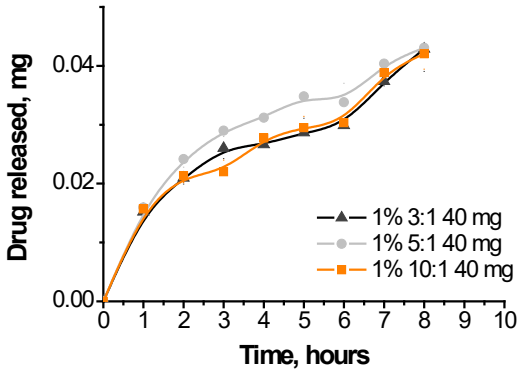

Fig. 6. TA release from casein nanoparticles with different crosslinker concentration

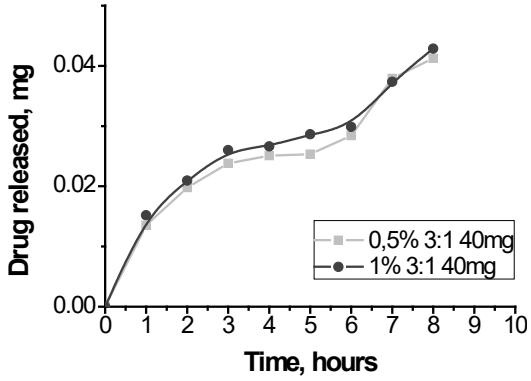

Fig. 7. TA release from casein nanoparticles with different protein concentration

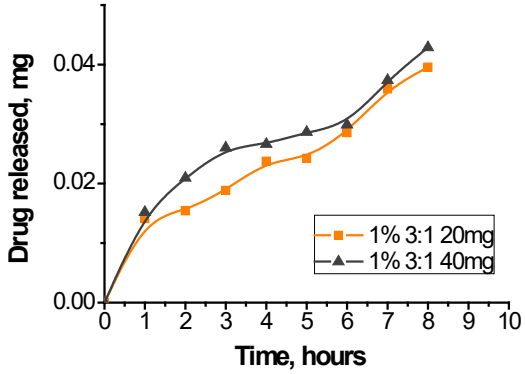

Fig. 8. TA release from casein nanoparticles at different drug concentration

The cumulative release of TA in different media at pH 7.4 and 37 °C for the first 8 hours is shown in Figures 6-8. The cumulative TA release is not more than 0.6 % during this period, demonstrating that no burst effect is realized. Hence, TA is well encapsulated in the casein gels. The slow release of TA could be due to its hydrophobic properties and its difficult dissolution in water media. The slower release is observed between the second and the fourth hour, when the nanoparticles are immersed in acetate buffer at pH 4. This pH is very close to the isoelectric point of casein and in this case the polymer is prone to precipitation [3]. Hence, the matrix is the densest and the drug diffusion is very difficult.

The increase of crosslinker concentration leads to delayed release (Fig. 6). Similar results have been reported by Baimark and Srisuwan [15] who found that the amount of released drug goes down when the concentration of Ca^{2+} crosslinker in alginate gels rose from 5% to 10% due to harder swelling of the alginate network.

Increasing the concentration of the casein solution from which the submicron gel particles were formed from 0.5% to 1% results in an increase in the TA release rate – Fig. 7. These results could be explained with the fact, that at the lower protein concentration the particles are less dense and part of the drug migrates from the bulk to the surface of the particles. This assumption is confirmed by the DSC experiments. Once the drug has come to the surface, its release is faster.

The dependence of the drug concentration on the release rate is presented in Fig. 8. The increase of the drug concentration leads to faster release as a result of the looser structure of the matrix.

The obtained results demonstrated that the TA release profile could be controlled by the concentration of casein and crosslinker.

CONCLUSION

TA-loaded casein gels crosslinked with $CaCl_2$ were investigated in this study. The average size of the gel particles varied from 140 nm to 380 nm, depending on the casein concentration and casein:crosslinker ratio. The loading of TA into the nanospheres did not lead to chemical interactions between the matrix and the drug. The loaded TA was predominantly amorphous, which increased its bioavailability.

Acknowledgements: *This work was financially supported by the Bulgarian National Science Fund, Project № KP-06-N 38/3. The authors thank the project BG05M20P001-1.002-0005, Personalized*

Innovative Medicine Competence Center (PERIMED) for the provided instrumentation used during the study.

REFERENCES

1. D. Verma, N. Gulati, S. Kaul, S. Mukherjee, U. Nagaich, *Journal of Pharmaceutics*, 2018:9285854, (2018), DOI: 10.1155/2018/9285854.
2. L. R. Brown, *Expert Opinion on Drug Delivery*, **2**(1), 29 (2005).
3. A. O. Elzoghby, W. S. A. El-Fotoh, N. A. Elgindy, *Journal of Controlled Release*, **153**(3), 206 (2011).
4. R. Basha, C. H. Baker, U. T. Sankpal, S. Ahmad, S. Safe, J. L. Abbruzzese, M. Abdelrahim, *Front Biosci. (Schol. Ed.)*, **3**(1), 797 (2011).
5. S. Rozou, S. Michaleas, E. Antoniadou-Vyza, *Pharmacy and Pharmacology Communications*, ***5***(2), 79 (1999).
6. M. Ouanezar, F. Guyomarc'h, A. Bouchoux, *Langmuir*, **28**(11), 4915 (2012).
7. S. Vino, K. R. Lokesh, S. Vijayaragavan, G. Jayaraman, A. R. Ghosh, *International Journal of Pharmaceutical Sciences and Research*, **2**(2), 383 (2011).
8. J. Raj, K. B. Uppuluri, *Biomedical and Pharmacology Journal*, **8**(1), 83 (2015).
9. M. Luo, J. Xiao, S. Sun, F. Cui, G. Liu, W Li, Y. Li, Y. Cao, *Food & Function*, **11**(6), 5284 (2020).
10. D. M. Byler, Jr, H. M. Farrell, *Journal of Dairy Science*, **72**(7), 1719 (1989).
11. S. Ahmed, M. A. Sheraz, I. Ahmad, *Excipients and Related Methodology*, **43**, 255 (2018).
12. A. Tsioulpas, M. J. Lewis, A. S. Grandison, *Journal of Dairy Research*, **74**(2), 167 (2007).
13. A. O. Elzoghby, M. W. Helmy, W. M. Samy, N. A. Elgindy, *European Journal of Pharmaceutics and Biopharmaceutics*, **84**(3), 487 (2013).
14. S. Ahmed, M. A. Sheraz, I. U. Rehman, *AAPS PharmSciTech*, **14**(2), 870 (2013).
15. Y. Baimark, Y. Srisuwan, *Adv. Powder Techn*., **25**(5), 1541 (2014).