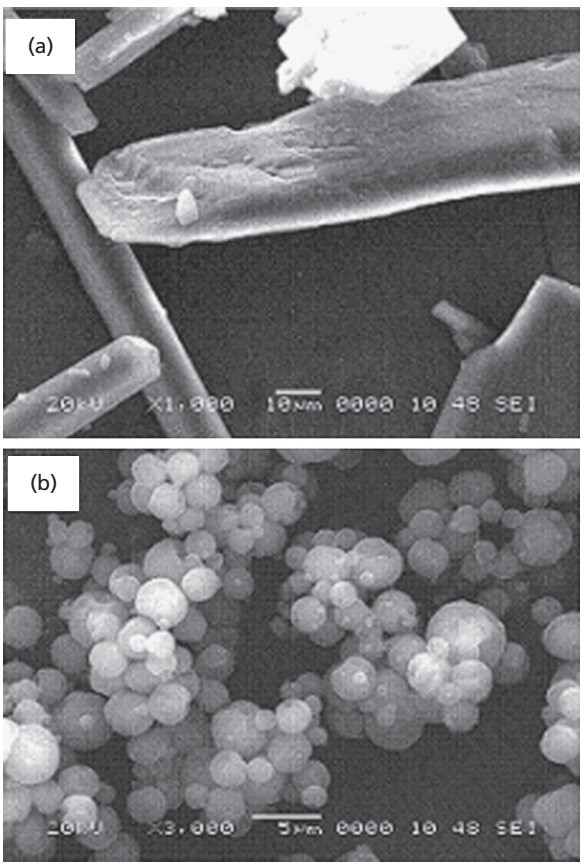

Figure 2 Scanning electron microscopy photographs of isolated andrographolide (a) and solid dispersion SD C (b).

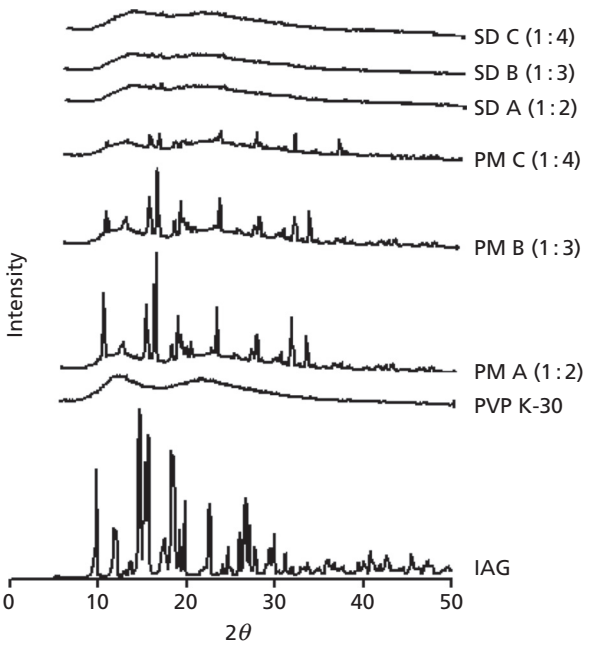

Figure 3 X-ray powder diffraction patterns of isolated andrographolide, physical mixtures and solid dispersions. IAG, isolated andrographolide; PM, physical mixture; SD, solid dispersion.

change in the physical mixtures. The solid dispersions showed no detectable andrographolide diffraction peaks, which indicated the existence of the amorphous form.

DSC thermograms of solid dispersions and physical mixtures displayed an endothermic peak (90–100°C) of water loss indicating the hygroscopic nature of PVP. The thermogram of isolated andrographolide showed a sharp endotherm at 235.35°C with ΔH 262.65 J/g. In physical mixtures, the drug endotherm broadened and shifted slightly to a lower temperature with decreased enthalpy, which was an inverse function of the amount of PVP. This reveals the salvation action of molten polymer. No peak was observed in the thermograms of PVP and solid dispersions indicating amorphous natures. The crystallization inhibition of PVP may also be attributed to hydrogen bonding between the drug and the polymer and the entrapment of drug molecules in the polymer matrix during spray drying.

FT-IR spectroscopy was carried out to study the possibility of chemical interaction between andrographolide and PVP. The FT-IR spectrum of isolated andrographolide showed a characteristic C=O absorption band at 1674.36 cm^{-1} and an OH stretch at 3395.02 cm^{-1} (Figure 4). Among the various bands, the spectrum of PVP at 2957.14 cm^{-1} (C-H stretch) and 1668.58 cm^{-1} is attributed to C-H stretch and C=O, respectively. The FT-IR spectra of physical mixtures showed all the characteristic peaks of drug and PVP, suggesting no chemical interaction, whereas solid dispersions showed almost complete disappearance of the hydroxyl band at 3395.02 cm^{-1} and the concomitant shift of the carbonyl peak

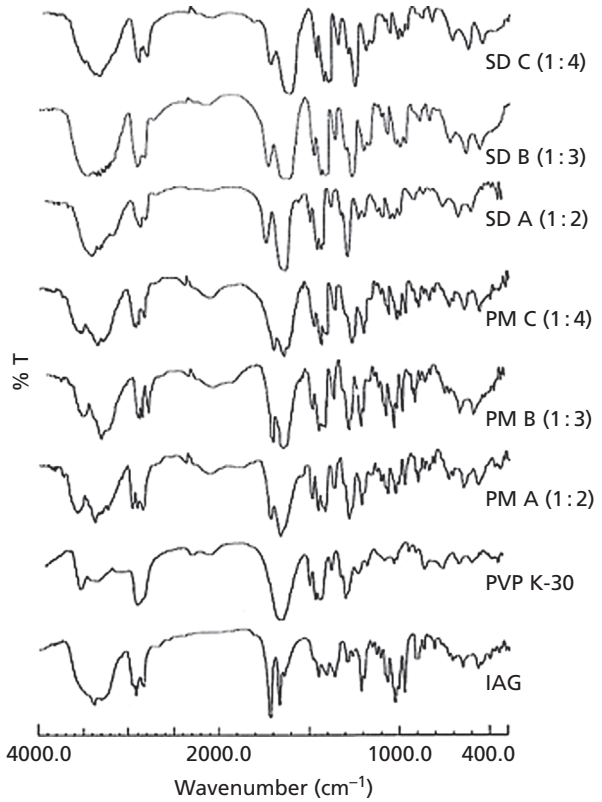

Figure 4 Fourier transform infrared spectra of isolated andrographolide, physical mixtures and solid dispersions. IAG, isolated andrographolide; PM, physical mixture; SD, solid dispersion.

from 1674.36 cm^{-1} to a lower frequency, 1643.50 cm^{-1}. This might be a consequence of intermolecular interactions, such as hydrogen bonding, which demonstrates the transformation of drug crystal into amorphous form.[17]

In-vitro drug release

In-vitro drug release of isolated andrographolide, physical mixtures and spray-dried product were carried out in 0.1 M HCl, phosphate buffer pH 6.8 and distilled water. Isolated andrographolide was characterized by only 2–10% drug release within 5 min. As shown in Table 2 and Figure 5, the dissolution rate of andrographolide from all physical mixtures was almost the same but was significantly higher than the dissolution rate of andrographolide alone. All solid dispersions showed higher values of Q_{5min} and lower values of $t_{75\%}$, which were greater than those for physical mixtures and andrographolide alone. Like saturation solubility, the rate of drug release in different media was in the order of 0.1 M HCl > phosphate buffer IP pH 6.8 > distilled water.

Stability studies

Many researchers have reported the re-conversion of drugs from the amorphous form into the crystalline form on storage.[18] Hence, solid dispersions were subjected for stability testing at 40 ± 2°C/75 ± 5% RH for 3 months. During the stability study, almost no significant difference in the dissolution profile in 0.1 M HCl and amorphous nature of the drug was observed over a period of 3 months as compared with freshly prepared solid dispersions.

Effect of solid dispersion on carrageenan-induced rat paw oedema

The acute toxicity study revealed that all solid dispersions were safe up to 2000 mg/kg, which is as per OECD guidelines,[19] whereas the reported acute toxic dose of isolated andrographolide was 11 460 mg/kg.[20] Based on the results of solubility, dissolution and stability study, batch SD C was selected for assessment of in-vivo performance in Wistar albino rats as determined by carrageenan-induced paw volume. The control group showed a high paw volume ($P < 0.01$) after carrageenan administration as compared with isolated andrographolide and SD C. The reduction in paw oedema produced in the SD C group was significantly higher ($P < 0.01$) than the control group and isolated andrographolide (Table 3). Isolated andrographolide produced 50% inhibition of paw volume at 3 h whereas the equivalent dose of SD C exhibited the same effect at 30 min. The marked increase in activity of SD C may be attributed to enhanced drug dissolution and bioavailability by the water-soluble surface active carrier PVP K-30.

Discussion

Andrographolide, a naturally occurring molecule, has a wide spectrum of pharmacological activity and medicinal properties. Pure andrographolide (95.9%) can be isolated by cold maceration and recrystallization from *Andrographis paniculata* leaves in a fast and effective manner. However, because poor aqueous solubility limits its bioavailability, solid

Table 2 Percentage release values of isolated andrographolide and physical mixtures and solid dispersions of andrographolide–PVP K-30

Parameter	Dissolution medium	Formulation IAG	PM A	PM B	PM C	SD A	SD B	SD C
Q_{5min}	0.1 M HCl	11.35 ± 1.75	16.26 ± 1.37	18.30 ± 1.42	23.14 ± 1.62	31.25 ± 1.59	68.43 ± 1.82	75.32 ± 1.58
	PB pH 6.8	5.83 ± 0.94	8.12 ± 0.73	10.38 ± 1.04	12.38 ± 1.38	30.16 ± 1.07	43.83 ± 1.47	61.80 ± 1.73
	Water	2.56 ± 0.73	4.78 ± 0.92	5.56 ± 0.64	6.86 ± 0.72	24.68 ± 1.12	36.21 ± 1.42	44.74 ± 1.82
$t_{75\%}$ (min)	0.1 M HCl	>120	>120	>120	>120	90	20	5
	PB pH 6.8	>120	>120	>120	>120	120	40	20
	Water	>120	>120	>120	>120	>120	>120	120

Q_{5min}, cumulative percentage release in 5 min; t75%, time required for 75% release; PB, phosphate buffer; IAG, isolated andrographolide; PM, physical mixture; SD, solid dispersion. Data are means ± SD, $n = 3$.

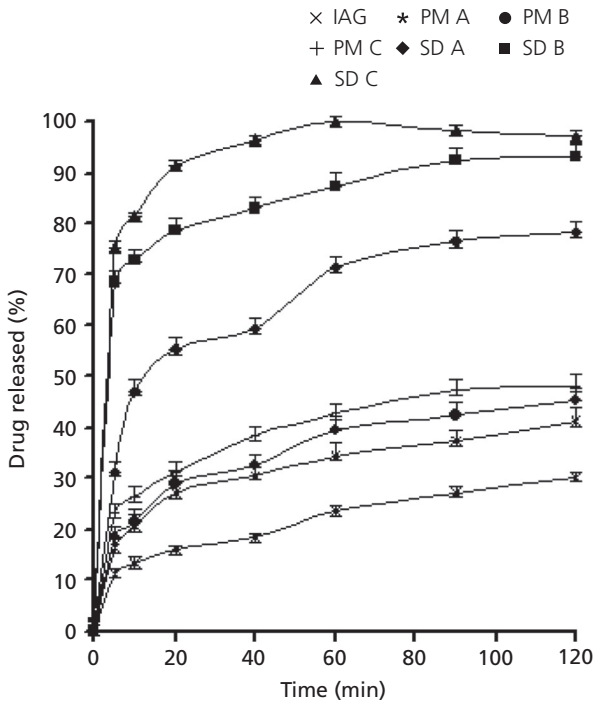

Figure 5 Dissolution profiles of isolated andrographolide, physical mixtures and solid dispersions in 0.1 M HCl. IAG, isolated andrographolide; PM, physical mixture; SD, solid dispersion.

dispersion using a spray-drying technique was attempted to overcome this problem. As indicated by thermal gravimetric analysis, the total residual solvents in solid dispersions was about 0.22% (w/w). The higher yield and low residual solvent content justifies use of the spray-drying technique to obtain solid dispersion.

The surface topography of the solid dispersions can be explained on the basis of formation of microparticles by solidification of droplets with preferential loss of solvent from the surface simultaneously with hardening in an inward direction. The surface of larger particles showed underdeveloped layers; this may be due to delayed solidification. Particle size determination of isolated andrographolide, physical mixtures and solid dispersions revealed the drastic reduction in particle size of the spray-dried product. The effect of PVP in size reduction can be explained on the basis of its surface tension-lowering effect that caused atomization of the liquid phase in tiny droplets and the crystal growth inhibition effect that restricted the crystal growth, producing smaller particles. In addition, the higher rate of evaporation of solvent from the droplets during spray drying induces formation of amorphous product.

The amorphous state was confirmed by XRPD and DSC patterns. In general, the process of crystallization of drug from supersaturated solution consists of two processes – creation of crystal nucleus and growth of the crystal. PVP might inhibit the association of the drug molecule to form the crystal nucleus and inhibit the crystal growth, thus the drug–PVP interaction is an inhibitory factor in the crystallization. In addition, hydrogen bonding between andrographolide and PVP inhibited drug recrystallization.[21]

Saturation solubility studies in different dissolution media reflected the poor solubility of the drug. Results of the dissolution experiments are in good agreement with the observation made in the solubility studies. The pure drug showed poor dissolution characteristics even in the most favourable dissolution medium. The dissolution rates of physical mixtures were higher than that of pure drug. This might due to the surface tension-lowering effect of PVP causing wetting of the hydrophobic crystalline surface of the drug, preventing the aggregation of drug, and by a local solublization effect in the diffusion layer. The improvement in the drug release from the solid dispersions might be due to the significant reduction in particle size of the drug during the formation of solid dispersion and the presence of the amorphous form of andrographolide.[22,23]

The improved stability of solid dispersions could be due to hydrogen bonding between the drug and the PVP carrier. Moreover the in-vivo study in Wistar rats also justified the improvement in the therapeutic efficacy of solid dispersions over isolated andrographolide.

Conclusions

This study demonstrates the utility of solid dispersion to improve the primary and secondary pharmaceutical properties of andrographolide using PVP K-30 as a carrier; this was reflected by the pharmacodynamic effect. Amorphous formulations of phytoconstituents could be used in the design of various dosage forms.

Table 3 Anti-inflammatory evaluation of andrographolide solid dispersion against carrageenan-induced paw oedema in rats

Group	Hind paw volume (ml)						
	BDA	0.5 h	1 h	2 h	3 h	4 h	6 h
Control	2.26 ± 0.06	3.87 ± 0.04	4.16 ± 0.03	4.83 ± 0.04	5.04 ± 0.05	4.25 ± 0.03	3.34 ± 0.04
Isolated andrographolide	2.22 ± 0.08	3.38 ± 0.07* (27.95%)	3.54 ± 0.04* (31.58%)	3.6 ± 0.02* (45.52%)	3.3 ± 0.03* (59.35%)	2.67 ± 0.04* (77.38%)	2.44 ± 0.03* (79.62%)
SD C	2.24 ± 0.05	2.93 ± 0.06$^{*\#}$ (57.14%)	2.73 ± 0.03$^{*\#}$ (74.21%)	2.31 ± 0.03$^{*\#}$ (97.27%)	2.25 ± 0.02$^{*\#}$ (99.64%)	2.24 ± 0.01$^{*\#}$	2.25 ± 0.03$^{*\#}$

BDA, before drug administration; SD C, solid dispersion C (andrographolide–PVP 1 : 4). All values are expressed as mean ± SD, $n = 6$. Percentage inhibition of paw volume is given in parentheses. $^{*}P < 0.01$, compared with control; $^{\#}P < 0.01$, compared with isolated andrographolide.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

The authors are thankful to All India Council for Technical Education, New Delhi, India for providing financial support in the form of a Quality Improvement Programme Fellowship.

Acknowledgements

The Authors are also thankful to Prof. S.L. Bodhankar and Sachin Vetal for their support during the in-vivo study.

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