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Brief/Technical Note

Stability Studies of Microparticulate System with Piroxicam as Model Drug

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INTRODUCTION

Non-steroidal anti-inflammatory agent, piroxicam (PIR), is particularly employed in treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, pain in musculoskeletal disorders, and acute gout (1). This drug has also been in clinical practice as an analgesic in dental, postoperative and postpartum pain (2). According to the biopharmaceutical classification system, PIR is included in class 2, having characteristics of low solubility and high permeability characteristics. The pharmacokinetic data reveal that this molecule undergoes slow and gradual absorption pattern when given orally. This leads to a delayed onset of anti-inflammatory and analgesic effect.

Poly(lactide-*co*-glycolide) (PLGA) has been the polymer of choice for injectable microparticulate systems due to its biocompatible nature (3). Release behavior from PLGA polymers are either by diffusion, erosion, or a combination of both (4). The dissolution kinetics can be affected by a number of significant factors like polymer molecular weight, copolymer ratio and crystallinity (5–8), properties related to the molecule (9–11), dissolution conditions employed in the study (12,13), and formulation factors like preparative conditions, particle size, and encapsulation efficiency (14–18). Depending upon the type of PLGA, the drug release profiles from PLGA microspheres can range from days to months. Hence, accelerated release testing of such systems especially for *in vitro* drug release kinetics becomes imperative. However, it should be noted that in ideal situation, the drug release from accelerated test and "real-time" study should follow the same release mechanism and a 1:1 correlation is expected. Exaggerated conditions like temperature, pH of media, etc. are generally employed in the test to achieve rapid release. It is possible that the release mechanism may alter due to these exaggerated conditions. Literature study shows that an increase in drug release from PLGA has been achieved either by increase in temperature (due to increased mobility of polymer and hence drug diffusion), by acid or alkali catalyzed hydrolysis, by addition of a surfactant that would enhance the dissolution, by addition of a cosolvent, or by radiation (19–24). Degradation of polylactic acid and PLGA polymers of different copolymer ratios and molecular weights at 37°C and 60°C with change in mass loss, molecular weight, and formation of respective acid components has been reported (25).

Stability of a pharmaceutical dosage form is very significant as it reflects the quality of the dosage form during its shelf life. The stability studies give proof on how the quality of a drug substance or product varies with time under the influence of environmental factors like temperature, humidity, and light. Regulatory bodies lay emphasis on performing stability studies and is a part of the product submission requirements. International conference on harmonization (ICH) has laid guidelines for stability studies. The ICH Q1A(R2) is for stability testing of new drug substances and products. ICH Q3A, Q3B, and Q3C are based on the impurities in drug substance, drug product, and residual solvents, respectively. The specifications, test procedures, and acceptance criteria for new drug substances and products are dealt in ICH Q6A. The stability studies should be an integral part of the development cycle for new dosage forms.

Often it is observed that the stability aspect is often neglected during the development of a new pharmaceutical product. To get more insight on how the product fairs during shelf life, we performed stability studies on the final system. Extensive literature search indicates that not much attention has been given to evaluate the quality of microsphere based products on storage for prolonged time period along with real-time release profiling for long-term release products. In the above context, a strong need was felt to conduct real-time stability studies for the developed PIR–PLGA microparticulate formulation. A sensitive stability indicating high-performance thin-layer chromatographic (HPTLC) method developed and validated in our lab was employed for estimation of drug. An attempt was made in the present investigation to focus on the stability aspects of PIR–PLGA microsphere system.

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MATERIALS AND METHODS

Materials

PIR was a gift sample from IPCA Laboratories Ltd. (Mumbai, India). Methyl cellulose (Methocel-400) was obtained from Colorcon Asia Pvt. Ltd. (Mumbai, India). Polyvinyl alcohol (partially hydrolyzed 88%), sodium azide, thiomerosal, potassium bromide, and potassium dihydrogen orthophosphate were purchased from s.d. Fine Chemicals (Mumbai, India). PLGA was gifted by Purac Biochem (Holland). Fluid thyoglycollate medium was purchased from Hi Media Pvt. Ltd. (Mumbai, India). Sodium dihydrogen phosphate (anhydrous), disodium hydrogen phosphate (anhydrous), polysorbate 80, and sodium chloride were purchased from Sigma Chemicals (USA). *Bacillus subtilis* ATCC No. 6633 (NCIM 2063) and *Candida albicans* ATCC No. 10231 (NCIM 3471) were obtained from National Chemical Laboratory (Pune, India). *Bacteroides vulgatus* ATCC No. 8482 (MTCC 1350) was procured from Institute of Microbial Technology (India). All other reagents used were of analytical grade and were purchased from Ranbaxy Chemicals (Mumbai, India).

Preparation of PIR–PLGA Microparticles

PIR loaded microparticles were formed by the modified in-water interrupted solvent evaporation technique (26). Briefly, polymeric emulsifiers, namely methyl cellulose (Methocel-400, 0.05% *w*/*w*) and polyvinyl alcohol (partially hydrolyzed 88%, 0.27% *w*/*w*), were dissolved in 200 mL purified water in a beaker. Poly(lactide/glycolide) 53/47 (intrinsic viscosity in chloroform at 25°C is 0.55 dl/g, 200 mg) and PIR (40 mg) were dissolved in 4 mL of dichloromethane and subsequently incorporated into the aqueous continuum. The emulsion thus formed was stirred at 23–24°C with a magnetic stirrer (Remi Motors, Mumbai, India). The embryonic microparticles were then decanted from the system and resuspended in stabilizer-free water (the suspension being stirred intermittently) wherein the methylene chloride evaporation proceeded to completion. The drug loaded microspheres were harvested, washed with water, and subjected to lyophilization at −0.004 mbar pressure and −40°C temperature using laboratory freeze dryer (Labconco Corporation, England). The PIR–PLGA system was subjected to gamma radiation sterilization at 2.5 Mrad dose (dose rate of 0.13 Mrad/h). This study was performed in Gamma Chamber GC-900 (Bhabha Atomic Research Centre, Mumbai, India).

Stability Studies of PIR–PLGA System

The PIR–PLGA microparticulate system sterilized by gamma radiation was subjected to stability studies. The stability protocol was designed as per ICH guidelines (27) with certain modification. For the long-term stability of drug products intended for storage in a refrigerator, the conditions of 5±3°C is suggested in guidelines. We used the same condition for real-time stability analysis of the microparticulate system. For accelerated study, the conditions of 25±2°C/60% RH±5% RH has been recommended. Literature studies show that a peculiar characteristic of PLGA microparticles is that they form aggregates at higher temperatures (28). We too encountered this phenomenon when the product was stored at 25±2°C/60±5% RH conditions. The irreversible aggregation has been attributed to the residual solvent migration and partial dissolution of the polymer on the superficial layers leading to coalescence. In view of this typical behavior, we did not conduct the stability at the accelerated conditions. In light of the above aspects, we found it worthwhile to conduct long-term stability studies for the PIR–PLGA microspheres. The microsphere samples were packed in amber colored (5 mL capacity) vials, stoppered with rubber closure, and crimped with an aluminum over-seal. The samples were stored in stability chamber stability chamber TH90G (Thermolab Scientific Equipments Pvt. Ltd., India). After initial analysis of PIR content, the vials were kept at 0°C and 5±3°C. The vials were periodically sampled at 1, 2, 3, 6, and 12 months time period. The stability samples were critically evaluated for physical appearance, particle size analysis, syringeability–injectability test, drug content, impurity (2-aminopyridine) profile, and real-time *in vitro* dissolution profile.

The samples were tested for different parameters. Physical characteristics of the samples were carefully observed for changes in color and clumping/aggregation behavior. Particle size was calculated by optical microscopic method. The possibility of any shriveling tendency in the microparticles was also examined. Individual samples were subjected to drug content and impurity analysis using a validated stability indicating HPTLC method. Moisture content in the samples was found by the Karl Fischer method. In the *in vitro* dissolution studies, static method was employed as described earlier.

Characterization of PIR–PLGA System

Particle Size Analysis

The particle size was determined by measuring the diameter of individual particle using optical microscope (Nikon YS100, Nikon Instruments Inc., USA). The diameter of 100 particles was measured and the average particle size determined. Microsieves corresponding to 420, 250, 180, 150, 105, 75, 53, 45, and 37 μm were stacked one above another, with the finest sieve at the bottom. Weighed quantity of microparticles was carefully placed on the topmost sieve. After subjecting the microsieves to mechanical agitation of 30 min using Retsch (Germany) mechanical sieve shaker, the microparticles retained on each of the sieves were carefully collected and each fraction was weighed. The percent retained and cumulative percent retained on each sieve was calculated. Percent finer was then computed. A plot of percent finer *versus* particle diameter was plotted, and the diameters at 10% (d0.1), 50% (d0.5), and 90% (d0.9) were extrapolated from this graph. The polydispersity was calculated by subtracting the value of ratio of d0.1/d0.5 from value of d0.9.

Moisture Content

The residual water content in the microparticles was estimated by the Karl Fischer (KF) technique using a Karl Fischer/Autotitrator (model 831 KF Coulometer, Metrohm, UK). Dehydrated methanol (Merck, 20 mL) was titrated to

the electrometric end point with the KF reagent (Merck). The microsphere sample was then carefully transferred to the titration vessel and after stirring for 1 min titrated again using the KF reagent till the characteristic end point was achieved. Since the degradation product of PIR is a hydrolytic product, the moisture content in final microspheres is important.

Surface Morphology

The surface morphology of microspheres was examined on a scanning electron microscope (model S-570, Hitachi, Japan). The drug-loaded microspheres were positioned on a metal stud, which was coated with adhesive label. The sample was sputter-coated with conductive gold palladium (Edwards sputter coater, model S 150B, Edwards High Vacuum International, England).

Fourier Transform Infra-red Spectroscopy

Fourier transform infra-red spectroscopy (FTIR) spectra were taken for PIR, PLGA, physical mixture of drug and polymer (1:5 ratio), and microparticles of PIR–PLGA. The aim was to detect any possibility of excipient–drug interaction. FTIR spectrophotometer (Jasco FT/IR 5300 instrument, Japan) was employed in this study using the potassium bromide disk method.

X-ray Diffraction Studies

The polymorphic state of the drug in the microparticulate system was examined by X-ray diffraction studies. X-ray diffractograms were obtained for PIR, PLGA, and PIR–PLGA microsphere system at a scanning rate of 2°/min. An automatic X-ray diffractometer (Siemens 5000, Germany) equipped with an X-ray generator was employed in the study. Nickel filtered Cu $K_{\alpha 1}$ radiation having a wavelength of 1.5106 Å, operating at 35 kW, and 20 mA in the range (2θ) of 5° to 70° was used.

Drug Encapsulation Efficiency, Drug Content, and Impurity Determination

Microspheres (10 mg) were accurately weighed and dissolved in 10 mL of dichloromethane. The polymer was precipitated using ethanol (volume made up to 25 mL). After centrifugation, the clear supernatant was subjected to stability indicating HPTLC analytical method (29) developed and validated in our laboratory. The encapsulation efficiency was calculated by the actual and theoretical drug loading values. The impurity; 2-aminopyridine was separated during the TLC plate development and estimated.

In Vitro Release Kinetics

Microsphere samples (25 mg) were taken in 100 mL stoppered conical flask. The dissolution medium (20 mL) that mimics saline (0.9% *w*/*v* sodium chloride in distilled water) was employed in the studies. To maintain adequate sink conditions, polysorbate 80 (0.01% *w*/*v*) was added. The microbial growth was arrested using sodium azide (0.02% *w*/*v*). Dissolution samples were subjected to agitation (80 strokes/min) using a constant temperature shaker water-bath (Shital Scientific Industries, Mumbai, India). The temperature of dissolution medium was maintained at 37±0.5°C. Microsphere-free samples (2 mL) were withdrawn at predetermined time intervals 4, 8, 12, 16, 20, 24, 28, and 32 days, using HPF Millex Filter units (Millipore, USA) and replacing with fresh medium after every sampling. The drug release was measured by a sensitive HPTLC method. Placebo microspheres (25 mg) were simultaneously subjected to dissolution studies to check excipient interference. High temperature *in vitro* dissolution study can be predictable for erosion-controlled systems but appears to be unsuitable method for the diffusion based PLGA based systems. The elevated temperature accelerated dissolution tests are not able distinguish the burst release phase for the PLGA microspheres, and hence, real-time study is recommended to determine the burst release phase (30,31). Keeping this important point in view, it was decided to perform the real-time *in vitro* dissolution studies to get the precise idea of the release kinetics on storage of the product.

Analytical Method (HPTLC)

Representative standard curve of PIR was constructed by plotting the peak area as well as peak height *versus* drug concentration. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation 0.9988; slope 11.4494 and intercept 6487.66 (peak area) and *r* 0.9981; slope 0.3763 and intercept 327.0312 (peak height) over the concentration range studied. The range of reliable quantification was set at 400 to 800 ng as no significant difference was observed in the slopes of the standard curves in this range (analysis of variance (ANOVA); $p>0.05$). The RSD for within-day and day-to-day analysis was found to be less than 2%. The limit of detection of PIR was estimated to be 40 ng where the drug could be detected without any noise. The limit of quantitation was found to be 130 ng. The degradation component 2 amino pyridine (2-AP) gave Rf value of 0.225±0.015 ($n=6$). The solvent system employed gave good separation between the degraded compound and the parent drug. Thus, the method proved to be a stability indicating one.

Residual Solvent Content

The residual solvent content in the PIR–PLGA microspheres was determined by head-space gas chromatography using CHEMITO 8610 HT gas chromatograph using BPX5 capillary column equipped with head-space system. The oven, injection port, and detector temperatures were kept at 40°C, 80°C, and 240°C, respectively. Nitrogen (flow rate 5 mL/min) was used as carrier gas, and a flame ionization detector was employed in the study. The microparticles (200 mg) were triturated and immediately taken in 5 mL of distilled water and sonicated for 5 min. The sample was then placed in the head-space tube in the heating block and heated at 80°C for 10 min, and the head space was subsequently injected. The standard employed in the analysis consisted of 0.1 mL (132.5 mg) of methylene chloride (99.5%) suitably diluted in distilled water to obtain a final concentration of 53 and 8.6 ppm. The above two standard solutions (4 mL) were taken separately in the head-space tube in the heating block and heated to 80°C for 10 min. The head space was injected, and gas chromatograms of samples and standards were

recorded. The residual solvent content was determined from the peak areas. This analytical method developed was validated for limit of detection, limit of quantitation, within-day precision, and day-to-day precision.

Syringeability–Injectability Studies

To evaluate the flow properties of the microparticulate system, syringeability–injectability studies were performed. Microspheres (sieve fraction 150/350, 50 mg) were suspended in 2 mL normal saline in a 5-mL-capacity glass vial. The resulting suspension was withdrawn into a syringe and subsequently injected out. In the study, three types of syringes, namely glass syringe, regular hypodermic disposable plastic syringe, and gas-tight syringe, were employed. A 22-gage needle was used in the study. The parameters studied included ease of withdrawal, freedom from clogging, foaming tendency, evenness of flow, and aspiration quality.

RESULTS

The particle size, drug encapsulation efficiency, and 2-aminopyridine level were estimated for initial microsphere sample. The polymer to drug ratio of 5:1 *w*/*w* gave an encapsulation efficiency of 42.79±2.23%. Polydispersity was calculated to be 0.72, which indicates wide particle size distribution. The range of particle diameter was found to be from approx. 7.24 to 27.52 μm size (with an average ± SD of 16.25±9.45 μm). We have incorporated this in the manuscript. The data given earlier were based on the optical microscopy method data in which we measured only 100 particles from the bulk microparticles. In sieve analysis, the entire batch was taken for the study.

Surface Morphology

The scanning electron photomicrographs of drug loaded PLGA system is shown in Fig. 1. The gamma-radiated microspheres exhibited a spherical shape. There were no free drug crystals observed on the surface of the microparticles. The photomicrograph reveals a smooth surface topography without any evidence of pores or invagination.

Fourier Transform Infra-red Spectroscopy

The characteristic absorption peaks in the IR spectrum of the drug, physical mixture of drug + polymer, and the microparticulate system did not indicate any change. The amide carbonyl stretch was observed at 1,620 cm^{-1}, second amide band stretch at 1,525 cm^{-1}, methyl at 1,350 cm^{-1}, –SO_2–N– at 1,150 cm^{-1}, and *ortho*-disubstituted phenyl at 770 cm^{-1} were sent for pure drug, physical mixture, and microspheres. The characteristic peaks representative of PIR are present in the IR spectrum of physical mixture of the drug and poly(lactide-*co*-glycolide) polymer. PIR–PLGA microsphere system also has the characteristic peaks of the drug. These results imply that there is no interaction between the drug and the excipients.

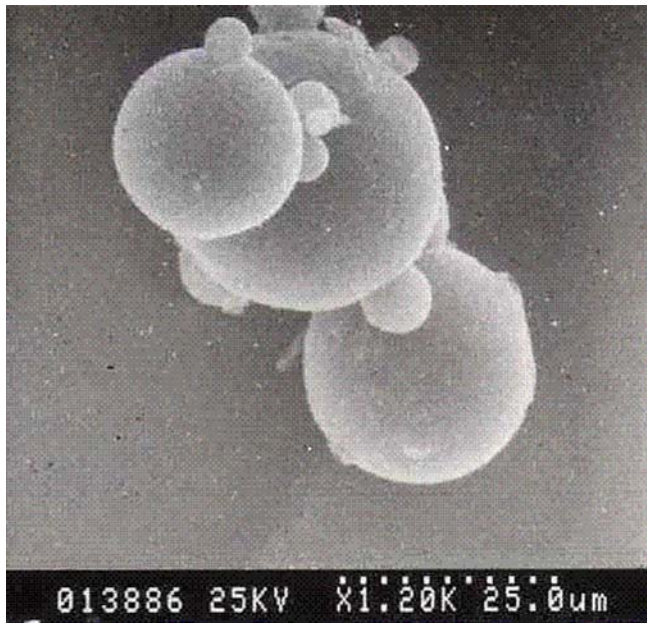

Fig. 1. Scanning electron photomicrograph of PIR–PLGA microspheres

X-ray Diffraction Studies

The X-ray diffractograms are shown in Fig. 2. The X-ray diffractogram of PIR showed a characteristic pattern of crystalline nature. With reference to X-ray diffraction pattern of PIR–PLGA system after gamma radiation, the characteristic X-ray diffraction peaks of the drug were absent. The drug in microparticulate system exists in the amorphous

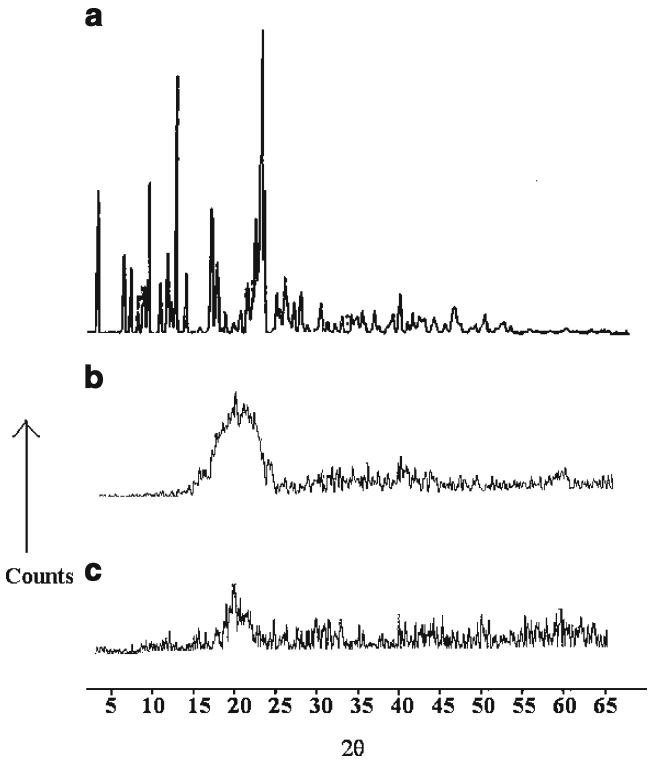

Fig. 2. X-ray diffraction spectra. *A* PIR, *B* PLGA, *C* PIR–PLGA microspheres

polymorphic state or might be due to low drug loading in the microspheres.

Residual Solvent Content

The residual methylene chloride content for initial sample was found to be 12 ppm, which is well below the ICH guidelines reported limit of 500 ppm.

Stability Studies of PIR–PLGA System

The physical observations of samples, particle size, drug content, impurity level, and moisture content in the PIR–PLGA formulation for initial and stability samples at 0°C are shown in the Table I and at 5±3°C condition in Table II. The product retained its spherical geometry and did not show shriveling tendency during the 12-month storage period.

Syringeability–Injectability Studies

The PIR–PLGA microparticles exhibited excellent flow properties. The system complied with the syringeability–injectability study for initial and all stability samples till 12 month storage time.

In Vitro Release Studies

The drug release data was fitted to various kinetic models *viz*. zero-order, first-order, and Higuchi kinetics. The corresponding coefficients of determination (r) and slopes were computed. The data are listed in Table III (for 0°C storage) and Table IV (for 5±3°C condition). The drug release profiles for the stability samples of 0°C and 5±3°C are shown in Figs. 3 and 4, respectively. The release data suggest that there was no major change in the release pattern during the storage period. All *in vitro* dissolution experiments were performed in triplicate and are expressed as mean values ± standard deviations. Both the drug release data plots at each time point and also the release rate constant calculated from individual plots were compared. Statistical significance was established using the ANOVA with a significance level α of 0.05.

DISCUSSION

The PIR–PLGA microsphere system was developed in a view to have a system for long-term treatment of inflammatory conditions like rheumatoid arthritis. In this study, an injectable system consisting of the Oxicam molecule taken as model drug was formulated by a solvent evaporation technique. Although a lot of literature is available on different microparticulate systems, stability aspects of such systems have not been studied. Stability studies play a major role in defining the safety aspects of the pharmaceutical product and also are a helpful tool in estimating the shelf life of the formulation.

PIR is stable under basic hydrolysis but yield 2-AP as one of the degradation products under acid hydrolysis. It is interesting to note that this impurity is also a precursor in the synthetic pathway of PIR. Hence, it is important to estimate the limits of this impurity in the dosage form till the shelf life of the product. The stability indicating analytical method used was able to separate the parent peak of PIR and 2-AP. The results indicate that there is no increase in the impurity levels of 2-AP in the formulation even after long-term storage.

In the *in vitro* dissolution studies, the Higuchi equation (32) describes the release of a drug from an insoluble matrix as the square root of a time-dependent process. This is essentially based on Fickian diffusion (Eq. 1).

$$Qt = 2DS\varepsilon(A - 0.5S\varepsilon)^{0.5} \times t^{0.5} = k_H\sqrt{t} \quad (1)$$

where Qt is the amount of drug released in time t, D is the diffusion coefficient, S is the solubility of drug in the dissolution medium, ε is the porosity, A is the drug content per cubic centimeter of matrix tablet, and k_H is the release rate constant for the Higuchi model. It has been documented that drug release from controlled drug delivery systems using typically hydrophilic matrices shows a time-dependent profile. The drug release decreases with time because of increased diffusion path length due to a gel layer formed on the surface that retards further the ingress of fluid and subsequent drug release (33). This inherent limitation leads to first-order release kinetics depicted in Eq. 2.

$$F = 100\left(1 - e^{-kt}\right) \quad (2)$$

where F is the percentage of drug released at time t. The release data were also fitted to Baker–Lonsdale kinetic model (34) (Eq. 3). This equation is for diffusion- controlled release and more specific from spherical matrix systems.

$$3/2(1 - F)^{2/3}F = kt \quad (3)$$

where F is the fraction of drug released, t is the time, and k is the rate constant. Zero-order kinetics is obtained when the drug

Table I. Stability Studies Evaluation of PIR–PLGA System (0°C)

Sampling time	Physical appearance	Average particle size (μm), avg ± SD	Syringeability–injectability test	Drug content (%), avg. ± SD	2-Amino pyridine (%), avg. ± SD	Moisture (% *w/w*)
Initial	Free flowing powder	16.45±9.45	Passes	98.25±1.20	0.15±0.003	0.15
1 month	Free flowing powder	17.55±10.25	Passes	96.52±1.58	0.17±0.002	0.14
2 months	Free flowing powder	14.24±9.89	Passes	98.08±1.25	0.22±0.005	0.14
3 months	Free flowing powder	18.22±8.54	Passes	96.55±1.98	0.25±0.009	0.13
6 months	Free flowing powder	16.89±8.22	Passes	97.88±1.20	0.28±0.008	0.15
12 months	Free flowing powder	15.29±7.89	Passes	98.23±1.52	0.29±0.007	0.16

All experiments were conducted in triplicate
2-Aminopyridine degradation product of piroxicam

Table II. Stability Studies Evaluation of PIR–PLGA System (5±3°C)

Sampling time	Physical appearance	Average particle size (μm)	Syringeability–injectability test	Drug content (%), avg. ± SD	2-Amino pyridine (%), avg. ± SD	Moisture (% *w/w*)
Initial	Free flowing powder	15.06±9.56	Passes	98.39±1.27	0.15±0.001	0.15
1 month	Free flowing powder	18.25±9.54	Passes	96.89±1.39	0.20±0.005	0.13
2 months	Free flowing powder	17.23±9.09	Passes	97.42±1.87	0.25±0.005	0.15
3 months	Free flowing powder	18.01±8.56	Passes	99.63±1.59	0.24±0.007	0.15
6 months	Free flowing powder	17.14±8.78	Passes	99.25±1.87	0.27±0.006	0.15
12 months	Free flowing powder	16.55±9.26	Passes	97.79±1.79	0.32±0.008	0.16

All experiments were conducted in triplicate
2-Aminopyridine Degradation product of piroxicam

release from a system is independent of the concentration of the drug (35). When the percent drug release was plotted as a function of time, a linear relationship was obtained with the "*r*" value closet to unity. The coefficients of determination fitted into different kinetics models suggest that the release followed zero-order kinetics.

United States FDA's guidance (36) recommends a metric that can be used to compare dissolution profiles of different formulations. This metric referred to as f_2 (as defined in Eq. 4) is called the similarity factor is used to compare multi-point dissolution profile.

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-05} \times 100 \right\} \qquad (4)$$

where R_t is the percentage dissolved at each time point for the reference formulation, and T_t is the percentage dissolved at each time point for the test formulation. It is noted that an f_2 value between 50 and 100 suggests that the two dissolution profiles under consideration are similar and indicates a point-to-point difference of 10% or less. In this study, the dissolution profiles of initial sample and the product stored for 12 months period were compared using this metric. The calculated f_2 value for 0°C storage condition sample was 63.62, and for 5±3°C, storage condition sample was 75.84. These values indicate no major difference in the drug release profiles at both the temperatures for the initial sample and the sample stored at long-term condition. No statistically significant differences in the dissolution profiles were observed between the formulations stored at accelerated conditions and initial or control samples (ANOVA, $p<0.05$). The dissolution characteristics of PIR in different pH conditions have been reported in the literature (37). Pure drug has very low solubility in the dissolution medium and also had the tendency to have a pH-dependent release profile (about 56% in pH 1.2 and lowering to about 80% in pH 7.5 in 120 min). However, it should be noted that there is no additional surfactant added to maintain sink conditions in these studies.

Syringeability is the ability of the suspension to pass easily through a hypodermic needle on transfer from the container prior to injection. Syringeability refers to the handling characteristics of a suspension while withdrawing it into and manipulating it in a syringe equipped with a properly sized needle. The particle size, density, viscosity, as well as the concentration of the solids in the suspension affects the syringeability of the suspension. Injectability refers to the performance of the suspension during the injection. This parameter includes evaluation of the pressure or force required for injection and evenness of flow. Both the syringeability and injectability are closely related to the viscosity and particle characteristics of the suspension. Clogging or blockage of syringe needles may be due to a single large particle or aggregates that occlude the lumen of the needle. Sometimes, clogging can occur due to "bridging" effect of the particles. This most often takes place when the suspension contains particles exceeding a certain size in relation to the needle diameter. Particle shape also plays a significant role in a similar type of blockade referred to as log-jamming. Long needle-like crystals are believed to produce this effect (38). As a general rule, it is advisable to avoid any particle greater than one fourth to one third the needle internal diameter. While administering a suspension, clogging of the needle is very disconcerting to the clinician and may be at times traumatic to the patient. Hence, every effort should be made to avoid this problem. In contrast to the blockade of the needle shaft, one might encounter clogging at or near the needle end. There is some separation of the vehicle from the suspended particles that are left deposited in the

Table III. Fit of Different Kinetic Models and Comparison of Coefficients of Determination for PIR–PLGA System (0°C)

Release kinetics	Initial	1 month	2 months	3 months	6 months	12 months
Zero order: *r* (rate)	0.965	0.981	0.968	0.963	0.975	0.955
	2.04	1.92	1.82	1.99	2.11	1.91
Higuchi: *r* (rate)	0.902	0.934	0.908	0.902	0.919	0.905
	15.32	14.64	13.79	15.07	15.97	14.77
First order: *r* (rate)	0.912	0.949	0.918	0.899	0.933	0.939
	−0.01	−0.01	−0.01	−0.01	−0.01	−0.01
Baker–Lonsdale: *r* (rate)	0.923	0.973	0.950	0.943	0.945	0.925
	0.01	0.01	0.01	0.01	0.01	0.01

Table IV. Fit of Different Kinetic Models and Comparison of Coefficients of Determination for PIR–PLGA System (5±3°C)

Release kinetics	Initial	1 month	2 months	3 months	6 months	12 months
Zero order: *r* (rate)	0.965	0.975	0.955	0.974	0.959	0.964
	2.04	2.13	2.15	1.95	2.04	2.12
Higuchi: *r* (rate)	0.902	0.919	0.889	0.918	0.898	0.908
	15.32	16.18	16.19	14.76	15.42	15.58
First order: *r* (rate)	0.912	0.923	0.903	0.917	0.916	0.907
	−0.01	−0.01	−0.01	−0.01	−0.01	−0.01
Baker–Lonsdale: *r* (rate)	0.923	0.967	0.922	0.953	0.904	0.937
	0.01	0.01	0.013	0.01	0.01	0.01

needle in the form of a dry plug. The type of vehicle used, wetting characteristics, particle size, shape and distribution, the suspension viscosity, and rheological properties, as well as the concentration of the suspension, may be the reasons contributing to clogging behavior. Drainage describes the ability of the suspension to break cleanly away from the inner walls of the primary container-closure system. There was ease of withdrawal and injection with no clogging or foaming tendency observed, which indicates that the PIR–PLGA microparticulate system passes the syringeability–injectability test for the storage samples.

PLGA microparticulate systems are known to degrade in hydrolytic conditions while passing through the random scissoring of ester linkages (39). This particular process liberates acidic degradants, which includes namely lactic acid and glycolic acid. The acids released facilitate further degradation of PLGA. When the encapsulated material is a protein, it is susceptible to these acidic microclimates, and it readily degrades by peptide bond hydrolysis and non-covalent aggregation (40). Few research groups have tried to incorporate an antacid like magnesium hydroxide into PLGA in order to neutralize this acidic microclimate (41). This formulation option might be useful in stabilizing certain susceptible molecules against the acidic degradation.

Although pH modifiers, mostly basic salts, have been incorporated in PLGA based formulations in an attempt to stabilize the microclimate pH, these techniques may not necessarily prevent degradation reactions that are both acid and base labile, such as deamidation. Alternatively, certain buffers and proton scavengers or "sponges" are possible alternatives to basic salts. Examples of proton sponges are amines that are more basic than the drug(s) and so are preferentially protonated without altering the overall pH of the formulation (42). Further, these buffers have the potential to neutralize the acidic monomeric products of PLGA degradation without producing a basic pH. Poloxamers and poloxamines have been incorporated into the nanoparticles during preparation (instead of adsorbing them onto the bare PLGA) of PLGA particles, which have given improved colloidal carriers (43). It has been documented that in the presence of these polymers, the acidity generated in the course of PLGA degradation is neutralized, thus preserving DNA structural integrity and thus its biological activity (44).

The intrinsic properties of the biodegradable polyesters greatly affect the degradation behavior and pattern. Certain polymer properties have been studied and include monomer structure (45), molecular weight (46), crystallinity (47,48), and shape (49). Further, the *in vivo* degradation of biodegradable polyesters commonly takes place by a chemical hydrolysis reaction of ester bonds in its backbone and results in the production of carboxylic acid end groups that act as a catalyst in the reaction (50).

The physico-chemical properties of encapsulated material also appreciably influence the resulting release patterns, especially at high initial drug loadings. For example, freely

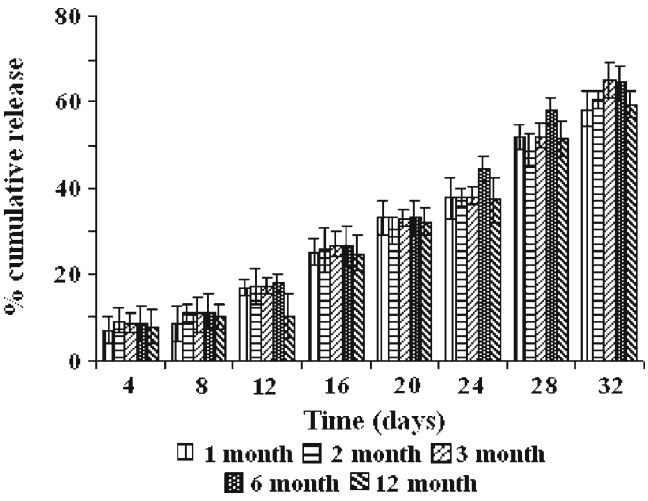

Fig. 3. *In vitro* dissolution drug release data at different time intervals for stability samples stored at 0°C. Each value represents the average ± standard deviation of $n=6$ units for each formulation under study

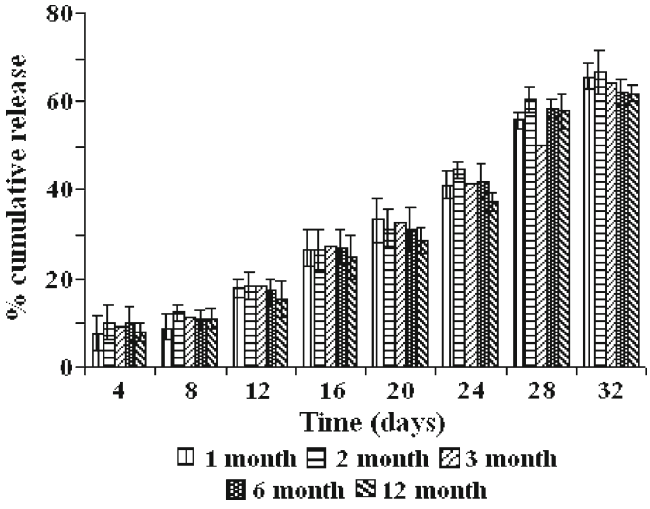

Fig. 4. *In vitro* dissolution drug release data at different time intervals for stability samples stored at 5±3°C. Each value represents the average ± standard deviation of $n=6$ units for each formulation under study

water-soluble drugs can assist water penetration and lead to the creation of highly porous polymer networks upon drug leaching. In contrast, drugs that are lipophilic in nature can hinder the diffusion process, thus slowing down polymer degradation. Further, in case of considerable amounts of acidic or basic drugs, additional effects on the PLGA degradation kinetics can be expected because of ester hydrolysis being catalyzed by acids and bases (51,52). Thus, these features of the degradation behavior of polymeric material used and the compatibility of encapsulating active pharmaceutical ingredient in the formulated system needs to be studied thoroughly from the system stability point of view.

CONCLUSIONS

The physical appearance of microsphere samples remained unaltered even after 12 months storage time period with drug content and drug impurity levels maintained within the acceptable limits. There was no clumping and/or aggregation behavior observed. The *in vitro* drug release did not alter as seen from the real-time dissolution studies. These studies have shown that the product quality is maintained with desired performance attributes throughout the one year of storage.

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