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Spectroscopic and Drug Delivery studies of Moxifloxacin Loaded Polylactic Acid/Polycaprolacton Polymeric Matrix for Medical Application.

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ABSTRACT

Polylactic Acid (PLA)/Polycaprolactone (PCL) blendloaded with different concentration of moxifloxacinwere prepared by casting method. Thebiocomposites were investigated by Fourier Transform Infrared spectroscopy (FTIR), Thermogravimetric Analysis (TGA) and Scanning Electron Microscope(SEM).The *in vitro* release profile of moxifloxacin from the biocomposite matrix showed a sustained release of the drug over a period of 25 hrs.Antimicrobial assay were performed against E.coli, Pseudomonas aeruginosa, and staphylococcus using the cup-plate method. Antimicrobialstudies showed equivalent zone of inhibition compared to marketed formulation. The results show successful interactionbetween moxifloxacin and PLA/PCL as observed from FTIR, Moxifloxacinimproved the thermal stability ofthe composites, as evidenced by thermogravimetric analysis and the SEM shows agood and uniform dispersion of the moxifloxacin particles in the polymer matrix.Thus the obtained results suggest that PLA/PCL loaded moxifloxacin appear promising for effective management of ocular infections.

Keywords: PLA/PCLcomposite, Moxifloxacin,FTIR,UV/Vis, Drug release.

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INTRODUCTION

The rising interest on new materials for fields of advanced such as packaging or biomedicine applications have increased the attention on bio-degradable polymers blends in years of recent[1-3].In general, the processing of polymer blends is an interesting goal forboth research groups and industrial companies because of it is an easy, low-cost, scalable way to enhance the properties of the pristine homopolymers[4, 5]. The mostcommercial polymer blends are immiscible, thus presenting two separated phase.However, still in this case, they have very useful properties[6]. Depending on thethermodynamics of the system, the processing conditions and the composition of theblends, they can present phase separation thus forming morphological structures, suchas elongated fibrous, or co-continuous structure and spherical droplet[7, 8].The properties of the polymer blends are strongly affected by the phase-separatedmorphology, and thus controlling this morphology it is possible to take advantage of thehomopolymer synergetic effects improving the final properties of the blend[9]. In this context, blends of PLA with other aliphatic polyesters, in particular with poly(caprolactone) (PCL), have attracted the attention of many research groups[10-12], dueto the fact that both polymers are biodegradable and biocompatible[13-15]withattractive synergic properties, referring to their thermal behavior, mechanical[16] andrheological properties[17, 18]or biodegradation rates[14, 19]. Moreover, anotherinteresting property to study when working with PLA and PCL-based materials is theshape memory effect[20, 21]. This particular behavior consists in the ability of thematerial to change its initial shape by the application of certain external stimulus,usually the temperature[22], and to recover its initial shape when the external stimulusis applied again. Generally, shape memory behavior is achieved by designing materialswith two or more phases, where one is responsible to fix the original shape, while theother one enables the modeling of the desired temporary shape[21].

In biopolymers, PCL and PLA had typical biodegradability and good biocompatibility, while they had variation characters. By comparison with PCL, PLA had slower degradation demeanor[23].

Moxifloxacin hydrochloride (HCl), a 4th generation fluoroquinolone, is anantibiotic of broad spectrum utilized in the treatment and prevention of a ocular infections variety[24]. new reports based on various in vivo studies have shown the moxifloxacin potency in preventing infections of anterior eye for example keratitis and bacterial conjunctivitis[25].Gadad AP and et al. [26] were studied efficient drug delivery to the region of ocular is an aim of challenging by Loading of the moxifloxacin on Polymer. The aim of this study was to develop and evaluate polymeric matrix containing moxifloxacin as potential drug delivery system

MATERIALS AND METHODS

Materials

The chemicals used were polylactic acid (PLA) pellets, with Mw=60,000 from Sigma-Aldrich, Polyε-caprolactone pellets (Mw~80,000, Sigma-Aldrich), Moxifloxacin (MOX) Hydrochloride obtained from Pvt, Ltd, Mumbai.

Preparations of PLA/PCL-Moxifloxacin

PLA/PCL (50/50 wt. %) with different concentration of moxifloxacin were prepared by casting method. PLA/PCL was dissolved in chloroform separately and then the solution was stirred continuously about 12 h at 25 ^{o}C until a homogenous solution was obtained. The moxifloxacin was dissolved in chloroform at the same condition. The resultingsolution of moxifloxacinwas added to the PLA/PCL blend with different content (0.1, 0.25, and 0.5wt%). The resulting solution of PLA/PCL-Moxifloxacinwas then cast to glass dishes at room temperature for about one day. After drying, thefilms were peeled from glass dishes. The samples have thickness ranging from 0.11 to 0.21 mm were obtained and kept in vacuum desiccators until use.

Instrumental Characterization

The FTIR data was obtained using (Nicolet iS10, USA) spectrometer at room temperature. UV-Vis-NIR spectrophotometer data was acquired using UNiCAM UV–vis spectrophotometer. Thermogravimetric analysis (TGA) was carried out in a Shimadzu system TGA. The films were scanned from 0 ^{o}C to 450 ^{o}C at a heating rate

of 10 °C/min in the presence of nitrogen.The morphology and drug dispersion in the biocomposite was scanned by SEM utilizing (JEOL 5300, Tokyo, Japan), operating at 30 KV accelerating voltage.

Drug Release Studies

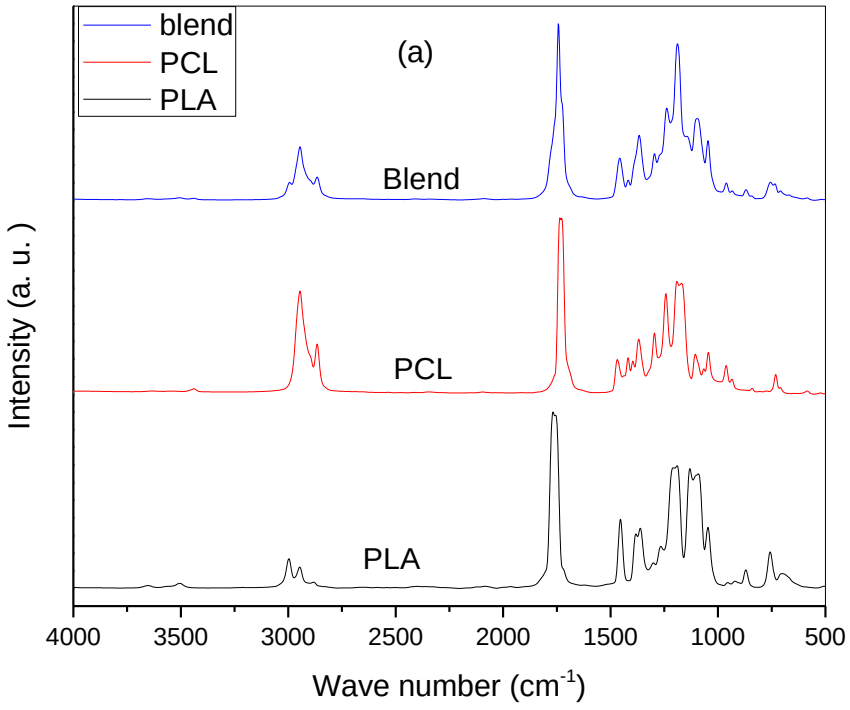
A piece of biocomposite film, that equivalent to 2mg of moxifloxacin drug, was calculated from each sample loaded with drug and then it was immersed into beaker containing 100ml of phosphate buffer saline (PBS) pH 7.4. Then the beaker was kept in a continuous shaking, maintained at 37°C. Then 2ml of drug release solution was withdrawn at constant time intervals of 1 hour,3,5,7,9,11,13,15,17,19,21 and 25 hours while replacing it with fresh 2ml of phosphate buffer saline. The withdrawn samples then filtered. From this filtered samples 1ml solution were withdrawn and diluted to 10 ml with PBS and the drug content was determined by UV spectrophotometer at 290nm. The cumulative % drug release was determined and a relation was plotted with cumulative % drug release against time.

Microbiological Investigations

The antimicrobial studies and the biological activity were carried out of the best formulation and of the marketed eye drops against microorganisms (E.coli, Pseudomonas aeruginosa and Staphylococcus) using a cup plat method. A mount of nutrient agar (40mL) seeded with the test microorganism (0.3mL) was allowed to solidify in the Petri plate. Three wells were prepared aseptically in each plate with the help of stainless steel borer Cups were made on the solidified agar layer with the help of a sterile borer (8 mm diameter). Then, an equal piece of 15mm diameter from the 0.5% sample and marketed eye drops containing equivalent amount of drug was separately poured into two cups. The plates were incubated at 37°C for 24 hours. The zones of inhibition were studied. The diameter of the zone of inhibition was measured by an antibiotic zone finder. Readings were taken in triplicate.

Results and Discussion

Fourier Transform Infrared (FT1R) Spectroscopy



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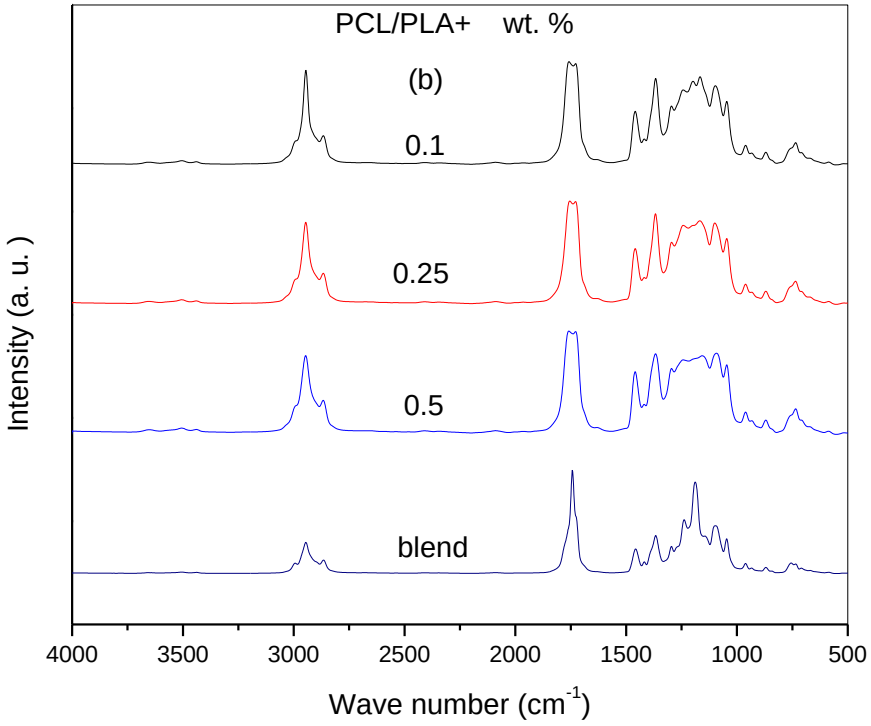

Figure (1):FTIR spectra of (a) Pure PLA, PCL and pure PLA/PCL blend (b) PLA/PCL blend doped with different concentration of moxifloxacin.

The spectral analysis of pure PLA, pure PCL and PLA/PCL blend are shown in Fig. (1a). The peaks at 2999, 2943 and 1755 cm^{-1} of PL A and 2942. 2859 and 1730 cm^{-1} of PCL were assigned to the vibrational stretching mode of –CH symmetric;–CH asymmetric, and vibrational mode of -C=O bonds, respectively. On the other hand in the blend matrix these characteristic peaks were found in the neutralized regions of 2950, 2866 and 1748 cm^{-1}[27].

The FTIR spectra of the pure polymer matrix and that filled with different concentration of moxifloxacin are shown in Fig. (2b). The spectrum of PLA/PCL/Mox show the peaks at 2950 and 2866 cm^{-1} which due to the C-H stretching symmetric and asymmetric. The peak for C=O bending observed at 1750 cm^{-1} and the peak for C-O bending is at 1154 cm^{-1} [28].

Thermogravimetric analysis (TGA)

TGA analysis detects multiple or single loss steps from room temperature to 450°C. This technique used to examine the thermal stability of the samples. The samples mass loss of the samples is monitored as a function of a temperature. The decomposition of pure PCL/PLA starts at about 255.39°C (fig.2). The decomposition is increase quicklybeyond this temperature and completed at 370.9 °C. About 99.11% the total weight loss of of pure PCL/PLA matrix was observed in its decomposition. This decomposition attributed to the completely dissolution of polymeric matrix. While the PCL/PLA-mox. begins to decompose at about 320.74°C and it completely decomposed at 430.86°C[29]. The decomposition of PCL/PLA-mox show lower rate of chain scission and a lower rate of volatile formation than that of pure PCL/PLA. Soafter addition of moxifloxacin the thermal stability of the polymeric matrix was improved.

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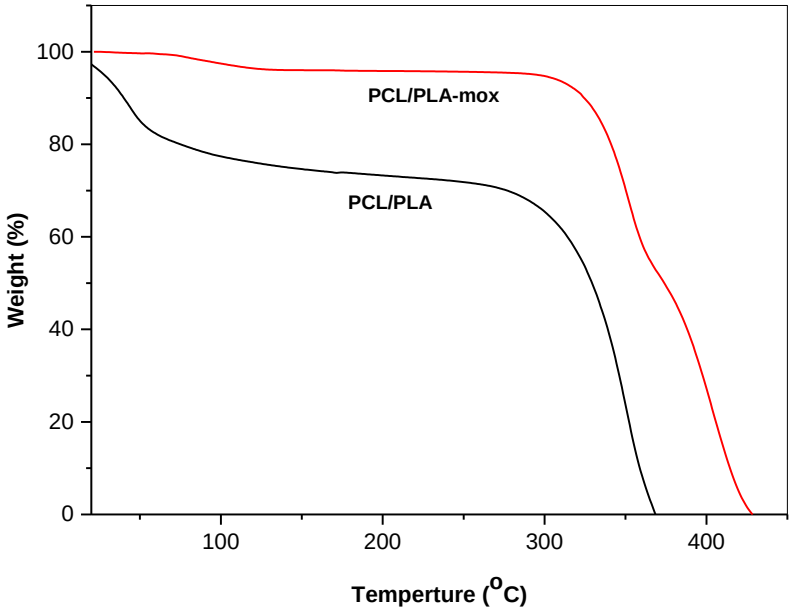

Figure (2): TGA thermograms of pure PCL/PLAandPCL/PLA-mox.

Scanning Electron Microscopy (SEM)

The morphology and dispersion of the drug in the biocomposite matrix (sample 0.5wt%) was characterized by scanning electron microscopy (SEM) fig. (3). The figure shows a good and uniform dispersion of the moxifloxacin particles in the polymeric matrix that confirm the good compatibility between the drug and the polymeric structure.

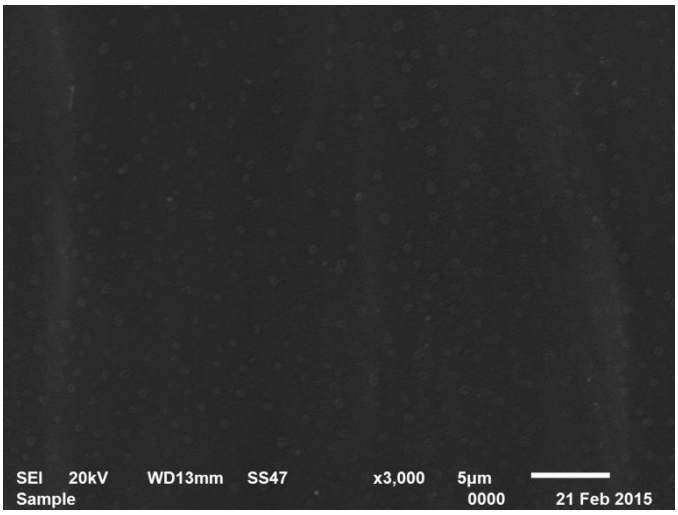

Figure (3): SEM of PCL/PLA-moxbiocomposite matrix

Drug release studies

From the UV-Vis analysis in fig. (4) it is also observed that an absorption peak appears around 290 nm that is characteristic for moxifloxacine. On the other hand, fig. (5) show the drug release rate as a function of time. By adding moxifloxacine with different (0.1, 0.25 and 0.5wt%) ratios to the biocomposites the absorbance value of the characteristic peak of moxifloxacine appear to be increases. All the drug loaded samples showed a cumulative% release between 57% and 67%. All the formulation shows bi-phasic release

pattern: initial burst release one followed by a slow second release phase (prolonged release). The initial burst drug release is important in terms of antimicrobial activity as it helps determine the drug therapeutic concentration in minimal time followed by constant release to achievecontrolled and sustained release of the drug. The next slow phase may be due to slow desorption of drug located inside of the polymer biocomposite matrixes[26].Among the three concentrations of biocomposite samples, the sample containing 0.5% of drug per polymer blend) showed maximum cumulative % drug release of 67%. It is concluded that PCL/PLA blend can serve as good drug carriers because it can decrease side effects of the drug by varying its drug release mechanism. That is meaning,the therapeutic agents not only could be delivered to specific location via targeted deliver but also must release at a specific time and certain conditions[30].

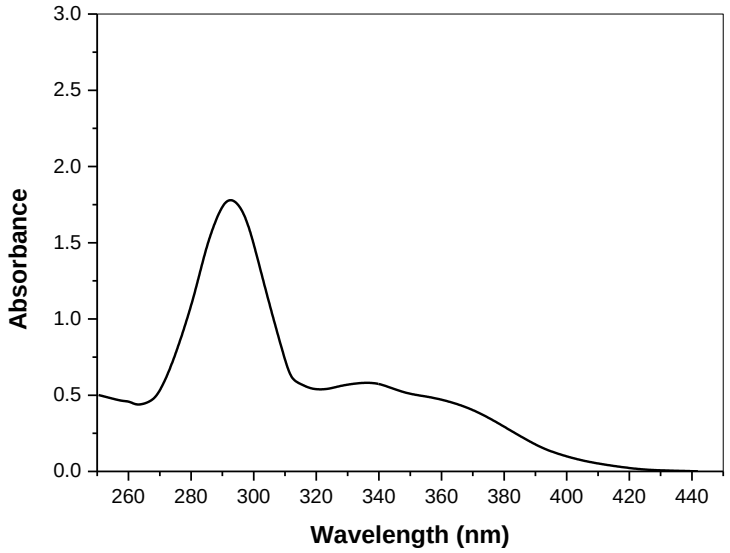

Figure (4): UV-Vis analysis for moxifloxacine at pH=7.4

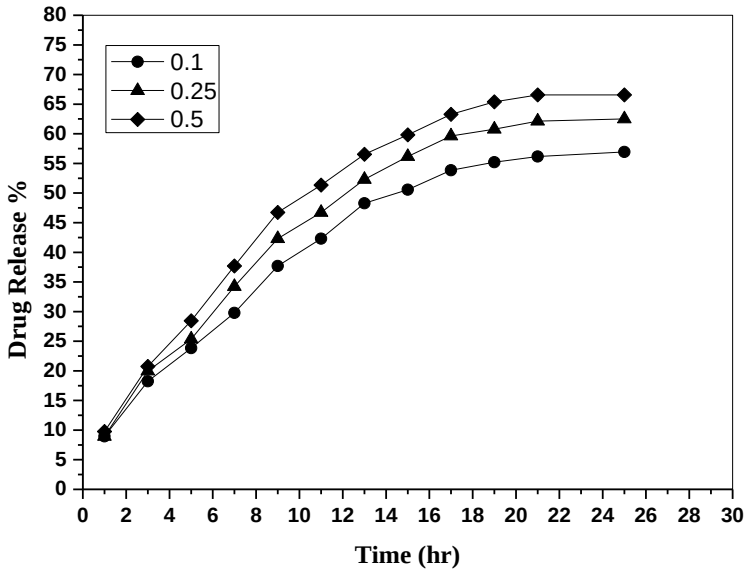

Figure (5): Release behavior of moxifloxacine loaded on PCL/PLA biocomposites with various concentrations of moxifloxacine.

Microbiological investigations

The microbiological test was carried out as qualitative estimation to compare antimicrobial efficacy of prepared samples with marketed formulation. The sample 0.5% wasexamined microbiologically by cup-plate technique. A Clear zone of inhibition was obtained for the sample against the test organisms namely E.coli, Pseudomonas aeruginosa and Staphylococcus. The results were shown in table **1**. Results show that the prepared composite is closely equivalent to marketed formulation in its antimicrobial action.

Table 1: Diameter of inhibition zone for the marketed formulation and the prepared samples for different organisms

	Diameter zone of inhibition(mm)	
	0.5% Sample	**Marketed Formulation**
E.coli	20±0.34	21±0.14
Pseudomonas aeruginosa	19±0.45	18±0.55
Staphylococcus	27±0.6	26±0.57

CONCLUSIONS

Polymeric matrix containing moxifloxacin as potential natural drug delivery carrier in terms of its physical structure, thermal stability, drug loading capacity, in vitro release characteristics and efficient antimicrobial effect. The release profile of moxifloxacin from the biocomposite matrix has shown a sustained release with a maximum cumulative % drug release of 67%. For the sample 0.5% drug loaded which serve as a good drug carriers. The antimicrobial investigation shows that the developed composite is equivalent to marketed formulation in its antibacterial action. The results confirm the effective use of moxifloxacin loaded PCL/PLA as a controlled release preparation for treatment of ocular infections.

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