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Poly(methyl vinyl ether-*co*-maleic acid) for enhancement of solubility, oral bioavailability and anti-osteoporotic effects of raloxifene hydrochloride

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ABSTRACT

Raloxifene HCl (RH) has poor water solubility and due to its extensive first pass metabolism; its bioavailability is only 2%. The purpose of the present study was to enhance the aqueous solubility, oral bioavailability and anti-osteoporotic effects of RH by electro-sprayed nanoparticles (NPs) in ovariectomized rats. NPs containing RH and different ratio of poly(methyl vinyl ether-*co*-maleic acid) (PMVEMA) were electrosprayed. The voltage, distance of needle to the collector, flow rate of the solution and polymeric percentage were optimized according to the size of NPs and drug solubility. The optimized formulation was characterized by SEM, XRD, DSC, and FTIR. The pharmacokinetic parameters were studies by oral administration of a single dose of 15 mg/kg in Wistar rats. The anti-osteoporotic effects were studied in female ovariectomized rats. Animals were treated with 6 mg/kg/day for 2 months then serum calcium, phosphorous and alkaline phosphatase levels were measured. RH loaded electrosprayed NPs showed 10-fold enhanced solubility compared to the free drug. Moreover, the XRD and SEM tests displayed an amorphous state of drug in the NPs. FTIR and DSC tests revealed no interaction between the polymer and the drug. Serum calcium, phosphorous and alkaline phosphatase levels were significantly decreased in ovariectomized rats receiving oral RH NPs ($P < 0.05$). No significant difference was detected between RH NPs and estradiol groups ($P > 0.05$). Oral bioavailability of NPs showed 7.5-fold increase compared to the pure drug. The electrosprayed PMVEMA nanoparticles can enhance solubility, bioavailability and antiosteoporotic effects of RH.

1. Introduction

Osteoporosis is a chronic world-wide disease and characterized by poor bone strength, low bone mass and micro-architectural deterioration of bone tissues (Roush, 2011). This is an age-related disease which occurs because of insufficient post-menopausal estrogen levels and misbalance between osteoblasts and osteoclasts (Sultan and Rao, 2011). Anti-resorptives are the major pharmacologic agents for osteoporosis which inhibit the development and action of osteoclasts (Jagadish et al., 2010). Numerous strategies such as hormone therapy, estrogen therapy, or a combination of estrogen and progesterone were frequently used in the past decades (Elsheikh et al., 2012). To date two major pharmacological approaches are reported for osteoporosis including anabolic agents such as parathyroid hormone which act via stimulating the bone formation process and administration of the anti-resorptive agents such as estrogen replacement therapy, calcitonin and RH which inhibits osteoclastic bone resorption (Silva and Bilezikian, 2011).

RH is the generic name for [6-hydroxy-2-(4-hydroxyphenyl) benzo-[*b*]thien-3-yl][4-[2-(1-piperidinyl)ethoxy]-phenyl]ethanone hydrochloride with a molecular weight of 510.05 g/mol (Bikiaris et al., 2009). It is a selective estrogen receptor modulator which is used for prevention and treatment of the osteoporosis (Tran et al., 2013). RH is also prescribed for breast cancer, prostate cancer, benign prostate hypertrophy and fibrocystic disease (Wempe et al., 2008). It belongs to class II of BCS (Biopharmaceutics Classification System) with poor water solubility due to its extensive first pass metabolism; the bioavailability of RH is only 2% (Shah et al., 2015). The solubility or dissolution of the drug in this category is therefore the rate-limiting step that determines the rate and extent of its absorption (Thakkar et al., 2011).

Approved dose of RH for oral administration for postmenopausal osteoporosis is 60 mg per day (Kushwaha et al., 2013). It is well documented there are cellular and molecular communications between estrogen and osteoblasts and osteoclasts activity (Uebelhart et al., 2009). Calciotropic and sex hormones are major regulators of bone growth, calcium and phosphate homeostasis (Uebelhart et al., 2009).

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Solubility of a drug is an important factor to increase its oral bioavailability (Patil et al., 2013). Enhancement of oral bioavailability of poorly water soluble drugs is the most challenging aspect of drug development (Patil et al., 2013). Scientists have enhanced the aqueous solubility and dissolution of the poorly water-soluble pharmaceutical agents by different nanotechnology techniques (Yousaf et al., 2016). Pharmaceutical NPs may be produced by polymeric compounds (Jia et al., 2011). Natural polymers are more preferable for NPs formation because of the effectiveness, biocompatibility and biodegradation characteristics (Reis et al., 2006). There are several methods to develop pharmaceutical NPs including solvent evaporation, emulsification, phase inversion, solvent displacement and spray drying (Yousaf et al., 2016). However, disadvantages such as low yield, chemical decomposition of the drug, stability problems and environmental problems are reported for solvents (Newa et al., 2008). One of the newly introduced methods in production of pharmaceutical NPs is electrospraying technique (Luo et al., 2011). In this method at first the drug and polymer are dissolved in a solvent and the obtained transparent solution is subjected to electrospraying (Luo et al., 2011) which provides spherical NPs containing evenly scattered drug molecules in the polymeric matrix (Jia et al., 2011). Poly(methyl vinyl ether-*co*-maleic acid) (PMVEMA) is a hydrophilic and biocompatible polymer with mucoadhesive advantageous properties (Shahbazi et al., 2014). Kerdsakundee et al. (2017) used this polymer to improve the oral bioavailability of curcumin as a low water soluble drug. They modified halloysite nanotubes (HNT) with PMVEMA to endow it with mucoadhesive function for promoting drug absorption, and solubility. Afterward, the curcumin-loaded HNT was encapsulated in hydroxypropyl methylcellulose acetate succinate, as a pH-responsive polymer. Their results showed that PMVEMA significantly enhanced the interactions of HNT with the intestinal Caco-2/HT29-MTX cells and the mouse small intestines, and increased the permeability of curcumin across the co-cultured Caco-2/HT29-MTX cell monolayers by about 13 times compared to the free curcumin (Kerdsakundee et al., 2017).

It is hypothesized that RH can be used as an effective drug against osteoporosis if its bioavailability is enhanced (Yousaf et al., 2016). Even though numerous researches was been done to enhance the solubility and bioavailability of RH, still it is required to design the new formulations to improve release and avoiding first pass metabolism of this drug. So, the purpose of the present study was to develop novel electrosprayed NPs of RH with enhanced aqueous solubility, bioavailability and anti-osteoporotic effects for oral delivery in ovariectomized rats. To our knowledge there is no report on the use of PMVEMA in enhancement of solubility of drugs with emphasis on RH.

2. Materials and methods

2.1. Materials

The Raloxifene hydrochloride powder was kindly gifted by Iran Hormone Research Laboratories (Tehran, Iran). Poly(methyl vinyl ether-*alt*-maleic anhydride) (PMVEMA) [Mw: 216,000, pH 2.5 in H_2O] was from Sigma Company (US). *N*,*N*-dimethyl formamide (DMF) and all other reagents and chemicals were of analytical grade and obtained from Merck Chemical Company (Germany).

2.2. Preparation of nanoparticles by electro-spraying technique

Four different variables of the electrospraying technique including; voltage (12–20 kV), the distance between the tip of the needle to the collector (12–20 cm), flow rate of the solution (0.3–1 ml/h) and the polymer concentration (12–17 g/100 ml) were studied according to the preliminary tests and a hybrid surface response design using Design Expert Software (Version 7.1, US) was applied to optimize the parameters of the electrospraying technique. Initially total amount of the polymer and drug were dissolved in DMF according to Table 1. For instance in formulation M1 seen in Table 1, for 1 ml of the solution 188.3 mg of the polymer and 6 mg of drug were dissolved in 1 ml of DMF.

Table 1
Different formulations of RH nanoparticles prepared by electrospraying technique in a hybrid design.

Formulation code	A: Voltage (kV)	B: Distance (cm)	C: Rate (ml/h)	D: Polymer concentration (g/100 ml)
M1	16.00	16.00	0.65	18.83
M2	16.00	16.00	0.65	13.83
M3	12.00	12.00	0.30	16.01
M4	20.00	12.00	0.30	16.01
M5	12.00	20.00	0.30	16.01
M6	20.00	20.00	0.30	16.01
M7	12.00	12.00	1.00	16.01
M8	20.00	12.00	1.00	16.01
M9	12.00	20.00	1.00	16.01
M10	20.00	20.00	1.00	16.01
M11	22.07	16.00	0.65	11.88
M12	9.93	16.00	0.65	11.88
M13	16.00	22.07	0.65	11.88
M14	16.00	9.93	0.65	11.88
M15	16.00	16.00	1.18	11.88
M16	16.00	16.00	0.12	11.88

The collector of the electrospraying device included a standard aluminum foil (17 × 21 cm^2) which was placed as a cathode. 1 ml of the clear solution of the polymer and the drug was filled in a syringe fitted by a stainless steel nozzle with 23 gauge which acted as an anode. The condition of electrospraying was set up as shown in Table 1 and two responses including; particle size and saturation solubility of the studied formulations were measured. The solutions were extruded by the nozzle using a syringe pump (WPI, USA). After electrospraying, the nanoparticles on the collectors were carefully gathered and then the dry powder products were transferred into microtubes for further studies. A typical schematic of the electrospraying setup which was used to produce nanoparticles is shown in Fig. 1.

2.3. Particle size analysis

One milligram of nanoparticles of each formulation was dispersed in 6 ml of ethyl acetate and the particle size and particle size distribution of the nanoparticles was measured by photon correlation spectroscopy (PCS) (Zetasizer, ZEN 3600, Malvern Instrument, UK). The nanoparticles size was gained via a He-Ne laser beam at 658 nm at a fixed

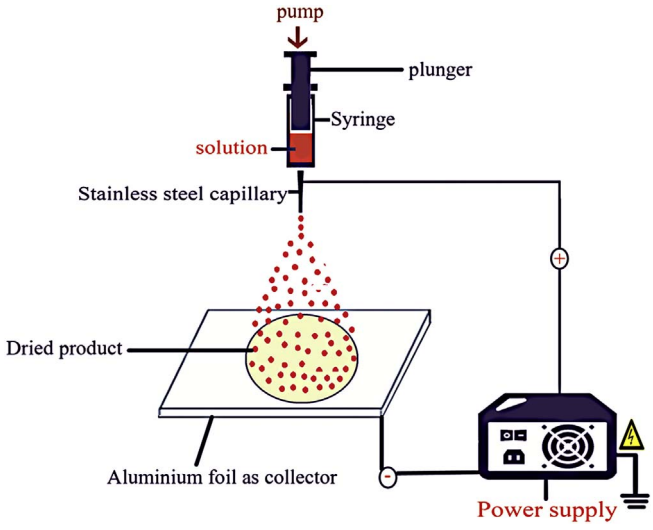

Fig. 1. Schematic representation of the electrospraying setup.