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Preformulation Studies on Imexon

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ABSTRACT Imexon is an aziridine containing iminopyrrolidone that, through aziridine ring opening, is able to induce oxidative stress resulting in apoptosis. The main objective of this research was to conduct extensive preformulation studies on Imexon in order to understand the factors that affect its stability. The results obtained indicate that the stability of Imexon is dependant on pH, ionic strength, temperature, buffer species, and initial concentration. Degradation of Imexon follows apparent first-order degradation kinetics with the primary degradation product resulting from opening of the aziridine ring. In order to maximize stability, ionic strength, temperature, and initial concentration should be minimized, with an optimal range pH between 7.2 and 9.0. Experimentation with other aqueous solutions indicates that Imexon has increased stability in D5W as opposed to normal saline, while it undergoes rapid degradation in 6% H_2O_2. Imexon is not ionizable between pH 5.0 to 8.5 and has an aqueous solubility of approximately 25 mg/mL over this range. Solid-state characterization has concluded that Imexon is a crystalline solid that begins decomposition at 165°C, prior to melting.

KEYWORDS Imexon, Preformulation studies, General acid/base catalysis, pH stability, Solubility

INTRODUCTION

Imexon, Fig. 1, (4-imino-1, 3-diazabicyclo-[3,1,0]hexan-one) is an aziridine containing iminopyrrolidone that is of significance due to its selective growth inhibitory potency against multiple myeloma (Dvorakova et al., 2002). While the exact mechanism of action for Imexon is not fully elucidated, data has shown that the opening of the aziridine ring moiety on Imexon can confer the ability to bind to sulfhydryl groups of cysteine residues. This binding causes a depletion of cellular thiols followed by the induction of oxidative stress and subsequent apoptosis (Dvorakova et al., 2000; Dvorakova et al., 2001; Iyengar et al., 1999; Iyengar et al., 2004).

Imexon's physiochemical properties, calculated LogP of –1.35 (CLogP, 1999) and a relatively high water solubility of 25.12 mg/mL, are contrary to the current trend of new drugs possessing a high LogP and a low water solubility (Gribbon and Sewing, 2005). While the raw drug is very stable as a crystalline solid at room temperature, under aqueous conditions the drug undergoes rapid

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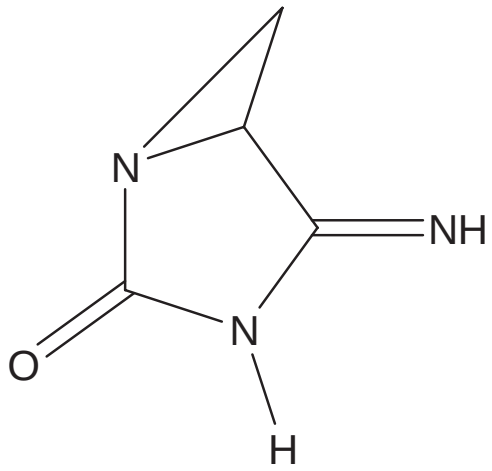

FIGURE 1 Structure of Imexon.

decomposition. Due to the fact that the solubility of Imexon is reasonable the main focus of preformulation experiments will be similar to those conducted by Ni et al. (2002), Zhu et al. (2002), and Anderson et al. (1987) and will focus on determining the formulation conditions under which the drug has sufficient solution stability. To this end, extensive preformulation studies have been conducted to determine the degradation of Imexon as a function of pH, ionic strength, temperature, buffer species, and initial drug concentration. Complimenting these data, solid state analysis and elucidation of a principle degradation product have been performed.

MATERIALS

Imexon was provided by AmpliMed Corp., Tucson, AZ, USA. Ammonium acetate ($C_2H_7NO_2$), monosodium phosphate (NaH_2PO_4), dipotassium phosphate (K_2HPO_4), sodium chloride (NaCl), boric acid (H_3BO_3), and hydrochloric acid (HCl) were obtained from Sigma Aldrich (St. Louis, MO, USA). Citric acid ($C_6H_8O_7$) was obtained from Spectrum Chemical (New Brunswick, NJ, USA). Sodium hydroxide (NaOH) was obtained from EM Science (Darmstadt, Germany). Hydrogen peroxide (H_2O_2), 30%, stock solution was obtained from Mallinckrodt (Hazelwood, MO, USA). Sterile water for Injection USP, 0.9% NaCl USP (normal saline), 0.45% NaCl USP (0.5 normal saline) and 5% dextrose USP for injection (D5W) were obtained from Baxter (Deerfield, IL, USA). HPLC grade acetonitrile (ACN) was obtained from EMD (Gibbstown, NJ, USA). A Millipore (Billerica, MA, USA) Milli-Q Ultrapure Water purification system with a 0.22 μm filter was utilized for water.

METHODS

High-Performance Liquid Chromatography Analysis

The HPLC system consisted of a Waters 2690 separation module (Waters, Milford, MA, USA) coupled with a Waters 996 Photodiode array (PDA) detector. Analysis was performed by a normal phase HPLC assay, using a 150 mm × 4.6 mm, Apollo Silica 5 μ column (Alltech Associates, Deerfield, IL), maintained at 30 ± 2°C. Ultraviolet detection was done at 234 nm. Mobile phase conditions were 90:10 (v/v) ACN:H_2O at a flow rate of 0.6 mL/min. Water was buffered with ammonium acetate at 0.1 M, with a pH of approximately 6.25. The injection volume was 5 μL. The parent compound had a retention time of 7.8 min.

Quantification was determined using peak area and calculated from a five-point standard curve prepared daily. Standards were prepared by volumetric dilution in purified water and stored at 4°C.

pH Measurements

All pH measurements were conducted on a Hanna Instruments Model HI 221 (Woonsocket, RI, USA) pH/mV meter with a model HI 1083 pH probe for aqueous samples. The pH of aqueous solutions was measured at the beginning, throughout the stability run, and upon completion of each stability trial. The instrument was calibrated each day with a two-point curve bracketing the expectant pH values.

Aqueous Stability

Effect of pH

The influence of pH on the stability of Imexon, at 0.12 mg/mL, was studied with a citrate buffer at pH 2.0 and 5.0, a phosphate buffer at pH 5.0, 7.2, and 9.0 and a borate buffer at pH 9.0 and 10.0. All buffers were made at a 0.1 M concentration and the pH was adjusted with NaOH or concentrated HCl. Ionic strength was adjusted to 0.2 M with NaCl. The influence of ionic strength was evaluated at pH 2.0 with a citrate buffer, pH 7.2 with a phosphate buffer, and pH

10.0 with a borate buffer by adjusting the ionic strength to 0.5 M with NaCl.

Effect of Temperature

Temperature influences on the degradation rates of Imexon were determined at four temperatures (4, 25, 37, and 48°C) at pH 5.0 with citrate buffer, pH 7.2 with phosphate buffer, and pH 9.0 with borate buffer. Analysis of the degradation rates of Imexon at different temperatures affords the assessment of activation energy.

Effect of Other Aqueous Solutions

In order to determine the susceptibility of Imexon to oxidation, experiments were performed with a 6% solution of hydrogen peroxide. In addition, degradation rates were evaluated in normal saline and D5W.

Effect of Initial Drug Concentration

The stability of Imexon as a function of concentration was conducted at 0.12, 1, 5, 10, and 18.5 mg/mL with phosphate buffers at pH 7.2 and 8.8. Phosphate buffers were prepared at 0.1 M and the ionic strength was adjusted to 0.2 M with NaCl. The effect of ionic strength was evaluated at 10 mg/mL by adjusting the ionic strength to 0.3 and 0.5 M with NaCl. Samples to determine the effect of drug concentration on Imexon stability were stored at 19°C.

In order to accurately determine the effect pH, temperature, other aqueous solutions, and initial drug concentration have on the degradation of Imexon, samples were prepared in duplicate and assayed for a minimum of six sample points, spanning three to six half-lives. In order to minimize the potential for self-catalysis (Waterman & Adami, 2005) samples were prepared at a dilute concentration of 0.12 mg/mL and stored at 25°C, unless otherwise stated. Sampling time varied due to large differences in degradation rates. The pH of each sample was measured at the beginning and completion of each stability trial.

Drug Degradation

Mass Spectroscopy

LC-MS analyses were performed with the Finnigan MAT TSQ 7000. The initial screening of Imexon was made in Q1 MS mode with the following parameters: scan type–full; polarity–positive; mass range: 50–500 m/z. Atmospheric pressure chemical ionization (APCI) source operating parameters: heated capillary temperature: 250°C; vaporizer temperature: 400°C; corona discharge current: 4.0 μA. The sample was introduced into the mass spectrometer via a Hewlett Packard 1050 HPLC system. Chromatographic separation was performed using the Apollo Silica column, previously described, with 12 min isocratic conditions of 80:20 (v/v) ACN:H_2O with a flow rate of 0.6 ml/min.

Individual fractions were collected utilizing the Waters HPLC system and method mentioned above with 100% isopropyl alcohol (IPA) at 0.6 mL/min. Fractions were analyzed in both Q1MS and MS/MS modes. The samples were reconstituted with methanol and introduced via flow shot injection.

As to Q1 MS, all the settings were the same as above. The instrument parameters for the MS/MS mode were as follows: scan type: full; polarity: positive; mass range: 10–150 m/z; set mass: 112.0; collision energy: 30V. APCI source settings remained the same as in Q1 mode.

Nuclear Magnetic Resonance

NMR samples were collected on a Bruker AM-250 NMR. Sample was dissolved in DMSO for collection of 64 scans in ^1H mode.

Solubility

The solubility of Imexon as a function of pH was conducted at seven pH values between 5.1 and 8.5 using phosphate buffer. Buffers were 0.1 M, and NaCl was utilized to adjust each to an ionic strength of 0.2 M. Each sample was saturated with Imexon raw drug substance and allowed to agitate for at least 30 min. Samples were visually inspected to ensure that solid drug was still in excess and then filtered through a 0.2 μm PTFE filter. The final pH of the buffered samples was then measured. The filtrate was then diluted appropriately with sterile water and assayed via the previously described HPLC assay. The solubility was also determined in 0.5 normal saline and sterile water for injection, at 12, 22, and 34°C. Prior to any solubility measurement studies were conducted to determine the appropriate agitation time.

Solid State Characterization

DSC/TGA

Thermal analysis was performed with a Q1000 Differential Scanning Calorimeter (DSC) (TA Instruments, New Castle, DE, USA) series with an auto sampler. Indium was used for the calibration of the DSC. Samples of 1 to 2 mg were weighed out and placed in an aluminum pan and crimped with an aluminum lid. Samples were equilibrated and isothermally heated at 30°C for 5 min, followed by heating at 5°C/min up to 300°C. A nitrogen purge was used at 40 mL/min for each sample.

Thermogravimetric analysis (TGA) was performed on a TA Instruments Q50 TGA. Samples of 1 to 2 mg were placed in an empty aluminum pan and heated at 5°C/min up to 300°C. Weight loss as a function of temperature was analyzed under nitrogen at 60 mL/minute purge.

X-ray Powder Diffraction

X-ray powder diffraction (XRD) was conducted with a PANalytical X'Pert PRO MPD (Panalytical Inc., Tempe, AZ, USA) system with copper (Kα) radiation (λ = 1.54 Å) at 50 KV (40 mA target current). High-resolution scans were conducted along the goniometer axis (θ/2θ) at a step size of ca. 0.0167°. A fixed divergent slit of 1/4° was used in the incident beam optics, followed by an anti-scattering slit of 1/2°. X'Celerator, a RTMS (real-time multiple strip) detector, was used in the diffracted beam optics. Approximately 4000 scans were taken between 2-Theta of 5.00 and 74.99°. Samples were placed on a silica zero background holder and rotated at 0.25 rps. Data was collected with X'Pert Data Collector version 2.0 and analyzed with X'Pert Pro version 1.4 software.

RESULTS AND DISCUSSION

Chromatography

The isocratic conditions, described in the Materials and Methods section, were maintained for 25 min in order to achieve separation of Imexon from one degradation peak and a second unknown peak. All peaks were well resolved and were of gaussian shape. Figure 2 shows a chromatograph of parent Imexon, 0.12 mg/mL, overlaid with a sample obtained at pH 2.0 after 10.0 min. The degradation peak elutes at ~6.8 min (Degradation Product A) and another peak at ~20.2 min. As seen in Fig. 2 the peak at ~20.2 min is present both in the standard and in the degraded sample.

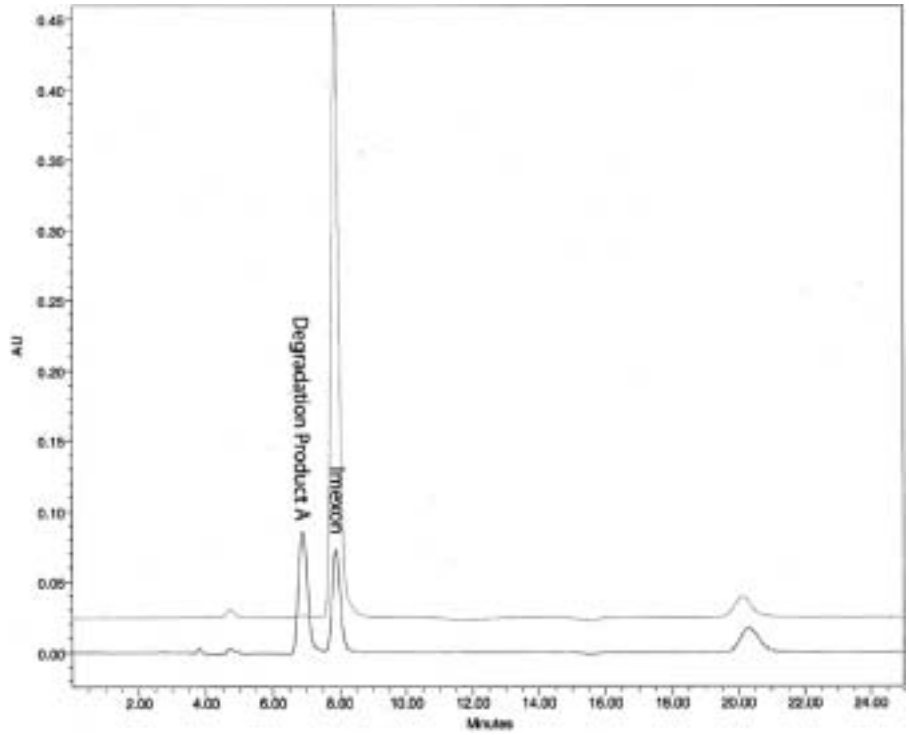

FIGURE 2 **Chromatographic Overlay of Parent Imexon with a Degraded Sample.**

Effect of pH

It is known that the pH of a solution can affect the degradation rate of many drugs. This change in degradation rate can be attributed to the catalytic effect of either the hydrogen ions or hydroxide ions in solution. The log percent of Imexon remaining versus time, for several pH values, is shown in Fig. 3. These results suggest that Imexon undergoes apparent first-order degradation (Carstensen and Rhodes, 2000). Degradation rates (k) were calculated from the slope of the linear best-fit line for the relationship between the logarithm percent drug remaining versus time. The rate constant was then used to calculate half-life (T_{50}), and usage life (T_{95}). These data are summarized in Table 1; note these values are all at 0.2 M ionic strength.

Preliminary studies on the degradation of Imexon indicated an increase in pH as the samples degraded. As a result, the buffer species described in the Material and Methods section was employed for stability samples. These buffers were found to hold pH constant throughout degradation studies. Figure 4 shows the pH rate profile for Imexon at 25°C and 0.12 mg/mL. The slope of this line deviates from −1 in the acid range and 1 in the basic range, indicating general acid/base catalysis. Imexon stability is pH dependant with an apparent maximal stability between pH 7.2 and 9.0.

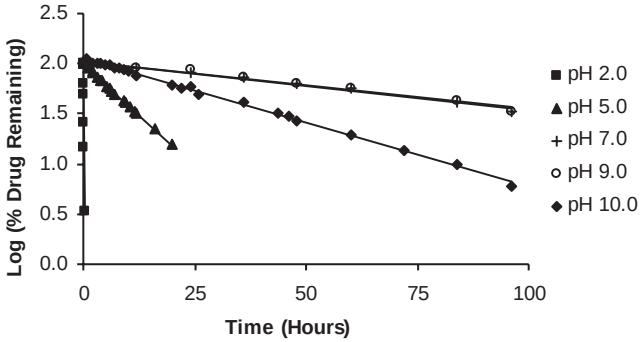

FIGURE 3 **Average (n = 2) Log % Drug Remaining vs. Time for Data Presented in pH Rate Profile. Citric Acid Buffer Represented for pH 5.0 and Borate Buffer Represented for pH 9.0.**

Effect of Buffer Species

The effect of buffer species was determined at pH 5.0 and 9.0 (0.2 M ionic strength). Imexon was found to undergo apparent first order kinetics for each of the buffer species evaluated. Table 1 shows the T_{50} values at pH 5.0 (citrate and phosphate buffers) and at pH 9.0 (phosphate and borate buffers). At pH 5.0 the T_{50} for the citrate buffer is about half of the value of the T_{50} for phosphate buffer, indicating that at lower pH, Imexon is more stable in a phosphate buffer. When buffered at pH 9.0, Imexon's T_{50} in the phosphate buffer is ~25% lower than that of the borate buffer. The differences in degradation rates for different buffers systems, at the same pH, further supports general acid/base catalysis.

Effect of Ionic Strength

The effect that ionic strength has on the degradation of Imexon was determined at three different pH values (2.0, 7.2, and 10.0) by adjusting the ionic strength of the buffers from 0.2 M to 0.5 M. Table 2 shows that the degradation of Imexon is accelerated by an increase in the ionic strength, at all three pH values. While the degree of acceleration is greater at low pH values, the trend is apparent for each of the pH values studied.

Effect of Temperature

Degradation rate constants are a function of temperature related through the Arrhenius equation:

$$k = Ae^{\frac{-Ea}{RT}} \quad (1)$$

TABLE 1 **Rate Constant (K), Standard Deviation (SD), T_{50} and T_{95} for Imexon at 25°C and 0.12 mg/mL**

pH	*k* (per hour)	SD	T_{50} (Hours)	T_{95} (Hours)
2.0 (Citrate)	10.381	2.9058	0.067	0.005
5.0 (Citrate)	0.095	0.0016	7.33	0.55
5.0 (Phosphate)	0.048	0.0017	14.41	1.07
7.2 (Phosphate)	0.010	0.0017	68.67	5.10
9.0 (Phosphate)	0.015	0.0002	47.15	3.50
9.0 (Borate)	0.011	0.0001	62.10	4.61
10.0 (Borate)	0.029	0.0007	23.70	1.76

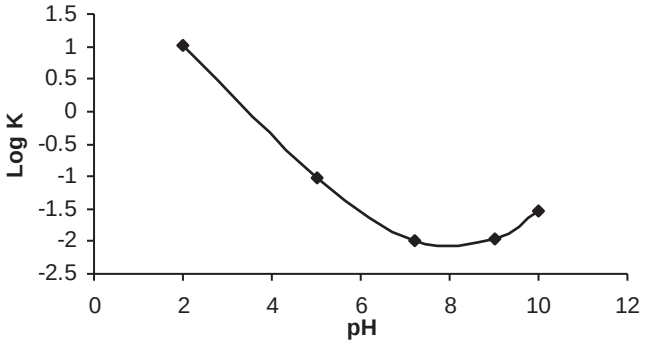

FIGURE 4 pH-Rate (log k vs. pH) Profile for Imexon at 25°C.

TABLE 2 Effect of Ionic Strength on the Degradation Rate of Imexon at 25°C for Three pH Values, 0.12 mg/mL Initial Concentration

pH 2.0			
Ionic Strength	*k* (per hour)	T_{50} (Hours)	T_{95} (Hours)
0.2	10.38	0.067	0.005
0.5	19.63	0.035	0.003
pH 7.2			
Ionic Strength	*k* (per hour)	T_{50} (Hours)	T_{95} (Hours)
0.2	0.010	68.7	5.1
0.5	0.017	41.9	3.1
pH 10.0			
Ionic Strength	*k* (per hour)	T_{50} (Hours)	T_{95} (Hours)
0.2	0.029	23.7	1.7
0.5	0.038	18.0	1.3

where k is the degradation rate constant, A is the frequency factor, E_a is the activation energy, R is the gas constant, and T is the temperature in Kelvin (Martin et al., 1983). The natural logarithms of the degradation rates were plotted as a function of the inverse of temperature for each of the three pH values. From this three linear plots are seen, as shown in Fig. 5, indicating that the degradation of Imexon increases as the temperature increase. The activation energies were calculated from the slopes for the degradation of Imexon at pH 5.0, 7.2 and 9.0 with a citrate buffer, phosphate buffer, and a borate buffer, respectively, and presented in Table 3.

In aqueous solutions of pH 7.2 and 9.0 the activation energy for the degradation of Imexon is 83.95 kJ/mol and 84.60 kJ/mol, respectively, while the activation energy at pH 5.0 is 59.87 kJ/mol. The equivalent activation energies for pH 7.2 and 9.0 are in alignment

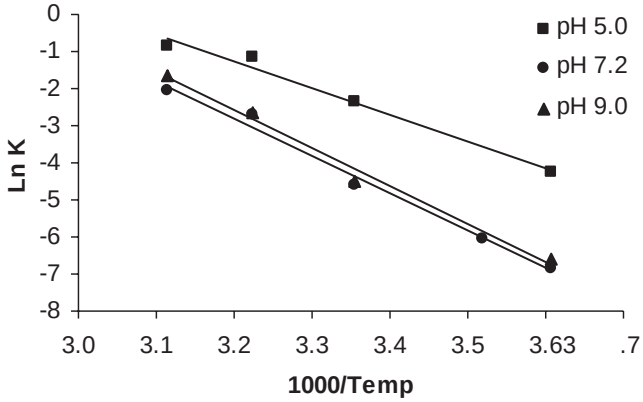

FIGURE 5 Arrhenius Plot (pH 5.0 citrate, pH 7.2 Phosphate and pH 9.0 Borate) Showing the Dependence of Degradation Rate at Four Temperatures for Three Different pHs.

TABLE 3 Activation Energies for Three Different pHs

Activation energy		
pH	Slope	Ea (kJ/mol)
5.0	–7.20	59.87
7.2	–10.10	83.95
9.0	–10.18	84.60

with the stability data in Fig. 4 and indicate the region in which Imexon is most stable. The decrease in activation energy at pH 5.0 can be attributed to hydrogen ion catalysis of Imexon as well as the citrate buffer system utilized for the study. From Fig. 4, pH clearly has an affect on degradation. In addition, buffer species have been shown to have some importance on degradation (Table 1), with Imexon degrading faster in citrate buffer as compared to phosphate buffer.

Effect of Other Aqueous Solutions

Analysis of Imexon degradation in 6% H_2O_2, normal saline, and D5W showed degradation to be apparent first-order degradation. Table 4 shows the effect of three aqueous solvents (6% H_2O_2, normal saline, and D5W) on the stability of Imexon at room temperature. These data allude to the effect of ionic strength on the degradation rate of Imexon. Normal saline, with an ionic strength of 0.154 M, has a T_{95} of 6.46 h while D5W, with an ionic strength of 0.0 M, has a T_{95} of 21.35 h. Since both solutions are at or near physiological pH and were kept at room temperature, the ionic strength is the main difference between the two solutions. Based on the effect

TABLE 4 Rate Constant (K), Standard Deviation (SD), T_{50} and T_{95} for Imexon at 25°C and 0.12 mg/mL in H_2O_2, Normal Saline and D_5W

	k (per hour)	SD	T_{50} (Hours)	T_{95} (Hours)
H_2O_2	0.252	0.0514	2.753	0.203
Normal saline	0.008	0.0018	87.86	6.466
D_5W	0.002	0.0007	290.1	21.35

changes in ionic strength had on the buffered samples it is hypothesized that it is also the cause of the increase in degradation rate in normal saline. H_2O_2 at 6% (pH 5.2) showed an increased degradation rate when compared to pH 5.0. This is indicative of the fact that Imexon is susceptible to oxidation catalyzed by peroxide.

Effect of Initial Drug Concentration

In order to determine the effect initial concentration has on the degradation rate of Imexon, samples were evaluated at 0.12, 1, 5, 10, and 18.5 mg/mL, for both pH 7.2 and 8.8. These data are shown in Fig. 6. Interestingly, all samples undergo apparent first-order degradation for each pH; however, the rates of the different initial concentrations are different from one another (p value <0.05, Stata 7.0 [Stata Corporation, College Station, TX, 2001]). A similar situation was observed by Fubara and Notari (1998) and alludes to the possibility that Imexon (or a moiety on the molecule) acts as a catalyst in the degradation pathway. No pH changes were noted for any of the samples, regardless of initial concentration or ionic strength. In addition, the data presented in Fig. 6 confirm the pH stability study, in which the stability of Imexon is the similar for pH 7.2 and 8.8.

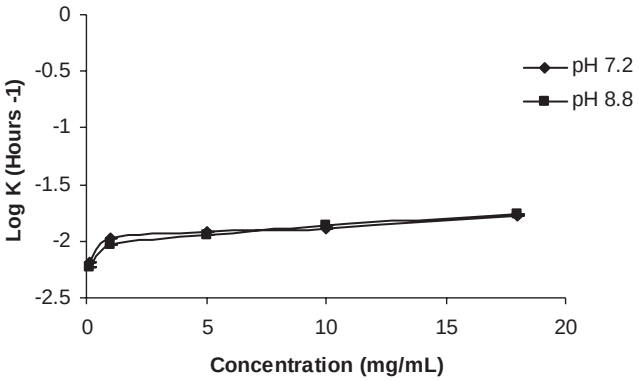

FIGURE 6 Concentration Rate Profile of Imexon, Indicating a Decrease in Stability of Imexon as Concentration Increases, Conducted at 19°C.

TABLE 5 Degradation Rate, T_{50} and T_{95} for pH 7.2 at Varied Ionic Strength

	10 mg/mL		
Ionic strength	*k* (per hour)	T_{50} (Hours)	T_{95} (Hours)
0.2 M	0.0130	53.49	3.98
0.3 M	0.0153	45.42	3.38
0.5 M	0.0206	33.72	2.51

The effect of ionic strength was concurrently studied at 10 mg/mL (pH 7.2) by adjusting buffer concentration to 0.3 M and 0.5 M, data shown in Table 5. As with the 0.12 mg/mL studies (Table 2), the degradation rate increased as the ionic strength of the buffer solution increased. Further analysis of the effect of ionic strength displayed that increasing ionic strength from 0.2 M to 0.5 M results in a ~37% decrease T_{50}, independent of initial concentration.

Degradation Products

Mass Spectrometry

Mass spectrometry was used to determine the m/z of Imexon and the degradation products, actual m/z are m+1 since positive ion spray was used to ionize samples. Under conditions described in the Material and Methods sections, an Imexon standard at 0.12 mg/mL was introduced to the mass spectrometer via flow shot and analyzed. The Imexon standard showed a clean mass spectrum with an m/z of 111.8 for the parent. Throughout MS analysis it was discovered that Imexon interacts with several of the solvents used in either the HPLC conditions or in sample preparation. Interactions with ACN, IPA, and MeOH were observed. The ACN, IPA, and MeOH showed m/z peaks of 152.8, 171.7, and ~142, respectively. When the same standard was analyzed in MS/MS mode, described in the Materials and Methods section, peaks were seen with m/z values of 42, 69, 84, and 95. The explanation for the fragmentation is as follows: m/z of 95 is the loss of the oxygen, m/z of 42, and m/z of 69 are complementing fragments resulting from fragmentation of two nitrogens, one carbon, and two hydrogens, and the m/z of 84 is the result of the loss of the carbonyl group.

Analysis of the potential degradation products was conducted in both MS mode and MS/MS mode. Initial characterization attempts were conducted

through LC/MS analysis of the degraded samples and analysis only of the retention time around the degradation peak. Analysis of Degradation Peak A showed two meaningful m/z peaks, 111.8 and 152.8. The m/z of 111.8 is an isomer of the parent while the 152.8 is the isomer forming the same complex with ACN that is seen with the parent. Analysis of the peak at 20.2 min yielded no meaningful m/z peaks. MS/MS analysis of the same degraded sample, injected via the HPLC system, displayed the same fragmentation ions seen in the flow shot injection of the standard for the Degradation Peak A and again no meaningful peaks for the peak at 20.2 min were observed.

In order to ensure accurate sampling of each component, a degraded sample of Imexon was separated using the Waters HPLC with 100% IPA as a mobile phase. While a mobile phase of 100% IPA provided better resolution of the degradation product from the parent peak, symmetry was substantially decreased. Five injections were collected as separate fractions and the IPA was evaporated. Fractions were reconstituted with methanol and injected via flow shot for MS/MS analysis. Degradation product A and the parent drug gave the same mass as seen with the standard. Analysis of the peak at 20.2 min showed the similar mass fragments; however, its signal was over three times lower than that of the other products and therefore was not meaningful.

Based on these data, the structure of degradation product A is proposed to be a constitutional isomer of the parent drug. It is hypothesized to have the structure shown in Fig. 7. Mechanism of degradation coincides with that proposed by Den Brok et al. (2005a and 2005b). Concurrent with the proposed structure is the nature of the peak seen via chromatography, assuming that the only interaction taking place on the column is the hydrophilic interaction of the molecule to the column then a molecule with a more nonpolar LogP would elute before a molecule with a more polar LogP. Noting that the proposed degradation product has a LogP of –1.21 (6) and Imexon's LogP of –1.35 it would be expected that the proposed degradation product would elute before Imexon (Fig. 2).

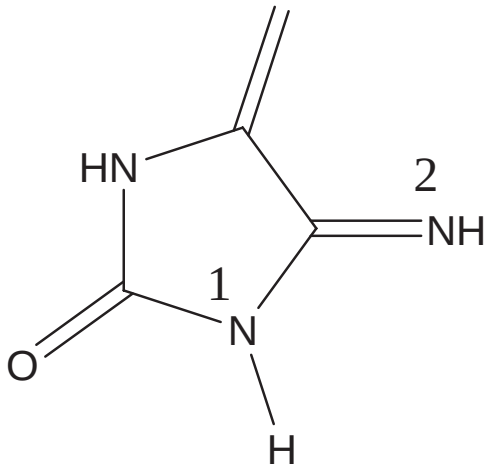

FIGURE 7 **Proposed Degradation Product A for Imexon.**

Nuclear Magnetic Resonance

Fractions of Degradation Product A were collected via HPLC separation with 100% IPA as the mobile phase. The organic solvent was evaporated off and the sample was reconstituted with DMSO. Resultant ^{1}H NMR data showed two doublets at 5.2 and 5.8 ppm. Theses peaks are consistent with the alkene shown in Fig. 7.

In addition to the previous results, visual inspection of the high-concentration stability samples (pH 7.2 and 8.8 at 10 and 18.5 mg/mL) revealed the formation of a precipitate as Imexon degraded. The precipitate was found to be more soluble in both high pH (~11.0) and low pH (~1.5), suggesting that the degradation product is a zwitter ion. Structural analysis of the proposed Degradation Product A (Fig. 7) suggests two potential ionizable functional groups; the nitrogen at position "1" being acidic and the nitrogen at position "2" being basic. However, due to the rapid degradation of Degradation Product A, confirmation of these pKa's via potentiometric titration was not possible. This observation further supports the structure of the Degradation Product A.

Solubility

The solubility of Imexon, as a function of pH, was investigated to explore the hypothesized presence of a basic pKa at or near pH 7.5. The solubility of a basic compound will increase as the pH of the solution decreases as described by the following equation:

$$S_{tot} = S_A\left(1+10^{(pKa-pH)}\right) \qquad (2)$$

where S_{tot} is the total solubility and S_A is the solubility of the un-ionized form (Yalkowsky, 1999). According to Eq. 2 the solubility of an ionizable compound one

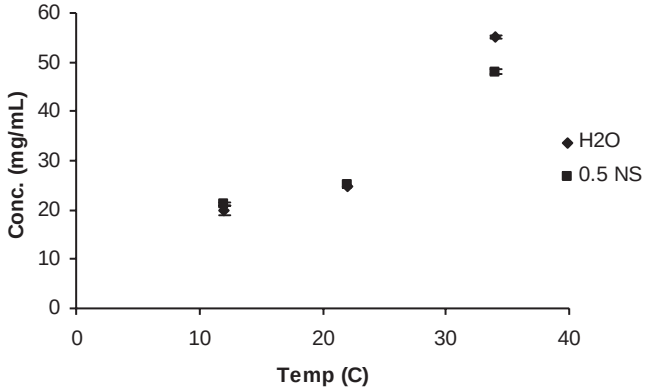

FIGURE 8 **Solubility of Imexon as a Function of Temperature in Both Sterile H_2O for Injection and 0.5 Normal Saline.**

pH unit lower than the basic pKa should result in a 10-fold increase in solubility.

Since it has been shown that Imexon can undergo rapid degradation as a function of pH, the required equilibration time needed for solubility determinations was balanced with degradation considerations. Fortunately, studies revealed that solid Imexon dissolves rapidly and that the solubility does not change appreciably after 30 min of agitation (data not shown). As a result, solubilities were able to be determined for all of the desired conditions utilizing this relatively short sampling time. From these studies it was found that the solubility of Imexon is constant for pH's 5.1, 6.5, 6.85, 7.54, 8.05, and 8.51 with an average solubility of 25.12 mg/mL (S.D. = 0.71). Due to the fact that the solubility of Imexon does not change in this pH range it is concluded that there is not a pKa in this pH range. The solubility of Imexon was also determined in 0.5 normal saline and sterile water for injection at three different temperatures (12, 22, and 34°C). As seen in Fig. 8, the solubility of Imexon increases as a function of temperature.

Solid-State Characterization

Analysis of pure Imexon via DSC showed an exothermal event initiating near 165°C and reaching a maximum at 200°C (Fig. 9). Integration of this peak gives an enthalpy of this event to be 920 J/g. Figure 9 also includes the TGA analysis of the Imexon raw drug substance. It can be seen that the onset of weight loss is at or near the same temperature of the thermal event seen on DSC (165°C). In order to further investigate this thermal event, Imexon was heated and isothermally held at 155°C for 15 min. No weight loss was detected, however, changes in the physical appearance of the material were observed. Upon cooling attempts were made to solubilize the material with a variety of solvents, including water, ethanol, acetonitrile, and tetrahydrofuran. The solubility properties of the isothermally held material were different than those of Imexon parent and subsequent HPLC analysis confirmed that the parent Imexon had been decomposed.

Analysis of pure Imexon drug substance via DSC displays an absence of an endothermic, indicating a melt, suggested the drug may not be crystalline. However, examination of Imexon crystallites, under magnification with cross polarized light, showed birefringence. Analysis utilizing X-ray Powder Diffraction confirmed that the Imexon does exist in a crystalline solid (Fig. 10). Upon determination that Imexon raw drug substance is crystalline analysis of DSC/TGA data indicates that Imexon decomposes prior to melting.

CONCLUSIONS

Imexon has been shown to undergo apparent first-order degradation via general acid/base catalysis in aqueous conditions independent of all variables studied. The stability of Imexon is dependant upon pH, ionic strength, temperature, and initial concentration. Maximal stability of Imexon would be achieved in aqueous conditions at a pH between 7.2 and 9.0 with low ionic strength, temperature, and initial concentration. Further analysis has shown stability to be increased in D5W, as compared to normal saline, presumably due to the lower ionic strength. The solubility of Imexon has been determined in 0.5 normal saline, sterile water, and as a function of pH. Aqueous solubility has been shown not to change with pH in the range of 5.1 to 8.5. Analyses have been conducted and a constitutional isomer of Imexon has been proposed as the primary degradation product. Solid-state characterization has shown Imexon to be a crystalline solid.

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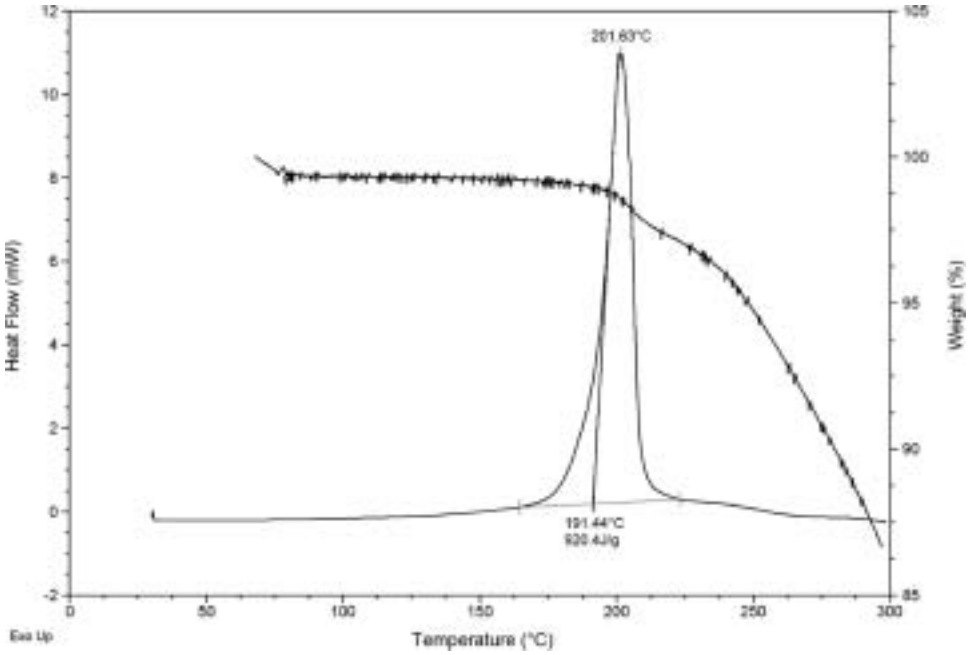

FIGURE 9 **DSC (Heat Flow) and TGA (Weight %) Overlay of Imexon Raw Drug Substance.**

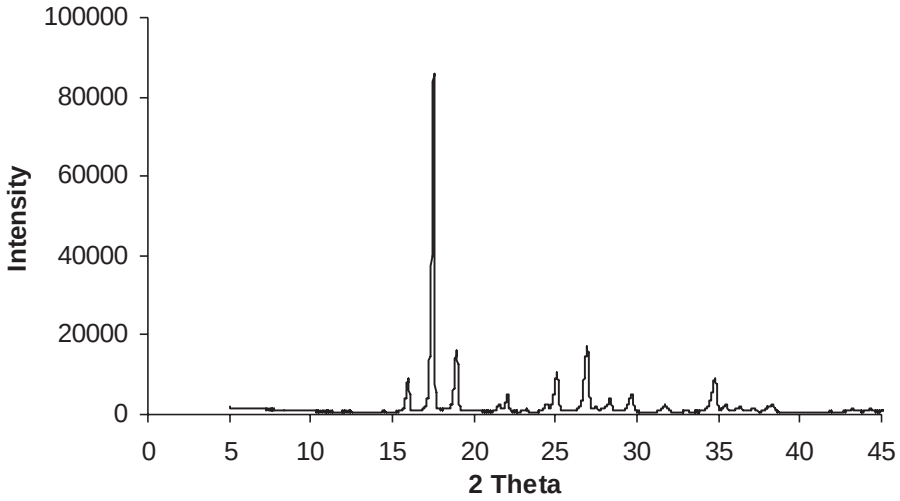

FIGURE 10 **XRD Analysis of Imexon Raw Drug Substance.**

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REFERENCES

Anderson, B. D., Wygant, M. B., Xiang, T., Waugh, W. A., & Stella. V. J. (1988) Preformulation solubility and kinetic studies of 2′,3′-dideoxypurine nucleosides: potential anti-AIDS agents. *Int. J. Pharm., 45*, 27–37.

Carstensen, J. T., & Rhodes, C. T. (2000) Solution Kinetics. *Drug Stability*, 3rd Ed. New York: Marcel Dekker Inc., 21–27.

ClogP for Windows, Version 4.0, 1995–1999 Biobyte Corp.

Den Brok, M. W. J., Nuijen, B., Hillebrand, M. J. X., Lutz, C., Opitz, H., & Beijnen, J. H. (2005a) LC-UV method development and validation for the investigational anticancer agent imexon and identification of its degradation products. *J. Pharm. Biomed., 38*, 686–694.

Den Brok, M. W. J., Nuijen, B., Lutz, C., Opitz, H., & Beijnen, J. H. (2005b) Pharmaceutical development of a lyophilized dosage form for the investigational anticancer agent imexon using dimethyl sulfoxide as a solubilizing and stabilizing agent. *J. Pharm. Sci., 94* (5), 1101–1114.

Dvorakova, K., Payne, C. M., Tome, M. E., Briehl, M. M., McClure, T., & Dorr, R. T. (2000) Induction of oxidative stress and apoptosis in myeloma cells by the aziridine-containing agent Imexon. *Biochem. Pharmacol., 60*, 749–758.

Dvorakova, K., Payne, C. M., Tome, M. E., Briehl, M. M., Vasquez, M. A., Waltmire, C. N., Coon, A., & Dorr, R. T. (2002) Molecular and cellular characterization of Imexon-resistant RPMI8226/I Myeloma Cells. *Mole. Cancer Ther., 1*, 185–195.

Dvorakova, K., Waltmire, C. N., Payne, C. M., Tome, M. E., Briehl, M. M., & Dorr, R. T. (2001) Induction of mitochondrial changers in myeloma cells by Imexon. *Neoplasia, 97* (11), 3544–3551.

Fubara, J. O., & Notari, R. E. (1998) A Kinetic Oxymoron: Concentration-Dependent First-order rate constants for hydrolysis of Ceftazidime. *J. Pharm. Sci., 87*(1), 53–58.

Gribbon, P., & Sewing, A. (2005) High-throughput drug discovery: what can we expect from HTS? *Drug Discovery Today, 10* (1), 17–22

Iyengar, B. S., Dorr, R. T., Alberts, D. S., Hersh, E. M., Salmon, S. E., & Remers, W. A. (1999) Novel antitumor 2-Cyanoaziridine-1-carboxamides. *J. Med. Chem., 42*, 510–514.

Iyengar, B. S., Dorr, R. T., & Remers, W. A. (2004) Chemical basis for the biological activity of Imexon and related Cyanaziridines. *J. Med. Chem., 47*, 218–223.

Martin, A., Swarbrick, J., & Cammarata, A. (1983) Solubility and distribution phenomena. *Physical Pharmacy*, 3rd Ed.; Philadelphia: Lea & Febiger.

Ni, N., Sanghvi, T., & Yalkowsky, S. H. (2002) Stabilization and preformulation of anticancer drug-SarCNU. *Int. J. Pharm., 249*, 257–264.

Waterman, K. C., Adami, R. C. (2005) Accelerated aging: prediction of chemical stability of pharmaceuticals. *Int. J. Pharm., 293*, 101–125.

Yalkowsky, S. H. (1999) Solubillization by pH control. In *Solubility and Solubilization in Aqueous Media*. New York: Oxford University Press.

Zhu, H., Meserve, K., & Floyd, E. (2002) Preforumlation Studies for an ultrashort-acting neuromuscular blocking agent GW280430A. I. Buffer and cosolvent effects on the solution stability. *Drug Dev. Int. Pharm., 28*(2), 135–142.