International Journal of Pharmaceutics 518 (2017) 50–58

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Hydrophilic drug encapsulation in shell-core microcarriers by two stage polyelectrolyte complexation method

Annalisa Dalmoro[a], Alexander Y. Sitenkov[b], Sara Cascone[c], Gaetano Lamberti[c], Anna Angela Barba[a,*], Rouslan I. Moustafine[b]

[a] Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, SA, Italy
[b] Department of Pharmaceutical, Analytical and Toxicological Chemistry, Kazan State Medical University, Butlerov Street 49, 420012 Kazan, Russian Federation
[c] Department of Industrial Engineering, Via Giovanni Paolo II, 132, 84084 Fisciano, SA, Italy

ARTICLE INFO

Article history:
Received 2 December 2016
Received in revised form 22 December 2016
Accepted 23 December 2016
Available online 26 December 2016

Chemical compounds studied in this article:
5-Fluorouracil (PubChem CID: 3385)
Sodium alginate (PubChem CID: 5102882)
Pluronic F127 (PubChem CID: 24751)
Calcium chloride (PubChem CID: 5284359)
Eudragit® E100 (PubChem CID: 107676)
Eudragit® RS100 (PubChem CID: 104931)
Eudragit® RL100 (PubChem CID: 104804)

Keywords:
Shell-core microparticles
5-Fluorouracil
Hydrophilic drug
Ultrasonic atomization
Polyelectrolyte complexation

ABSTRACT

In this study a protocol exploiting the combination of the ultrasonic atomization and the complexation between polyelectrolytes was developed to efficiently encapsulate a hydrophilic chemotherapeutic agent essentially used in the treatment of colon cancer, 5-fluorouracil, in enteric shell-core alginate-based microcarriers. The atomization assisted by ultrasound allowed to obtain small droplets by supplying low energy and avoiding drug degradation. In particular microcarriers were produced in a home-made apparatus where both the core (composed of alginate, drug, and Pluronic F127) and shell (composed of only alginate) feed were separately sent to the coaxial ultrasonic atomizer where they were nebulized and placed in contact with the complexation bulk. With the aim to obtain microstructured particles of alginate encapsulating 5-fluorouracil, different formulations of the first complexation bulk were tested; at last an emulsion made of a calcium chloride aqueous solution and dichloromethane allowed to reach an encapsulation efficiency of about 50%. This result can be considered very interesting considering that in literature similar techniques gave 5-fluorouracil encapsulation efficiencies of about 10%.

Since a single complexation stage was not able to assure microcarriers gastroresistance, the formulation of a second complexation bulk was evaluated. The solution of cationic and pH-insoluble Eudragit® RS 100 in dichloromethane was chosen as bulk of second-stage complexation obtaining good enteric properties of shell-core microcarriers, i.e. a 5-FU cumulative release at pH 1 (simulating gastric pH) lower than 35%. The formation of interpolyelectrolyte complex (IPEC) between countercharged polymers and the chemical stability of 5-FU in microcarriers were confirmed by FTIR analysis, the presence of an amorphous dispersion of 5-FU in prepared microparticles was also confirmed by DSC. Finally, shell-core enteric coated microcarriers encapsulating 5-fluorouracil were used to prepare tablets, which can be potentially used as oral administration dosage systems for their 5-fluorouracil slower release.

1. Introduction

Colorectal cancer is the third most common cancer in men and the second in women worldwide, occurring for almost 55% of the cases in more developed regions (Ferlay et al., 2015). The therapeutic strategies commonly used in the clinical practice for colorectal cancer treatment are surgery, radiation, biologic therapies (immunotherapy and hormonal therapy), and chemotherapy. In particular, cytotoxic drugs are very heterogeneous chemical compounds with severe side effects since they attack both healthy and target cells for the lack of specificity of their systemic bio-distribution, moreover their in-vivo degradation implies the necessity of high dose administration (Arias, 2008). Among chemotherapeutic compounds, 5-fluorouracil (5-FU) is one of the most widely used agents for the treatment of colorectal cancer. Chemically, 5-FU is a diprotic acid with pKa values of 8.0 and 13.0, highly polar in nature (Pendekal and Tegginamat, 2013). For its structural resemblance to natural pyrimidines, 5-FU interferes with nucleic acid synthesis, inhibits DNA synthesis, and eventually halts cell growth. The common method of administration of 5-FU is intravenously, that requires high doses (400 mg/(m^2 day) for 5 days by intravenous bolus injection) to

* Corresponding author.
E-mail address: aabarba@unisa.it (A.A. Barba).

http://dx.doi.org/10.1016/j.ijpharm.2016.12.056
0378-5173/

reach therapeutic drug levels (He et al., 2008), moreover it is characterized by severe toxic effects of gastrointestinal (vomiting, nausea, poor appetite), hematological, neural, cardiac, and dermatological type (Rahman et al., 2006). A site-specific delivery of 5-FU may reduce the systemic side effects and provide effective and safe therapy for colorectal cancer, thus a suitable carrier for 5-FU should be produced with particular properties: physical stability; small size to assure capillary distribution to the target; ability to encapsulate high amounts of drug by using biodegradable and biocompatible excipients and to control its release rate; ability to protect drug from degradation (Arias, 2008; Barba et al., 2014). For example, polymeric nanoparticles can penetrate pathological vasculature of an unhealthy tissue without being eliminated, taking advantage from passive targeting by enhanced permeability and retention (EPR) effect (Sayari et al., 2014). Another advantage deriving from active agent protection by a nanocarrier might be the reduction in the formation of cardiotoxic 5-FU degradation compounds in the basic medium of the injected vials (Arias, 2008). However, patients prefer oral rather than intravenous therapy, with oral treatment potentially more convenient and less costly (Rahman et al., 2006). Researcher are continuously working on ways to deliver the drug more effectively to the colon via the GI tract, where it can target the tumor tissues (Glavas Dodov et al., 2009), trying to: ensure the appropriate therapeutic dose at colon, avoid dose and activity loss of the drug during the GI transit, minimize its adverse side effects derived from absorption at non-specific tissue locations (Agarwal et al., 2015). Only few approaches for an oral administration of 5-FU have been described in literature. They were essentially focused on the use of natural polymers, such as alginate, chitosan, and pectins for their free availability, non-toxicity and biodegradability features. Matrix tablets of chitosan–sodium alginate interpolyelectrolyte complex encapsulating 5-FU were produced (Pendekal and Tegginamat, 2013), however this delivery system may be not susceptible to colonic anaerobic microflora for the high coat weight and the drug release may be incomplete when it is not readily disintegrated (He et al., 2008). Multiparticulate formulations, such as microvectors, present several advantages on tablets: less influence by food; more consistent absorption compared to single unit systems (Barba et al., 2013), avoided local drug concentration and lowered risk of toxicity by an uniform spread throughout the gastrointestinal tract (Dalmoro et al., 2010); possibility to insert them in capsules or smart enteric tablets in order to obtain a more controlled release (Dalmoro et al., 2016). Poly(acrylamide-methylmethacrylate) core-shell microspheres were produced by the free radical emulsion polymerization, by loading 5-FU during in situ polymerization or by absorption and adsorption technique, with an encapsulation efficiency of 80–85% (Babu et al., 2006). Glavas Dodov et al. (2009) efficiently produced lectin-conjugated chitosan–Ca–alginate microparticles loaded with acid-resistant hydroxyl propyl methyl cellulose particles of 5-fluorouracil (5-FU) by a spray-drying technique. More examples of enteric microspheres encapsulating 5-FU, produced by solvent evaporation/extraction from emulsion, were found in literature: Eudragit® P-4135F microspheres (Lamprecht et al., 2003), core microspheres of alginate coated with Eudragit® S-100 (Rahman et al., 2006), microvectors made of polymer complexes between chitosan and cellulose acetate phthalate (Thakker et al., 2014), with variable encapsulation efficiency in the range 20–100%. Despite the good encapsulation efficiencies of the above mentioned techniques, they present several drawbacks, such as the use of high amount of solvents, the possibility of drug degradation, the long process times and hardworking protocols. Ionic gelation is instead a solvent-free and low-temperature process, in particular alginates can form gels in presence of bivalent or polyvalent metal ions obtaining the "egg-box" structure, responsible for the entrapment of active principles, and their bioadhesive properties are useful for vehiculating drugs to mucosal tissues, such as the GI tract (Barba et al., 2015). Beads of alginate alone or together with other polymers, cross-linked with divalent ions, were produced with the aim to encapsulate 5-FU, but they presented very low encapsulation efficiencies and sometimes undesirable release properties (Agarwal et al., 2015; Arıca et al., 2002; Gupta and Aggarwal, 2007; Olukman and Solak, 2012; Yu et al., 2008) due to mainly 5-FU hydrophilic feature and small size.

In this work a protocol to efficiently encapsulate 5-fluorouracil in enteric shell-core alginate-based microparticles has been developed. The protocol was based on the coupling of the technique of ultrasonic atomization (for obtaining small droplets by low energy supply and avoided drug degradation) followed by ionic gelation and polyelectrolyte complexation stages to assure drug high encapsulation efficiency and to enhance the microparticles gastroresistance features. Microcarriers loaded with 5-fluorouracil have been then used to prepare tablets which can be potentially used as oral administration dosage systems.

2. Materials and methods

2.1. Materials

Manugel GHB sodium alginate, AL, (medium molecular weight, FMC Bio-polymers, Milan Italy) was used as encapsulating matrix for both core and shell solutions for its ability to dissolve at pH > 7 (ideal for enteric formulations). 5-Fluoruracil, 5-FU, (Sigma Aldrich, Milan Italy) was used as active ingredient to encapsulate. It is a hydrophilic and very small model molecule: solubility in water at 25 °C of 12 mg/ml, molecular mass of 130 g/mol and Stokes radius of 0.28 nm (www.chemspider.com; Sigma Aldrich srl data sheet). Pluronic F127, PF127 (Sigma Aldrich srl, Milan Italy) was used as alginate mesh size reducer to avoid an easier drug leakage during preparation.

Emulsions constitute by water solutions of calcium chloride ($CaCl_2$) and dichloromethane (both Sigma Aldrich srl, Milan Italy) were selected as cross-linker for the alginate chains reticulation (or first complexation of alginate). Moreover, for other formulations of the first complexation bulk, ethanol and 2-propanol (Sigma Aldrich srl, Milan Italy) were used as solvents.

The cationic Eudragit® E100 (E 100) was used as polycation for complexing with the anionic alginate in the first complexing bulk. Dichloromethane solutions of the cationic Eudragit® RS100, E RS 100, and Eudragit® RL100, E RL 100, were as used as complexing agent for alginate in the second complexation step. All used Eudragits® were kindly donated by Rofarma Italia (Milan Italy) and utilized as received.

The solutions at different pH values, simulating the gastrointestinal or physiological conditions, were prepared for release tests by using hydrochloric acid, sodium phosphate tribasic dodecahydrate, potassium dihydrogen phosphate, and sodium hydroxide (Sigma Aldrich srl, Milan Italy).

2.2. Methods

2.2.1. Microparticles production and characterization

Microparticles were produced in a home-made apparatus described in a previous work (Dalmoro et al., 2014) and then modified to allow the two stage polyelectrolyte complexation method (Dalmoro et al., 2016). Briefly, both the core and shell feed were separately sent to the coaxial ultrasonic atomizer (25 kHz ultrasonic frequency), where they were nebulized (2 min) and placed in contact with the first cross-linking solution (5 min), mixed in a beaker (at constant magnetic stirring, speed 400 rev/min). AL was chosen as encapsulating matrix for both core and shell solutions in similar concentrations for its biocompatibility,