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It is our great pleasure to present this Supplement Issue on "Macedonian Pharmaceutical Bulletin" to the scientific and professional community. This supplement includes the short communications from the Sixth Congress of Pharmacy in Macedonia with International participation, as the largest gathering for the pharmacy profession held in the Republic of Macedonia. The main theme of the Congress was "Modern pharmacist - bridging science with practice".

A broad spectrum of topics within the pharmaceutical sciences and practice carefully selected for this special occasion in order to build up a highly interesting and comprehensive program were covered. The contributions submitted to the Congress included 6 plenary lectures, 84 section lectures, and more that 240 posters. This Congress, followed the excellent international tradition, was attended by close to 1000 domestic and foreign participants. We received 326 short paper submissions from more than 25 countries. These numbers show that our Congress is aiming for the highest scientific standards, and that it can be considered a well-established venue for researchers in the broad fields of Pharmaceutical sciences and practice.

We would like to thank all internationally prominent researchers for their contribution to reinforcing the overall quality of the Congress. They give the state of the art of the recent advances in the field of pharmacy research.

Sincere thanks to the hosts of the Sixth Congress of Pharmacy in Macedonia with International participation, Macedonian Pharmaceutical Association and Faculty of Pharmacy, Ss Cyril and Methodius University in Skopje for their vision and commitments.

We acknowledge the sponsoring companies: the platinium sponsor AD ALKALOID, Skopje, the golden sponsor PLIVA, the silver sponsor EUROFARM and the bronze sponsor SEPTIMA, for the permanent support to our efforts during the organization.

We would also like to thank our members of the Scientific Committee for their volunteer time and dedication to the critical peer review process and in the organization of the program. We also wish to thank all the members of the Organizing Committee, whose work and commitment was invaluable.

On behalf of the Advisory and Scientific Committees, we would like to especially thank the authors, whose work was the essential part of the congress and contributed to a very successful event. Besides the many academic staff and professionals who contributed to the success of the Congress, we are grateful to the students who participated with oral presentations and posters.

The pharmaceutical sciences continue to grow as dynamic scientific interdisciplinary fields. We believe that published short communications will be an excellent source of scientific material in the fast evolving fields in Pharmaceutical sciences and practice.

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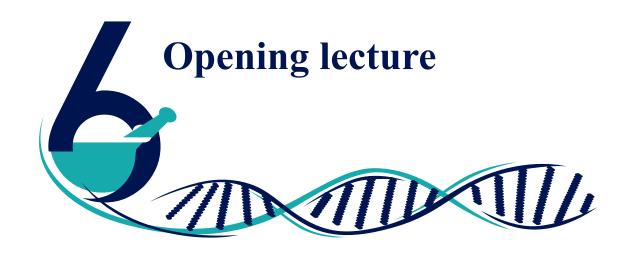
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The present issue of Macedonian Pharmaceutical Bulletin is a special issue of the 6th Congress of Pharmacy
in Macedonia with international participation. This issue of <i>Macedonian Pharmaceutical Bulletin</i> contains short papers accepted by the scientific committee
for the presentation at the Congress. The authors are fully responsible for the contents of their short papers.
All reviewers that were involved in the short papers revision process are sincerely acknowledged.



Short communication

Preparation of curcumin loaded nanoparticles: physicochemical characterization and in vitro evaluation

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Introduction

Curcumin is the active principle of the spice turmeric, produced by the rhizome of Curcuma longa (Zingiberaceae), which is widely used in traditional eastern medicine as a hepatoprotective, anti-infectious and anti-inflammatory remedy (Shehzad et al., 2010). A compelling body of recent evidence has shown that curcumin is endowed by pleiotropic antineoplastic effects, due to modulation of NFkB and other cell signaling pathways, implicated in cell survival, apoptosis and angiogenesis (Shehzad et al., 2010). Regretfully, the enormous therapeutic potential of curcumin can't be exploited in clinical practice, due to its extremely unfavorable physicochemical and pharmacokinetic characteristics, and also due to the instability in systemic circulation (Singh and Khar, 2006). The contribution is focused on newly-synthetized octopus-shaped macromolecules, consisting of hydrophobic calix[4] arene core and four arms of hydrophilic poly(ethylene oxide) chains as platform for delivery of curcumin.

Materials and methods

Two methods for preparation of inclusion complexes were used:

Heating method described by (Loftsson et al., 2005) with slight modifications. Briefly, to aqueous solutions of increasing concentrations of CX[4]PEG polyoxyethylatedtertbuthylcalix[4]arene) (2 mg/ml - 12 mg/ml) a constant amount of curcumin (1 mg/ml) that exceeded its aqueous solubility (11 ng/ml) was added. The vials were closed and heated at 50 °C for two hours. After that, the samples were left at room temperature for 24 h. Then the samples were subjected to centrifugation at 5000 rpm for 10 minutes. The clear transparent supernatants containing the inclusion complexes were collected and the amount of the curcumin was analyzed using a validated UV/VIS

ing a fixed concentration of curcumin (1 mg/ml) and increasing concentrations of CX[4]PEG (2-12 mg/ml) were prepared in absolute ethanol, and evaporated to dryness using a Buchi rotation-type vacuum evaporator (R-215, Sigma-Aldrich). The concentrations of CX[4]PEG were chosen on the basis of its critical micelar concentration (CMC) of 7.7 mg/ml (or 0.24 µmol/ml) (Momekova et al., 2012). Thereafter the dried CX[4]PEG/curcumin containing films were hydrated with deionizaed water and were left for 2 h at 50 °C and then in dark at room temperature for 24 h. Then the samples were centrifuged for 10 minutes at 5000 rpm. The transparent vellow supernatants containing the curcumin-CX[4]PEG complexes were analyzed for curcumin content using a validated UV/VIS spectrophotometric method. Phase-solubility profiles were obtained by plotting the solubility of drug versus the excipient concentration.

Characterization of the CX[4]PEG-curcumin complexation

UV/VIS spectroscopy

The UV/VIS spectra of curcumin (in absolute ethanol and 10% ethanol solution) and its CX[4]PEG complex (in deionized water) were recorded on JASCO V570 UV-Vis-NIR spectrophotometer equipped with thermostatic cell holder (Huber MPC-K6 thermostat with precision 1 °C).

Fourier transform infrared (FT-IR) spectroscopy analysis

Samples of pure curcumin, pure CX[4]PEG, their physical mixture, and a lyophilized complex were characterized by an IRAffinity-1 Shimadzu FT-IR spectrophotometer. The scanning range was between 4000 and 400 cm-1.

spectrophotometric method at 427 nm. Solvent evaporation method: series of samples contain-

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Dynamic light scattering (DLS) analysis

The size, size distribution patterns and zeta-potential of curcumin loaded supramolecular CX[4]PEG aggregates were investigated by ZetaSizer NanoZS (Malvern Instruments), equipped with a 633 nm laser. The parameters were evaluated from the measurements at the scattering angle of 175 ° at 25 °C.

In vitro curcumin release

The *in vitro* curcumin release from supramolecular BEC-X aggregates was evaluated by regular membrane dialysis at 37 °C against phosphate buffered saline (PBS). 1 ml of tested formulations was placed in dialysis membrane tubing (MWCO 10,000). The dialysis bag was then placed in a temperature controlled vessel, containing 100 ml of PBS (pH 7). At various time intervals aliquots were taken from the released medium and assayed for curcumin by UV–VIS spectroscopy.

Results and Discussion

Phase solubility studies

The phase solubility studies of curcumin with BEC-X were performed using the procedure utilized for the evaluation of cyclodextrin inclusion complexes by Higuchi and Connors (Higuchi and Connors, 1965). Due to their amphiphilic nature, polyoxyethylated calyx(4)arenes (CX[4] PEG) can self-associate in water by forming well-defined spherical nanoparticles. At concentration below the CMC, CX[4]PEG drastically increased curcumin solubility by formation of inclusion complexes with high stability constant (Kc). A significantly higher solubility enhancement of curcumin was observed at concentration exceeding the critical micellar concentration, attributed with additional solubilization of curcumin into the hydrophobic domains of the supramolecular aggregates by non-covalent interactions.

UV/VIS characterization

In order to characterize the spectral behavior of curcumin and its inclusion complex, absorption spectra of pure curcumin in absolute ethanol and 10% ethanol are compared with the absorption spectrum of the inclusion complex in water. The characteristic absorption peak of curcumin at 427 nm is identical in the three media under investigation, which demonstrates that the inclusion complex is formed by non-covalent hydrophobic interactions. An interesting finding is the appearance of a shoulder at 361 nm in the spectrum of pure curcumin dissolved in 10% ethanol which cannot be seen in spectra of curcumin in absolute ethanol and in the inclusion complex of curcumin in water. The shoulder can be attributed to the shifting of the tautomeric equilibrium from keto—enol to diketo-form.

FT-IR analysis

FT-IR spectroscopy is a useful tool for characterization of inclusion complexes. Characteristic combination of a sharp peak at 3508 cm⁻¹ and a broad peak at 3293 cm⁻¹ in the curcumin spectrum implies the presence of aromatic OH group stretching vibrations (Kolev et al., 2005) and the intensive sharp peaks at 1626 cm⁻¹ and 1601 cm⁻¹ corresponding to mixed C=O and C=C vibrations and symmetric aromatic ring (C=C) stretching vibrations, respectively, did not interfere with the vibrations in BEC-X spectra and can be used as marks for description of curcumin in inclusion complex.

DLS analysis

Physicochemical characteristics of the nanoparticles (size, size distribution and zeta potential were evaluated by DLS and the results revealed particles of app. 180 nm with monomodal distribution (PDI below 0.2) and zeta potential of -20 mV suitable for systemic application.

In vitro curcumin release

The in vitro curcumin release profiles from supramolecular CX[4]PEG aggregates were studied under simulated physiological conditions for different incubation periods from 2, 4, 6, 8, 10 and 24 hours. The results showed initial burst release of curcumin, followed by slower drug release.

Conclusion

Thus on the grounds of the excellent in vitro biocompatibility profile and the favorable physicochemical and drug loading characteristics of the tested liposomal nanoparticles, and their ability to retain the intrinsic pharmacological properties of encapsulated drug they could be considered promising drug delivery platforms for lipophilic curcumin.

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