Enhancement of vaginal penetration of celecoxib via formulation of chitosan-coated polymeric nanocapsules

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BACKGROUND

Vaginal administration of drugs suffers from many limitations, e.g., the short contact time of the drug with the mucosa or continuous carrier wash-out. Thus, the development of new carriers for gynecological use is necessary. Celecoxib, a selective cyclo-oxygenase-2 inhibitor, has been administered orally as an anti-inflammatory drug. It is a poorly water-soluble drug with oral bioavailability of around 40%. Besides, long-term oral administration of celecoxib produces gastrointestinal side effects. The hypothesis of the present work was to augment the permeation of celecoxib through the vaginal mucosa via its incorporation within chitosan-coated polymeric nanocapsules formulation.

METHODS

1- Fabrication of Celecoxib-loaded polymeric nanocapsules

Celecoxib-loaded polymeric nanocapsules were prepared using nanoprecipitation technique. Briefly, celecoxib (20 mg), lecithin, oleic acid and CTAB, were dissolved in the internal organic phase. The internal organic phase consisted of ethanol and acetone at ratio of 1.5:1. The aqueous phase (20 ml) containing hyaluronic acid polymer and a hydrophilic surfactant tween 40 was then added dropwise using a syringe to 10 ml organic phase. The prepared nanocapsules were then kept for one hour under magnetic stirring at room temperature to ensure evaporation of organic solvents. The prepared nanocapsules were stored at temperature 4–8 °C for 24 hr. The chitosan solution used for optimized nanocapsule coating was prepared in 1% (v/v) glacial acetic acid. The chitosan solution was added dropwise to an equal volume of nanocapsule dispersion, under controlled magnetic stirring at room temperature for 1 h, followed by probe sonication for 10 minutes (pulse 2 sec on, 2 sec off), then incubation in the refrigerator overnight.

2- Characterization of Celecoxib-loaded polymeric nanocapsules

2.1. Encapsulation Efficiency.
The encapsulation efficiency (EE%) was calculated indirectly by measuring unincorporated celecoxib spectrophotometrically using (Shimadzu, model UV-1601 PC, Kyoto, Japan), at λ = 255 nm, after cooling centrifugation of nanocapsules suspension at (15000 rpm, 4 °C) for 60 min.

2.2. Particle Size and Zeta Potential.
The particle size distribution was determined at 25 °C by dynamic laser light scattering (DLS) technique using the Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK), equipped with a 4mW helium/neon laser operating at (λ = 633 nm) and a thermoelectric temperature controller. The zeta potential of nanocapsules suspension was measured using electrophoretic mobility data. Measurements were performed in triplicate.

2.3. Morphology.
Optimized Celecoxib-loaded polymeric nanocapsules and Chitosan coated celecoxib nanocapsules dispersion were imaged using a scanning electron microscope (SEM, JSM-5400 LV, JEOL, Tokyo, Japan).

2.4. In vitro drug release study.
In vitro drug release from the optimal celecoxib-loaded polymeric nanocapsules and chitosan coated celecoxib nanocapsules compared to free drug dispersion was studied. The tested formulations were placed over a previously soaked cellulose membrane (molecular weight cutoff 12,000–14,000) fitted at the lower end of a glass cylinder. The glass cylinder was then dipped in a beaker containing phosphate buffer (pH 5.5, 50 mL, with 1% propylene glycol) at 37 ± 0.5 ◦C, 50 rpm using a thermostatically controlled water bath. Aliquots were removed and substituted with a fresh medium. The drug content was estimated for 24 h.

2.5. Ex vivo vaginal permeation studies.
Fresh vaginal tissues were carefully removed from rabbits. The vaginal membrane was mounted at the lower end of a glass cylinder, dipped in a beaker containing phosphate buffer (pH 5.5, 50 mL, with 1% propylene glycol). The donor cell filled with celecoxib-loaded polymeric nanocapsules, chitosan coated celecoxib nanocapsules, or free drug dispersion. The diffusion cells were kept at water bath, 37 ± 0.5 ◦C, 50 rpm. At specified time intervals, aliquots were withdrawn and replaced with fresh media, and the amount of permeated drug was determined spectrophotometrically at λ = 255 nm.

RESULTS

Table 1. Experimental runs and their observed responses.

FORMULA CODE	Drug (mg)	Lecithin (% w/v)	Oleic acid (% w/v)	CTAB (mg)	Hyaluronic acid (mg)	Tween 40 (%w/v)	CS (%w/v)	Particle size (nm) (±SD)	PDI (±SD)	Zeta potential (mV) (±SD)	EE (%) (±SD)
F1	20	0.1	2.5	7.5	10	0.15	0	170.7 ± 0.634	0.347 ± 0.08	-54.5 ± 2.1	91.0 ± 1.74
F2	20	0.1	2.5	7.5	---	0.15	0.1	193 ± 2.1	0.265 ± 0.012	+32.8 ± 0.5	82.7 ± 0.2
F3	20	0.1	2.5	7.5	---	0.15	0.3	574.9 ± 13.06	0.572 ± 0.088	+34.56 ± 3.65	88.54 ± 0.18
F4	20	0.1	2.5	7.5	---	0.15	0.6	1387 ±27.87	0.692 ± 0.062	+67.7 ± 1.92	70.8 ±1.28

Hyaluronan nanocapsules and chitosan coated nanocapsules appeared as a homogenous dispersion of well-identified spherical particles and smooth surface with an obvious shell and core structure.

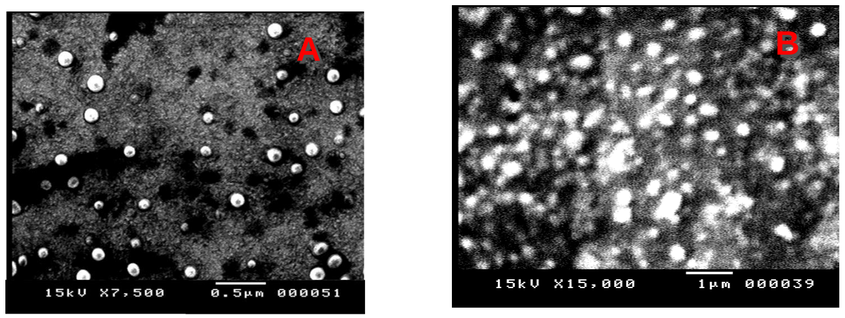

Fig.1. Representative SEM images of panel (A); un-coated celecoxib loaded hyaluronan nanocapsules (x7500), and panel (B); Optimized celecoxib loaded chitosan coated polymeric nanocapsule (× *15000*).

Free celecoxib dispersion showed in vitro drug release percentage of (83.4±0.04%), after 24 h. Hyaluronic nanocapsules showed (29.5±0.02%) drug release after 6 h, followed by a prolonged release of (62.7±0.02%), after 24 h. Chitosan coated nanocapsules showed (46.5±0.07%) after 6 h followed by a prolonged release of (76.8±0.02%), after 24 h.

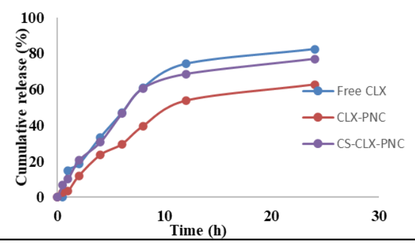

Fig.2. Cumulative in vitro release profiles of optimized celecoxib polymeric nanocapsules coated with chitosan compared to un-coated celecoxib loaded hyaluronan nanocapsules, and free celecoxib dispersion in phosphate buffer, pH 5.5, 1% propylene glycol at 37 °C (results presented as mean ± SD, n = 3).

RESULTS (continued)

- Chitosan coated nanocapsules and hyaluronic acid polymeric nanocapsules showed significantly higher permeability coefficients (p_{app}= 5.6±3.2, and 4.8 ±2.6 x10^{-6} cm/sec), respectively, compared to the control free drug dispersion (p_{app}= 1.3 ±0.08 x10^{-6} cm/sec)
- The chitosan coat and polymeric nanocapsules increase the mucoadhesion and the amount of lipophilic drug that penetrates into the vaginal tissue, leading to a greater effect compared with the free drug.

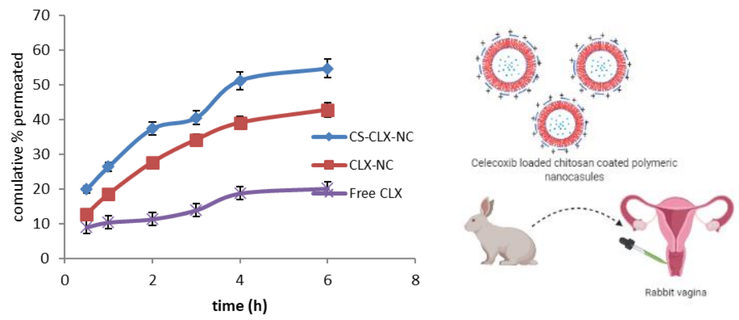

Fig.3. Ex vivo permeation profile of optimized celecoxib polymeric nanocapsules coated with chitosan compared to un-coated celecoxib loaded hyaluronic acid polymeric nanocapsules, and free celecoxib dispersion in phosphate buffer, pH 5.5, 1% propylene glycol at 37 °C (results presented as mean ± SD, n = 3).

CONCLUSION

- Formulations of celecoxib nanocapsules coated with chitosan showed high encapsulation efficiencies. The size and polydispersion of the particles were greater for NC coated with chitosan. The zeta potential of the formulation was shifted from negative to positive with the addition of chitosan, indicative of changes in the conformation of the polymeric wall. The release profile of celecoxib was altered by the presence of chitosan on the surface of the nanocapsules, compared to uncoated NC.
- The nanoencapsulation of a hydrophobic celecoxib in positively charged nanocapsules, could increase the penetration into the vaginal mucosa, improving the drug's efficacy. Moreover, an increase in penetration could also enable a reduction of dose.
- The developed chitosan-coated polymeric nanocapsules might provide a promising carrier for vaginal drug delivery and for improved control of inflammation.

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