# TOWARDS PROPER SIZING EXPERIMENTS: ANALYSIS OF THE IMPACT OF MEASUREMENT CONDITIONS AND SAMPLE PREPARATION ON THE SIZE ESTIMATION OF THE NANODROPLETS AND NANOPARTICLES

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Physicochemical properties of many active ingredients jeopardize their pharmacological activity. To overcome identified obstacles, nanosystems as carriers for delivery of actives have been recognized as promising tools, with highly posted expectations. The nanotechnology-based approach in drug formulation is much more than just another step in size miniaturization. Given the complexity of nanosystems, challenges encountered in their characterization, and evident lack of testing protocols, relevant European research/regulatory bodies have issued guidelines, summarizing important parameters for nanosystem characterization. Size/size distribution (*per se* and in biorelevant environment) are essentially important, representing critical quality attributes (1). The aim was to show how significantly different results could be obtained under different measurement conditions/sample preparation methods, offering optimal protocol for size estimation applying dynamic light scattering (DLS), with complementary analysis through atomic force microscopy. Hydrophilic nanoemulsion and aqueous dispersion of polymeric nanoparticles were used as test samples. Ultrapurified water, phosphate buffer saline (PBS, pH 7.4) and biorelevant medium with serum proteins were used for dilution. Measurements were performed applying batch-mode DLS (ZetasizerNano), and AutoProbe CP-Research microscope. Significant differences in obtained nanodroplet/nanoparticle size were observed depending on the type of medium and dilution level. Protein corona formation could not be confirmed with certainty. Preference was given to PBS as dispersant. The optimal level of dilution for nanoparticles was 1:10 (*Z-ave*=59.16±0.46nm), and for nanoemulsion 1:100 (*Z-ave*=73.5±0.75nm). For proper interpretation, it is necessary that the DLS measurement report, in addition to the *Z-ave* and the polydispersity index, contains at least the data on the attenuation and correlation function intercept.

**References**

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# U SUSRET PRAVILNOM ODREĐIVANJU VELIČINE NANOSTRUKTURA: ANALIZA UTICAJA USLOVA MERENJA I PRIPREME UZORKA NA RAZULTATE PROCENE VELIČINE NANOKAPI I NANOČESTICA

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Fizičkohemijske osobine mnogih aktivnih supstanci otežavaju ostvarivanje farmakoloških efekata. Kako bi se identifikovane prepreke prevazišle, visoka očekivanja postavljena su pred nanosisteme - nosače za isporuku aktivnih supstanci, uz značajna ulaganja u njihov razvoj. Nanotehnološki pristup formulaciji leka mnogo je više od pukog smanjenja veličine. Imajući u vidu kompleksnost nanosistema, izazove koji se sreću u njihovoj karakterizaciji, te evidentan nedostatak protokola ispitivanja, relevanta evropska istraživačka/regulatorna tela izdala su vodiče, sumirajući značajne parametre karakterizacije nanosistema. Veličina/distribucija veličina (*per se* i u biorelevantnim uslovima) od suštinske su važnosti za postizanje očekivanih performansi, predstavljajući kritične atribute kvaliteta (1). Cilj rada bio je pokazati kako se pri različitim uslovima merenja/načinima pripreme istog uzorka dobijaju značajno različiti rezultati procene veličine, te ponuditi optimalan protokol merenja dinamičkim rasipanjem svetlosti (DLS), uz komplementarno ispitivanje mikroskopijom atomskih sila. Kao test uzorci korišćeni su hidrofilna nanoemulzija i vodena disperzija polimernih nanočestica, a kao medijumi za razblaživanje visokoprečišćena voda, izotonični fosfatni pufer (PBS, pH 7,4) i biorelevantni medijum obogaćen proteinima seruma. Merenja su sprovedena primenom *batch-mode* DLS, (ZetasizerNano), i na mikroskopu AutoProbe CP-Research. Primećene su značajne razlike u vrednostima veličine nanokapi/nanočestica zavisno od tipa medijuma i nivoa razblaženja. Nije se moglo sa sigurnošću potvrditi formiranje protein korone nakon inkubacije u biorelevantnom medijumu. Za procenu veličine *per se*, prednost je data PBS-u kao disperzantu. Optimalan nivo razblaženja za nanočestice bio je 1:10 (*Z-ave*=59,16±0,46nm), a za nanoemulziju 1:100 (*Z-ave*=73,5±0,75nm). Radi adekvatnog tumačenja, potrebno je da izveštaj DLS merenja, pored Z-ave i indeksa polidisperznosti, sadrži barem još podatke o atenuaciji i odsečku korelacione funkcije.

**Literatura**

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