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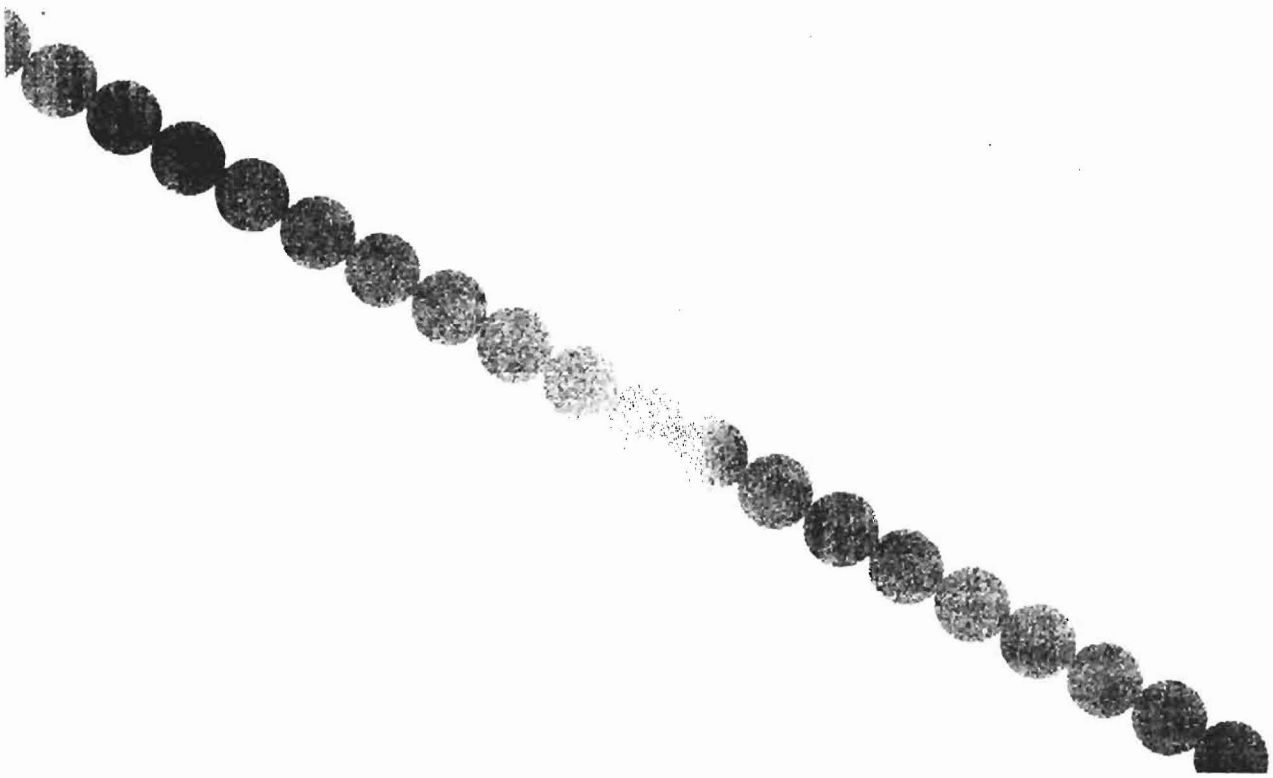

problem cause analysis are limited. Deferring from the empirical approach, QBD/PAT implementation represents a systematic, multidimensional, CQAs determination within design space. Defining design space depends on QTPP and available resources, using prior scientific knowledge and design of multivariable experiments, to better understanding of input materials and process qualitative attributes. Product quality is reflected in the approved design space. Safety and efficacy data in this phase are obtained by performing in vitro studies and IV/IVC correlations. Specification in compliance with regulatory requirements and risk assessment of CQA, results in: determination of CQAs should be tested and PAT tools should be used. Production processes and scale up to industrial facilitates are flexible, easier and faster. QbD product almost never fails the bioequivalence study and transfer to commercial manufacturing is predictable. Ongoing quality management leads to processes validation through the life cycle. This approach focuses on robust processes and determination of scientific and risk based control strategy. PAT enables statistical analysis and quality monitoring at real time, as a base for proactive quality management. Quality performances are improved through the life cycle using QbD/PAT.

DISSCUSION

Major focus of the generic industry is components selection, formulation, manufacturing process and control strategy development, for easier and faster commercialization of consistent quality product. There are no strict requirements for QbD/PAT application. Depending on needs and possibilities for built in quality, QbD implementation should be optimized. Robust processes and understanding of variables justifies the shift of control. QbD/PAT Implementation is innovative challenge for generic industry with great opportunities for cost reduction, lowered rate of batch failures and science based regulatory assessment.

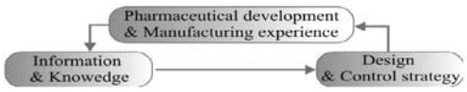

Fig. 1: QbD (ICHQ8)

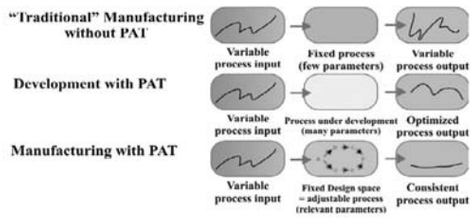

Fig. 2: PAT, source: adopted from K. Ho, CHMP Biologics Working Party, EMA, presentation

CONCLUSIONS

Implementation of QbD/PAT is challenge and opportunity for the generic industry in manner of technological, financial and quality improvement.Investment in the development enables better understanding of product and processes leading to: easier improvement of the built in quality, faster and easier regulatory assessment. Due to economic competition, wider design space leads to faster commercialization and reduced post-approval variations. QbD as systemic approach to pharmaceutical development supported with PAT tools is cost and time benefit for generic industry.

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INFLUENCE OF FORMULATION VARIABLES ON SURVIVAL OF L. CASEI LOADED IN CHITOSAN-Ca-ALGINATE MICROPARTICLES PREPARED BY SPRAY-DRYING

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INTRODUCTION

Few methods of microencapsulation are widely used to enhance the viability of probiotics in pharmaceutical and food products and during the passage in the GIT (1). Functional properties of microparticles for effective colon delivery of viable cells depend to great extent on the type of the encapsulating materials. Although different protective materials are applied during the microencapsulation process (2), natural biopolymers alginate and chitosan are of continuous interest due to their biocompatibility, potential for effective preservation of probiotics and targeted release of viable cells in the colon (3, 4).

The aim of this study was to evaluate the effect of encapsulating materials in given ranges of concentrations on viability of probiotic *L. casei* in simulated *in vivo* conditions using polynomial regression model at 2nd level.

MATERIALS AND METHODS

Materials

Freeze-dried probiotic culture of *Lactobacillus casei* was purchased from Chr. Hansen, Denmark. Prebiotic fructo- oligosaccharide (FOS) was supplied from Sigma-Aldrich, USA. As encapsulating agent, alginate-LF 10/60 (Protanal, FMC Biopolymers, UK) was used. For additional coating of spray-dried microparticles, chitosan (Chitine, France) and for cross-linking procedure, $CaCl_2$ (Merck, Germany) were used.

Preparation of microparticles and determination of physicochemical properties

The microparticles were prepared by modified spray-drying method, previously used for microencapsulation of drugs (5) and for the first time for micro-encapsulation of probiotic cells. An aqueous dispersion of alginate, FOS and *L. casei* was spray-dried (nozzle diameter 0.7 mm, aspirator pressure 90%, flow rate 6 ml/min, inlet and outlet temperature, 120 °C and 60 °C, Büchi Mini Spray Dryer B-290, SW) to obtain microparticles, which were subsequently cross-linked and coated in solution of $CaCl_2$ and chitosan in 1% *w/w* acetic acid. Prepared microparticles were cured for 3 h, separated and freeze-dried (-50 °C, 0.070 mbar, 24 h, Freeze-Dryer, Labconco, USA). Following physicochemical properties were determined: particles size (Master-sizer Hydro-2000S, Malvern Instruments Ltd., UK), Ca-content (AES-ICP, Varian, USA), zeta-potential (Zeta-sizer Nano ZS, Malvern Instruments Ltd., UK) and cell viability into the microparticles (plate-count method).

Experimental modelling

The cross-linking procedure and poly- electrolyte complexation were carried out at concentration limits of alginate (1% and 4% *w/w*), chitosan (0.1 and 0.5% *w/w*) and $CaCl_2$ (0.5 and 5% *w/w*). The cell load in the feed suspension was ca. 11-12 log_{10} cfu/g. The plan matrix included 11 batches.

Viability of microencapsulated *L. casei* in simulated *in vivo* conditions

To determine the viability of encapsulated probiotic, experiments were performed in simulated gastric juice for 3 h (0.08 M HCl with 0.2% NaCl, pH 1.5), bile salts solution for additional 3 h (0.05 M KH_2PO_4 with 1% bile salt, pH 6.8) and in colonic pH 7.4 (0.1 M KH_2PO_4) up to 24 h.

RESULTS AND DISCUSSION

Microparticles with d_{vs} ranging from 6.7 to 12.5 µm, zeta-potential -22.6 to 30.3 mV, Ca-content 3.5 to 12.5% and survival rates of probiotic within the particles from 6.91 to 11.43 log_{10} cfu/g were obtained. Viability of microencapsulated *L. casei* in simulated GI conditions is presented in Fig.1.

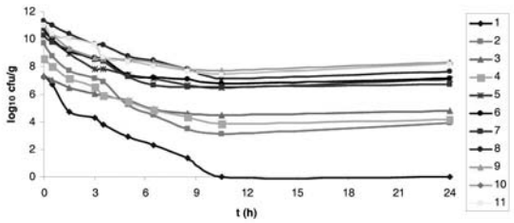

Fig.1: Viability of microencapsulated *L. casei* (log_{10} cfu/g) in simulated GI conditions in different series generated with experimental design

Overall effects of the formulation variables pointed to the dominant influence of the concentration of $CaCl_2$ on survival rate of the microencapsulated cells of *L. casei* in all pH media tested. Higher content of Ca^{2+} in the microparticles resulted in increased viability of *L. casei*. The relationships between variable factors and responses were plotted by holding constant one of the three variables. Chitosan concentrations produced only minor influence on probiotic's viability in simulated gastric and intestinal conditions and by fixing its value, survival rate of probiotic cells increased with increased alginate concentration. At constant level of chitosan, the viability of microencapsulated probiotic cells would increase to 9.1 log_{10} cfu/g or higher and 7.35 log_{10} cfu/g or higher, for alginate concentrations of 2.64-4% and 1-4% (*w/w*) and for $CaCl_2$ in range of 4.64-5% and 3.58-5% (*w/w*) in gastric juice and bile salts solution, respectively (Fig 2).

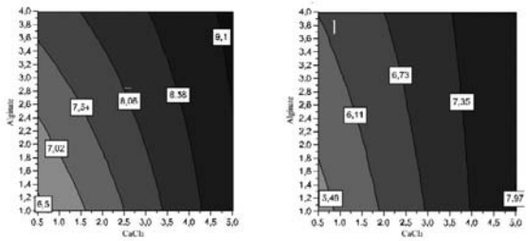

Fig.2: Response surface plot for viability of microencapsulated *L. casei* (log_{10} cfu/g) showing the effects of alginate and CaCl2 for constant level of chitosan, 0.5% (w/v); a) pH 1.5; b) pH 6.8

Considering the influence of the formulation variables on the physico-chemical properties of the microparticles and on the viability of *L. casei* in simulated GI conditions, an optimal formulation was prepared, with 4% alginate, 0.5% chitosan and 5% $CaCl_2$. The data for the viability of *L.casei* confirmed the predicted values, 9.62 log_{10} cfu/g in pH 1.2, 8.46 log_{10} cfu/g in pH 6.8 and 7.67 log_{10} cfu/g in pH 7.4.

In conclusion, the prepared microparticles showed potential for effective colon delivery of live probiotic cells.

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THE INFLUENCE OF SHELL FORMING PHASE ON EFFICIENCY OF SMES ENCAPSULATION INTO POLYMERIC MATRIX

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INTRODUCTION

The ability to develop an effective oral dosage form is crucial for the successful launch of a new active pharmaceutical ingredient (API) in the marketplace. One of formulation strategies recently employed for APIs with poor biopharmaceutical properties are self-microemulsifying drug delivery systems (SMES) (1). In our previous study (2,3) liquid SMES was successfully transformed to solid microcapsules by technique of liquid jet co-extrusion by vibrating nozzle technology. Obtained microcapsules merge the advantages of SMES with those of a solid dosage forms, and thus provide a promising alternative to ensure successful oral delivery of drugs with poor biopharmaceutical properties.

Key steps for the successful encapsulation of the furosemide-loaded SMES by above mentioned technology are to prevent mixing between the core (SMES) and the shell forming phase (polymer solution with additives) during microcapsules production and drying process. The main scope of the current study was therefore to optimize the properties of shell forming phase with respect to encapsulation efficiency and microcapsules morphology.

MATERIALS AND METHODS

SMES was prepared by blending 88 % of the Labrasol® and Plurol oleique® (2:1) mixture (both from Gattefosse, France) with 12 % of Mygliol 812® (Hüls, Germany). Afterwards 0.5 % of $CaCl_2$ was added to promote shell hardening from the inside out as soon as the capsules are formed. Finally, SMES was loaded with 5 % furosemide and thickened with 4 % of colloidal silica (Aerosil 200, Degussa, Germany) (3).

Shell forming phase was prepared by mixing 2 % Na-alginate solution (Low viscosity Na-alginate, Sigma, Germany) with 2 % pectin solution (Genu® pectin type LM-104 AS-Z, CP Kelco, Denmark) in 3:1 ratio (4). Afterwards 5-20 % of hydrophilic filling agent (lactose, trechalose, mannitol, or sorbitol) was added to the aqueous solution of polymers.

SMES was microencapsulated within the polymeric shell by coextrusion of liquid jet by using an Inotech IE-50R encapsulator (Inotech, Switzerland). Microcapsules were than hardened according to different procedures: (a) 15 min incubation in 0.5M $CaCl_2$ (*$CaCl_2$*) followed by (b) 5 min incubation in 1mg/ml chitosan solution (*$CaCl_2$+C-LV*) or (c) 1mg/ml chitosan solution with 0.5M $CaCl_2$ (*$CaCl_2$+C-LV with $CaCl_2$*) to apply additional chitosan coating;