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Note

Spray dried Eudragit microparticles as encapsulation devices for vitamin C

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Abstract

The aim of the present paper was to study production of methacrylate microparticles for the delivery (administration) of ascorbic acid via the oral route. Vitamin C is an important antioxidant that may be involved in the reduction of the risk of certain types of cancer, such as colorectal cancer. As polymers different acrylic compounds were considered, namely Eudragit® RL, L and RS. Spray-drying was used as preparation method of vitamin C/Eudragit® microspheres. Microspheres were first characterized by size and morphology by scanning electron microscopy, then in vitro release kinetics by mean of dialysis method were studied. Although the produced microparticles were unable to slow down the release of the drug with respect to the free form of ascorbic acid, these microspheres showed a good morphology and size distribution that permit to propose them as candidate for the delivery of vitamin C as associated therapy in the treatment of colorectal cancer by oral route.

Keywords: Microspheres; Spray-drying; Vitamin C; Antioxidants; Eudragit

Vitamin C is an important antioxidant that may reduce the risk of cancer by neutralising reactive oxygen species or other free radicals that can damage DNA (Jacobs et al., 2001). The prevention and treatment of cancer considers different mechanisms of vitamin C activity, such as (a) enhancement of the immune system by increased lymphocyte production (Brennan and Hannigan, 1996; Vohra and Khan, 1990), (b) stimulation of collagen formation necessary for 'walling off' tumors (Shklar and Schwartz, 1996), (c) inhibition of hyaluronidase (Shklar and Schwartz, 1996), (d) inhibition of oncogenic microorganisms (Zhang and Wakisaka, 1997), (e) correction of an ascorbate deficiency, often seen in cancer patients (Dyke and Craven, 1994), (f) enhancement of the effect of certain chemotherapy drugs (i.e. tamoxifen or cisplatin) (Kurbacher et al., 1996; Wells et al., 1995; Chiang et al., 1994), (g) reduction of the toxicity of other chemotherapeutic agents (i.e.

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doxorubicin) (Shimpo et al., 1991; Kurbacher et al., 1996), (h) prevention of cellular free radical damage (Flagg et al., 1995) and (i) neutralization of carcinogenic substances (Block, 1991).

In addition, a number of in vitro and in vivo experiments have been performed in order to evaluate the ability of ascorbic acid to prevent the adverse effects, increase the effects of, and decrease resistance to chemotherapeutic agents (Shils et al., 1999). For instance, co-treatment of vitamin C with doxorubicin gave a reduction in the toxicity of doxorubicin both in mice and guinea pigs. This effect was not ascribed to a decreased effect of doxorubicin, but to an increased life span of the drug compared with the treatment of sole doxorubicin (Kurbacher et al., 1996). With respect to colorectal cancer, ascorbic acid has shown to inhibit this type of cancer in rodents (Logue and Frommer, 1980). Nevertheless, the use of vitamin C supplement could substantially reduce the risk of colorectal cancer, also if the stability and release of ascorbic acid are relatively low after administration. In order to obviate these drawbacks the possibility to obtain microparticle devices containing vitamin C was considered.

The aim of the present study was to produce and characterise methacrylate microparticles prepared by spray drying for the delivery of ascorbic acid by oral route.

The spray dry technique has been an important and widely applied technique in the pharmaceutical and biochemical fields (Esposito et al., 2000). Particularly, spray drying can be applicable either to both heat resistant and heat sensitive drugs either to both water soluble and water insoluble drugs or to both hydrophilic and hydrophobic polymers. Spray drying is characterized by important features such as reliability, reproducibility and possible control of particles size and drug release. In addition, it is a one stage continuous process, easy to scale-up and only little dependent on the solubility of drug and polymer (Masters, 1991). Furthermore, the polymeric drug delivery systems produced by spray drying have a potential to provide new types of administered routes, such as oral dosage forms (dry powders, granules or agglomerates), targeting systems to organs and tissues and long-acting parenteral biodegradable systems (He et al., 1999).

The principle of spray drying is based on the nebulization through a desiccating chamber of a polymer solution containing the active ingredient as solute or in suspension. The solvent is rapidly evaporated by a stream of heated air transforming the small droplets in solid microparticles. Microparticles obtained by spray drying are usually organic solvent free with respect to other preparation methods often resulting in particles possibly contaminated by toxic organic solvents (Masters, 1991; Palmieri et al., 2001).

Methacrylate copolymers (Eudragit®) represent interesting candidates for the production of microparticles by spray drying since they are inert and freely soluble in organic solvent (Lehmann et al., 1989). Among the different types of commercialized Eudragit®, L100 type is a pH dependent enteric polymer composed of methacrylic acid–methacrylic acid methyl ester copolymers soluble from pH 6 (Weiss et al., 1993). Due to this behaviour, Eudragit® L is insoluble in the mouth and in the stomach and it starts to be soluble in the duodenum (pH around 6). Since the pH in the colon is around pH 7.5, Eudragit® L microparticles can be used for the delivery of ascorbic acid in this lower part of the intestine and also in the jejunum and in the ileum. On the other hand, Eudragit® RS100 and RL100 are copolymers based on neutral methacrylic acid esters. In particular Eudragit® RS is able to form permeable films (Lehmann et al., 1989) since it contains only 5% w/w of hydrophilic units and it exhibits a very low permeability, enabling sustained release formulation manufacture (Otsuka et al., 1993; Kim et al., 1994).

Ascorbic acid containing microparticles were produced as follows. Twenty millilitre of aqueous suspension of 30 mg/ml Eudragit® RL, L, RS/L or RL/L (1:1 by weight) were fed at 600 l/h (inlet temperature 105 °C) by mean of a peristaltic pump and sprayed, through a 0.7 μm nozzle, in the drying chamber of the instrument by means of a flow of compressed air. The solvent evaporation by a flow of heated air (80 °C) aspirated by a pump, induced the formation of solid microparticles from the drops. The obtained particles separated in a cyclone and settled down into a collector.

A spray dry preformulative study performed on Eudragit® microspheres (Esposito et al., 2000) demonstrated that: (a) low feed rates of polymeric suspension enabled the obtaining of the best microparticles in term of morphology; (b) the concentration of the polymer affected both morphology and dimensions of microparticles; (c) an increase of air drying temperature induced a reduction of microparticle size and recovery and (d) changes in flow nebulization did not affect microparticle characteristics (i.e. size, surface characteristics, porosity).

Taking into account these results, in the present work the influence of polymer type and ascorbic acid concentration was studied on microparticle morphology, size distribution, recovery and drug release. Particularly, electron microscopy was employed in order to analyse microparticle morphology and size distribution, whilst a dialysis method was used to determine the release kinetics of vitamin C from the microspheres.

By using the experimental parameters above reported, all the acrylic microparticles obtained by spray drying showed high encapsulation efficiencies, comprised between 98–100%. In addition, the produced particles present a spherical geometry and a smooth surface, as demostrated by scanning electron microscopy (SEM) photographs (Fig. 1). Frequency distribution plots of Eudragit® RL, L, RL/L and RS/L particles, deter-

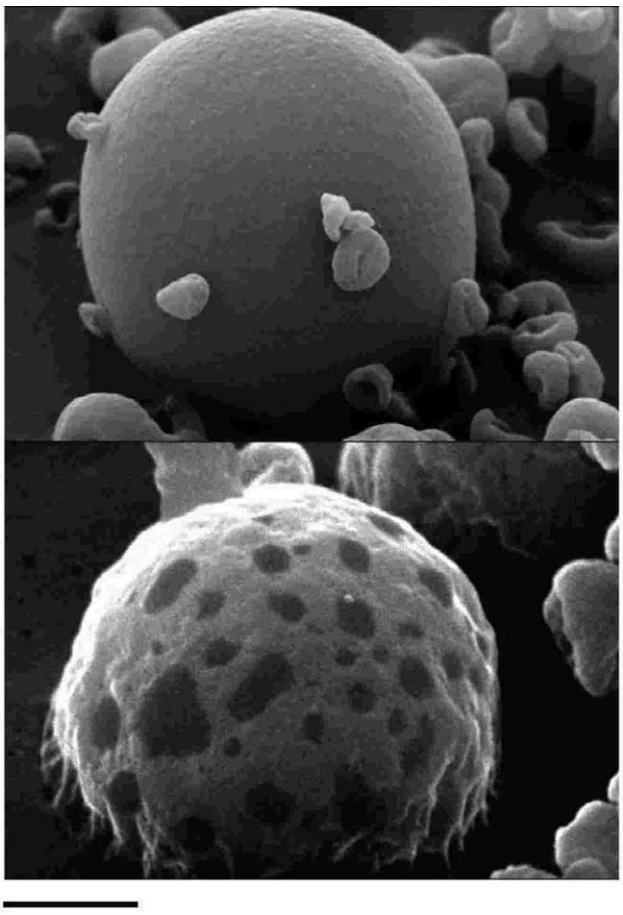

Fig. 1. SEM photographs of empty (panel A) and vitamin C (panel B) containing Eudragit RL microparticles obtained by spray drying. Bar corresponds to 3 and 6 μm, in panel A and B, respectively.

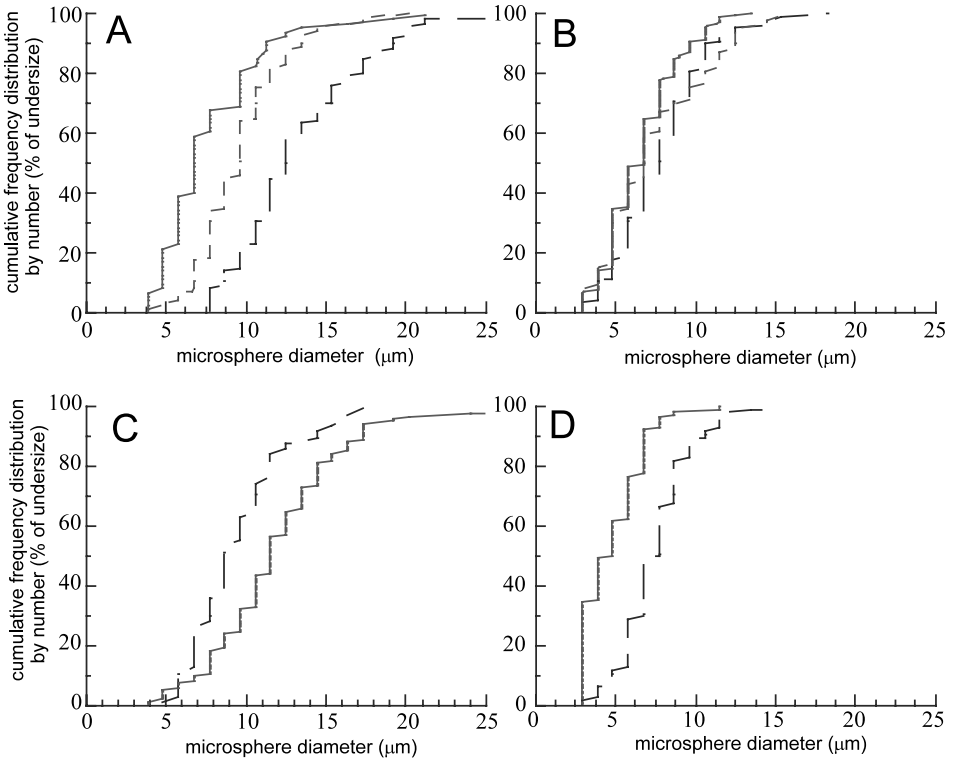

Fig. 2. Size distribution of empty (full line) and vitamin C (dashed line) containing Eudragit microparticles obtained by spray drying. Panel A: Eudragit RL microparticles. Panel B: Eudragit L microparticles. Panel C: Eudragit RL/L microparticles. Panel D: Eudragit RS/L microparticles.

mined from SEM microphotographs, are reported in Fig. 2. As showed from data reported in Table 1, Figs. 1 and 2, the selection of appropriate parameters enabled to obtain spray dried Eudragit microparticles containing vitamin C with good morphology and size distribution, however the presence of ascorbic acid influenced both mean size and surface characteristics of the particles. The surface of ascorbic acid containing microspheres is, in fact, characterised by the presence of brown plaques that gave a leopard appearance to the particles. On the contrary, the percentage of microparticle recovery was quite low, being comprised between 35 and 55% with respect to the total amount of polymer used.

Drug release kinetics from microparticles can be usually determined by in vitro experimental approaches predictive of in vivo 'real' situation. In the present investigation a dialysis method was used, since it reproduces a situation where predominate non-sink conditions (Washington, 1990). In the case of colon delivery, in fact, non-sink conditions are prevalent due to the relatively slow motility of the colon and long transit and residence time (Hardy, 1993). In addition, in order to mimic colonic situation a receiving buffer with a pH 7.0 was used. This pH value was chosen since it is comprised between colon mucosal microclimate (pH 6.8) and lumen (pH 7.5) (Edwards, 1993).

Fig. 3 reports the ascorbic acid release kinetics from Eudragit® RL, L, RL/L and RS/L microparticles, determined by dialysis method. The amount of vitamin C released (expressed as a percentage of the amount of total drug content) is plotted versus time. Release profile data indicate that vitamin C is released from microparticles with a rate adequate to an enteric delivery; in addition, the release pattern of the drug is slowly

Table 1
Composition, recovery efficiency and mean size of the prepared Eudragit® microparticles

Type of Eudragit	Amount of polymer (mg)	Amount of vitamin C (mg)	Microsphere recovery[a] (%)	Microsphere mean diameter (μm)
RL	600	/	55	7.88
RL	600	250	36	9.63
RL	600	500	35	19.43
L	600	/	48	6.56
L	600	250	68	7.30
L	600	500	78	7.80
RL/L	300/300	/	43	9.46
RL/L	300/300	500	26	11.86
RS/L	300/300	/	40	4.73
RS/L	300/300	500	25	7.47

[a] % of total amount of polymer.

influenced by Eudragit® type or mixture used for microparticle production (Fig. 3). As clearly appreciable, the release profiles demostrated that a plateau is reached between 4 and 5 h.

Differently from other studies (Esposito et al., 1997) were the release kinetics of the encapsulated drug is clearly dependendent from the different solubility of the polymers at the pH of the receiving buffer, in this case the release rate of the drug does not seem to be heavily affected by the nature of the polymer. In the case of Eudragit® L microparticles, in fact, 60% release of ascorbic acid is reached in about 100 min; whilst in the case of Eudragit® RL/L, 60% release is reached almost in 75–77 min. Finally, the microparticles composed of Eudragit® RL released the 60% of ascorbic acid within 82 min whilst Eudragit® RS/L microparticles showed a release behaviour superimposable to that of free ascorbic acid.

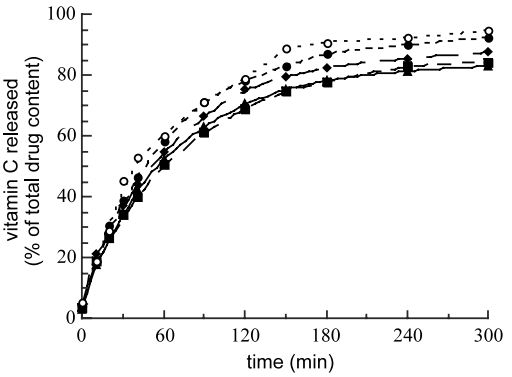

Fig. 3. Release of Vitamin C from L (■), RL (▲), RL/L (◆) and RS/L (●) Eudragit microparticles determined by dialysis. As comparison the release of free vitamin C (○) is reported.

In conclusion, the results here reported indicated that Eudragit microparticle encapsulating vitamin C could represent a candidate for its sustained delivery after in vivo administration.

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