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Devising Interactive Dissolution Experiment for Pharmacy Students (part II): Use of Dialysis Bag Method to Evaluate Effect of Dialysis Bag Length on Drug Release from Novel Drug Delivery Systems

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Abstract
Laboratory experiments of pharmaceutics (5 years pharmacy graduation degree, Pharm D and 2 years postgraduate degree, M.Phil) include dissolution experiments of conventional release tablets and capsules. This paper reports an interactive dissolution experiments for niosomes by dialysis bag method which may be adapted to find interesting observation with minor modification. Dialysis tubes of different length were taken and filled with drug loaded niosomes. Dissolution studies were conducted at 25°C and 50 rpm stirring rate in 500 ml distilled water. Dialysis rate was initially influenced by the length of the tube i.e. drug release rate was faster from longer tubes. Later, drug release rate became independent of length and difference in release profile was negligible. These results demonstrate that dialysis tube cab affect drug release behavior independent of drug delivery system. However, this effect may not be significant in case of sustained or extended release. It was also observed that participant students were involved actively in the laboratory experiment and able to relate these observations theoretically with Fick's law of diffusion and Noyes-Whitney equation.
Keywords: Dialysis bag, dissolution, niosomes, diacerein, pharmacy education.

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INTRODUCTION

Laboratory experiments are considered an efficient tool to enhance student's knowledge in science studies (Berry *et al.,* 1999). Experiments help students get hand on with science, which in turns will enable them to translate the science into technology. As the field of pharmacy education grows, researchers have tried to overcome the hurdles in students understanding during laboratory work by proposing new strategies. One strategy, put forward by academia and researches alike, is to enhance students understanding by clearly defining "aims and purpose" of the study (Hart *et al.,* 2000). This can be achieved by providing comprehensive knowledge of background science and related theories, so that students can foresee the expected outcomes of the experiments. In addition, making a laboratory experiment interactive will help to enhance student's involvement and promote active learning (Roche, 2007). In Pakistan, laboratory experiments are compulsory part of pharmacy education course work for pharmacy graduation (5 years doctor of pharmacy or Pharm D degree) and M.Phil Pharmaceutics (2 years post-graduate degree) (HEC, 2016). Pharmaceutics is a branch of pharmacy which deals with the science and art of drug delivery. In pharmaceutics, *in vitro* dissolution studies are employed in drug delivery research to surrogate the *in vivo* behavior of drug release. Therefore, it is desirable to design an interactive dissolution experiment which can attract attention of students and help them correlate the experiment with theory (Philip *et al.,* 2015; Yu-Chihd *et al.,* 2015; Herdeiro *et al.,* 2014). As authors, consisting of highly experienced academia and postgraduate students, consider it pertinent to first establish theoretical background, definitions of all relevant phenomenon are also discussed in the introduction section.

Diffusion involves the movements of molecules from the area of higher concentration to the area of lower concentration (Shargel *et al.,* 2007). Molecules have the tendency to move randomly across all the available volume. In living systems mostly diffusion is involved for the

transportation of molecules. Dialysis is the process of separation of different molecules, small molecules are separated from large molecules by selective diffusion in a solution through a semipermeable membrane (Misra *et al.,* 2008). It has acquired wide biomedical applications. Dialysis works on the principle of modified phenomenon of diffusion. In dialysis process, semipermeable membrane with small pore size is used. Larger molecules cannot pass through the membrane so can be separated. While small molecules, buffers, salts and ions easily cross the semipermeable membrane during dialysis. In hemodialysis, rate of dialysis for removing wastes from blood has been found to be directly proportional to the surface area of the membrane and inversely proportional to its thickness (Mandolfo *et al.*, 2003). As the surface area of semipermeable membrane is increased the separation process becomes faster. The reason is larger number of molecules are in direct contact with membrane at any instant and can be effectively spread over the membrane. Whereas if thickness of the membrane is increased then process slows down. Membranes normally used for laboratory dialysis applications are 0.5 to 1.2 mm (12 to 30 µm) thick, providing good diffusion rate as well as structural integrity (Moreno-Bautista and Tam, 2011). Some modern dialysis machines have been designed to effectively increase the surface area relative to volume. Examples are High-performance dialysis products e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes, and MINI Devices and Flask (TFSI, 2016). However, they are beyond the scope of this paper and are not discussed in detail. Dialysis bags have been employed in pharmaceutical research for the separation and/or purification of different compounds. Dialysis bags and dialysis tube are synonyms and used interchangeably. However, term dialysis bag will be used throughout this manuscript for dialysis bag method. In pharmaceutical nanotechnology, dialysis tube has been used for the dissolution studies of novel nano-scale drug delivery systems (DDS) with different shape and chemistry such as nanospheres, nanocapsules, nanoemulsions, niosomes, liposomes and dendrimers etc. (Madni *et al.,* 2014; Rehman *et al.,* 2015). It involves encapsulation of DDS in dialysis tube which allows drug particles to pass through the membrane, into dissolution medium, but retains the DDS itself so they are not removed during sampling. Dialysis tubes are made of selectively permeable cellulose tubing perforated with microscopic pores (Klinkmann and Vienken, 1995). Microscopic pore size is usually defined in terms of molecular weight cut off value (Daltons or killo Daltons). Evidently, the selection of appropriated dislysis tube depends upon size of molecule to be separated. Generally, it is recommended to select a molecular weight cut off value should be (SLI, 2016);

i. half the size of the molecular weight of the species (nanoparticles) to be retained
ii. at least 10 times larger than twice the size of the molecular weight of the species intended to pass through.

Although the molecular weight of drugs is available in the literature yet the determination of molecular weight of nanoparticles may be difficult to the researchers. The following figure 1 can be very helpful to researchers for selection of appropriate dialysis tube for the given drug and DDS.

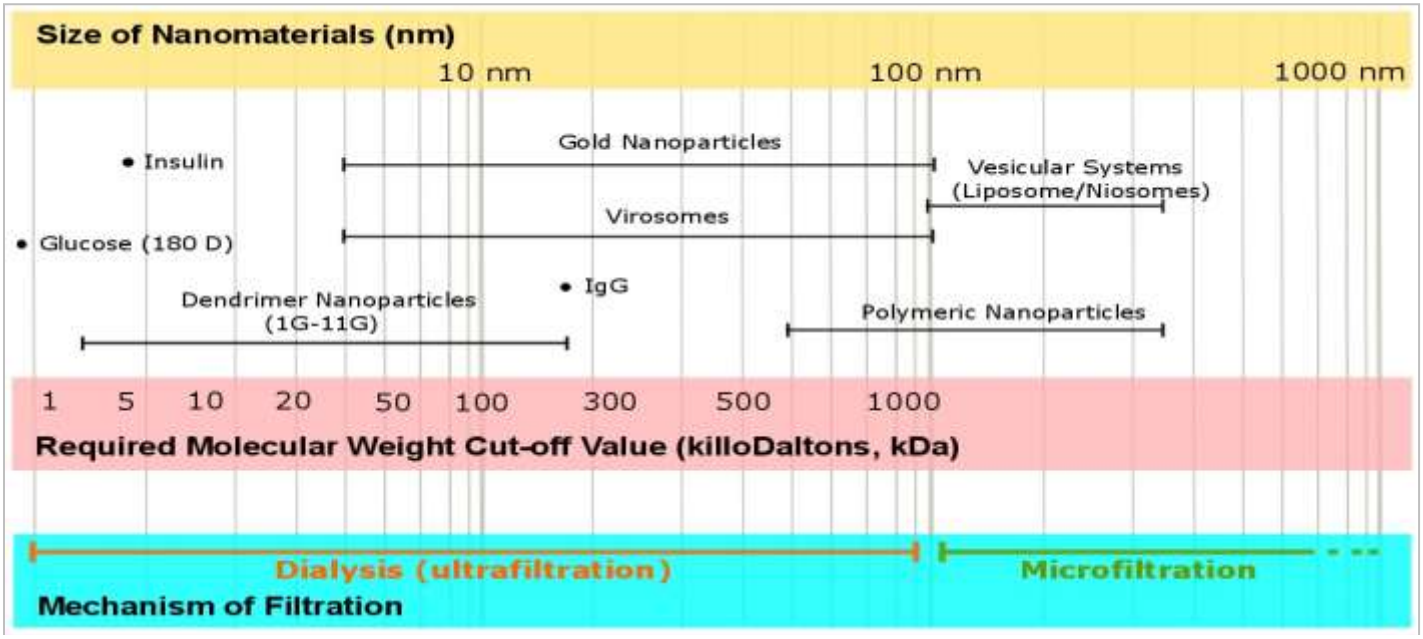

Fig. 1. Nanoparticles of different size used in biomedical research and required molecular weight cut-off value of dialysis tube for their separation or dissolution studies.

It is widely reported in the literature that dialysis method can be self-sustaining as indicated by dissolution studies of different formulations and free drug solution (Seleem *et al.,* 2009; Diao *et al.,* 2011; Dia-Long *et al.,* 2011). In this case, the delayed release of free drug from dialysis tube has been attributed to the formation of stagnant layer inside dialysis tube, which is not minimized by stirring the dissolution medium (D'Souza and DeLuca, 2006). Formation of stagnant layer has been directly correlated with the diameter of tube. A dialysis tube of greater diameter will significantly delay the drug release, resulting in misleading results. However, little is known about the effect of dialysis tube length on the drug release behavior. Therefore, the main objective of this interactive experiment was to use dialysis tube of different lengths to identify its effect on dissolution profile of same niosomes formulation containing model drug diacerein.

MATERIALS AND METHODS

Materials

Diacerein was received as generous gift sample from CCL Pharmaceutical Laboratories (Lahore, Pakistan) as a gift sample. Cellulose ester dialysis tube (10 kDa) was purchased from BioMembranes, Germany. Diacerein containing niosomes were received as generous gift from Dr. Asadullah Mani's laboratory, The Islamia University of Bahawalpur, Pakistan. Distilled water was used for dissolution studies.

Preparation of Diacerein loaded Niosomes

The niosomes obtained as gift sample were prepared by reverse phase evaporation method in the said laboratory (Khan *et al.,* 2015). Briefly, surfactant and cholesterol added to 20 ml of chloroform/ethanol mixture, separately. The, rotary evaporator was used to slowly evaporate organic solvent under reduced pressure (Heidolph, Germany) at 60°C. Removal of organic left behind a thin layer of surfactant and cholesterol. 20 ml ether was added to this system to resuspend thin film. To prepare drug loaded niosomes, drug solution was added to organic phase and sonicated for about 5 minutes. The flask was swirled by hand for a few minutes and again sonicated for 2 minutes. This resulted in the formation of gel. The gel was disrupted by evaporation of ether from this system. Finally, 10 ml phosphate buffer of pH 7.4 was added. Evaporation was continued until diethyl ether was evaporated and niosomes were formed (Khan *et al.,* 2015).

Preparation of Standard Curve

Serial dilutions of Diacerein from 1ug/ml to 10ug/ml were analyzed spectroscopically at 340nm. The absorbance of each dilution was measured three times and average of three absorbance values was recorded as absorbance of dilution. Concentration of various dilutions and their respective UV absorbance has been given in the table. Using MS excel, the concentration of dilutions was plotted against the absorbance to prepare the standard curve of aspirin (Varshosaz *et al.,* 2014). The trend line along with its equation as well as value of regression coefficient (R^2) was added using MS excel 2010.

In vitro dissolution studies

The in vitro release study for the Diacerein formulation was performed by employing dialysis tube method. A dialysis tube of cellulose ester and molecular weight cut off value of 10 kDa was filled with niosomes formulation equivalent to 1 mg drug was added over the dialysis membrane. Filled dialysis tube was placed in 500 ml of distilled water in a beaker. Dissolution experiment conditions were: stirring rate of 50 rpm by help of magnetic stirrer and temperature at 37°C±0.5°C. Absorbance of all samples was recorded at 340 nm with the help of UV spectrophotometer. The calculation of drug concentration was done by using calibration curve. The standard curve was plotted between the absorbance of known concentrations of diacerein in distilled water and their corresponding absorbance values (Khan *et al.*, 2015).

The diacerein loaded noisome formulation was filled in dialysis tube of two different lengths i.e 7.5 cm and 10.5 cm. After filling, dialysis tubes were tied at 1 cm from either end. Thus, remaining shorter tubes was 5.5 cm and longer tube was 8.5 cm (1.5 times longer than former). The minimum length of 5.5 cm was selected because it was the shortest length to contain noisome formulation according to manufacturer protocols. Samples of around 1 mL volume were taken and equal amount of fresh medium was added to supplement dissolution medium. Sampling was done for 5 hrs.

RESULTS AND DISCUSSION

The primary aim of the study was to expand the understanding of how distinctive lengths of dialysis tube, 7.5cm and 9.5 cm, influence the diacerein release from nanoparticles systems. For this purpose, an interactive dissolution test was designed. In this test, the standard condition for dissolution test i.e. stirring rate at 50 rpm and temperature of 37°C±0.5°C for 3 hours. This will help to complete the experiment in shorter time which is generally allotted for practical work in teaching institutions.

Diacerein standard curve was prepared with coefficient of determination R^2 = 0.9843. A R^2 estimation of 1.0000 show perfect relationship which was essentially unrealistic to accomplish. Nevertheless, our estimation of R^2 was near to 1.0000 which demonstrated that our standard curve was suitable to calculate amount of drug released from the niosomes (Rehman *et al.,* 2015). Following standard equation was derived from standard curve and used for the calculation of amount of drug released (x) using absorbance of sample (y) at any time point (Figure 2);

$$y = 0.0505x + 0.0029 \quad (1)$$

$$\mathrm{x} = (\mathrm{y} - 0.0029)/0.0505 \quad (2)$$

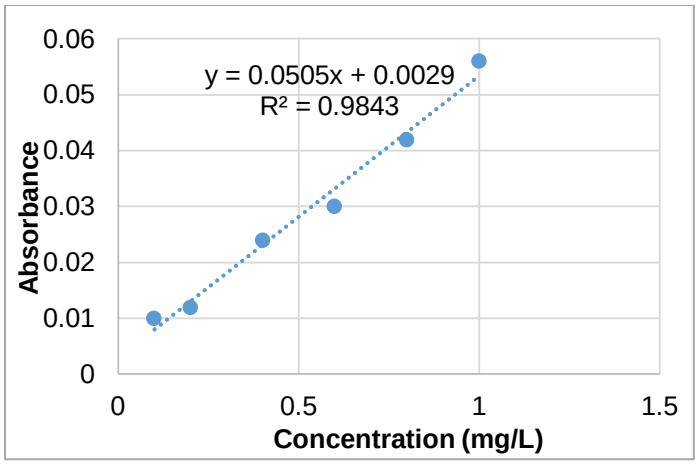

Fig. 2. Standard curve of Diacerein for calculation to estimate the effect of dialysis bag length on drug release from novel drug delivery systems.

Faster release of drug was observed in both cases i.e. short tube (5.5 cm) and long tube (8.5 cm) (Figure 3). This burst release has been attributed to the amount of unentrapped drug and drug loosely bound to the noisome surface (Mahato and Narang, 2011).

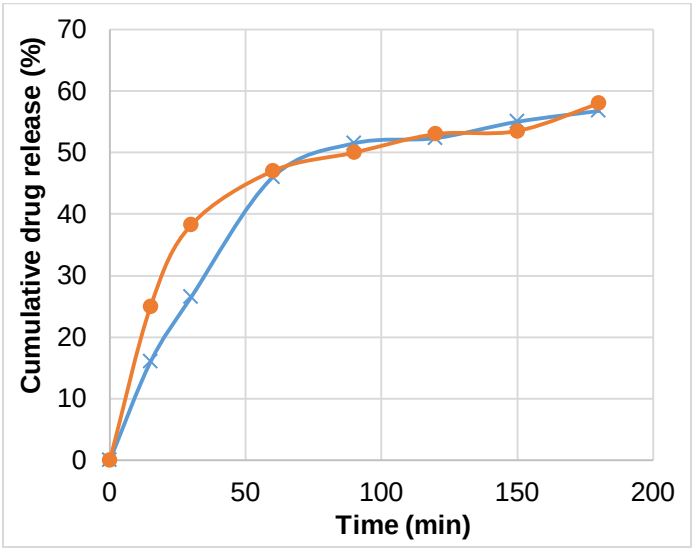

Fig. 3. Drug release profile of different lengths of dialysis tube i.e 8.5cm (in red) and 5.5 cm (blue line) with respect to time change.

In first hour, *in vitro* dissolution characteristics of diacerein showed variable results with respect to different lengths of dialysis bags used. The drug showed rapid rate of diffusion into the dissolution medium from the longer dialysis tube i.e. 8.5 cm as compared to that of the shorter one i.e. 5.5 cm. The possible consideration for the rapid release of diacerein from the longer dialysis tube was its larger surface area. Such relationship between diffusion and surface area has been reported in hemodialysis studies aimed to increase rate of purification of blood (Mandolfo *et al.,* 2003). Increase in surface area lead to increased volume inside dialysis tube available for diffusion. This assumption is supported by Noyes-Whitney equation (dM/dt = D S (Cs-C) / h) which shows that dissolution rate (dM/dt) is directly proportional to surface area (S) for discussion (Rhoades and Bell, 2012). Similarly, our results are also in agreement with Fick's law which states that rate of diffusion is directly proportional to the surface area for diffusion (Jeetah *et al.* 2014). As the amount of free or released drug is higher inside dialysis tube, the rate of drug released is solely controlled by Fick's law and drug release was faster from longer dialysis tube.

However, there was no significant difference in drug release in next two hours. This may be due to the fact that drug release rate was slower and more sustained subsequently from niosomes, after initial burst release during first hour. This has been shown elsewhere by Khan *et al.*, in separate publication that niosomes can sustain the drug release rate for up to 24 hours (Khan *et al.,* 2015; Sohail *et al.,* 2015). In this case, amount of free or released drug was lower inside dialysis tube at any instance and rate limiting step was the drug release from niosomes, not the dialysis membrane. These results suggest that length of dialysis tube can affect the rate of drug appearing in the dissolution only when rate of drug release is faster. In these conditions, drug release will be slower from shorter dialysis tube and they may act as self-sustained systems. However, drug release from dialysis tube will be independent of the dialysis tube length when amount of released drug is lower, such as sustained release drug delivery systems. As most of novel drug delivery systems are designed to sustain the drug release rate as one of their main objective, the effect of dialysis tube length may be negligible. Nevertheless, it is suggested that researchers should conduct experimentation to find suitable dialysis tube in terms of type and dimensions to ensure that their results are not influenced by process parameters such as dialysis tube length. Moreover, pharmacy academia is encouraged to design such interactive experiments that can enhance students understanding of the theories behind different experimental processes and the factors affecting it (Madni *et al.,* 2016).

CONCLUSION

Drug release rate from niosomes was faster from longer dialysis tubes during first hour of the study due to presence of unentrapped and loosely surface bound drug. Later on, there was no difference in drug release from longer and shorter dialysis tubes. Thus, length of dialysis tube may influence drug release rate in case of immediate release dosage forms while this effect will be negligible for controlled release dosage forms.

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CONFLICT OF INTEREST

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Conflict of interest: none.

REFERENCES

Berry, A., Mulhall, P., Gunstone, R., Loughran, J., 1999. Helping students learn from laboratory work. Aust. Sci. Teach. J., 45(1): 27-31.

Dai-Long, F., Chen, Y., Xu, B., Ren, K., Zhi-Yao, H., Li-Li, H., Lei, Y., Chun-Mei, F., Xiang-Rong, S. 2014. Development of Lipid-Shell and Polymer Core Nanoparticles with Water-Soluble Salidroside for Anti-Cancer Therapy. Int. J. Mol. Sci., 15(3):3373-3388.

Diao, Y.Y., Li, H.Y., Fu, Y.H., Han, M., Hu, Y.L., Jiang, H.L., Tsutsumi, Y., Wei, Q.C., Chen, D.W., Gao, J.Q. 2011. Doxorubicin-loaded PEG-PCL copolymer micelles enhance cytotoxicity and intracellular accumulation of doxorubicin in adriamycin-resistant tumor cells. Int. J. Nanomed., 6:1955-62.

D'Souza, S.S., DeLuca, P.P. 2006. Methods to Assess in Vitro Drug Release from Injectable Polymeric Particulate Systems. Int. J. Pharm. Res., 23(3): 22-24.

Hart, C., Mulhall, P., Berry, A., Loughran, J., Gunstone, R. 2000. What is the purpose of this experiment? Or can students learn something from doing experiments? J. Res. Sci. Teach., 37(7):655-75.

Herdeiro, M.T., Rodrigues, T.A., Ferriera, M., da Cruz e Silva, O.A.B., Fradilha, M. 2014. Multimethod Active learning approach: improving the learning experience in pharmaceutical drug development. Ind. J. Pharm. Educ. Res., 48(3):16-25.

Higher Education Commission (HEC). Curriculum of Doctor of Pharmacy. Government of Pakistan. Internet: http://www.hec.gov.pk/InsideHEC/Divisions/AECA/CurriculumRevision/Documents/Draft-Pharmacy-2011.pdf. (Accessed 15 April, 2016).

Jeetah, R., Bhaw-Luximon, A., Jhurry, D. 2014. Polymeric nanomicelles for sustained delivery of anti-cancer drugs. Mutat. Res., 768: 47-59.

Khan, M.I., Madni, A., Ahmad, S., Mahmood, M.A., Rehman. M. 2015. ATR-FTIR Based Pre and Post Formulation Compatibility Studies for the Design of Niosomal Drug Delivery System Containing Nonionic Amphiphiles and Chondroprotective Drug. J. Chem. Soc. Pak., 37(3): 527-534.

Klinkmann, H., Vienken, J. 1995. Membranes for dialysis. Nephro. Dial. Transplant., 10(3):39-45.

Madni, A., Kanwal, R., Tariq, M., Baloch, A., Shah, K., Jabbar, A., Khan, M.I. and Rehman, M., Devising Interactive Dissolution Experiment for Pharmacy Students (part I): Use of USP Type II Apparatus for Comparison of Immediate Release and Enteric Coated Tablets in Time Varying pH Conditions. PSM Biol. Res., 1(2):83-87.

Madni, A., Sarfraz, M., Rehman, M., Ahmad, M., Akhtar, N., Ahmad, S. 2014. Liposomal Drug Delivery: A Versatile Platform for Challenging Clinical Applications. Int. J. Pharm. Sci., 17(3):4014-426.

Mahato, R.I., Narang, A.S. 2011. Pharmaceutical dosage forms and drug delivery. CRC Press, New York, United States.

Mandolfo, S., Malberti, F., Imbasciati, E., Cogliati, P., Gauly, A. 2003. Impact of blood and dialysate flow and surface on performance of new polysulfone hemodialysis dialyzers. Inter. J. Arti. Org., 26(2):113-120.

Misra, S.K., Dybowska, A., Berhanu, D., Luoma, S.N., Valsami-Jones, E. 2012. The complexity of nanoparticle dissolution and its importance in nanotoxicological studies. Sci. Total Environment., 438:225-232.

Moreno-Bautista, G., Tam, K.C. 2011. Evaluation of dialysis membrane process for quantifying the invitro drug release from colloidal drug carriers. Colloids Surf. A., 389(1-3):299-303.

Philip, A., Stephens, M., Mitchell, S.L., Watkins, E.B. 2015. Design and Implementation of a Laboratory-Based Drug Design and Synthesis Advanced Pharmacy Practice Experience. Am. J. Pharm. Educ., 79(3). 43.

Rehman. M., Madni. A,, Ihsan. A., Khan, W.S., Khan, M.I., Mahmood, M.A. 2015. Solid and liquid lipid-based binary solid lipid nanoparticles of diacerein: in vitro evaluation of sustained release, simultaneous loading of gold nanoparticles, and potential thermoresponsive behavior. Int. J. Nanomed., 10:2823-46.

Rhoades, R.A., Bell, D.R. 2012. Medical Physiology: Principles for Clinical Medicine. LWW., 2012.

Roche, V.F. 2007. Improving pharmacy students' understanding and long-term retention of acid-base chemistry. Am. J. Pharm. Educ., 71(6):122.

Seleem, M.N., Jain, N., Pothayee, N., Ranjan, A., Riffle, J.S., Sriranganathan, N. 2009. Targeting Brucellamelitensis with polymeric nanoparticles containing streptomycin and doxycycline. FEM Microbio. Let., 2009;24-31.

Shargel, L., Wu-Pong, S., Yu, A.B. 2007. Applied Biopharm & Pharmacokin: McGraw-Hil/.

Sohail, M., Ahmad, M., Minhas, M.U., Ali, L., Khalid, I., Rashid, H. 2015. Controlled delivery of valsartan by cross-linked polymeric matrices: Synthesis, in vitro and in vivo evaluation. Int. J. Pharm., 487(1):110-19.

Spectrum Laboratories Inc (SLI). Fundamentals of Membrane Dialysis. Internet:

http://www.spectrumlabs.com/dialysis/Fund.html. (Accessed on February 02, 2016).

Thermo Fisher Scientific Inc (TFSI). Dialysis Method for Protein Research. Internet: https://www.thermofisher.com/pk/en/home/life-science/ protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/ dialysis-methods-protein-research.html. (Accessed on April 24, 2016).

Varshosaz, J., Taymouri, S., Pardakhty, A., Asadi-Shekaari, M., Babaee, A. 2014. Niosomes of Ascorbic Acid and α-Tocopherol in the Cerebral Ischemia-Reperfusion Model in Male Rats. BioMed. Res. Int., Article ID 816103.

Yu-Chihd, H., Lee, Y.W., Sung-Hui, T., Shu-Chen, C., Ching-Chiung, W. 2015. Learning the fundamentals of traditional Chinese medicines by using computer software: A preliminary study. Ind. J. Pharm. Educ. Res., 49(1):18-25.