AMERICAN JOURNAL OF PHARMTECH RESEARCH

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# Sustained Release of Riboflavin via Microencapsulation using Polylactic acid/Chitosan Blend

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## ABSTRACT

Blending is an important method for improving the properties of polymers. PLA has been blended with many other polymers to improve its biodegradation as well as its mechanical properties. In the presented study; poly L(+)lactic acid [PLLA] has been blended with chitosan [Cs] as a biodegradable polymers to improve the release profile. PLLA was prepared via direct polycondensation reaction as mentioned in our previous publications. Riboflavin [RF] was chosen as a model drug to evaluate the release behavior through PLLA.Cs polymeric device. Different ratios of blends (1:1, 2:1 PLLA:Cs) were prepared and compared with PLLA alone. The prepared PLLA and PLLA.Cs blends were characterized and evaluated via microencapsulation of RF. The prepared microcapsules were characterized in terms of morphology and encapsulation efficiency [E.E.]. In vitro release profiles and kinetics studies were performed. The release profiles were investigated by the measurement of the riboflavin concentration in the release medium at various time intervals. It has been found, that the matrix degradation and riboflavin release profiles were high in case of (1:1 PLLA: Cs ratio) rather than 2:1 ratio and pure PLLA. The highest encapsulation efficiency E.E. was obtained in case of 1:1 PLLA:Cs blend (99%).

**Keywords:** PLLA, Chitosan, Biodegradation, Riboflavin, blend, and Microcapsules.

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Received 1 March 2017, Accepted 17 March 2017

Please cite this article as: Adawy Sh *et al.*, Sustained Release of Riboflavin via Microencapsulation using Polylactic acid/Chitosan Blend. American Journal of PharmTech Research 2017.

# INTRODUCTION

PLA has been blended with many other polymers to tailor its biodegradation as well as its mechanical properties[1]. Gajria et al [2] examined the effect of blending PLA with poly (sebacic anhydride) (PSA) or polymandelic acid (PMA) for controlled drug delivery systems. The release of drugs on tested blends of PLA and PSA increased by 50% [2]. Further studies have investigated the miscibility and biodegradability of blends of PLA with polyvinyl acetate [3,4], and PLA with polyethylene-co-vinyl acetate [4] and PLA with chitosan [5]. These studies found that PLA and PVA are miscible by using DSC analysis. The blends exhibited a single Tg for all blend compositions. A similar study has been performed by blending poly D-lactic acid with poly ethylene/vinyl acetate for controlled drug release applications [4,6]. This experiment examined the processing history of the polymer blends as well as the degradation rate. Also many studies have been carried out for blending PLA with polyethylene oxide (PEO), polyethylene glycol (PEG), polycaprolactone (PCL), polyhydroxybutyrate (PHB) and (PHBV) to obtain biodegradable materials with flexible mechanical properties [6,7] and with polypropylene to obtain better resistance to biodegradation [8] . The ultimate goal of the present study is to improve the release behavior of the prepared polymer through blending with Cs and evaluate via microencapsulation of RF as model drug.

# MATERIALS AND METHOD

## Materials

L(+)lactic acid (assay 79-81% lactic in water) from FLUKA (Germany), zinc oxide from Dr. Paul Lohmann GMBH KG, Riboflavin USP from BASF(Germany), Polyvinyl alcohol (PVA) (Medium Viscosity from 40 to50 mpa's per 4% w/w solution) with molecular weight 130,000 from CHANG CHUN PETROCHEMICAL CO., LTD. (TAIWAN), Xylene from MERCK (Germany), Chloroform, Diethyl ether, absolute ethyl alcohol, and Methylene chloride from El-Nasr pharmaceutical Co. for Chemicals (Egypt).

## Equipment

Dean & Starck apparatus equipped with round bottomed flask, condenser, and measuring cylinder. Filtration unit with Millipore type 0.45 µm HV Model DURAPORE MEMBRANE FILTERS and vacuum pump. Rotatory evaporator Model Heidolph WBECO (Germany), vacuum oven (L O. Vacuum-1 Model HERAEUS) (Germany). Homogenizer Model ULTRA-TURRAX T25, mechanical stirrer Model Heidolph (Germany), sonication apparatus Model JP Selecta (Spain), centrifugation apparatus Model Meditronic Selecta (Spain), rotatory bottle apparatus Model Varian (USA), Automatic polarimeter Model ATAGO AP-300(England), UV/Vis.

Spectrophetometer Model PERKIN ELMER Precisely Lambda 35(USA), FTIR Spectrometer Model PERKIN ELMER (USA), oven Model HERAEUS (Germany), Scanning electron microscope (JEOL – JSM-T 330A) and GPC Agilent 1100 series for molecular weight measurements, Germany, Detector: Refractive Index plgel particle size (5µm), pore type (100, 104, 105 A°) on series, length 7.5x300 mm (1000, 5000000), and TEM JEOL (JEM-1400 TEM) at the candidate magnification. Images were captured by CCD camera model AMT, optronics camera with 1632 x 1632 pixel formate as side mount configuration. This camera uses a 1394 fire wire boared for acquision.

**Methods**

**Preparation of poly L(+)lactic acid[9,10]:**

L(+)lactic acid (11.26 gm) were mixed with 30 ml of xylene and (0.02gm) zinc oxide for each experiment in a round bottomed flask fitted with Dean&Stark apparatus. The reaction mixture was subjected to reflux for about 10 hr or more until all the estimated water was separated from the reaction medium. The polymeric adduct [PLLA] was washed and purified. Polylactic acid product was purified by dissolving in chloroform and filtering to isolate catalyst and precipitating by diethyl ether using separating funnel, remnants of chloroform and diethyl ether were evaporated from polylactic acid by rotatory evaporator. The polymer was washed by absolute ethyl alcohol and dried in vacuum oven at 50º C.

**Preparation RF-PLLA microcapsules [11]:**

Polymer [PLLA] was dissolved in methylene chloride [$CH2Cl_2$] by sonication; polyvinyl alcohol [PVA] was dissolved in distilled water and added to polymer solution. Riboflavin [RF] was added to the polymer/PVA mixture. This solution was stirred at 700 rpm and the temperature was maintained at 25ºC and poured into 40 ml distilled water at 70º C with stirring. Stirring was continued for 5 minutes. The temperature was rapidly raised between (40-45ºC) resulting rapid solvent evaporation and consequent formation of microcapsules. This mixture was allowed to settle in a water bath maintained at 40°C for 15 minutes, cooled to room temperature and the supernatant water was discarded. The microcapsules were further washed with distilled water several times and freeze dried.

**Preparation of RF-PLLA.Cs microcapsules:**

RF microcapsules were prepared using solution casting technique. Cs was dissolved in the least amount of 1% acetic acid and mixed with PLLA solution in acetone. Slight stirring was used to expedite the dissolution and to homogenize the solutions for 20 minutes. RF was added during homogenization. Salting out of polymers was carried out by adding an equal amount v/v to 1%

acetic acid of 4% Sodium lauryl sulfate at 50° C. three formula were prepared via this method as shown in table (1).

**Table 1: Riboflavin microcapsules prepared via o/w emulsion solvent evaporation and solution casting technique.**

| Formula | RF (gm) | PLLA(gm) | Cs(gm) |
|---|---|---|---|
| F1 | 0.500 | 1.0 | 0.500 |
| F2 | 2.0 | 1.0 | 1.0 |
| F3 | 0.250 | 0.250 | ---- |

## RESULTS AND DISCUSSION

### Preparation of poly L(+)lactic acid

Poly L(+)lactic acid was prepared by the condensation reaction of L(+)lactic acid in presence of zinc oxide as catalyst using Dean&Starck apparatus to separate water from the reaction medium. The yield percentage of the polymerization reaction was over 90%. Poly L(+)lactic acid was prepared and characterized via FTIR, XRD, GPC and acid value. Figure (1) show the mechanism of polymerization using direct polycondensation of L(+)lactic acid. Figure (2) and Table (2) show the characteristic FTIR bands for PLLA. Acid value of PLLA was 10.12[12] and average Molecular weight of 6000 g/mole and polydispersity of 1.84 $M_w/M_n$.

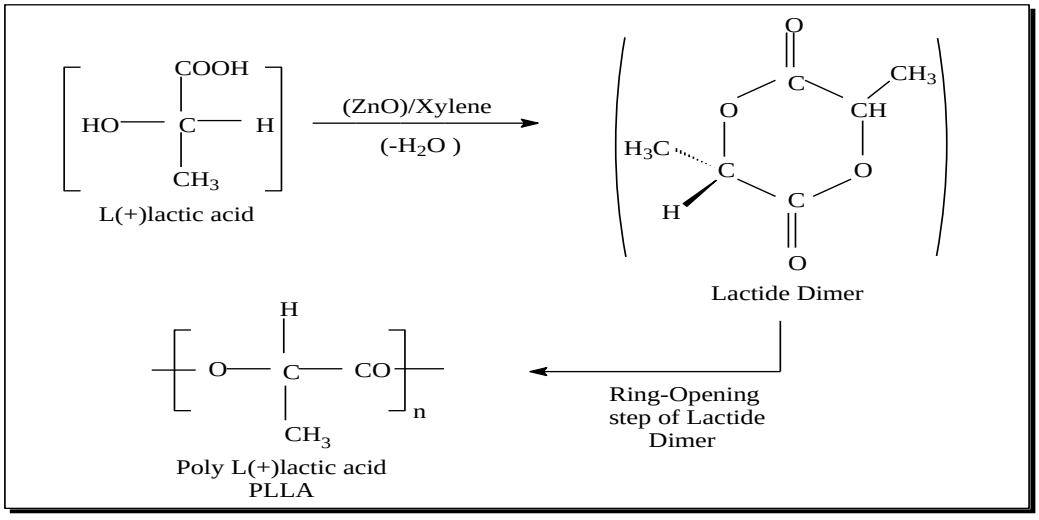


**Figure 1: Mechanism of synthesis of poly L(+)lactic acid [PLLA].**

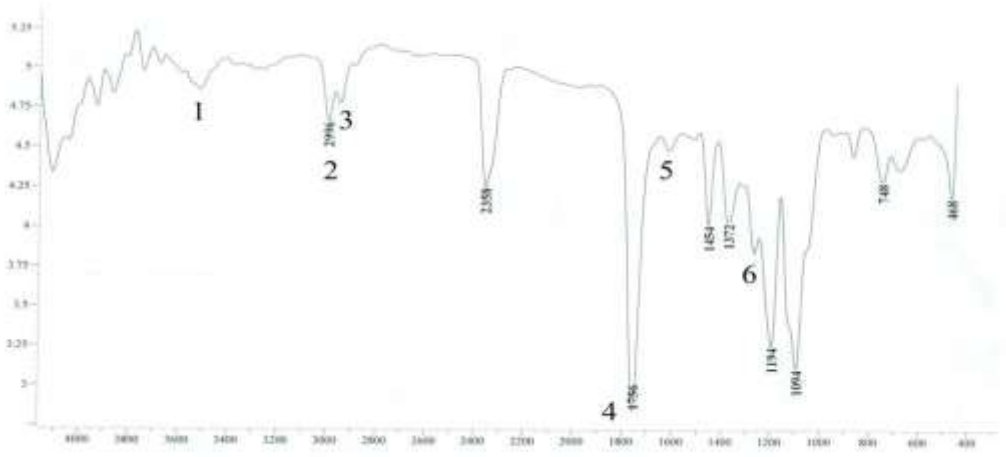


**Figure 2: FTIR spectra of PLLA in chloroform.**

**Table 2: Characteristic FTIR bands of poly [L(+)lactic-acid]**

| Bands No. | $Cm^{-1}$ | Intensity | Assignments |
|---|---|---|---|
| 1 | 3512 | Broad | OH of COOH |
| 1 | 3487 | Broad | OH of COOH |
| 4 | 1756 | Very strong | C=O of COO |
| 4 | 1754 | Very strong | C=O of COO |
| 2 | 2996 | Medium | CH of $CH_3$ |
| 2 | 2994 | Medium | CH of $CH_3$ |
| 3 | 2946 | Medium | CH |
| 3 | 2949 | Medium | CH OF $CH_2$ |
| 5 | 1647 | Medium | COO |
| 5 | 1650 | Medium | COO |
| 6 | 1268 | Medium | C—O |
| 6 | 1272 | Medium | C—O |

The prepared RF microcapsules were characterized in various methods in terms of morphology and entrapment efficiency (E.E.)[13]. Different microscopic pictures were taken during microencapsulation and after microencapsulation as shown in figure (3 a, b). Also SEM, TEM of RF microcapsules were performed as shown in figure (3 c, d and e). Entrapment efficiency of RF microcapsules was 99.0%, 96.0% and 94.5% for F1, F2 and F3 respectively [13].

**FTIR of RF microcapsules:**

Before studying the performance of the prepared RF microcapsules, we should study if there is chemical interaction between polymer and RF. The compatibility of the prepared microcapsules

was evaluated verses physical mixtures and RF alone via FTIR analyses. It was found that; there is no shift in characteristic bands of drug and polymers as seen in figure (4).

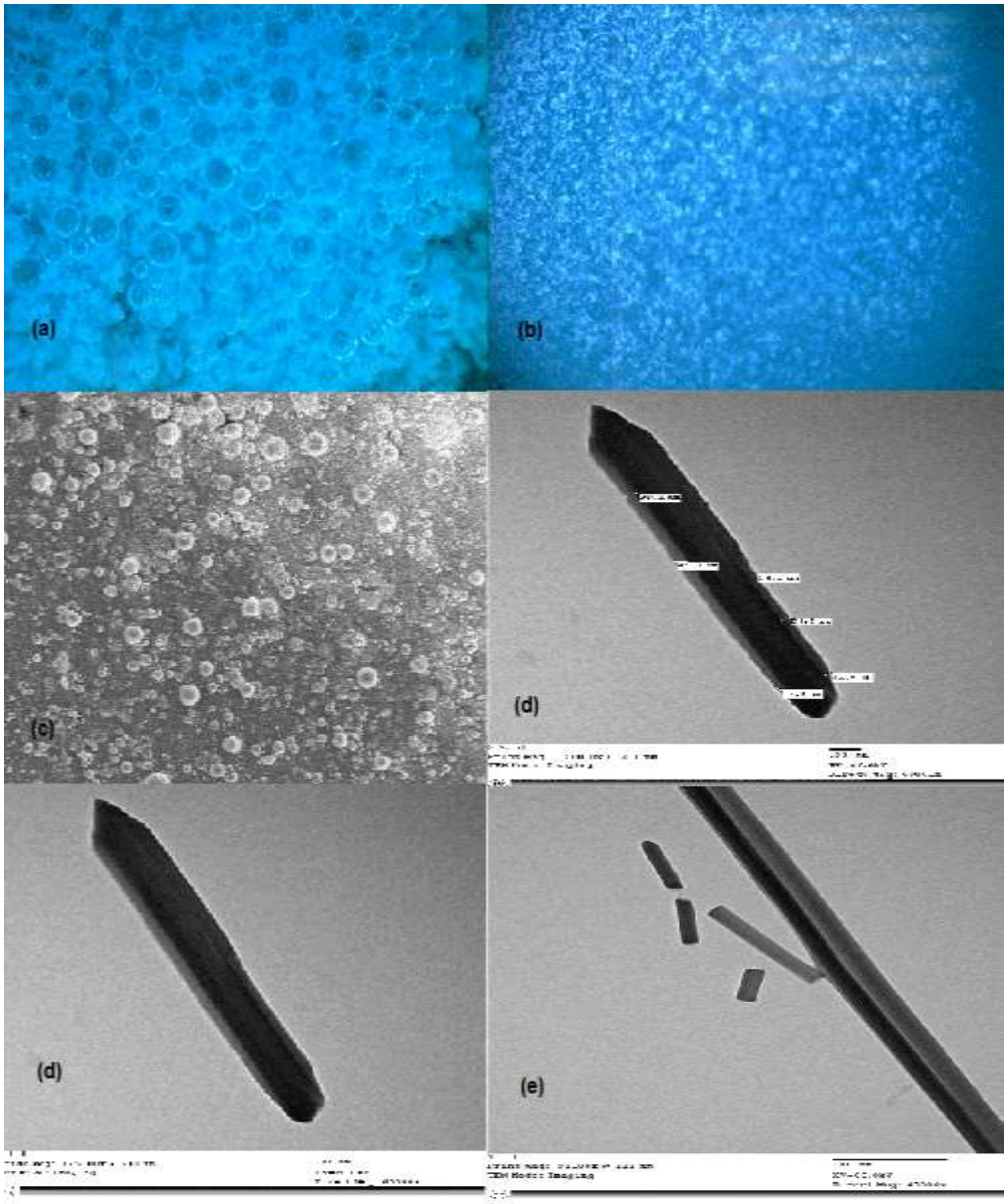


**Figure 3: (a) Microscopic picture during step coacervation of polymer droplets on RF core (Before evaporation of solvent), (b) after complete evaporation of solvent, (c) Scanning electron micrographs of RF microcapsules prepared with O/W method. (d, e) TEM of RF microcapsules.**

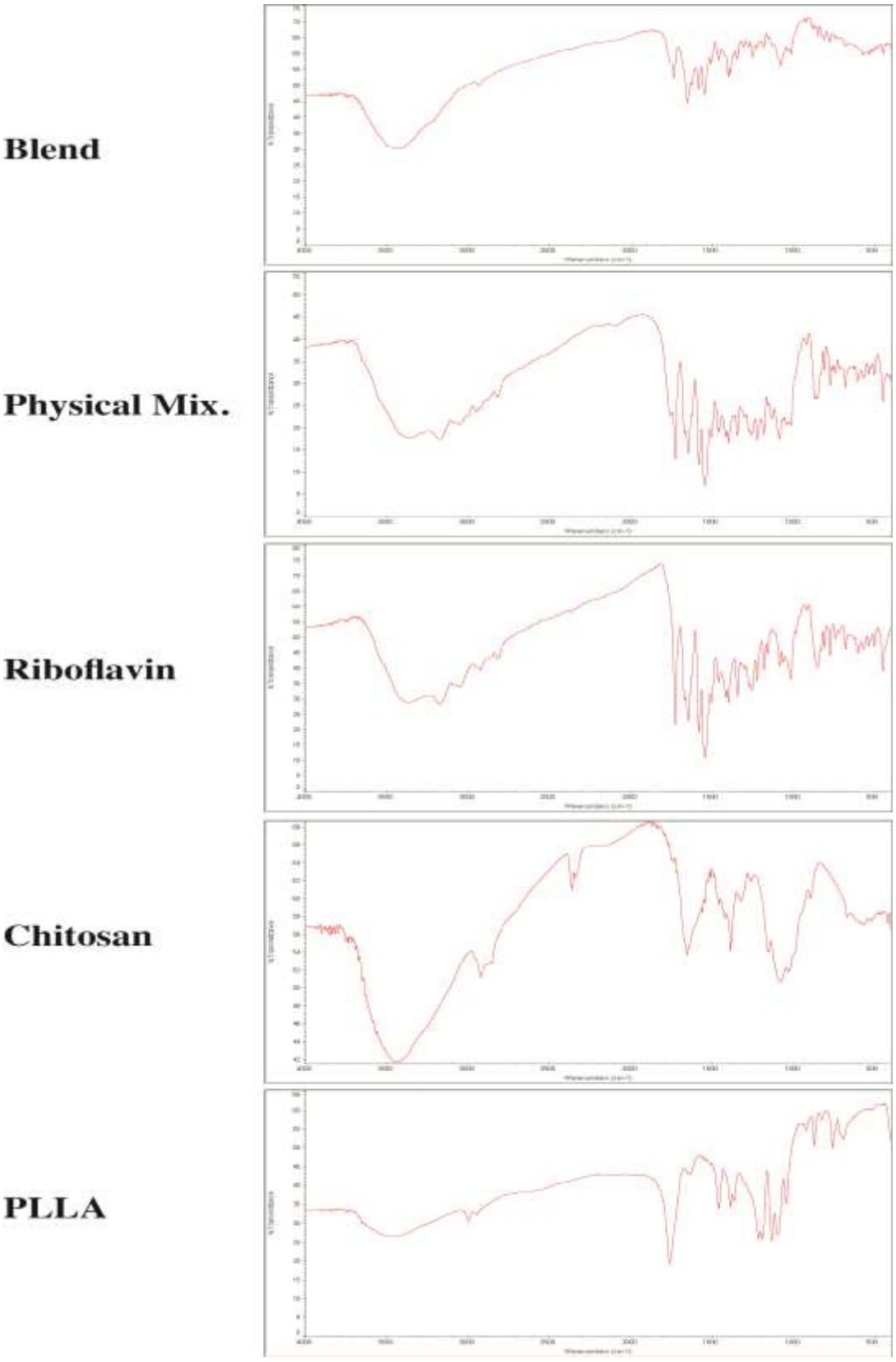


**Figure 4: FTIR spectra of PLLA, Cs, RF and their physical mixture and the prepared microcapsules (Blend).**

**In vitro RF release from its microcapsules in distilled water:**

Samples of prepared riboflavin microcapsules were placed in dissolution bottles of rotatory bottle apparatus (50ml distilled water at 37±0.5° C and maintained at 44 rpm for 48.00 hours). Riboflavin released from microcapsules into distilled water was determined from the measurement of absorbance at 270 nm [9] and a standard curve in distilled water.

Figure (5) show the percent release of riboflavin samples from microcapsules against riboflavin polymer free at the same time. These results are the mean of three experiments. The riboflavin release profiles from microcapsules consist of a burst release followed by a gradual release phase over the 2 days study period. The extent of the riboflavin burst release of all samples of the microcapsules at the initial phase is about 54%, 59% and 50 for F1, F2 and F3 respectively.

The gradual release rate of riboflavin from the microcapsules prepared by PLLA.Cs (1:1) and (2:1) is greater than those prepared by PLLA, this due to Cs which less hydrophobic than [PLLA][13,14] which increase the diffusion of the release medium and erosion of the polymer wall. The cumulative riboflavin release from these microcapsule formulations at the end of 2 days was 94 %, 100% and 74% for F1, F2 and F3 of the initial riboflavin loading. It was concluded that the riboflavin burst release could be controlled and sustained. This means that, a gradual release could be obtained by the biodegradable microcapsule system.

The release involved two different mechanisms: diffusion of riboflavin molecules and degradation of the polymer matrix. The burst release of the riboflavin is associated with those riboflavin molecules dispersing close to the microcapsule surface, which diffuses out in the initial incubation time. The diffusion rate was greater incase of PLLA.Cs blend than of pure PLLA that's due to the hydrophilic groups in Cs. In gradual release stage the release of PLLA.Cs blends was greater than pure PLLA that's because the fastest the degradation of Cs compared to PLLA [14].

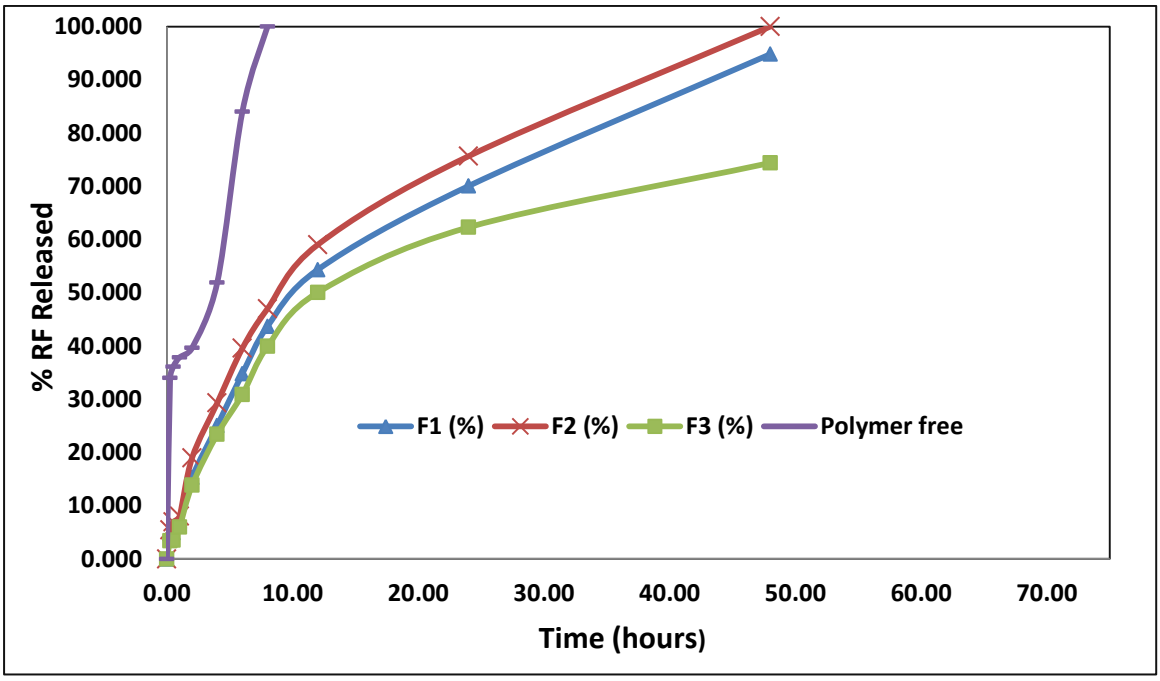


**Figure 5: In vitro RF Release in distilled water; microcapsules (F1, F2 and F3) verses RF-polymer free.**

## CONCLUSION

In the present study; we prepare PLLA in a novel, easy, simple and economic pathway via direct polycondensation reaction. The prepared polymer exceeds the characterization analyses. RF was chosen as a model drug to evaluate the prepared polymer. Microcapsules loaded PLLA pass through two stages; burst release followed by gradual release. Burst release can be controlled by increasing polymer: dug ratio and by blending with more hydrophilic polymer than PLLA as shown in the present study. The E.E. was improved by blending with Cs. The degradation rate of Cs was greater than of PLLA due the presence of primary amine in Cs chain.

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