Accepting the challenge of scale-up – A new approach for the targeted treatment of colon cancer based on nanoparticles overcomes the limitations of batch size

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ABSTRACT SUMMARY
Eudragit® RS 100-nanoparticles were manufactured in laboratory scale by nanoprecipitation technique as described by Bodmeier et al. [1] Scale-up to a continuous flow process was achieved using a microreactor-assisted approach. Particle properties could be preserved in spite of the scale-up procedure.

The photosensitizer meso-tetra(hydroxyphenyl)chlorin (mTHPC) was encapsulated into the polymeric matrix and cell culture-based evaluations including efficacy and uptake were performed. Nanoparticles entered the cytoplasm of Caco-2 cells as confirmed by transmission electron microscopy (TEM) after embedment of cell layers into a resin and subsequent slicing. Laser illumination of A-253 cells treated with mTHPC confirmed that efficacy of the photosensitizer was not altered, but the general toxicity could be reduced by encapsulation into the polymer.

EXPERIMENTAL METHODS
Nanoprecipitation was achieved by pumping an aqueous polysorbate 20-solution (0.01%) to a continuously stirred solution of Eudragit® RS 100 dissolved in ethanol at a concentration of 15%. For the incorporation of the photosensitizer, mTHPC was added to the organic solution at a ratio of 1 part API per 20 parts polymer. A microjet reactor sytem connected to a nitrogen supply served as experimental setup for accomplishing manufacture of Eudragit® RS 100-nanoparticles in a continuous flow process. Conditions for the achievement of corresponding particle properties to the small scale approach were established. Particle characteristics (diameter, size distribution, and zeta potential) were determined by dynamic light scattering with the help of a Malvern Zetasizer Nano ZS equipped with a backscatter detector at an angle of 173°, and a microelectrophoresis unit.

A-253 cells were incubated with mTHPC-loaded nanoparticles and the pure API, respectively. Irradiation with a laser was performed inducing cell death of the treated cancer cells. Toxicity was evaluated by MTT assay and compared to the dark control that was conducted in the absence of light.

Moreover, Caco-2 cells were seeded on a cover glass and incubated with Eudragit® RS 100-nanoparticles. Embedment into Araldite® resin was achieved. Slicing with a microtome was performed, as well as a uranyl acetate and lead citrate-based double contrasting procedure of the polymer. TEM served for visualization.

RESULTS AND DISCUSSION
Eudragit® RS 100-nanoparticles were characterized by a size of 193.1 ± 9.5 nm when prepared in small scale. Size distribution was adequate as indicated by polydispersity indices of 0.167 ± 0.007. These parameters were not altered.

INTRODUCTION
Recently, extensive research activities have been focused on nanosized drug delivery systems due to their manifold advantages that include the option for crossing biological barriers, drug targeting and improving bioavailability. [2] Nevertheless, most nanosized dosage forms do not obtain access into the pharmaceutical market. One reason is the lack for simple and effective translation procedures to large scale manufacturing. [3]

Photodynamic therapy (PDT) is an upcoming treatment option for colon cancer that has the principle of inducing cell death in malignant tissues by illumination of a photosensitizing agent with infrared or visible light. Remarkable is the low toxicity of the active pharmaceutical ingredients (APIs) used for PDT in unilluminated conditions compared to cytostatic drugs.

The present study provides a profound evaluation of an innovative peroral drug delivery system for PDT utilizing Eudragit® RS 100, a sustained release polymer, as the matrix forming material. Special interest was also given to the translation of the laboratory scale technique to a medium scale approach. This was implemented in terms of a continuous flow process with two fine jets of liquid that strike each other under specified conditions in a chamber (microreactor-assisted nanoprecipitation).
significantly when mTHPC was encapsulated into the polymer. Scale-up with the microreactor-assisted technology revealed a strong dependency on a multitude of process parameters including speed of desolvation that could be adjusted by variation of the pumping speed of polymer and stabilizer solution, as well as their ratio. Increasing the absolute flow rate yielded particles of reduced diameters (see Figure 1). A similar effect was recognized when the ratio of the described liquids was increased in favor of the stabilizer solution. Other influence coefficients were the pressure of nitrogen supply and the concentration of polymer in the organic phase. Particles that were comparable to their counterparts prepared by the small scale approach in size, size distribution, zeta potential, and shape were gained when the process was carried out with flow rates of 0.1 mL/min for the polymer solution and 0.3 mL/min for the stabilizer solution. Drug loading efficiency of mTHPC was determined indirectly by HPLC analysis and found to be 78.65 ± 4.79% for the laboratory scale approach. It could be increased to values higher than 90% when the continuous flow process was applied.

**Figure 1.** Impact of absolute flow rate (summation of stabilizer and polymer flow) on particle size (○) and polydispersity index (●) of Eudragit® RS 100-particles generated with the microreactor technique. The experiments were conducted in triplicate. The error bars represent the standard deviation.

The toxicity assay with A-253 cells in the presence of mTHPC revealed a significantly reduced cell viability after exposure to red light. This is in accordance with the mode of action of this API, since singulet oxygen is generated when a photosensitizing agent is brought to the excited state. Consequently, cell death in the tumor tissue is induced. [4] The nanoparticulate formulation with identical amounts of encapsulated mTHPC was tested and exhibited a similar cytotoxic effect as the pure API. Moreover, the performed dark control confirmed that the Eudragit®-based formulation was able to lower the toxicity of mTHPC for the unilluminated conditions. Thus, the developed nanocarriers allow distinct control of the endurance and spot of the photodynamic effect. Hence, the toxic potential and therewith side effects of PDT can be reduced. Uptake into the cytoplasm of Caco-2 cells was observed by TEM after slicing cell layers horizontally. Nanoparticles were found in the cytoplasm (see Figure 2), suggesting that the developed nanosized formulation does not require any further coatings or antibody binding to achieve its full efficacy in the treatment of colon cancer.

**Figure 2.** TEM picture of Caco-2 cells after incubation with Eudragit® RS 100 nanoparticles for 2 hours. Uranyl acetate and lead citrate were used for staining of the polymer.

**CONCLUSION**

The developed mTHPC-loaded Eudragit® RS 100-nanocarriers showed high potential for the treatment of colon cancer as indicated by cell culture investigations in various cell lines. Particles were effectively taken up into the cytoplasm without the need for further modification of surface properties. Moreover, full efficacy of the API could be preserved and toxicity in unilluminated conditions reduced as shown in investigations with A-253 cells. Favorable particle properties were achieved in the conventional small scale approach, as well as in a continuous flow process (microreactor-assisted nanoprecipitation), exhibiting the high potential of this particle system for translation to industrial scale.

**REFERENCES**


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