Paclitaxel delivery by micro/nano encapsulation using layer-by- layer assembly

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Introduction

Recently an extensive effort has been made in the development of smart, functional, organized delivery system by layer-by-layer (LBL) self-assembling technique for micro/nano-encapsulation of bioactives, Caruso [2001]; Decher and Schlenoff [2003]. The encapsulation of bioactive materials into porous microparticles of inorganic origin have a great potential to allocate the drug in their nanopores (nanoreservoir) and have features to impart biological stability along with sustained release properties. We have made an attempt to develop a novel formulation of paclitaxel (PTX) by providing multilayer assembly over drug loaded porous $CaCO_3$ microparticles ($CaCO_3$ MP) by using combination of biocompatible and biodegradable polyelectrolytes (PE's). There has been a paradigm shift in the delivery of PTX and research is being focused to eliminate intrinsic problem associated with drug itself and toxicity associated with excipients used in the existing formulation.

Materials and methods

Sodium poly (styrene sulfonate) (PSS, Aldrich, Mwt. 70,000), protamine sulfate (PRM, Sigma), Sodium alginate (SA, Sigma,) paclitaxel (PTX, Dabur Research Foundation, India), $CaCl_2 \cdot 2H_2O$ and Na_2CO_3 (Hi media, Mumbai) were used without further purification. The water used throughout the experiment was purified with a Milli Q system from Millipore Co., USA.

Preparation of PTX loaded $CaCO_3$ MP and multilayer assembly

Porous and monodisperse core $CaCO_3$ MP were prepared using co-precipitation technique by mixing equimolar concentration of calcium chloride dihydrate and sodium carbonate containing appropriate concentration of PSS. PTX was loaded into these porous $CaCO_3$ MP by incubating ethanolic solution of PTX. The PTX loaded $CaCO_3$ MP ($CaCO_3$-PTX) was collected by repeated washing with ethanol and centrifugation. The multilayer assembly over $CaCO_3$-PTX was effectuated by alternate deposition of protamine sulfate (PRM) and sodium alginate (SA) using LBL technique $CaCO_3$-PTX- $(PRM/SA)_5$ followed by subsequent core removal PTX- $(PRM/SA)_5$.

Characterization

Powder X-ray diffraction (XRD) patterns of porous $CaCO_3$ MP, PTX and $CaCO_3$-PTX were recorded using a Rigaku D/max- 3A instrument (monochromatic Cu-K radiation).Typically; the diffractogram was recorded in a 2θ range of 5–25 °C. The morphology of the prepared $CaCO_3$ MP and $CaCO_3$-PTX- $(PRM/SA)_5$ were examined on Gemini Leo VP 435 scanning electron microscope (SEM) operated at 3 KeV. Infrared (IR) spectra of porous $CaCO_3$ MP, PTX and $CaCO_3$-PTX were measured with Perkin Elmer on carefully dried samples embedded in KBr pellets. Thermo gravimetric analysis (TGA) curves of porous $CaCO_3$ MP, PTX and $CaCO_3$-PTX were collected with a thermo analyzer (Diamond TG/DTA, Perkin Elmer, Germany) within a temperature range of 30–900 °C and with the rate of increasing temperature of 10 °C /min. TGA has also been used to ensure core removal in composite system ($CaCO_3$-PTX- $(PRM/SA)_5$ according to protocol described previously. Layer-by-layer growth was determine by the ζ-potential of each adsorbing layer on the $CaCO_3$-PTX dispersed in milli Q water was using Zetasizer Nano ZS (Malvern zetasizer, 3000 HS). The ζ-potential value was the average of three successive measurements. The pay load efficiency was determined by removing free PTX by extensive washing of $CaCO_3$-PTX

and subsequently sonicating at 20% amplitude for 1 min. Briefly, the dried $CaCO_3$-PTX was sonicated with ethanol and PTX was extracted from $CaCO_3$-PTX. The preparation was centrifuged at 5000 rpm for 5 min and the PTX concentration in the supernatant was determined with RP-HPLC using UV detector at 225 nm as method reported by Wang *et al.* [2004] with slight modification. The release profile of $CaCO_3$-PTX, PTX- $(PRM/SA)_5$ and marketed formulation of PTX (PTX-M) was determined in simulated intestinal fluid (SIF pH=7.4) using dialysis membrane (Cut off. Mwt. 12,000, by Sigma, USA).

Results and Discussion

A novel microencapsulation technology based on layer-by-layer assembly of oppositely charged PE's has been established to form supramolecular multilayer assemblages of PE's using biofriendly core particles of inorganic origin prepared by co-precipitation method. Addition of PSS often plays a crucial role to avoid polydispersity and aggregation of porous $CaCO_3$ MP and moreover helps to prevent any polymorphic changes occurred during 6 months of storage in water. The diffraction peaks observed in case of PTX is almost absent in XRD of $CaCO_3$-PTX appearing at low 2θ as shown in fig 1. Similarly the diffraction pattern of $CaCO_3$ MP has been found to be similar to $CaCO_3$-PTX. This indicates that PTX encapsulated/adsorbed into the nanopores of $CaCO_3$ MP in amorphous state without crystallization, otherwise the diffraction peaks of PTX would have been observed as Charnay *et al*. [2004] reported similar observation with ibuprofen. The IR spectrum of the $CaCO_3$-PTX reflects the characteristic absorption bands of PTX and $CaCO_3$ without obvious new bands in fig 2, which indicates that PTX encapsulated/adsorbed in nanopores of $CaCO_3$ is of purely physical in nature as supported by the study of Wang *et al.* [2006].

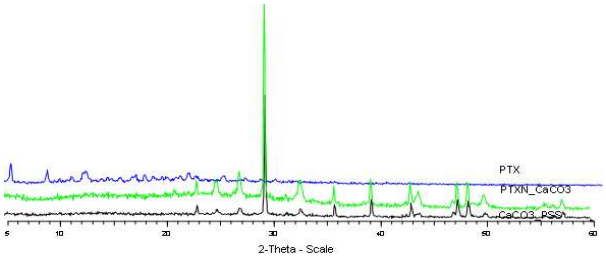

Fig. 1. XRD comparison chart between PTX $CaCO_3$-PTX, and $CaCO_3$ MP.

Fig. 2. IR spectra of A. $CaCO_3$ MP B. PTX and C. $CaCO_3$-PTX.

$CaCO_3$-PTX was found to be highly stabilized against thermal decomposition as evidenced by thermo gravimetric analysis (TGA, TG/DTA, Perkin Elmer, Germany) indicating decomposition at 600°C and 250°C for $CaCO_3$-PTX and PTX respectively as represented in fig 3. The increase in thermal decomposition temperature of PTX by 2-3 times due to entrapment in $CaCO_3$ MP indicates that some interaction would have occurred between PTX and $CaCO_3$, which may be attributed to the formation of ceramics, since mass transfer of heat is uniform throughout the MP as reported by Wang et. al [2006]. Core removal study by TGA indicates that thermal decomposition temperature remained higher for $CaCO_3$-PTX $(PRM/SA)_5$ compared to PTX $(PRM/SA)_5$ shells as shown in fig 4. Had the $CaCO_3$ remained in PTX $(PRM/SA)_5$ composite particle the decomposition temperature would have been higher. The mean particle size & size distribution of $CaCO_3$ MP and $CaCO_3$-PTX $(PRM/SA)_5$ were determined by a Malvern Zetasizer NanoZS (Malvern 3000HS, France). The data has been represented in fig.5A and B. It reveals that the all the microparticles and fabricated systems are in the range of 3-5 μ and distribution is monodisperse.

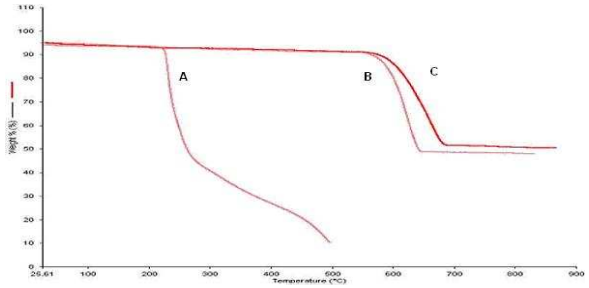

Fig. 3. TGA curve of A. PTX; B. $CaCO_3$-PTX, and C. $CaCO_3$ MP.

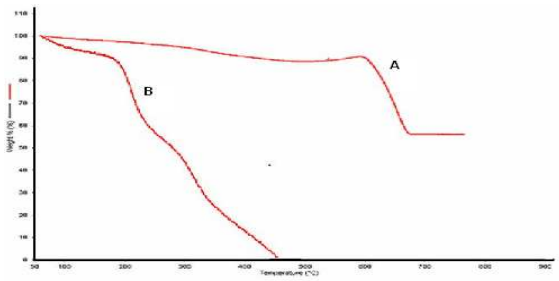

Fig. 4. TGA curve of A. $CaCO_3$-PTX $(PRM/SA)_5$, and PTX $(PRM/SA)_5$

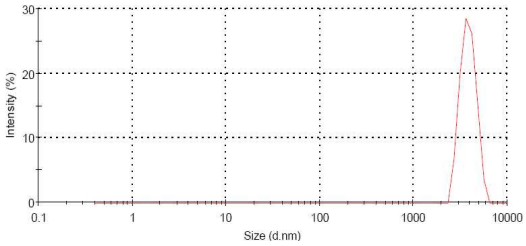

Fig. 5A. Mean particle size & size distribution of $CaCO_3$ MP (Average (d.nm):3558)

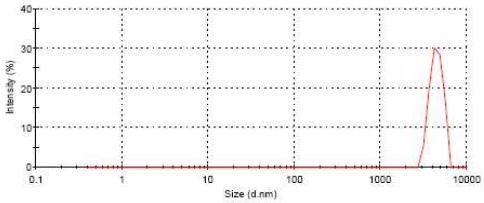

Fig. 5B. Mean particle size & size distribution of $CaCO_3$-PTX $(PRM/SA)_5$ (Average (d.nm):4.110)

To verify the surface morphology, scanning electron microscopy (SEM) was performed using Gemini Leo VP 435 electron microscope. It is evident that, a thick capsule shell has been formed due to presence of inner, cross-linked alginate layer. It is known that the PE's multilayer assembled by the LbL technique offer rough morphology. The SEM image reveals that $CaCO_3$ MP are perfectly spherical building blocks having dense nanopores with a typical diameter of approx 50–100 nm (qualitatively on the basis of observation) as shown in fig 6A. Fluffy projected impression over the surface of $CaCO_3$-PTX $(PRM/SA)_5$ is ascribed to PE's assembly over core particle as shown in fig 6B. The amount of PTX encapsulated has been expressed as % PTX /g $CaCO_3$. The maximum drug loading was found to be 78.98±2.14%. LBL growth was confirmed by successful recharging of the particle surface with each deposition cycle. Stepwise PE's assembly onto these initially negatively charged particles has been monitored by ζ-potential reversal data as shown in fig. 7. The zeta potential of capsules varied between -26.75 mV and +11.04 mV having final coating of SA and PRM respectively.

Fig. 6A. SEM image of porous $CaCO_3$ MP. Scale bar 1µm.

Fig. 6B. SEM image of fabricated $CaCO_3$-PTX $(PRM/SA)_5$. Scale bar 1µm.

The release data of PTX-$(PRM/SA)_5$ was comparable with marketed formulation of PTX (PTX-M) and $CaCO_3$-PTX when performed in simulated intestinal fluid (SIF pH=7.4). The release profile of PTX-(PRM/SA)5 indicates that PEs based multilayer matrix is capable to provide barrier to PTX release as it has been found to follow first order matrix diffusion kinetics (r^2= 0.9973) with 72±4.8% release within 24 hrs. The $t_{50\%}$ of PTX-M, $CaCO_3$-PTX and PTX-$(PRM/SA)_5$ was found to be 70, 90 and 480 minutes respectively. Observed data was shown in fig. 8.

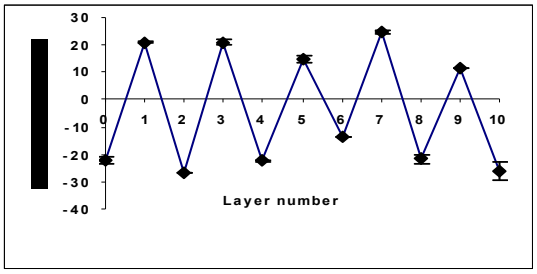

Fig. 7. Zeta potential study at different layering.

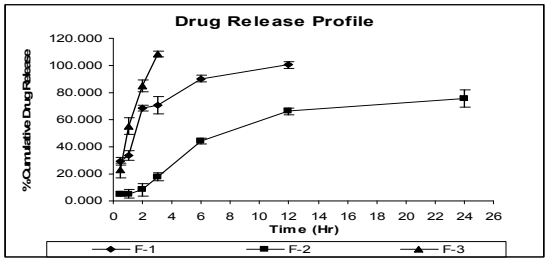

Fig. 8. Drug release profile of formulations in SIF (pH=7.4): F-1:$CaCO_3$-PTX; F-2: PTX $(PRM/SA)_5$ and F-3: PTX-M.

Conclusions

This alternative delivery system of PTX disguised in the form of LBL assembly could have immense application for the treatment of metastasized mammary glands vis-à-vis existing formulation of PTX which is by and large criticized for having certain toxic excipients to be given parentrally. Moreover, the proposed system provides ample of opportunity to modify the surface for targeted application of PTX. Further investigations are still underway to gather toxicity profile of the proposed formulation.

Acknowledgements

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