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Combined Poly(alkyl cyanoacrylate)/Cyclodextrin Nanoparticles

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Abstract

The use of nanoparticles is of great interest for oral or parenteral administration. In fact, nanoparticles not only can protect the active ingredient, but they confer on it a large contact surface with biological membranes and a possible improvement in bioavailability. Biodegradability of nanoparticles, which can be considered as a pre-requisite in the case of parenteral administration, is valuable in the case of oral administration because of their capture by Peyer's patches. Among the different biodegradable polymers used for the preparation of nanoparticles, poly(alkyl cyanoacrylate) (PACA) has the main advantage of a possible preparation by anionic polymerisation in aqueous medium without addition of any initiator, which could have toxic effects. However, in such conditions a major drawback is the difficulty of encapsulating poorly water-soluble drugs. In order to overcome this problem we have investigated the use of cyclodextrins in the preparation of PACA nanoparticles.

Feasibility and characteristics of combined poly(alkyl cyanoacrylate)/cyclodextrin nanoparticles

Nanoparticle preparation

Nanoparticles were prepared by emulsion anionic polymerisation of either isobutyl or isohexyl cyanoacrylate (IBCA or IHCA) in a 0.01 M HCl aqueous solution (pH 2) containing 1% (w/v) poloxamer 188 and variable amounts of α-, β-, or γ-cyclodextrin (CDs), their hydroxypropyl derivatives (HP-CDs), the sulphobutyl ether of β-CD (SE-β-CD), or dimethyl βCD(Me-β-CD). The polymerisation medium is stirred for 6 h and then filtered on a 2-μm porosity membrane. When an active ingredient (series of steroids or saquinavir) is added, an inclusion compound is pre-prepared and added to the preparation medium in place of pure cyclodextrin [1, 2].

Blank nanoparticles characteristics

PIBCA blank nanoparticles were prepared in the presence of natural CDs or their HP derivatives, as well as SE-β-CD [3]. In all cases it was possible to obtain nanoparticles with a diameter varying from 230 to 370 nm, except with HP-β-CD (103 ± 6 nm) and HP-γ-CD (87 ± 3 nm). An increase in the cyclodextrin (SE-β-CD) concentration, from 5 to 20 mM, resulted in a particle size decrease from 252 to 126 nm [1]. Zeta potential, which is about –40 mV in the absence of CDs, is increased in the presence of neutral CDs up to -8.6 ± 0.9 and 2.6 ± 2.3 mV for HP-β-CD and HP-γ-CD respectively, indicating the presence of CDs at the nanoparticle surface. With SE-β-CD, a decrease to -45.4 ± 2.4 mV is observed due to the negative charge of this CD. The content in CDs is always noticeable, from 20 to 30% of the nanoparticle total weight.

Steroid loaded HP-β-CD nanoparticles

PIBCA/HP-β-CD nanoparticles were prepared with a series of steroids as model drugs: danazol, hydrocortisone, megestrol acetate, prednisolone progesterone, spironolactone, testosterone [1, 2]. For all these steroids a 7 (hydrocortisone, spironolactone), to 28-fold (progesterone) or 130-fold (prednisolone) increase is observed. The amount of active ingredient loaded increases with an increase in HP-β-CD concentration in the preparation medium.

DSC analysis carried out on progesterone nanoparticles, demonstrated that a true inclusion is obtained and that the drug loses its crystallinity in nanoparticles whatever its concentration, progesterone exists in either the molecular or amorphous state [1, 2]. The drug (progesterone) release occurs very rapidly in pH 8.4 buffer solution, but reaches a plateau depending on the nanoparticle size (50–60% for 70 nm diameter, 30% for 150 nm diameter). The complete release is obtained in the presence of esterases capable of degrading the PIBCA polymer [1, 2].

Active ingredient localisation and role of cyclodextrins

The previous results (high amount of HP-β-CD linked to nanoparticles, modification of zeta potential, fast release of progesterone followed by a plateau and complete release after enzymatic degradation) suggest the following mechanism of nanoparticle formation and loading.

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The cyclodextrin hydroxyl groups of the different cyclodextrins and especially HP-CDs which can be slightly dissociated, could behave like IBCA polymerisation initiators. The high CD content in the nanoparticles and their localisation at the nanoparticle surface (as demonstrated by their fast release) suggest their role as steric stabilisers. Loading in active ingredient theoretically could occur according to three different processes. First, the drug/CD inclusion compound can be simply adsorbed at the nanoparticle surface (rapid release). Secondly, the inclusion compound can be entrapped in the nanoparticle core during the polymerisation process. However, due to the high external hydrophilicity of inclusion compounds, this second process is rather improbable. But a third process most probably occurs by dissociation of the inclusion compound leading to free molecules of drug and cyclodextrin:

$$\text{steroid/HP-}\beta\text{-CD} \Leftrightarrow \text{steroid} + \text{HP-}\beta\text{-CD}$$

the free drug molecules can be easily entrapped in the nanoparticle during the polymerisation process as a function of their partition coefficient in favour of the polymer. In fact, by replacing the polymer/water drug partition coefficient by its octanol/water partition coefficient (logP {polymer/water} = A $\times$ logP {octanol/water} = b), we demonstrated a linearity between the nanoparticle loading capacity in steroids and the product: initial concentration $\times$ logP [4]. Finally, the active ingredient can be adsorbed at the nanoparticle surface either in the form of an inclusion compound, or as free molecules, and it can be entrapped in molecular state in the nanoparticle core.

Improvement in bioavailability

Taking advantages of the previous results, we investigated the effect of combined PACA/CD nanoparticles on the oral bioavailability of saquinavir [5].

Saquinavir, a HIV_1 and HIV_2 protease inhibitor, has a low water-solubility (35.8 μg/ml), suffers from hepatic first pass effect and P-gp expression, and has a very poor oral bioavailability. By increasing the water-solubility and nanoparticle loading capacity, we could overstep the hepatic first pass effect. Furthermore, we investigated the role of additional cylodextrin as absorption enhancer on Caco-2 cell monolayers.

Saquinavir combined PACA/CD nanoparticles

By using HP-β-CD, it was possible to prepare saquinavir PIBCA or PIHCA nanoparticles with a 20-fold increased content compared with nanoparticles in the absence of Cds [5]. Saquinavir release in reconstituted gastric (or intestinal medium) is very rapid and complete in less than 4 h from combined PIBCA or PIHCA/HP-β-CD nanoparticles and occurs more rapidly than in the absence of HP-β-CD [6].

Working on Caco-2 cell monolayers [7], we confirmed that transport of saquinavir from basolateral to apical compartment was higher than the one existing from the apical to the basolateral side. This phenomenon can be attributed to the expression of P-glycoprotein (P-gp) efflux pump present at the apical side of Caco-2 cells. Saquinavir/HP-β-CD or Me-β-CD inclusion compounds were not able to improve the absorption of the drug. At the opposite, association of saquinavir to combined PIBCA/CD nanoparticles promoted a faster transport of the drug whatever the direction of the transport, without any change in the concentrations transported after 4 h of incubation. Nevertheless, addition of free Me-β-CD (2.5%) to the complexes or to the saquinavir loaded combined PIBCA/CD nanoparticles modified the amount of saquinavir transported through Caco-2 cells. Increase in the amounts of saquinavir recovered in the basolateral chamber was correlated to a decrease of the transport from the basolateral to the apical chambers [7]. Combined PIBCA/CD nanoparticles associated to free Me-β-CD increased the kinetics of absorption of saquinavir, suggesting that Me-β-CD inhibits saquinavir efflux.

Conclusion

The feasibility of combined PACA/CD particles was demonstrated, and more especially the advantage of using the hydroxypropyl derivatives which lead to small nanoparticles. The loading capacity of nanoparticles in poorly water-soluble drugs is significantly increased as shown on a series of steroids and on saquinavir. The presence of methyl β-cyclodextrin increases saquinavir absorption through Caco-2 cells. Depending on the active ingredient and the polymer nature , it is possible to modify the drug release. Combined poly(alky cyanoacrylate)/HPβ-cyclodextrin, associated to methyl-β-cyclodextrin, is a promising drug delivery system for poorly water-soluble and poorly bioavailable drugs.

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