International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 3, 2011

Research Article

EVALUATION OF POLYMORPHS IN CEPHALEXIN MEDICINES BY ^{13}C SOLID STATE NMR

DANIEL L.M. DE AGUIAR[1], ROSANE A.S. SAN GIL[1*], LEANDRO B. BORRE[1], MONICA R.C. MARQUES[2], ANDRE L. GEMAL[1]

[1] Universidade Federal do Rio de Janeiro, Instituto de Química, Laboratório de RMN de Sólidos, Av. Athos da Silveira Ramos 149, bl.A, s.605, Ilha do Fundão, CEP 21941-909 RJ, Brazil, [2] Universidade do Estado do Rio de Janeiro, Instituto de Química, Rio de Janeiro, Brazil Email: rsangil@iq.ufrj.br

Received: 03 May 2011, Revised and Accepted: 04 Jun 2011

ABSTRACT

In this study the presence of cephalexin polymorphs in four commercial medicines was evaluated. The samples were analyzed using X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FTIR) and solid state ^{13}C NMR spectroscopy (CPMAS NMR). The crystalline pattern obtained by XRPD analyses for two medicines are very similar to the cephalexin monohydrate reference sample. FTIR was not capable to distinguish between the medicine samples, although one was showed to be amorphous by XRPD. In contrast, ^{13}C solid state NMR evidenced the differences between the samples studied and confirmed that in those medicines the cephalexin monohydrate is the polymorph present in higher amount.

Keywords: Cephalexin, ^{13}C CPMAS NMR, XRD, FTIR, Polymorphs

INTRODUCTION

The oral absorption of a drug depends on physicochemical and physiological factors such as drug solubility, lipophilicity, dissolution rate, formulation, food composition and gastric emptying time.[1, 2, 3].Delivery of drugs by using oral ingestion is a common route of drug delivery due to the ease of administration, patient compliance and flexibility in formulation.[4] The major impact of solid state physicochemical properties of drugs on their medicines properties is that they may not have the same bioavailability.[5] One of the possible causes of the difference in the bioavailability of medicines is the polymorphism of pharmaceuticals or excipients.[6] Polymorphism is the ability of a named substance to form two or more crystalline states, in this way polymorphs are different crystalline forms that possessing the same chemical structure.[7] Polymorphism includes all the solid forms of the same molecule that have the same vapor, liquid or solution phase, i.e., amorphous pharmaceuticals and solvates were also included in this definition.[8]

The key of polymorphic research are recrystallization techniques, and different crystallization methods can lead to different polymorphic systems with distinct solubilities.[9, 10] In this field of research a number of techniques has been used to characterize the polymorphism in pharmaceuticals: X-ray powder diffraction (XRPD)[11,12] , thermal analysis[13], and spectroscopic techniques as Raman and Fourier transform infrared spectrometry (FTIR)[14,15] are some examples. Solid state NMR has also experienced a welcome breakthrough in polymorphism study since the early 1990s with the pioneer work of Christopher et al.[16] It constitutes a complementary technique to X-ray diffraction for solid structure studies and holds especially for the pharmaceutical compounds which are amorphous, and therefore not amenable to X-ray diffraction.

Beta-lactam antibiotics form a bulky family of zwitterionic drugs that are potent inhibitors of bacterial wall biosynthesis, which account for approximately 60% of commercial antibiotics formulations.[17] The inhibition of that reaction leads the bacteria to death.[18] The optimal efficiency of beta-lactam antibiotics are related with serum concentrations above the minimum inhibitor concentration (MIC) of at least 50 or 60% of the dosing interval. In this manner, spread variations of serum concentration are by far unwelcome,[19] and the study of the factors that could affect the efficiency of beta-lactam based antibiotics are of importance to public health.

Cephalexin, (6*R*,7*R*)-7-[[(2*R*)-2-amino-2-phenylacetyl]amino]- 3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene- 2-carboxylic acid (structure given in Figure 1) is one of the most used first generation cephalosporin based antibiotics to treat infections due to its broad antibacterial activity. Its crystalline structure was published by Kennedy et al.[20] Distinct formulations can be found commercially and are available to public. However no information is available related to the polymorph present in those medicines. This work aims to investigate the nature of the cephalexin polymorph present in four distinct medicines by using XRPD, infrared spectroscopy and ^{13}C solid state NMR, and was compared with that on the reference cephalexin monohydrate sample. To our knowledge solid state NMR data for cephalexin monohydrate and some of its medicines have not been previously published.

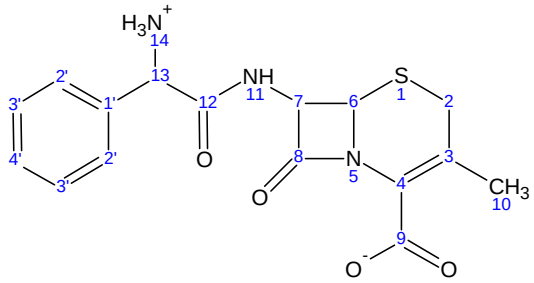

Fig. 1: Chemical structure of cephalexin

MATERIALS AND METHODS

Samples

Cephalexin monohydrate (PSA) was purchased from Sigma Aldrich Inc; the suspension sample (CSM) was purchased from local markets; the capsule (CCC) and the powder for oral suspension (CSH) samples were kindly donated by the Brazilian Public Health System (SUS); the raw powder (PMD) sample, was provided by Pharmantiga (Linhares, Brazil), purchased from DEG (India). The samples were used as received, except the suspension sample CSM, that was centrifuged at 3500 rpm for 10 minutes. Then the supernatant was removed and 125 mL of H_2O were added. The washing and centrifugation processes were repeated for three times, followed by liofilization.

Characterization Methods:

X-ray powder diffraction (XRPD)

X-ray powder diffraction patterns were obtained using a Rigaku MiniFlex powder diffraction system. The X-ray source was nickel-filtered K-α emission of copper (1.54056 Å), operating at 30 kV and 15 mA, and employing a scanning step of 0.05º/s in 2θ range from 2º to 60º.

Fourier transform infrared spectroscopy (FTIR)

Fourier Transform Infrared spectra (FTIR) were recorded in the range 4000-400 cm^{-1} on a ABB Inc. FTIR system model FTLA 2000-100 (Quebec, Canada), with resolution of 4 cm^{-1}, scanning from 4,000 to 400 cm^{-1} at room temperature. The samples were crushed by mixing with potassium bromide (1%) and pressed to disks under conditions to avoid the waffle hydration.

^{1}H and $^{13}C\{^{1}H\}$ solution state nuclear magnetic resonance

^{1}H and $^{13}C\{^{1}H\}$ were obtained to evaluate the presence of impurities in the samples studied. The spectra were acquired on a Bruker Avance DPX-200 NMR spectrometer (4.7T), operating at 200MHz (^{1}H) and 50MHz (^{13}C) resp. The samples (5 mg) were dissolved in deuterium oxide (0.6 mL); dioxane was added for referencing the ^{13}C spectra. The $\pi/6$ pulse length was 12 µs (^{1}H) and 11 µs (^{13}C), the recycle delay was 2s and 4s for ^{1}H and ^{13}C respectively and the spectra were transformed after accumulation of 256 (^{1}H) and 512 (^{13}C) scans. Residual H_2O signal (4.74 ppm) and dioxane (67.4 ppm) were used as internal chemical shift references for ^{1}H and ^{13}C spectra, resp.

^{13}C Solid state nuclear magnetic resonance (^{13}C CPMAS NMR)

^{13}C solid state NMR spectra were recorded using a Bruker Avance DRX-300 NMR spectrometer (7.05T), operating at 75.46 MHz. A CPMAS 4mm probehead was used to spun ZrO_2 rotors at 6 KHz. Cross polarization magic angle spinning combined with high power proton decoupling was used as pulse sequence (CPMAS). The contact time was optimized to 2000 µs. The recycle delay was 4s, and $\pi/2$ pulse length was 5 µs. The spectra was transformed after accumulation of 512 scans. The line broadening used to process the data was 20Hz. Hexamethylbenzene (CH_3 at 17.3ppm) was used as external chemical shift reference.

RESULTS AND DISCUSSION

X-ray powder diffraction (XRPD) analyses

Figure 2 shows XRPD patterns obtained for cephalexin medicines CCC (capsules), PMD (powder for suspension), CSM (lyophilized sample) and CSH (raw powder) and for the cephalexin reference sample (PSA). All the samples, except the liophylized (CSM) showed well defined diffraction peaks, comparable with those observed in the XRPD of the cephalexin monohydrate, with intense diffraction peaks at 2θ 17° and 22.5°. The results suggest that the polymorph in medicine samples presents the same crystalline structure, but distinct to those indicated by Stephenson[21] and Otsuka.[22] In the other side, the XRPD obtained for a liophylized sample (CSM) showed no diffraction peaks, evidencing the amorphous structure of this material. The XRPD obtained for the oral suspension sample (CSH) showed a great number of diffraction peaks in the range $35°<2\theta<60°$, absent in the reference sample, that can be assigned as corresponding to excipients.

No extra peaks between $2° < 2\theta < 35°$ (with exception of CSH) were observed for CCC and PMD, evidencing the purity of the solid form present in these medicines. However in the case of CSH medicine the XRPD was not able to clearly distinguish between the peaks of the active phase and the excipients in the range $2° < 2\theta < 35°$. Also in the case of the amorphous drug, XRPD was not able to provide any information about the structure present.

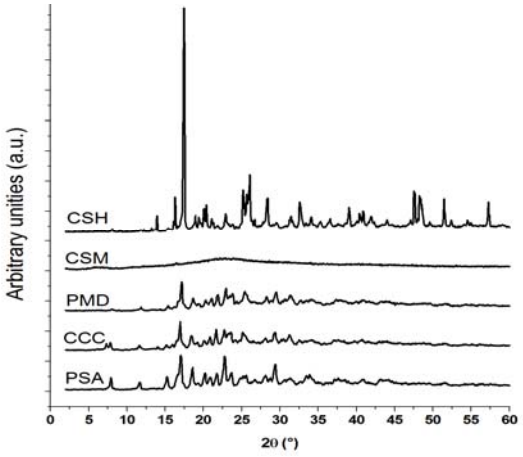

Fig. 2: Comparative X-ray powder diffraction data obtained for cephalexin based medicines: PSA (reference), CCC (capsules), PMD (powder for suspension), CSM (lyophilized sample) and CSH (raw powder)

Fourier Transform Infrared Spectra (FTIR)

The FTIR spectra are shown in Figure 3. The spectrum obtained for cephalexin monohydrate reference sample (PSA) shows major bands at 3425 cm^{-1}, 3190 cm^{-1}, 2610 cm^{-1}, 1767 cm^{-1}, 1689 cm^{-1} and 1593 cm^{-1} (Table 1), similar to that reported by Di Stefano et al.[23] When compared with the standard cephalexin monohydrate (PSA), CCC, PMD and CSM had the same FTIR profile although CSM was amorphous following XRPD.

The broad band at ~2600 cm^{-1} due to stretching mode of NH_3^+ is present in all medicines, confirming the presence of Zwitterionic form. The principal differences in the spectra were observed in the region 3500-2900 cm^{-1}. CSH showed an intense band at 2980 cm^{-1}, and a well defined band at 3550 cm^{-1}, corresponding to the C-H stretching and isolated O-H stretching modes resp., due to excipients (for example carboxymethyl cellulose and sucrose). No differences could be found when comparing the FTIR spectra in the region below 1800 cm^{-1}. FTIR data is in accordance with the XRPD, i.e. both techniques indicate the same polymorphic system in those medicines.

^{1}H and ^{13}C solution state nuclear magnetic resonance

Solution state ^{1}H NMR spectra (Figure 4) were carried out to monitoring the components present in the studied medicines. No extra peaks were observed in CCC and PMD spectra, compared with reference sample (PSA). However for CSM and CSH several peaks assigned as excipient signals were observed. In the case of CSH, the 2a proton signal (3.0 ppm) is overlapped by excipient peaks, but this did not affect the identification of cephalexin, since the hydrogens are diateroisotopic. No chemical shift displacement was observed for the cephalexin protons comparing the medicines's spectra, thus indicating the absence of intermolecular interaction with the excipients. By integrating the signals at 5.4 ppm (O-CH-O from carbohydrate moiety) and at 5.7 ppm (CH from cephalexin molecule)

it was possible to calculate the concentration of cephalexin, as been 17.4 mol% in CSH medicine. The solution state NMR of CSM also shows low intensity signals at around 2.2 ppm and 1.0 ppm that could be assigned as from excipients. A summary of ^{1}H NMR data is showed in Table 2.

The solution state ^{13}C NMR spectrum was obtained only for reference sample PSA (Figure 5), and was carried out in order to obtain reliable assignment of ^{13}C NMR resonances, compared with literature data.[24]

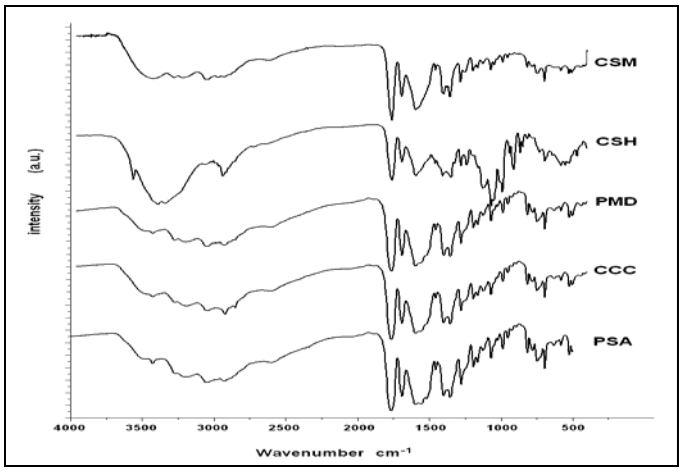

Fig. 3: Fourier transforms infrared spectra of PSA, CCC, PMD, CSH and CSM cephalexin samples

Table 1: Absorption frequencies observed for cephalexin monohydrate and cephalexin medicines

Absorption freq. cm^{-1}	Absorption mode	Absorption freq. cm^{-1}	Absorption mode
3425	ν_{O-H}	1689	$\nu_{C=O}$ (amide I) + ν_{C-N}
3190	ν_{N-H} (amide)	1593	ν_{COO} (asym)
2610	ν_{NH3}^{+}	1519	δ_{N-H} (amide II)
1766	$\nu_{C=O}$ (β-Lactam)	1396	ν_{COO} (sim)

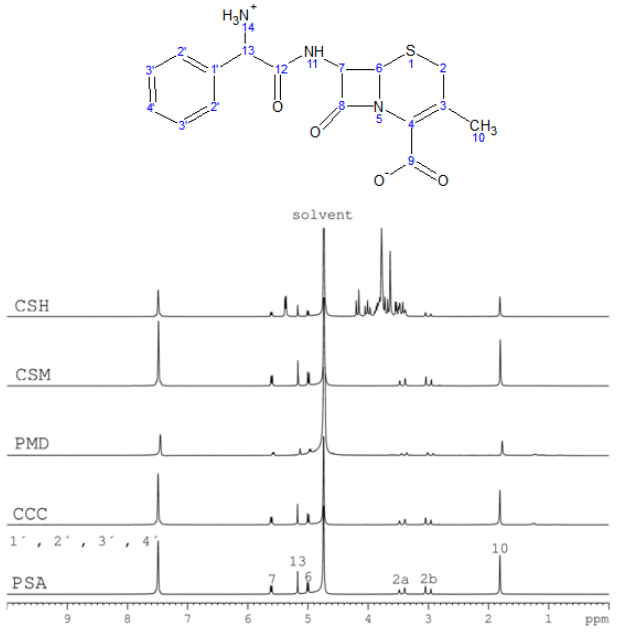

Fig. 4: ^{1}H NMR spectra (D_2O) obtained for PSA, CCC, PMD, CSM and CSH

Table 2: ^{1}H chemical shifts observed for cephalexin monohydrate and medicines

Hydrogen	δ(ppm)			
	PSA	CCC	CSM	CSH
2a	3.0(d)	3.0(d)	3.0(d)	3.0(d)
2b	3.4(d)	3.4(d)	3.4(d)	3.4(d)
6	5.0(d)	5.0(d)	5.0(d)	5.0(d)
7	5.6(d)	5.6(d)	5.6(d)	5.6(d)
10	1.8 (s)	1.8 (s)	1.8 (s)	1.8 (s)
13	5.17(s)	5.17(s)	5.17(s)	5.17(s)
1'	7.49(s)	7.49(s)	7.49(s)	7.49(s)
2'	7.49(s)	7.49(s)	7.49(s)	7.49(s)
3'	7.49(s)	7.49(s)	7.49(s)	7.49(s)
4'	7.49(s)	7.49(s)	7.49(s)	7.49(s)
O-CH-O*	-	-	-	5.37
CH_3/CH_2*	-	1.25	0.8; 1.25	-
CH_2O/CHO*	-	-	-	3.4to 4.2

*From excipients

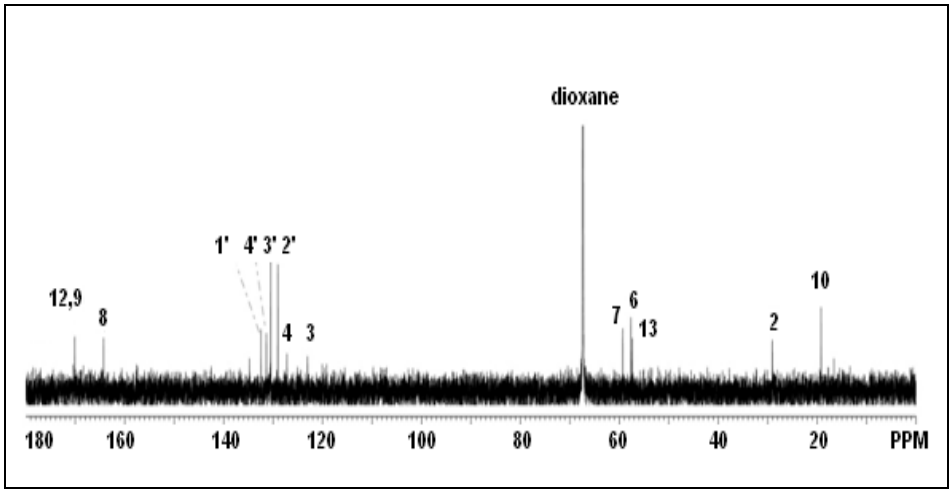

Fig. 5: Solution state (D_2O+dioxane) ^{13}C NMR spectrum of cephalexin monohydrate

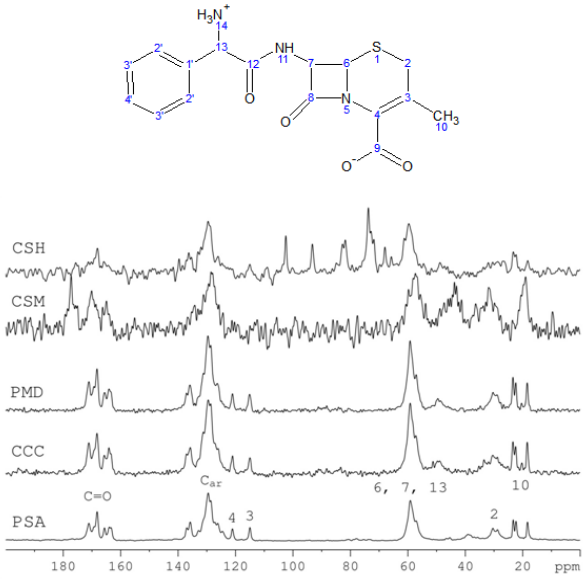

Fig. 6: Solid state ^{13}C CPMAS NMR spectra of cephalexin samples

Solid State ^{13}C NMR spectroscopy

The ^{13}C CPMAS spectrum obtained for reference sample (Figure 6, entry PSA) was assigned on the basis of liquid state one, following the same sequence of chemical shifts. Surprisingly some resonances were not consistent with the solution data, and show ^{13}C signals splitted, due the occupation of non-crystallographically equivalent positions.[25] That finding confirmed the existence of distinct cephalexin molecules in the unitary cell of cephalexin monohydrate, as suggested by Kennedy et al.[20] Some signals can be readily assigned, but there are some ambiguities, for example in the aromatic and carbonyl region, which are too complex for any assignments to be made with confidence. It was possible to see three split methyl carbons (δ 17.8, 21.8 and 22.8 ppm), indicating that in this sample at least three distinct cephalexin molecules are present in higher amounts, besides a fourth methyl carbon in low intensity at δ 19.9 ppm, detected both in reference sample and in CCC and PMD medicines, but absent in CSH sample (Figure 6).

CCC and PMD spectra are very similar to the reference sample, and the resonances at 136 and 137 ppm showed to be slightly more resolved in PSA than in CCC and PMD spectra. In contrast with the crystalline medicines, whose linewidths were typically 40 to 70 Hz, in the amorphous medicine CSM spectrum it was possible to detect a number of resonances, but the linewidths are of the order of hundreds Hz. This difference is attributed to the existence of a great number of molecular conformations and or intermolecular interactions in the amorphous material, which give rise to a spread of chemical shifts for each signal, as stated in the literature.[26,27,28] In contrast with the XRPD, ^{13}C MAS spectrum provides molecular structure information for amorphous medicine sample. The resonances corresponding to cephalexin in the CSH spectrum showed low intensity compared with those of the excipients, thus the commercial medicine used to prepare the suspensions possess greater amount of excipient, compared with the other medicines studied. In the other side, the signals in the range 16 to 23 ppm can be clearly seen, confirming the presence of cephalexin monohydrate. The chemical shifts and assignments proposed to cephalexin monohydrate in solution and in the solid state, and for the medicines CSH and CSM are given in Table 3. From the results it is concluded that in the studied medicines the cephalexin monohydrate is the polymorph present in higher amount.

Table 3: Solution and solid state ^{13}C NMR assignments of cephalexin monohydrate

Carbon	δ(ppm)			
	PSA		CSH	CSM
	Solution[a]	Solid		
2	29	28.4 / 29.8	28.3/29.7	31.9
3	123	114.4	115	absent
4	127.2	120.5	absent	absent
6	57.6	56.6 / 58.5	59.6/61.3	57.5
7	59.4	56.6 / 58.5	59.6/61.3	57.5
8	164.2	162.75/163.5/165.0	168.1	165.1
9	170.1	167.5 / 168.8 / 170.4 / 172.0	168.1	170.4
10	19.2	17.8 / 19.9 / 21.8 / 22.8	18.1/22.4/23.3	19.4
12	170.1	167.5 / 168.8 / 170.4 / 172.0	168.1	170.4
13	57.4	56.6 / 58.5	59.6/61.3	57.5
1'	132.4	123.9 - 136.37	129/136	128.6
2'	129	123.9 - 136.37	129/136	128.6
3'	130.5	123.9 - 136.37	129/136	128.6
4'	131.4	123.9 - 136.37	129/136	128.6
O-CH-O[b]	-	-	93.2/102.7	-
CH_3/CH_2[b]	-	-	-	43.4
CH_2-O/CHO[b]	-	-	65.5/68.3/72.5/73.3/73.9/81.7/82.8	-
C=O	-	-	-	177.8

[a]D_2O + Dioxane, [b]Excipients chemical shift

CONCLUSION

In conclusion by a combination of powder XRPD measurements, FTIR and solid state NMR we have identified the presence of cephalexin monohydrate and excipients in some of the medicines studied. Solid state NMR showed to be powerful tools in investigation of medicines, mostly in the cases was its amorphous nature precludes the evaluation by XRPD techniques.

ACKNOWLEDGEMENT

The authors thank S. Caldarelli (Université Paul Cèzanne, France) for helpful discussions. D.L.M. Aguiar thanks CNPq (Conselho Nacional de Pesquisas, Brazil) for scholarship.

REFERENCES

1. Chu KA, Yalkowsky SH. An interesting relationship between drug absorption and melting point. Int J Pharm 2009; 373(1-2):24-40
2. Wagh MP, Patel J. Biopharmaceutical classification system: Scientific basis for biowaiver extentions. Int J Pharm Pharm Sci 2010; 2(3): 12-19
3. Patel J, Aneja K, Tiwari R. A review on bioavailability and bioequivalence trials and its necessity. Int J Pharm Pharm Sci 2010; 2(3):1-8
4. Shinde AJ, Waghule AN, Paithane MJ, More HN. Formulation and in vitro evaluation of sustained release floating tablet of cephalexin using hydrophilic polymers. Int J Pharm Pharm Sci 2010; 2(2):58-65
5. Paluch KJ, Tajber L, McCabe T, O´Brien JE, Corrigan OI, Healy AM. Preparation and solid state characterization of chlorothiazide sodium intermolecular self-assembly suprastructure. Eur J Pharm Sci 2010; 41(5):603-611
6. Kobayashi Y, Ito S, Itai S, Yamamoto K. Physicochemical properties and bioavailability of carbamazepine polymorphs and dehydrate. Int J Pharm 2000; 193(2):137-146
7. Seifee M, Inamdar N, Dhamecha DL, Rathi AA. Drug Polymorphism: A Review. International Journal of Health Research 2009; 2(4):291-306
8. Yu L, Reutzel SM, Stephenson GA. Physical characterization of polymorphic drugs: an integrated characterization strategy. Pharmaceutical Science & Technology Today 1998; 1(3):118-127.

9. Vippagunta SR, Brittain HG, Grant DJW. Crystalline Solids. Advanced Drug Delivery Reviews 2001; 48(1):3-26
10. Bauer J, Spanton S, Henry R, Quick J, Dziki W, Porter W, Morris J. Ritonavir: An Extraordinary Example of Conformational Polymorphism. Pharm Res 2001; 18(6):859-866
11. Shete G, Puri V, Kumar L, Bansal AK. Solid state characterization of commercial crystalline and amorphous artovastatin calcium samples. AAPS Pharm Sci Tech 2011; 11(2):598-609
12. Német Z, Sajó I, Demeter A. Rietveld Refinement in routine quantitative analysis of famotidine polymorphs. Pharm Biom Anal 2010; 51(3):572-576
13. Nguyen TNP, KIM KJ. Transformation of monohydrate into anhydrous form of risedronate monosodium in methanol-water mixture. Ind Eng Chem 2010; 49(10):4842-4849
14. Tripathi R, Biradar SV, Mishra B, Paradkar AR. Study of polymorphs of progesterone by novel melt sonocrystallization technique: A technical note. AAPS Pharm Sci Tech 2010; 11(3):1493-1498
15. Brittain HG. Solid state fluorescence of Trihydrate phases of ampicillin and amoxicillin. AAPS Pharm Sci Tech 2005; 6(3):444-448
16. Christopher EA, Harris RK, Fletton RA.Assignment of solid-state ^{13}C resonances for polymorphs of cortisone acetate using shielding tensor components. Solid State NMR 1992; 1(2):93-101
17. Lima APCS, Gallini NR, Toledo MI, Lopes LC. Utilização de um sistema de gerenciamento de benefícios farmacêuticos (PBM) para a caracterização do perfil de prescrição e aquisição de antibióticos. Braz J Pharm Sci 2008; 44(2):215-223
18. Kumar I, Pratt RF. Transpeptidation Reactions of a Specific Substrate Catalyzed by the Streptomyces R61 DD-Peptidase: Characterization of a Chromogenic Substrate and Acyl Acceptor Design. Biochemistry 2005; 44(30):9971-9979
19. Cars O. Efficacy of Beta-lactam Antibiotics Integration of Pharmacokinetics and Pharmacodynamics. Diagn Microbiol Infect Dis 1997; 27(1-2):29-33
20. Kennedy AR, Okoth MO, Sheen DB, Sherwood JN, Teat SJ, Vrcelj RM. Cephalexin: a chanel hydrate. Acta Cryst 2003; C59(11):o650-o652
21. Stephenson GA, Grolean EG, Kleemann RR, Xu W, Rigsbu DR. Formation of isomorphic desolvates. Creating a molecular vacuum. J Pharm Sci 1998; 87(5):536-542
22. Otsuka M, Fukui Y, Otsuka K, Kim HJ, Ozaki Y. Determination of cephalexin crystallinity and investigation of formation of its amorphous solid by chemoinformetrical near infrared spectroscopy. J Near Infrared Spectrosc 2006; 14(9):9-16
23. Di Stefano R, Scopelliti M, Pellerito C, Casella G, Fiore T, Stocco GC, Vitturi R, Colomba M, Ronconi L, Sciacca ID, Pellerito L. Organometallic complexes with biological molecules. XVIII. Alkyltin (IV) cephalexinate complexes: synthesis, solid state and solution phase investigations. J Inorg Biochem 2004; 98(3):534-46
24. Tori K, Nishikawa J, Takeuchi Y. ^{13}C spectra of cephalosporins. Signal assignments of free acids and esters. Tetrahedron Lett 1981; 22(29):2793-2796
25. San Gil RAS, Albuquerque MG, Alencastro RB, Pinto AC, Gomes FES, Dantas TNC, Maciel MAM. Solid state NMR and molecular modeling studies on Croton cajucara bent. J Mol Struct 2008; 885(1-3):82-88
26. VanderHart DL, Earl WL, Garroway AN. Resolution in 13C NMR of organic solids using high-power proton decoupling and magic-angle sample spinning. J Magn Reson 1981; 44(2):361-401
27. Harris RK. NMR studies of organic polymorphs & solvates. Analyst 2006; 131(3): 351-373
28. Mousavi M, Yu SSF, Tzou DLM. A ^{13}C solid-state NMR analysis of vitamin D compounds. Solid State NMR 2009; 36(1):24-31