Integration of Liposomes as a new detection particle into a test strip format and evaluation of its performance character

microcoat

Author: Carolin Schrötel, Barbara Kaiser

Company: Microcoat Biotechnologie GmbH, Am Neuland 3, 82347 Bernried

Mentor: Dr. Mark-Steven Steiner (☏: 08158 / 9981 17, ✉: m.steiner@microcoat.de)

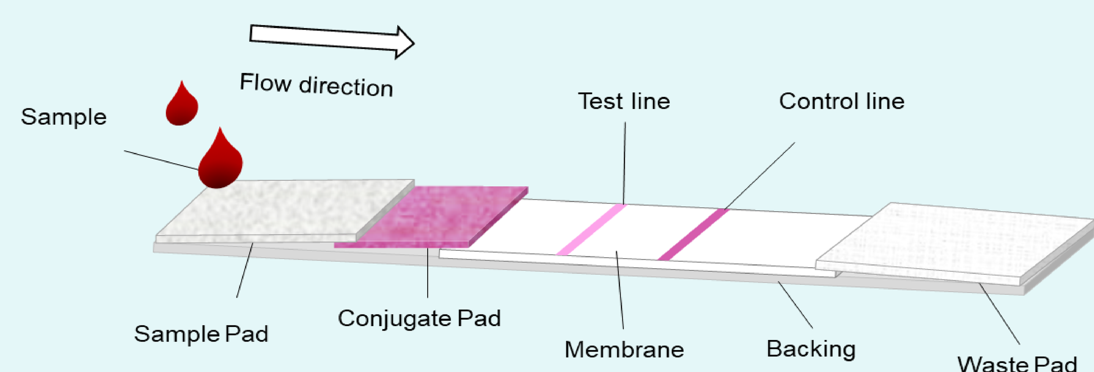
Introduction

Lateral Flow Assay (LFA) based point of care (POC) devices are rapidly growing strategies for qualitative and quantitative analysis [1]. A big advantage over other detection tests are the short time till the result is visible (<30min) and the low costs. As detection particle, liposomes were established and compared to usually used colloidal gold particles. The major difference of liposomes compared to colloidal gold is, that liposomes consist of a phospholipid bilayer forming a hollow space, where aqueous solution can be encapsulated. Different detection methods can be applied by encapsulating (1) varying concentrations of sulforhodamine B (SRB) (colorimetric and fluorescent) or (2)m-caboxy luminol (chemiluminescent). The aim of this work was to improve sensitivity with different detection methods of liposomes and the implementation of a sucessfull drying method including stability studies.

Principles

Principle of a Lateral Flow Assay

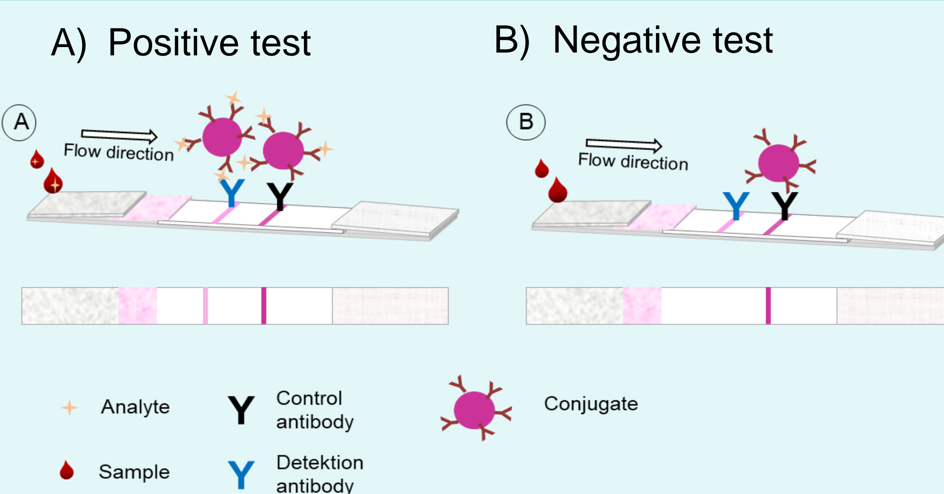
The sample containing the analyte moves via capillary forces from sample pad to conjugate pad. There, the dried detection conjugate particles are dissolved and bind to the analyte. This complex moves than over the nitrocellulose membrane and in a sandwich format it will bind to the test line, where detection antibodies are bound. Conjugate particles can also bind to the control line, where antispecies specific antibodies capture the antibodies immobilized on the detection particle [2].

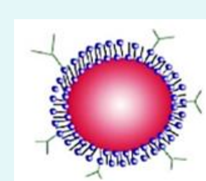


Sandwich format

A) Positive test

B) Negative test



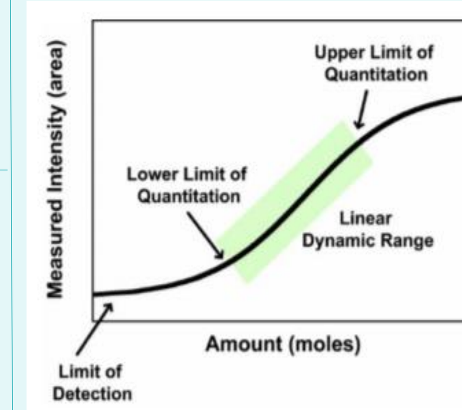


As an analyte, Interleukin 6 was used. Detection particles were liposomes coated with anti-Interleukin 6.

parameters for sensitivity

LOD is defined as the lowest concentration of an analyte which can be detected, but not essentially quantified, while LOQ is defined as lowest concentration of an analyte that can be determined with acceptable accuracy [3].

LOQ can be divided into the lower limit of quantification (LLOQ) an upper limit of qualification (ULOQ). All measurements between these two limits form the dynamic range [4].



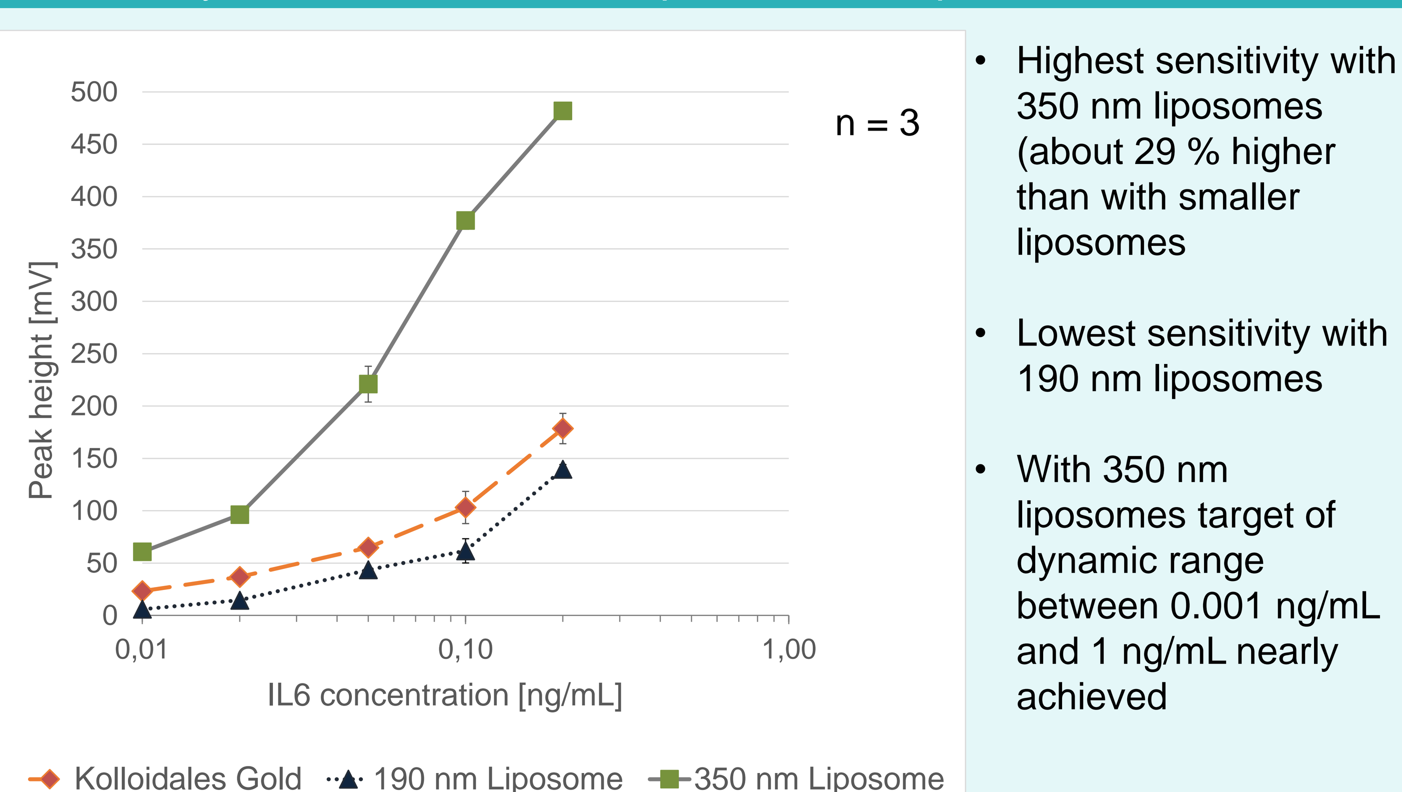
$$LOD = A_{negative\ test} + 3 * SD_{negative\ test}$$

A = Average

SD = Standard Deviation

Results

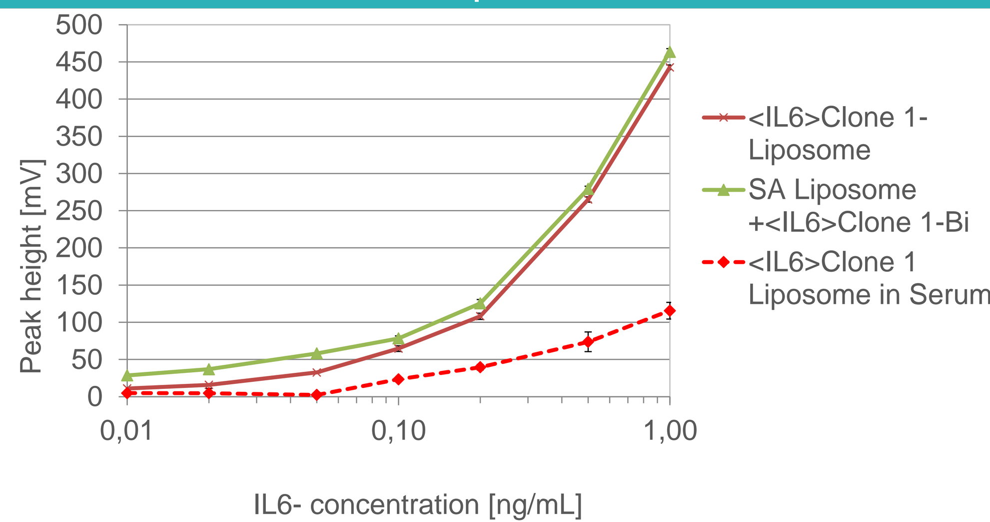
Sensitivity of colorimetric SRB Liposomes compared to colloidal Gold

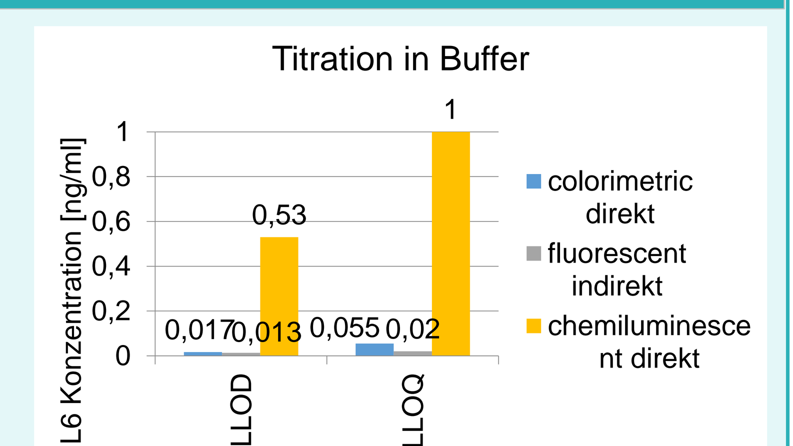


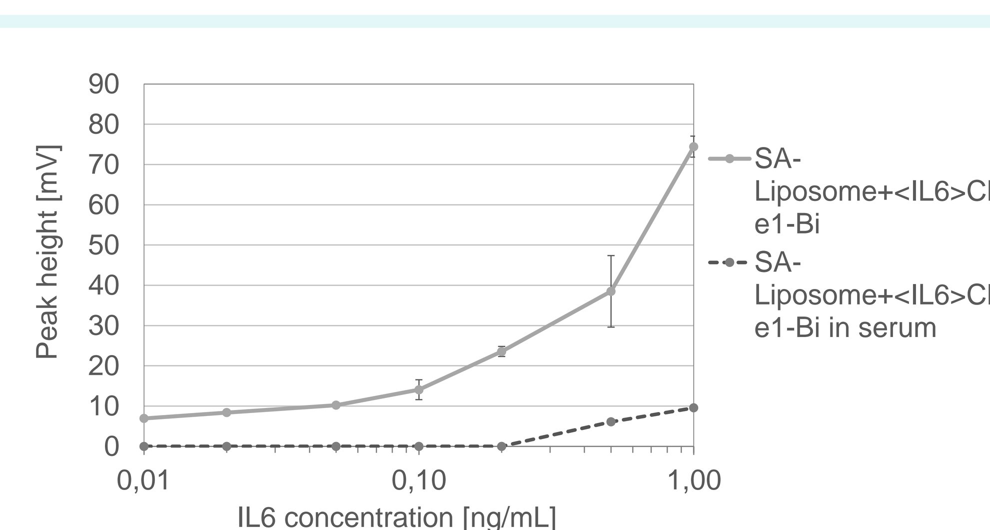
- Highest sensitivity with 350 nm liposomes (about 29 % higher than with smaller liposomes
- Lowest sensitivity with 190 nm liposomes
- With 350 nm liposomes target of dynamic range between 0.001 ng/mL and 1 ng/mL nearly achieved

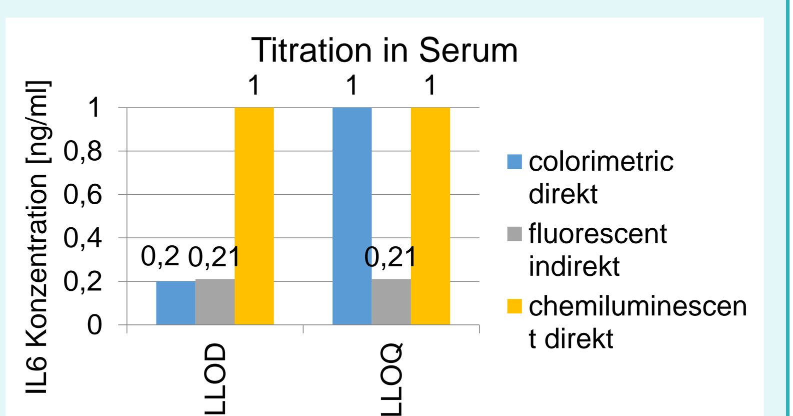
Colloidal Gold:	Test line signal of LLOD = 25 mV → 0.0034 ng/mL IL6 Test line signal of LLOQ = 51 mV → 0.021 ng/mL IL6
190 nm Liposomes:	Test line signal of LLOD = 23 mV → 0,03 ng/mL IL6 Test line signal of LLOQ = 59 mV → 0,09 ng/mL IL6
350 nm Liposomes:	Test line signal of LLOD = 27 mV → 0.0006 ng/mL IL6 Test line signal of LLOQ = 51 mV → 0.0065 ng/mL IL6

Titration test of liposomes with different detection Methods in Buffer and Serum



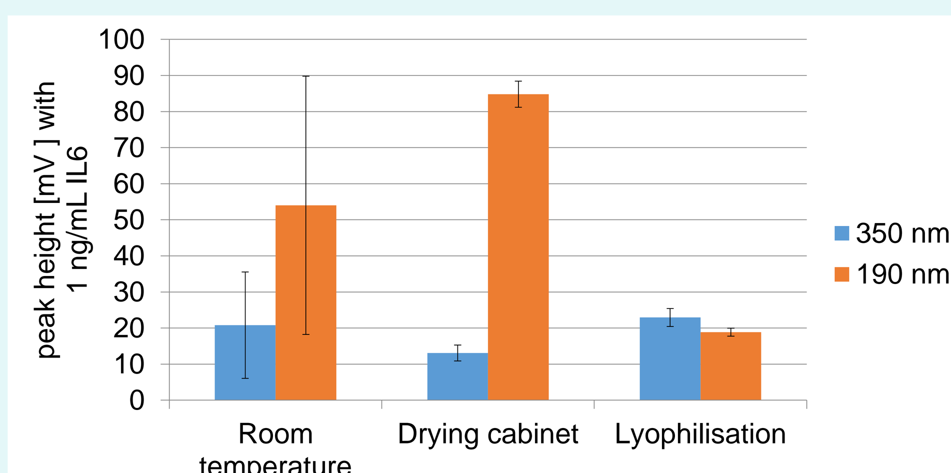




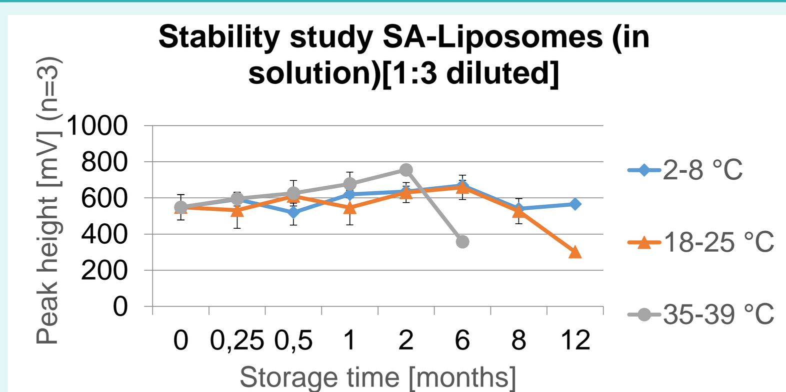


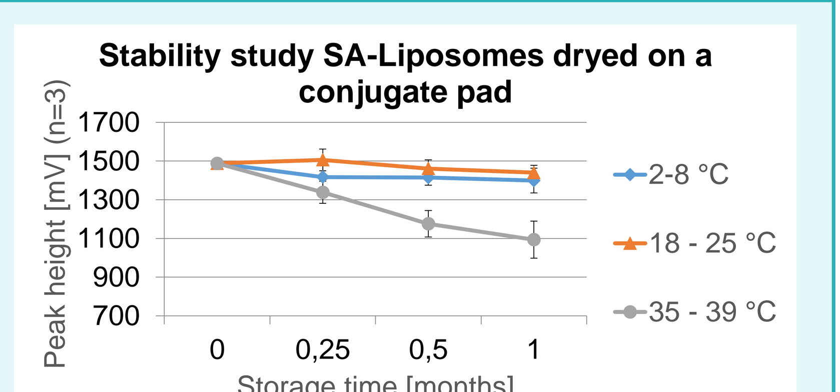
Drying of liposome conjugate onto the conjugate pad

- For liposomes with diameter of 190 nm, most effective drying method is drying for 30 min in a drying cabinet(40 mOD/Test)
- For liposomes with diameter of 350 nm, lyophilisation with slow freezing time is the most effective drying method (40mOD/Test)-but results show that the process needs to be optimized



Stability Studies





- Liposome conjugates in solution and dried on a conjugate pad showed a decrease of 80% signal for the storage at 35-39 °C
- Liposome conjugates in solution also showed a signal loss of 50 % after 8 months for the storage at 18-25 °C

→ Recommend storage temperature for liposome conjugates in solution and for test strips with conjugate pad is 2-8 °C

Outlook

Because 350 nm liposomes revealed in a higher sensitivity than 190 nm liposomes, a titration test for colorimetric and fluorescent liposomes with a diameter of 350 nm should be performed in buffer and in serum. For chemiluminescent liposomes, a specific reader with photodiodes and the possibility to measure time-gated is necessary. Additionally, the indirect format using SA-liposomes should be tested for freeze-drying 190 and 350 nm liposomes.

Conclusion

Titration test in serum lead – not unexpectedly – to a higher LLOD and LLOQ for all detection methods compared to the measurement in buffer. In testing with Assay Buffer 1 the lowest values for LLOD were reached with fluorescent liposomes with a 0,013 ng/mL IL6. LLOQ was also best with measurement with fluorescent Liposomes with a value 0,02 ng/mL IL6. For liposome stability, a drying of SA-Liposomes or small liposomes via lyofilization showed the lowest LLOD and LLOQ, while drying at Room temperature was the best drying method for big liposomes. Even though in liquid measurement big liposomes showed a much higher signal height, after drying the signals of small liposomes were higher. Stability study showed, that in liquid condition, liposome conjugates do not lose activity within the first 6 months, if they are stored at 2-8°C. Storage in dried format is possible for 12 month.

Literatur:
[1] M. Sajid, A.-N. Kawde, M. Daud, *Journal of Saudi Chemical Society* **2015**, *19 (6)*, 689 – 705. DOI: 10.1016/j.jscs.2014.09.001.
[2] B. Ngom, Y. Guo, X. Wang, D. Bi, *Analytical and bioanalytical chemistry* **2010**, *397*, 1113–1135.
[3] A. Shrivastava, V. B. Gupta, *Chronicles of young scientists* **2011**, *2*, 21.
[4] J. R. Johnson, L. L. Summit Analytical, *Methods for handling concentration values below the limit of quantification in PK studies*, **2019**

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