DIFFERENTIAL SCANNING CALORIMETRY AS A SCREENING TECHNIQUE IN COMPATIBILITY STUDIES OF ACYCLOVIR EXTENDED RELEASE FORMULATIONS

F. Barboza,[1] D. D. Vecchia,[1] M. P. Tagliari,[2] M. A. S. Silva,[2] H. K. Stulzer[1,2,*]

Acyclovir (ACV) has been investigated during the past years, mainly due to its antiviral activity. Assessment of possible incompatibility between an active component and different excipients along with the evaluation of thermal stability are crucial parts of a normal study prior to the final formulation setting of a medicine. Thermal analysis studies were used as important and complementary tools during pre-formulation to determine the compatibility of drug-excipients with the purpose of developing an acyclovir extended release formulation. Fourier transform infrared spectroscopy (FT-IR) and x-ray powder diffraction (XRPD) analyses were also realized. The results showed that ACV only exhibited interaction that could influence the stability of the product in the binary mixtures of ACV/magnesium stearate.

Key words*:* acyclovir, compatibility studies, extended release formulations

INTRODUCTION

Acyclovir (ACV), chemically known as [9-(2-hydroxyethoxylmethyl)guanine], a synthetic purine nucleoside analog derived from guanine (Fig. 1), is the most widely used antiviral agent. It is effective in the treatment of herpes simplex virus (HSV), mainly HSV-1 and HSV-2, and varicella-zoster virus. In humans, ACV showed poor and variable oral bioavailability (15 – 30%) and a short circulation half-life ($t_{1/2}$, 2.5 h) [1, 2].

Due to its relative short half-life and low solubility, researchers have been developing different systems containing ACV [3 – 10]. Oral-controlled release technologies have been developed in an attempt to improve patients' quality of life by reducing the inconvenience caused by the frequent dosing of conventional tablets [11 – 14].

Matrix technologies have often proven popular among the oral controlled drug delivery technologies because of their simplicity, ease in manufacturing, high level of reproducibility, stability of the raw materials and dosage form, and ease of scale-up and process validation [15].

The successful formulation of a stable and effective solid dosage form depends on a careful selection of the excipients. Studies of drug-excipient compatibility represent an important phase in the pre-formulation stage for the development of all dosage forms [16 – 18].

Excipients are known to facilitate administration and release of an active component, as well as to protect it from the environment. While most of them have no direct pharmacological action, they do perform either useful tasks or damaging actions such as speeding up degradation of the drug [19 – 20]. In fact potential physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of drugs and, consequently, their therapeutic efficacy and safety. In this sense, devising a quick and accurate method to test and select the best candidates for stable dosage forms would constitute a real breakthrough in the pre-formulation pharmacy.

Differential scanning calorimetry (DSC) is widely used to evaluate physical properties of drugs, as well as to study compatibility and stability of the components of pharmaceutical preparations. DSC allows a rapid evaluation of possible incompatibilities by revealing changes in appearance, shift or disappearance of endothermic or exothermic peaks, and/or variations in the relevant enthalpy values in thermal curves of drug-excipients mixtures [21 – 22].

This study evaluated the thermal stability of ACV and mixtures of ACV/excipients by a thermoanalytical technique (DSC), with the support of x-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FT-IR) techniques. Furthermore, the excipients used in the prototype formulation of the tablet extended-release matrix were selected and analyzed.

[1] Department of Science Pharmacy, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.
[2] Department of Science Pharmacy, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil.
* Corresponding author: Hellen Karine Stulzer
Universidade Estadual de Ponta Grossa
Centro de Ciências da Saúde
Departamento de Ciências Farmacêuticas
Campus Universitário – Uvaranas – Ponta Grossa, PR, Brazil
CEP 84030-900

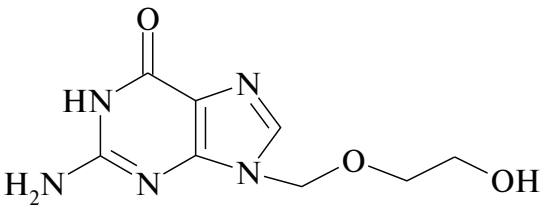

Fig. 1. Chemical structure of ACV.

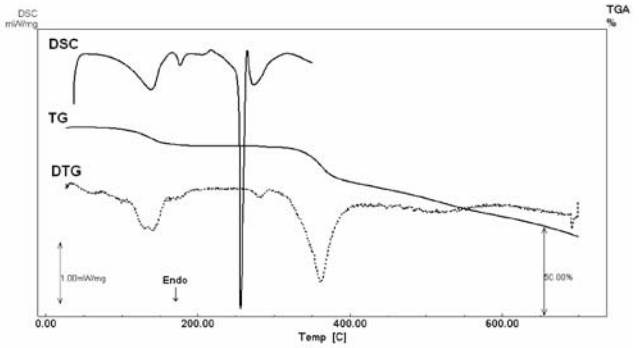

Fig. 2. DSC and TG/DTG curves of ACV.

EXPERIMENTAL PART

Materials

ACV was received from Shenyang Fine Chemical Co., China, Methocel, Polyox, and Ethocel were donated by Colorcon Brazil®, lactose and Microcel® 101 were provided by Blanver®, sodium starch glycolate was obtained from Unipex DMV, sodium croscamellose was obtained from FMC Europe, Aerosil® was obtained from Degussa, and magnesium stearate and stearic acid were purchased from Galena Brazil®.

Methods

Sample preparation

All the analyses were performed using samples of ACV, single excipients, and binary mixtures formed by ACV and each excipient (1:1; w/w) separately. The prototype formulas were analyzed at the same conditions.

Differential Scanning Calorimetry (DSC)

DSC curves were obtained in a DSC-60 cell (Shimadzu) using aluminum crucibles with about 2 mg of samples, under a dynamic N_2 atmosphere (flow rate: 50 ml min^{-1}).The temperature range was from 25 to 500°C with a heating rate of 10°C min^{-1}. An empty aluminum pan was used as reference. The DSC cell was calibrated with indium (mp 156.6°C; ΔH_{fusion} = 28.54 J/g) and zinc (mp 419.6°C).

Thermogravimetry (TG)

The TG experiments were measured on a Shimadzu thermobalance TGA-50 in the temperature range of 25 to 600°C, using platinum crucibles with approximately 4 mg of sample, under a dynamic N_2 atmosphere (flow rate: 50 ml min^{-1}). The equipment was previously calibrated with calcium oxalate standard.

X-ray powder diffraction (XRPD)

For characterization of powder crystallinity, x-ray diffraction patterns were obtained on a Siemens model D 5000, with a tube of CuK_a, voltage of 40 kV, and current of 40 mA, in the range of 3–65 (2θ), using the powder XRD method.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra were recorded on a Perkin-Elmer Model 1600 apparatus using KBr discs in the range of 4000 – 400 cm^{-1}.

Preparation of extended release tablets

Matrices tablets were prepared by direct compression of physical mixtures of the drug, hydrophilic polymers, and other excipients, maintaining a total mass of 600 mg. The drug, polymer, and diluents were preliminarily mixed homogeneously in a plastic bag for 15 min. The final mixtures were compressed into a single punch tablet press using a set of punches number of 13 mm.

RESULTS AND DISCUSSION

The thermoanalytical curves (DSC and TG) of ACV are shown in Fig. 2. The DSC curve of ACV showed a first endothermic event between 245 and 265 °C (ΔH_{fusion} = –135.4 J/g), with a melting temperature of 243.36°C, in agreement with the literature values [23, 24]. The TG/DTG curves exhibited 20.9% of mass loss between 303 and 390°C.

Excipients have been classified according to the functions that they perform in a formulation, although many excipients perform multiple functions. The excipients chosen for this study are described in the European Phamacopeia [24]. Moreover, they are usually employed in pharmaceutical process technology. The commercial names, functions, and suppliers are presented in Table 1.

These excipients were selected based on their potential suitability for the development of the intended extended-release matrix formulation. The polymers were selected as matrix-forming materials suitable for direct-compression due to their good flow properties and binding capacities. Moreover, they have hydrophilic and swelling characteristics that are particularly desirable for the developed formulation, considering the limited solubility of the drug. For the same reason, a highly soluble excipient was selected to dissolve the ACV quickly in aqueous medium. Finally, two different lubricants were elected for the formulation. Three different polymers were selected as candidates to matrix forming to produce ACV sustained release tablets, i.e., HPMC, PEO, and ethylcellulose. DSC of ACV and their respective 1:1 w/w

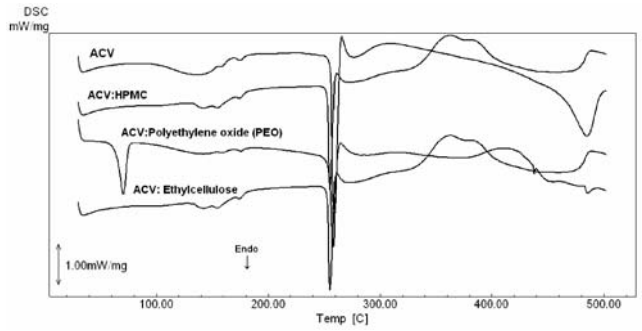

Fig. 3. DSC curves of ACV and 1:1 w/w blends as simple physical mixtures with HPMC, PEO, and ethylcellulose.

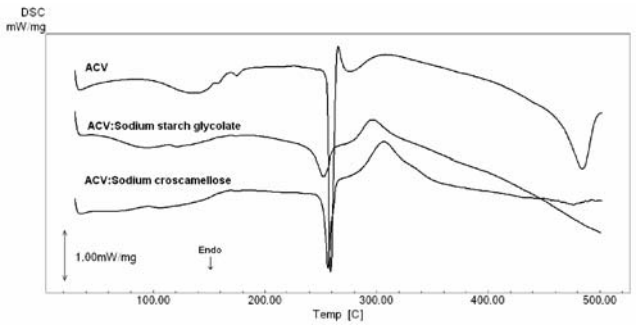

Fig. 5. DSC curves of ACV and 1:1 w/w blends as simple physical mixtures with sodium starch glycolate and sodium croscamellose.

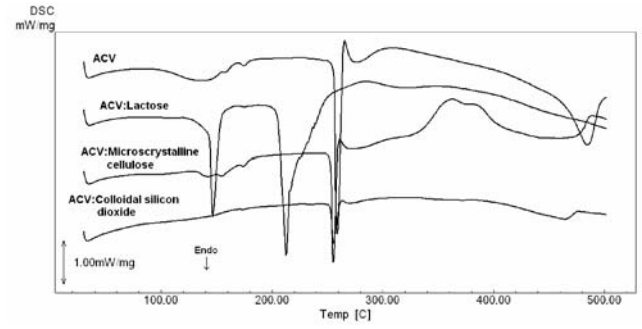

Fig. 4. DSC curves of ACV and 1:1 w/w blends as simple physical mixtures with lactose, microcrystalline cellulose, and colloidal silicon dioxide.

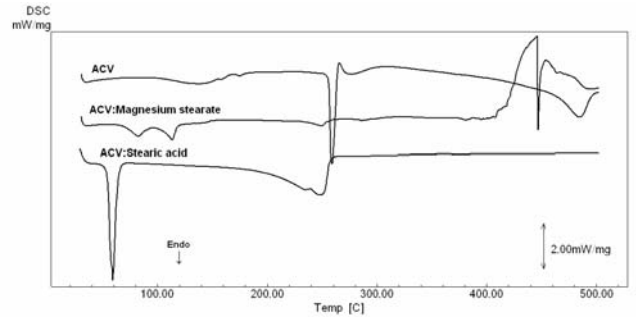

Fig. 6. DSC curves of ACV and 1:1 w/w blends as simple physical mixtures with magnesium stearate and stearic acid.

combinations are shown in Fig. 3. The sharp melting peak of pure ACV at 243°C (ΔH_{fusion} = –135.4 J/g) was visualized in DSC curves of physical mixtures, which indicate the absence of incompatibility between the drug and each excipient analyzed.

Lactose and microcrystalline cellulose were the diluents selected to evaluate the compatibility with ACV. Colloid silicon dioxide was chosen as glidant agent. This last one is widely used in pharmaceutical products due to its small particle size and large specific surface area, which provide its desirable flow characteristic. The DSC curve of ACV and the lactose physical mixture (Fig. 4) presented an endothermic event corresponding to dehydration of the bound water from the excipient at 145°C. In the temperature around 220°C an endothermic peak occurred corresponding to excipient fusion.

The interactions between ACV and lactose might be physical in nature due to their similar melting temperature ranges, which can displace the drug fusion point. In fact,

TABLE 1. Pharmaceutical excipients used in the compatibility studies

Excipient	Commercial name	Function	Supplier
Hydroxypropylmethylcelulose (HPMC KM15)	Methocel®	Binder	Colorcon
Polyethylene oxide $M_{w:}$ 7×10^6 (PEO)	Polyox®	Binder	Colorcon
Ethylcellulose	Ethocel®	Binder	Colorcon
Lactose		Diluent	Blanver
Microcrystalline cellulose 101	Microcel®	Diluent	Blanver
Colloid silicon dioxide	Aerosil®	Glidant	Degussa
Sodium starch glycolate	Primojel®	Disintegrant	Unipex DMV
Sodium croscamellose	AcDiSol®	Disintegrant	FMC Europe
Magnesium stearate		Lubrificant	Galena
Stearic acid		Lubrificant	Galena

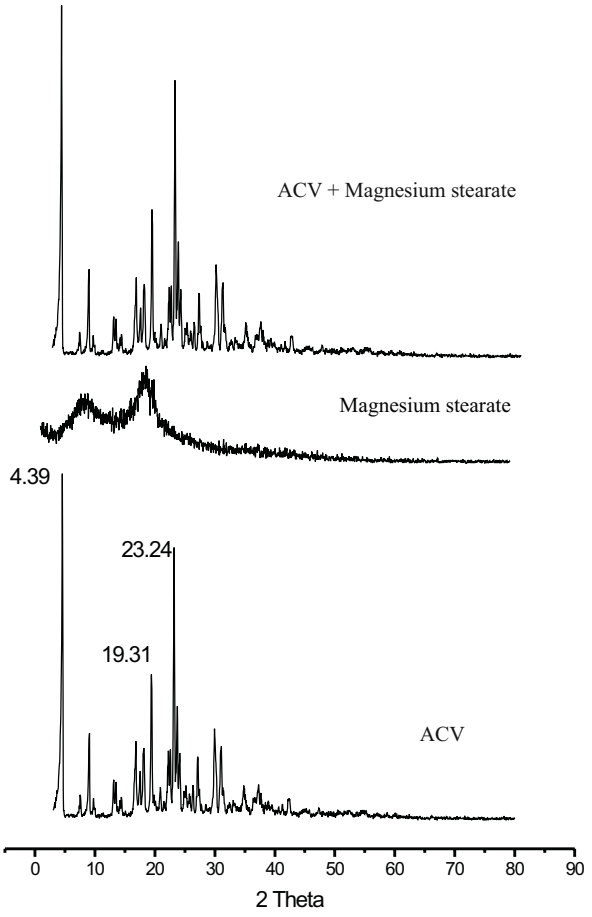

Fig. 7. X-ray diffraction spectra of ACV, magnesium stearate, and 1:1 blends as simple mixtures of ACV and magnesium stearate.

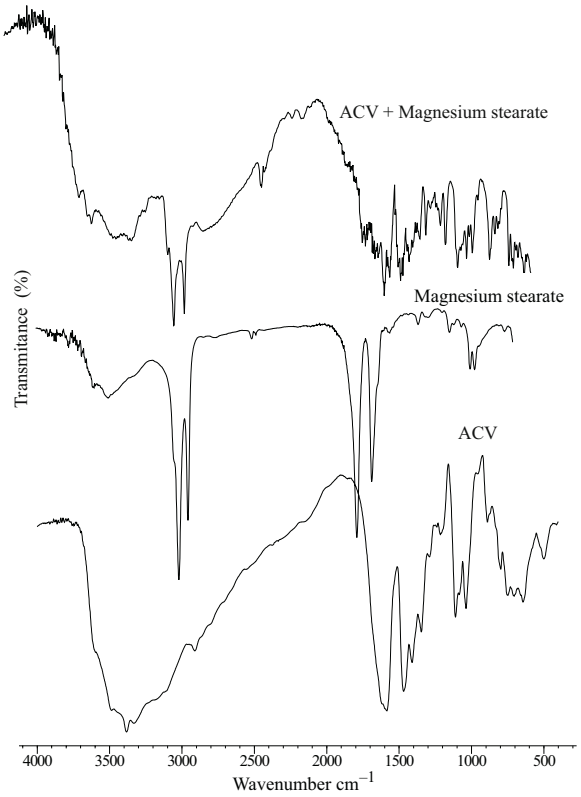

Fig. 8. FT-IR spectra of ACV, magnesium stearate, and 1:1 blends as simple mixtures of ACV and magnesium stearate.

TABLE 2. Thermoanalytical data of ACV and drug:excipients physical mixtures

Samples	DSC		Enthalpy (fusion), J/g
	T_{onset} (fusion), °C	T_{peak} (fusion), °C	
Drug			
Acyclovir	256.35	258.91	–135.4
Drug/excipient			
Hydroxypropylmethylcellulose	253.44	255.39	–61.72
Polyethylene oxide	254.96	258.26	–62.02
Ethylcellulose	256.38	254.72	–64.94
Lactose	207.82	212.70	–290.02
Microcrystalline cellulose 101	253.37	255.39	–47.92
Colloidal silicon dioxide	253.30	255.39	–51.74
Sodium starch glycolate	251.76	252.5	–45.98
Sodium croscamellose	255.39	253.30	–55.89
Magnesium stearate			
Stearic acid	239.7	247.9	–52.9

similar results have been observed for other amines and amides such as glibenclamide [25], glipizide [26], and glimepiride [27].

The DSC curves of the physical mixture ACV:microcrystalline cellulose and ACV:colloid silicon dioxide can be considered to be the superposition of the DSC curves of the two individual components. These results show that physical interactions of the components did not occur within the mixture.

Sodium croscamellose and sodium starch glycolate were the disintegrants selected to evaluate the compatibility with ACV. These excipients are recommended for tablets prepared by either direct-compression or wet-granulation processes. The thermal behavior of ACV:sodium croscamellose and ACV:sodium starch glycolate indicated a light displacement of the ACV fusion peak, which is indicative of a strong interaction, but not necessarily corresponding to incompatibility (Fig. 5).

Magnesium stearate and stearic acid are used as lubrificants to improve flowing properties of the mixture and reducting the friction during compression. The DSC curve of ACV and magnesium stearate (Fig. 6) presented two endothermic events in the range of 8 – 112°C, which is related to the dehydration process of this excipient. In the DSC curve of binary mixture was evidenced the disappearance of char-

acteristic ACV fusion peak, which can be related to incompatibility between these compounds.

The literature describes interactions between different drugs and the excipient magnesium stearate, for example, glibenclamide, atenolol, and captopril [27 – 29]. The DSC curve of ACV and stearic acid (Fig. 6) showed an endothermic peak at 59.07°C (ΔH = -193.88 J/g), characteristic of excipient melting. In agreement with the thermoanalytical curves of the physical mixture, a slight displacement of the ACV melting peak was evident, which does not seem to indicate incompatibility.

The results taken from DSC curves of ACV and binary mixtures are collected in Table 2.

To investigate the possible interaction between ACV and magnesium stearate, x-ray diffraction and FT-IR studies were then performed in order to obtain more information and support for the DSC and TG results. The results obtained by x-ray diffraction (Fig. 7) indicated that ACV presented crystalline characteristics. In the x-ray diffractograms the more evident peaks appear at $2\theta = 4.39$, 19.31, and 23.44. The magnesium stearate did not present peaks, which is characteristic of an amorphous compound. The XRPD did not evidence incompatibility between ACV and the magnesium stearate, since the diffractions peaks remained unaltered in the physical mixture.

The FT-IR spectra of ACV, magnesium stearate, and the binary mixture ACV:magnesium stearate are shown in Fig. 8. The ACV spectrum was in accordance with the literature, which describes in the region of 3500 cm^{-1} a large band attributed to the OH group present in the ACV molecule. The region of 3382 and 3332 cm^{-1} showed bands corresponding to primary and secondary amines. Thus, in the 1584 cm^{-1} region we visualized a band with high intensity attributed to R-NH vibrations. The low-intensity absorption band of secondary amine is in the 2912 cm^{-1} region. The CH group has a characteristic band in the 1346 cm^{-1} region, and the methylene group in the 1468 cm^{-1} region. The 1584 and 1036 cm^{-1} regions are represented by bands corresponding to amide and primary alcohol, respectively. Magnesium stearate presents a strong CH_2-CH_3 vibration in the region of 2918 up to 2850 cm^{-1}. In the 1567 and 1464 cm^{-1} regions it showed an asymmetric stretch corresponding to the COO^- group. The main difference observed in the FT-IR of binary mixtures of ACV:stearic acid was the significant decrease of the band attributed to the OH group, indicating a possible chemical interaction between these compounds. The hydrogen from magnesium stearate interacts with OH from the ACV molecule, forming a hydrogen bond.

The subsequent phase of the study was the evaluation of the compatibility of some model matrix-tablet formulations containing all the components together, in their actual weight proportions and after compression.

Based on the results from the preliminary screening described above, POE and HPMC were selected as direct compression matrix forming materials. The lubrificant chosen

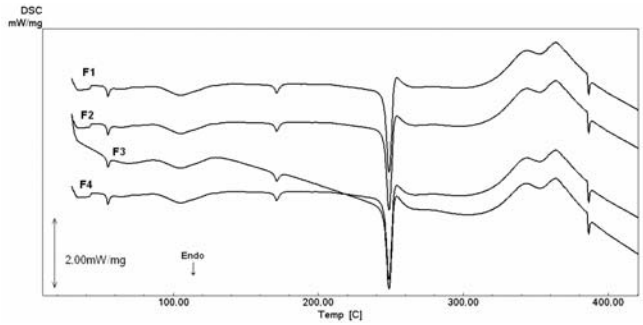

Fig. 9. DSC curves of formulations F1, F2, F3, and F4.

was stearic acid, which proved to be compatible with ACV. Sodium starch glycolate and sodium croscamellose were discarded since preliminary studies of dug release from matrix tablets showed that, within the polymer concentration range employed in these formulations, the disintegrant effect prevailed, giving rise to complete disaggregation of tablets after 15 min in simulated gastric fluid. Finally, microcrystalline cellulose 101 and colloid silicon dioxide were the diluent and glidant agents selected, respectively. The prototypes formulations are described in Table 3.

The DSC data obtained from different formulations before compression are shown in Fig. 9. The results indicate that all formulations maintained the characteristic ACV peak fusion, with ΔH values for F1 (–72.04 J/g), F2 (–68.59 J/g), F3 (–66.64 J/g), and F4 (–65.65 J/g). In this way, the model formulations developed showed excellent compatibility among all excipients used.

CONCLUSIONS

As a part of an ongoing project aimed at the development of an ACV extended release formulation, different excipients were tested to verify their compatibility with the drug. The results confirmed the utility and reliability of thermal analysis as quick and accurate methods to test and select the best candidates for stable dosage forms, constituting a real breakthrough in preformulation pharmacy. Moreover, the DSC technique offers significant advantages in saving time, sub-

TABLE 3. Composition of different formulations (mg)

Formulation	F1	F2	F3	F4
Acyclovir	200	200	200	200
PEO M_w: 7×10^6	300		180	
HPMC KM 15		300		180
Microcrystalline cellulose 101	82	82	202	202
Colloidal silicon dioxide	6	6	6	6
Stearic acid	12	12	12	12

stance, and money to detect compatibility/incompatibility in binary mixtures. Based on the DSC results, supported by FT-IR and XRPD analyses, all the excipients tested were compatible with ACV, except magnesium stearate, which was replaced by stearic acid in the developed formulations.

REFERENCES

1. P. K. Gosh, R. J. Majithiya, M. L. Umerethia, et al., *AAPS Pharm. Sci. Tech.*, **7**, 1 – 6 (2006).
2. I. A. Attia, S. A. El-Gizawy, M. A Fouda, et al., *AAPS Pharm. Sci. Tech.*, **8**, 1 – 7 (2007).
3. S. C. Chen, Y. C. Wu, F. L. Mi, et al., *J. Control. Rel.*, **96**, 285 – 300 (2004).
4. S. J. Cheu, R. R. L Chen, P. F. Chen, et al., *J. Microencapsul.*, **18**, 559 – 565 (2001).
5. E. G. Jalón, M. J. Blanco-Príeto, P. Ygartua, et al., *J. Control. Rel.*, **75**, 191 – 197 (2001).
6. S. L. Law, K. J Huang, and C. H. Chiang, *J. Control. Rel.*, **63**, 135 – 140 (2000).
7. Z. P Pavelic, N. Skalko-Basnet, J. Filipovic-Grcic, et al., *J. Control. Rel.*, **106**, 34 – 43 (2005).
8. C. V. Rossel, J. S. Carrenõ, M. R. Baeza, et al., *Quím. Nova*, **23**, 749 – 752 (2000).
9. C. M. Sancho, R. H. Vanrell, and S. Negro, *J. Microencapsul.*, **20**, 799 – 810 (2003).
10. H. K. Stulzer, L. Lacerda, M. P. Tagliari, et al., *Carbohydr. Polym.*, **73**, 490 – 497 (2008).
11. R. K. Verma, D. M. Krishna, and S. Garg, *J. Control. Rel.*, **79**, 7 – 27 (2002).
12. R. K. Verma and S. Garg, *Pharm. Technol.*, **25**, 1 – 14 (2001).
13. A. Bernardos, E. Aznar, C. Coll, et al., *J. Control. Rel.*, **131**, 181 – 189 (2008).
14. D. A. Fluri, C. Kemmer, M. Daoud-El Baba, et al., *J. Control. Rel.*, **131**, 211 – 219 (2008).
15. M. V. S Verma, A. M. Kaushal, A. Garg, et al., *Am. J. Drug Deliv.*, **2**, 43 – 57 (2004).
16. K. Jackson, D. Young, and S. Pant, *Pharm. Sci. Technol. Today*, **3**, 336 (2000).
17. P. Mura, M. T. Faucci, A. Manderioli, et al., *J. Pharm. Biomed. Anal.*, **18**, 151 (1998).
18. P. C. Mora, M. Cirri, and P. Mura, *J. Pharm. Biomed. Anal.*, **42**, 3 – 10 (2006).
19. A. R. Fassihi and P. H. R. Persicaner, *Int. J. Pharm.*, **37**, 167 – 170 (1987).
20. P. Crowley and L. Martini, *Pharm. Technol. Eur.*, **13**, 26 – 34 (2001).
21. M. Tomassetti, A. Catalani, V. Rossi, et al., *J. Pharm. Biomed. Anal.*, **37**, 949 – 955 (2005).
22. M. N. Freitas, R. Alves, J. R. Matos, et al., *J. Pharm. Biomed. Anal.*, **87**, 905 – 911 (2007).
23. A. C. Moffatt, M. D. Osselton, and B. Widdop, *Clarke's Analysis of Drugs and Poisons*, The Pharmaceutical Press, London (2004).
24. COE, *European Pharmacopoeia*, TSO (2007).
25. R. K. Verma and S. Garg, *J. Pharm. Biomed. Anal.*, **38**, 633 (2005).
26. L. C. S. Cides, A. A. S. Araújo, M. Santos-Filho, et al., *J. Therm. Anal. Calorim.*, **84**, 441 – 445 (2006).
27. G. G. G. Oliveira, H. F. G. Ferraz, and J. R. S. Matos, *J. Therm. Anal. Calorim.*, **79**, 267 (2005).
28. G. Pyramides, J. W. Robinson, and S. W. Zito, *J. Pharm. Biomed. Anal.*, **13**, 103 (1995).
29. H. K. Stulzer, P. O. Rodrigues, and T. M. Cardoso, *J. Therm. Anal. Calorim.*, **91**, 323 – 328 (2008).