ORIGINAL ARTICLE

Improving of pelB-Secreted MPT64 protein released by *Escherichia coli* BL21 (DE3) using Triton X-100 and Tween-80

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ABSTRACT

pelB has been known as a successful signal peptide to translocate the protein target extracellularly in the *Escherichia coli* system. However, in our previous study, the yield of MPT64 protein extracellular recovery was still low and plenty of this protein was remain trapped in cytoplasm and periplasm. Recently, nonionic surfactants were efficiently reported to secrete recombinant protein extracellularly. Nonetheless, it must be clarified whether the surfactant supplementation can improve the yield of MPT64 extracellular protein significantly without giving impact on the structure of isolated MPT64 protein and can minimized the cell lysis effect. MPT64 protein secretion was carried out by comparing the effects of surfactants Tween 80 and Triton × 100 at various concentrations. Triton × 100 was able to increase the extracellular MPT64 protein gain up to 3 times higher than Tween 80 and it was in line with the greater level ratio of cell leakage of Triton × 100 compared to that of Tween 80. Similarly, the viable cell of the cultures decreased dramatically. However, both surfactants did not interfere the structure of MPT64 protein. In conclusion, Triton × 100 can be chosen as the supporting surfactant to assist the act of peptide signal in improving the resulting of MPT64 extracellular protein.

Key words: Extracellular, MPT64, pelB, Triton × 100, Tween 80

INTRODUCTION

The strategy to evade the host's immune system is an important virulence factor of *Mycobacterium tuberculosis* in maintaining the multiplication of this bacterium in infected host cells. Several *M. tuberculosis* secreted proteins facilitate this bacterium by regulating antiapoptotic and proapoptotic molecules to inhibit the apoptosis of infected macrophages.[1,2] MPT64 is one of the proteins secreted from *M. tuberculosis* with antiapoptosis mechanism on RAW264.7 macrophages by upregulating the Bcl-2, miRNA21, and nuclear factor-kappa B (NF-κB).[2] The most interesting of this protein is its specification to distinguish the *M. tuberculosis* complex from other mycobacteria bacterial species. This protein has great potential for *M. tuberculosis* identification using immune chromatographic methods through the antigen–antibody binding process.[3,4] Hence, this protein must be present in adequate concentration to generate antibodies against MPT64 to be constructed in immunochromatographic diagnostic kit. However, MPT64

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is difficult to be conventionally isolated from the original source due to the abundant of other secreted tuberculosis proteins which difficult to purify, and it is grouped as high-risk air infect pathogen.

In our previous study, this protein was produced by expressing the synthetic gene coded for the MPT64 protein in *Escherichia coli* BL21 (DE3) and secreted extracellularly by constructing the gene fused with pelB signal peptide.[5,6] We found that the pelB success to translocate the MPT64 extracellularly, but the yield was still low and plenty of this protein was remain trapped in cytoplasm and periplasm. Thus, to enhance the secretory potential, the growth medium of *E. coli* BL21 (DE3) was augmented with surfactants, which allows the production of highly efficient extracellular target proteins in *E. coli* but does not impair the activity of the target protein.[7,8] Triton × 100 and Tween 80 were reported effectively secreted protein targets in high levels compared to other surfactants such as $CaCl_2$, sodium dodecyl sulfate (SDS), and glycine in *E. coli*.[8] Therefore, in this study, the optimized conditions related to the secretion process of MPT64 protein were conducted and the extracellular MPT64 structure was evaluated to validate the structural integrity of the protein required to maintain antigen epitopes for antibody production.

MATERIALS AND METHODS

Materials

The materials used are *E. coli* BL21 (DE3) transformant, Luria Bertani (LB) (Himedia), L-rhamnose (Sigma-Aldrich), Tween 80 (Sigma-Aldrich), Triton × 100 (Sigma-Aldrich), and SDS PAGE components.

Optimization of recombinant MPT64 protein secretion

Optimization of MPT64 protein secretion was carried out by comparing the effects of surfactants Tween 80 and Triton × 100 at various concentrations as follows: 0%, 0.1%, 0.2%, and 0.5%. A total of 6 mL of bacterial starter were inoculated into four Erlenmeyer flasks containing 294 mL of liquid LB, then 300 L of kanamycin (100 g/mL) were added to each of the Erlenmeyer flasks and incubated at 37°C, 180 rpm, optical density OD600 (0.6–0.8). After that, the bacterial culture was measured for its OD at a wavelength of 600 nm (t0) and 10 mL of the bacterial culture was taken and centrifuged at 6000 g at 4°C for 20 min. The cell culture was then induced with the addition of Rhamnose to a concentration of 4 mM, then incubated again at 37°C, 180 rpm for 24 h. After 24 h, the OD600 of bacterial culture was remeasured (t24) and 10 mL of cell culture was taken for centrifugation at a speed of 6000 g at 4°C for 20 min. Then, the surfactant was added to each Erlenmeyer flask with each concentration of 0, 0.1, 0.2, and 0.5%, and incubated at 37°C, 100 rpm for 6 h. After that, the bacterial culture OD600 was measured again (t30) and 10 mL of cell culture was taken and centrifuged at 6000 g at 4°C for 20 min. Before the isolation of the media fraction, phenylmethylsulfonyl fluoride was added to each culture until the final concentration was 0.1 mM. A total of 10 mL of the culture taken at t0, t24, and t30 were centrifuged to separate the supernatant from the pellets. Proteins in the media fraction were characterized using the SDS-PAGE method and quantified using ImageJ software.

Effects of surfactants

Ten milliliters of overproduced cultures at t0, t24, and t30 were centrifuged at 10,000 g, 4°C, for 5 min. Then, its absorbance at OD260 was checked to measure the DNA concentration in the transformant cultures. The higher the absorbance, the higher the concentration of DNA in the sample, which indicates the number of cells undergoing lysis. As confirmation of cell viability, each sample at t0, t24, and t30 was inoculated as much as 10 L on the surface of solid LB medium containing kanamycin. The culture on solid media was then incubated at 37°C for 24 h and the number of colonies observed was counted.

RESULTS AND DISCUSSION

In our previous study, the optimal conditions for MPT64 gene expression have been conducted, but the acquisition of extracellular MPT64 protein as a protein target was still low.[9] In line with this, it has been widely reported that the challenge to express extracellular proteins in *E. coli* is always distributed in the periplasm.[10] To overcome this drawback, the acquisition of extracellular MPT64 protein can be increased by improving the leakage of specific host cell membranes by constructing the cell host into a leaky phenotype. Such phenotypes can be induced through several ways, as follows: mutation or deletion of membrane components, addition of permeability enhancers (glycine, calcium, Triton X-100, etc.), or coexpression of proteins with lytic activity.[11-15]

Triton × 100 and Tween 80 are the most widely used nonionic surfactants to increase the permeability of living cell membranes.[16-20] Therefore, this evidence based bring this study to investigate the effect of both surfactants in improving the yield of MPT64 extracellular recovery. As seen in Figures 1 and 2, the thickness of the protein band in each cell fraction described the different protein concentrations at various surfactant concentrations. The protein concentrations were demonstrated in Tables 1 and 2 and the different effects of each surfactant in various concentrations on the MPT64 extracellular protein recovery can be described clearly in Figures 3 and 4. The supplementation of Triton × 100 (0.5% v/v) into the growth medium could increase the extracellular MPT64 protein gain up to 3 times higher than using Tween 80 at the same concentration. The different effects of both surfactants on membrane permeability were predicted by the fact that nonionic surfactants do not dissociate when dissolved in

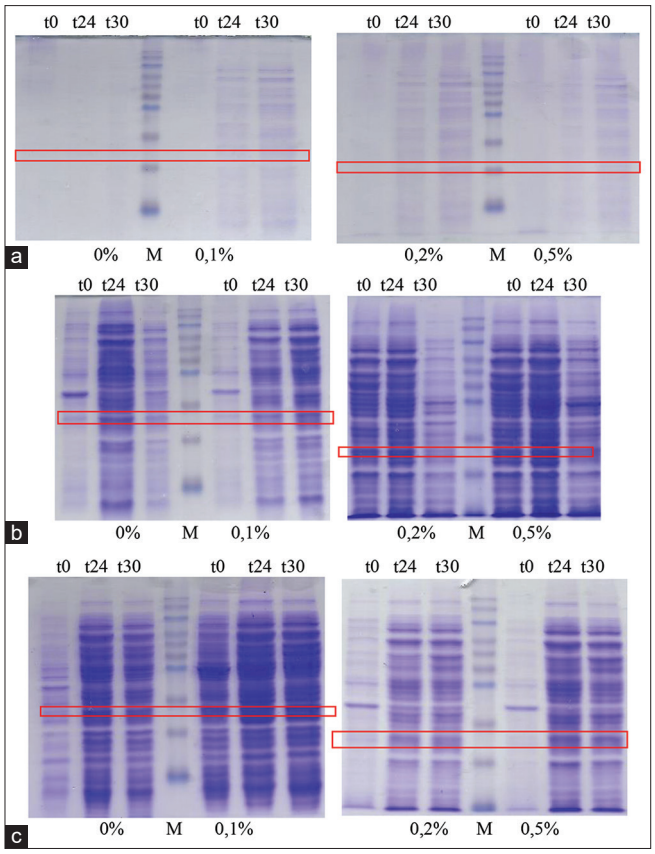

Figure 1: Effect of Tween 80 on MPT64 protein gain in different fractions (a) medium (b) periplasm (c) cytoplasm

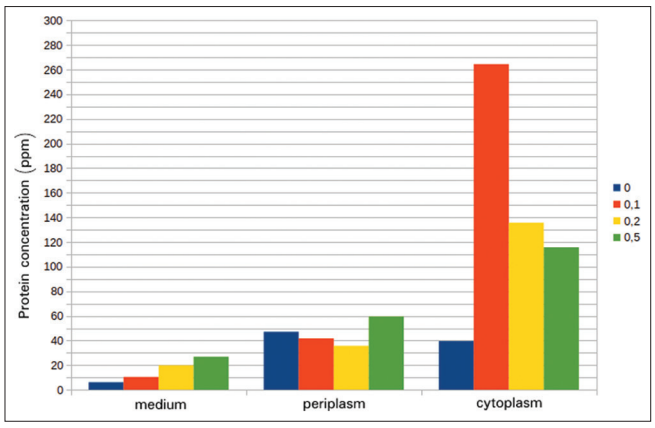

Figure 3: Distribution of MPT64 production after Triton × 100 treatment in different concentrations

water and have the widest range of properties depending on the hydrophilic-lipophilic equilibrium ratio (HLB).[21] If the HLB value of the surfactant is higher than 10, then the nonionic surfactant tends to be more hydrophilic. As a result, the surfactant layer lowers the surface tension of the water more than the surface tension of the oil. Surfactants with low HLB values are more soluble in oil (lipophilic), while surfactants with higher HLB values are more soluble in water (hydrophilic). This supports the evidence that Triton × 100 (HLB = 13.5) with a lower HLB value than

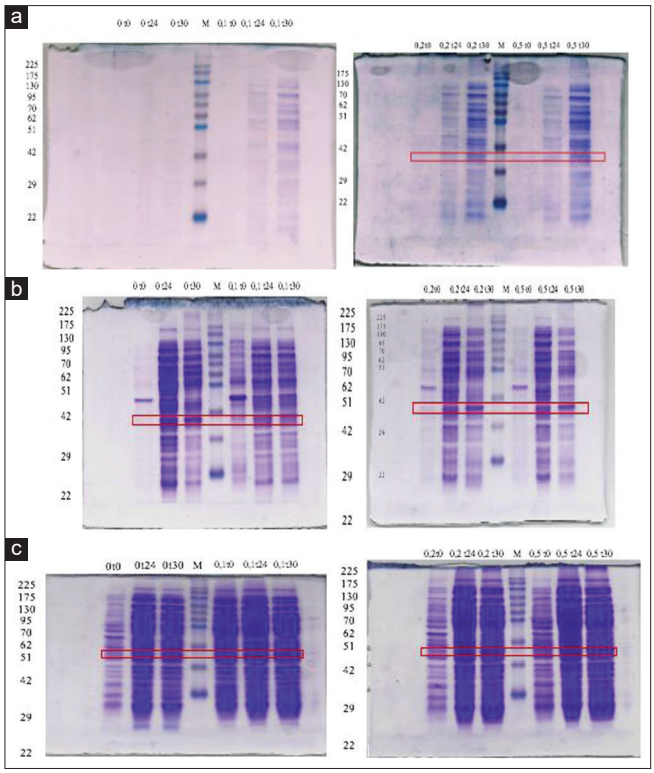

Figure 2: Effect of Triton × 100 on MPT64 protein gain in different fractions (a) medium (b) periplasm (c) cytoplasm

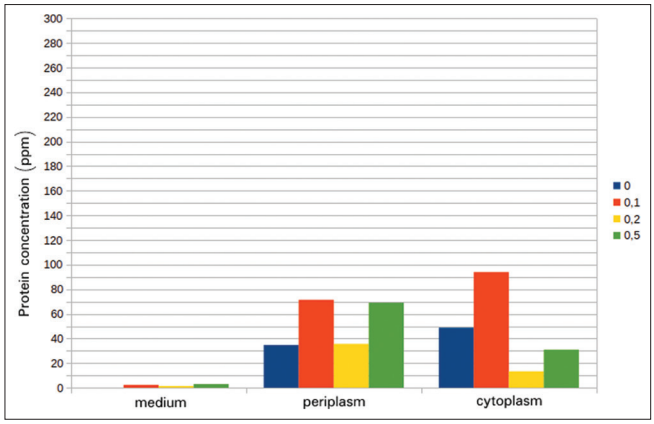

Figure 4: Distribution of MPT64 production after Tween 80 treatment in different concentrations

Tween 80 (HLB = 15.0) is able to interact more strongly with bacterial cell membranes composed of phospholipids.[22] In addition, we must confirm the effect of the surfactants to the MPT64 structure. From Figure 5, we can see that the antibody of the kit diagnostics can capture the MPT64 successfully. It is indicated that both surfactants did not interfere the MPT64 structure. Thus, the overproduction of MPT64 protein can be combined with Triton × 100 surfactant at the late stage of incubation.

However, the concentration used must be considered because several studies have reported the occurrence of cell

death after prolonged exposure to these surfactants.[23-25] From this study, both surfactants were shown to be able to release higher extracellular MPT64 protein compared to untreated cells, presented in Table 3. The level ratio of cell leakage between Triton × 100 (0.5%) and Tween 80 (0.5%) against the control cell was 86.07% and 66.35%, respectively. From Table 4, at the 30th h after the addition of surfactant, the culture added with Triton × 100 experienced a large decrease number of viable cells for about 8%–20% at 24 h. Therefore, we hypothesized that both surfactants could interact with the cell membrane, but the high level of MPT64 protein is still trapped in the cytoplasm and periplasm. We predicted that this was due to the ineffectiveness of the pelB signal peptide used in translocating MPT64 protein

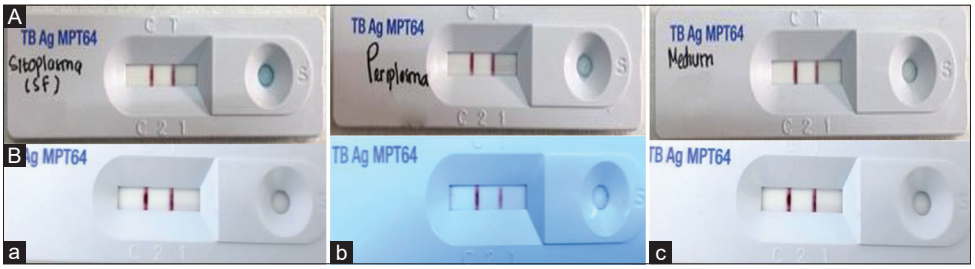

Figure 5: The identification of secreted MPT64 protein influenced by (A) Triton-×100; (B) Tween 80 (a) cytoplasm (b) periplasm, (c) medium

Table 1: Effect of Tween 80 on MPT64 protein levels

Surfactant concentration (%v/v)	Time (h)	Quantification of protein content at various concentrations of Tween 80					
		Medium		Periplasm		Cytoplasm	
		Band area	Concentration (ppm)	Band area	Concentration (ppm)	Band area	Concentration (ppm)
0	t0	0	0.00±0.000	22.282	22.48±0.002	23.717	24.16±0.001
	t24	0	0.00±0.000	78.863	88.61±0.001	50.809	55.82±0.002
	t30	0	0.00±0.000	32.805	34.78±0.002	44.78	48.78±0.001
0.1	t0	0	0.00±0.000	16.834	16.11±0.001	51.879	57.07±0.002
	t24	4.108	1.24±0.004	54.446	60.07±0.004	68.531	76.54±0.001
	t30	5.07	2.37±0.001	64.184	71.46±0.001	83.422	93.94±0.001
0.2	t0	0	0.00±0.000	54.633	60.29±0.002	53.396	58.85±0.001
	t24	3.997	1.11±0.001	52.447	57.74±0.001	62.834	69.88±0.001
	t30	4.254	1.41±0.002	33.571	35.68±0.001	14.481	13.36±0.001
0.5	t0	0	0.00±0.000	60.404	67.04±0.001	56.267	62.20±0.000
	t24	3.906	1.00±0.001	67.631	75.48±0.002	60.856	67.57±0.001
	t30	5.633	3.02 ± 0.004	62.166	69.10 ± 0.001	29.476	30.89 ± 0.001

Table 2: Effect of Triton X-100 on MPT64 protein levels

Surfactant concentration (%v/v)	Time (h)	Quantification of protein content at various concentrations of Triton X-100					
		Medium		Periplasm		Cytoplasm	
		Band area	Concentration (ppm)	Band area	Concentration (ppm)	Band area	Concentration (ppm)
0	t0	1.66	−0.89±0.001	3.342	2.10±0.000	12.474	18.36±0.004
	t24	5.391	5.75±0.001	32.794	54.53±0.001	51.287	87.45±0.001
	t30	5.648	6.20±0.000	28.631	47.12±0.000	24.374	39.54±0.004
0.1	t0	1.478	−1.21±0.001	12.503	18.41±0.001	16.521	25.56±0.004
	t24	5.675	6.25±0.000	29.644	48.92±0.002	81.168	140.65±0.001
	t30	8.052	10.48±0.002	25.595	41.71±0.001	150.719	264.47±0.001
0.2	t0	4.541	4.23±0.001	12.091	17.67±0.001	16.452	25.44±0.000
	t24	12.859	19.04±0.001	23.695	38.33±0.001	69.303	119.53±0.001
	t30	13.327	19.87±0.001	22.226	35.72±0.002	78.345	135.63±0.001
0.5	t0	5.191	5.39±0.001	10.984	15.70±0.000	33.447	55.69±0.001
	t24	8.925	12.04±0.001	34.819	58.14±0.001	68.55	118.19±0.001
	t30	17.257	26.87 ± 0.001	62.166	59.47 ± 0.001	67.15	115.69 ± 0.001

Table 3: Release of intracellular components analysis

Sample	Surfactant	DNA concentration (μg/ml)
1	0%	3.55±0.001
2	Triton 0.1%	14.55±0.001
3	Triton 0.2%	15.90±0.000
4	Triton 0.5%	25.50±0.001
5	Tween 0.1%	6.10±0.002
6	Tween 0.2%	8.60±0.001
7	Tween 0.5%	10.55±0.000

Table 4: Effect of surfactants on the viable cell

Surfactant	Incubation time (h)		
	0	24th	30th
0%	2.05×10^{11}	4.59×10^{12}	3.84×10^{11}
Triton 0.1%	2.05×10^{11}	2.91×10^{12}	5.10×10^{10}
Triton 0.2%	3.84×10^{11}	4.14×10^{12}	4.46×10^{10}
Triton 0.5%	2.78×10^{11}	2.75×10^{12}	7.10×10^{10}
Tween 0.1%	2.53×10^{11}	2.46×10^{12}	5.00×10^{11}
Tween 0.2%	3.66×10^{11}	2.68×10^{12}	2.81×10^{11}
Tween 0.5%	2.34×10^{11}	2.26×10^{12}	2.15×10^{11}

from the cytoplasm to the periplasm, so that the acquisition of MPT64 extracellular protein is still not optimal. In the future, it is possible to optimize the use of signal peptides to increase the acquisition of extracellular MPT64 protein with the support of surfactants to release the accumulated MPT64 protein from periplasm to medium.

CONCLUSION

This study showed the efficiency of using Triton × 100 in supporting the action of the pelB signal peptide in translocating the extracellular MPT64 protein.

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Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Russell DG. *Mycobacterium tuberculosis*: Here today, and here tomorrow. Nat Rev Mol Cell Biol 2001;2:569-77.
2. Wang Q, Liu S, Tang Y, Liu Q, Yao Y. MPT64 protein from *Mycobacterium tuberculosis* inhibits apoptosis of macrophages through NF-kB-miRNA21-Bcl-2 pathway. PLoS One 2014;9:e100949.
3. Tomiyama T, Matsuo K, Abe C. Rapid identification of *Mycobacterium tuberculosis* by an immunochromatography using anti-mpb64 monoclonal antibodies. Int J Tuberc Lung Dis 1997;1:326-32.
4. Kanade S, Nataraj G, Suryawanshi R, Mehta P. Utility of MPT 64 antigen detection assay for rapid characterization of mycobacteria in a resource constrained setting. Indian J Tuberc 2012;59:92-6.
5. Kusuma SA, Parwati I, Subroto T, Rukayadi Y, Fadhlillah M, Rostinawati T, *et al*. Construction and expression of synthetic gene encoding mpt64 as extracellular protein in *Escherichia coli* BL21 (DE3) expression system. J Pharm Sci Res 2018;10:2659-65.
6. Kusuma SAF, Parwati I, Subroto T, Rukayadi Y, Fadhlillah M, Rostinawati T, *et al*. Optimization of culture conditions for *Mpt64* synthetic gene expression in *Escherichia coli* BL21 (DE3) using surface response methodology. Heliyon 2019;5:e02741.
7. Duan X, Zou C, Wu J. Triton X-100 enhances the solubility and secretion ratio of aggregation-prone pullulanase produced in *Escherichia coli*. Bioresour Technol 2015;194:137-43.
8. Yang L, Xu Y, Chen Y, Ying H. Efficient extracellular expression of phospholipase d in *Escherichia coli* with an optimized signal peptide. IOP Conf Ser Mater Sci Eng 2018;301:1-9.
9. Kusuma SAF, Parwati I, Subroto T, Rukayadi Y, Rostinawati T, Yusuf M, *et al.* Real-time monitoring of rhamnose induction effect on the expression of mpt64 gene fused with pelB signal peptide in *Escherichia coli* BL21 (DE3). J Adv Pharm Technol Res 2020;11:69-73.
10. Choi JH, Jeong KJ, Kim SC, Lee SY. Efficient secretory production of alkaline phosphatase by high cell density culture of recombinant *Escherichia coli* using the Bacillus sp. endoxylanase signal sequence. Appl Microbiol Biotechnol 2000;53:640-5.
11. Mergulhão FJ, Summers DK, Monteiro GA. Recombinant protein secretion in *Escherichia coli*. Biotechnol Adv 2005;23:177-202.
12. Rigi G, Mohammadi SG, Arjomand MR, Ahmadian G, Noghabi KA. Optimization of extracellular truncated staphylococcal protein a expression in *Escherichia coli* BL21 (DE3). Biotechnol Appl Biochem 2014;61:217-25.
13. Voulgaris I, Finka G, Uden M, Hoare M. Enhancing the selective extracellular location of a recombinant *E. coli* domain antibody by management of fermentation conditions. Appl Microbiol Biotechnol 2015;99:8441-53.
14. Li ZF, Li B, Liu ZG, Wang M, Gu ZB, Du GC, *et al.* Calcium leads to further increase in glycine-enhanced extracellular secretion of recombinant alpha-cyclodextrin glycosyltransferase in *Escherichia coli*. J Agric Food Chem 2009;57:6231-7.
15. Zou C, Duan X, Wu J. Enhanced extracellular production of recombinant *Bacillus deramificans* pullulanase in *Escherichia coli* through induction mode optimization and a glycine feeding strategy. Bioresour Technol 2014;172:174-9.
16. Gennuso F, Fernetti C, Tirolo C, Testa N, L'Episcopo F, Caniglia S, *et al*. Bilirubin protects astrocytes from its own toxicity by inducing up-regulation and translocation of multidrug resistance-associated protein 1 (Mrp1). Proc Natl Acad Sci U S A 2004;101:2470-5.
17. Rajagopal A, Pant AC, Simon SM, Chen Y. *In vivo* analysis of human multidrug resistance protein 1 (MRP1) activity using transient expression of fluorescently tagged MRP1. Cancer Res 2002;62:391-6.
18. Ashfaq M, Shah S, Rasul A, Hanif M, Khan HU, Khames A, *et al.* Enhancement of the Solubility and Bioavailability of Pitavastatin through a Self-Nanoemulsifying Drug Delivery System (SNEDDS). Pharmaceutics 2022;14:482.
19. Bae CS, Yang DS, Lee J, Park YH. Improved process for production of recombinant yeast-derived monomeric human G-CSF. Appl Microbiol Biotechnol 1999;52:338-44.
20. Bahrami A, Shojaosadati SA, Khalilzadeh R, Mohammadian J, Farahani EV, Masoumian MR. Prevention of human granulocyte colony-stimulating factor protein aggregation in recombinant Pichia pastoris fed-batch fermentation using additives. Biotechnol Appl Biochem 2009;52:141-8.
21. Williams JJ. Formulation of Carpet Cleaners. In Handbook for Cleaning/Decontamination of Surfaces. New York: Elsevier; 2007.
22. Miller, R. Emulsifiers: Types and Uses. Encyclopedia of Food and

Health. USA: Elsevier, Inc.: 2016.

23. Borner MM, Schneider E, Pirnia F, Sartor O, Trepel JB. Myers CE. The detergent Triton X-100 induces a death pattern in human carcinoma cell lines that resembles cytotoxic lymphocyte-induced apoptosis. FEBS Lett 1994;353:129-32.
24. Benoit J, Cormier M, Wepierre J. Comparative effects of four surfactants on growth, contraction and adhesion of cultured human fibroblasts. Cell Biol Toxicol 1988;4:111-22.
25. Dayeh VR, Chow SL, Schirmer K, Lynn DH, Bols NC. Evaluating the toxicity of Triton X-100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability. Ecotox Environ Safe 2004;57:375-82.