Determination of encapsulation efficiency of fluorescent tetanus toxoid in microsphere and Liposome drug delivery systems by spectrofluorimetery method

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Introduction: Microspheres and liposomes are frequently used for encapsulation of antigens, peptides and proteins. The general approach for measurement of encapsulated protein is spectrophotometric protein assay methods. However, in many cases some of existing materials interact with these methods. Another method for protein assay is preparation of fluorescent product of protein and measurement of encapsulation efficiency by spectrofluorimetry method. The objective of this study was to compare one spectrophotometry protein assay method with spectrofluorimetry for determination of amount of encapsulated TT in alginate microspheres and lecithin-cholestrol liposomes.

Methods: Tetanus toxoid (TT) was conjugated with fluprescein isothiocyanate (FITC) as fluorescent material. For this purpose specific amounts of TT and FITC were gently mixed in carbonale buffer pH 9.5 at 4^0C in the darkness for 18h. Dialysis and ultrafiltration methods were used for separation of buffer salts and unconjugated FITC. Alginate microspheres encapsulated with FITC – TT were prepared by emulsification- internal phase gelation method, and lecithin–cholesterol liposomes encapsulated with FITC–TT were prepared by solvent evaporation method. The amount of encapsulated TT in microsphere was directly determined in solution of microspheres in citrate buffer. The amount of encapsulated TT in liposomes were measured by determination of unencapsulated protein in supernatant (indirect method) by Bradford protein assay and spectrofluorimetry methods.

Results: The encapsulation efficiencies (EEs) for microspheres were %38.33 + 6.11 and % 21.33 + 2.82 by Bradford and fluorimetry method, respectively ($P<0.01$). The EEs for liposomes encapsulated with FITC–TT resulted by Bradford and fluorimetry methods were %46.33 + 6.23 and %56.0 + 3.0, respectively ($P< 0.05$).

Conclusion: Dissolved blank microspheres and supernatant of blank liposomes resulted high absorbance in Brodford method, resulted from interaction of particle forming materials with this method. However, absorbances were very low in spectroflourimetry, so measurements by this method were more satisfactory. The lower EE in microspheres (direct method) and higher EE in liposomes (indirect method) was due to lower interaction potential with spectroflourimetry method. So the spectrophotometric methods can be substituted by spectroflourimtry method, when high interactions are encountered in spectrophotometric protein assay methods.

Keywords: Alginate microsphere; Liposome; FITC–TT; Encapsulation efficiency; Spectrofluorimetry

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