World Research Journal of Pharmaceutical Research
ISSN: 2322-0562 & E-ISSN: 2322-0570 Volume 1, Issue 2, 2013, pp.-028-032.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000294

PROSPECTIVE PLANNING FOR DEVELOPING CONTROLLED AND EXTENDED RELEASE ORAL MUCOADHESIVE MICROCARRIERS

SAIKH M.A.A.*
Department of Pharmaceutical Sciences, Faculty of Health Sciences, Shiats, Allahabad- 221 007, UP, India.
*Corresponding Author: Email- atharodi@gmail.com

Received: November 10, 2013; Accepted: December 09, 2013

Abstract- Nowadays oral mucoadhesive microcarriers (MMs) were exploited extensively to improve the performance of orally administered delivery system and the patient compliance through controlling and extending release profile of drug. Present review work is aimed to explore the aspects of mucoadhesives, techniques of devising and evaluating MMs that will aid in designing an efficient controlled and extended release oral system based on MMs. Informative literatures on prospective planning for developing them are rare. In this regard literatures were studied and presented as handy reference. Presented information wills insight on prospective planning for their development. This will cover development and evaluation process to be adopted, during processing and evolution. Presented data will help pharmaceutical scientists for getting a prospering product with wished performance.

Keywords- Controlled, extended, microcarrier, mucoadhesion, mucoadhesive, oral

Introduction

Historically oral route of administration had been used for both conventional as well as novel drug delivery systems. Oral route is preferred due to self medication, flexibility in formulation, ease of administration and improved patient compliance; their compact nature, stability and low cost; and ease of packaging, transport, and manufacture. However, performance of oral conventional dosage form can be improved with controlled/extended-release (CER) or targeted-delivery products. These CER products minimizes dosing frequency; improves patient convenience/compliance, safety margin and efficacy; reduces intensity of local or systemic side effects, health care costs, and expenses and complications involved in marketing new drug entities; and many more to list [1-5].

Multiparticulate (microcarriers) based systems consensus to be promising for having oral CER products. Exploited microcarriers particles are pellets, beads, microcapsules, microspheres, solid-lipid microparticles, lipospheres, and so on. These systems modulate release and absorption characteristics of the drug(s), to achieve CER and or targeting of drug. Their short GI retention/transit time is the major drawback that decreases their performance and success. Said drawback can be improved by amalgamating them with mucoadhesive properties [2-8].

Short GI retention/transit time of microcarriers can be improved by developing microcarriers with mucoadhesive property. Microcarriers embedded with mucoadhesive property referred as “mucoadhesive microcarriers”. Systems containing MMs may localize delivery system in selected regions of GI tract [2-9].

However available literatures are merely describe prospective planning to be adopted for developing MMs based orally administered CER product. This manuscript is aimed to provide information for complying stated issue. Factors influencing their development and performance also discussed.

Mucoadhesive

Diverse classes of polymers have been investigated for their potentiality to be used as mucoadhesives. Polymers that can be used to form MMs include soluble, insoluble, non-biodegradable, biodegradable polymers of natural or synthetic origin; or their blend. These polymers can be hydrogels, thermoplastics, homopolymers, copolymers, and so on. Category and example of mucoadhesive polymers provided with [Table-1] [5,9,10].

Table 1- Category and example of mucoadhesive polymers

Category	Example
Synthetic	Poly (acrylic acid), polycarbonates, poly(methylacrylate) derivatives, sodium carboxymethylcellulose, polyvinyl alcohol, hydroxypropyl methylcellulose, hydroxypropyl cellulose, polymethylmethacrylic acid, polyalkylene glycols, polyamides, polyvinyl ethers/esters/halides, methylcellulose, and so on.
Biocompatible	Cellulose-based polymers, ethylene glycol polymers and its copolymers, oxyethylene polymers, polyvinyl alcohol, polyvinyl acetate, esters of hyaluronic acid, and so on.
Biodegradable	Chitosan, poly (lactides), poly (glycolides), polyorthoesters, poly (lactide-co-glycolides), polyalkyl cyanoacrylates, polyanhydrides, polyethylene oxide, polycaprolactones, polyphosphoesters, polyphosphazenes, and so on.
Natural	Sodium alginate, pectin, tragacanth, gelatin, carrageenan, and so on.
Hydrophilic	Methylcellulose, hydroxyethyl cellulose, chitosan, sodium carboxy methylcellulose, plant gums, carbomers, hydroxypropyl methylcellulose, polyvinylpyrrolidone, methylcellulose, sodium carboxy methylcellulose, hydroxypropyl cellulose, other cellulose derivative, and so on.
Hydrogels	Poly (acrylic acid-co-acrylamide) copolymers, carrageenan, sodium alginate, guar gum, modified guar gum, and so on.
Thermoplastic	Polyanhydrides, polylactic acid, and so on.
Novel (under trial & development)	Copolymer of poly (acrylic acid-co-acrylamide) and PEG monoethylether monomethacrylate, AB block copolymer of oligo (methyl methacrylate) and poly (acrylic acid-co-acrylamide), PEGylated poly (acrylic acid-co-acrylamide), cysteine grafted or PEGylated polyvinylpyrrolidone, and so on.

Mucoadhesive polymers upon hydration becomes adhesive thereby network with mucosal layer casing the mucosal epithelial surface and the mucin. This facilitates their intimate contact with the absorption surface and aid in prolonging gastric residence time. The type of polymer used to practise MMs influences their surface characteristics, force of mucoadhesion, release pattern and clearance of drug [5,10].

Mucoadhesive Microcarriers

MMs intended for oral use include microspheres, microbeads, and microcapsules. These are devised either exclusively from mucoadhesive polymer or by laying an outer coat of mucoadhesive polymer on the preformed microcarriers. They improve absorption and bioavailability of the drug(s), and reduce dosing frequency and improve patient compliance [1,5-8,10].

MMs had potentiality to be employed for devising targeted and or CER products. They have high surface to volume ratio and enhanced intimate contact with the mucus layer. Their targeting to the absorption site can be done with tailored and or site-specific MMs [5-8,11,12].

Tailored MMs offers the possibilities of localized as well as controlled release of drug(s). These prepared with bioerodable polymers, undertake selective uptake by the M cells of Peyer's patches in GI mucosa. They can be employed for delivering protein and peptide, antigens, and plasmid DNA. Non-invasive single shot vaccine developed from MMs offers CER of antigens, and fantastic application in mucosal immunization [5-8,11,12].

Site-specific MMs increase therapeutic benefit through site-specific delivery of drugs. These can be developing with polymers having affinity for the site or by grafting their surface with mucus or cell-specific ligands. Useful ligands may be of lectin, antibodies, bacterial adhesive moiety, or amino acid sequences. Lectin improves adherence of microcarriers to the intestinal epithelium thus may be used for targeting different gut components or even different cells [5-8,11,12]. Classes and example of ligands along with their site of targeting is provided in [Table-2] [5].

Table 2- Classes and example of ligands with their site of targeting

Name of the ligand	Category	Site of targeting
Complex-specific lectins	Lectins	Parietal cells [34] and GI tumour cells [35]
Morniga G lectins		Tumour-associated T/Tn antigen [36]
Fucose-specific lectins and lectins from *Aleuria aurantia*		M cells [37, 38]
Tomato lectins		Enterocytes [39] and Peyer's patches [40]
Wheat germ agglutinin lectins		Brain [41, 42]
Lectins from *Arachis hypogea*, *Lens culinaris*, *Dolichus biflorus*, *Solanum tuberosum* and *Triticum vulgare*.		Human colonocytes and monolayer-forming Caco-2 and HT-29 cells [5]
Lectins from *Bryothamnion triquetrum* and *B. Seaforthii*.		Colon carcinoma cell ([43])
Lectins from *Abrus precatroius*, *Agaricus bisporus*, *Pandeiraea simplicifolia*, *Anguilla anguilla*, *Arachis hypogaea*, *Polygonatum cyrtonema*, *Bauhinia purpurea* and *Phaseolus vulgaris*.		Under study[5, 44-48]
Tetanus toxoid, influenza antigen and Mucosal adjuvant-LTK 63	Antibodies	IgG antibody or immune response [5]
Fimbriae	Bacterial adhesives	Epithelial surfaces [5, 12]
Arg-Gly-Asp	Amino acid sequences	Specific cell surface glycoprotein [5]

Polymer Selection

Ideal mucoadhesive polymers should have sufficient number of hydrogen bonding chemical groups (-OH and -COOH), anionic surface chain, high molecular weight, high chain flexibility. They should have adequate surface tension that will provoke their spreading into the mucus layer favouring the creation of bonds that are either of chemical or mechanical origin [5,13].

Methods or Techniques of their Preparation

MMs can be prepared following assorted methods or techniques. Important are spray drying method, spray freeze-drying method, hydrogel method (impinging aerosol method, dispersion and ionic gelification method, dispersion and enzymatic gelification method, extrusion method), complex coacervation method, inter-polymer complex encapsulation with supercritical carbon dioxide (ICESCF), fluidized bed coating and agglomeration, hot melt microencapsulation, direct compression encapsulation, and so on. However, coating of preformed microcarriers with mucoadhesives can be done following pan coating methods, vibrational nozzle methods, air-suspension coating methods, spray-drying methods, and so on [5, 10,14-17]. Glimpse on the method of preparing MMs provided with [Fig-1].

Prospective Planning for Developing

Spray drying requires continuous spraying of aqueous dispersion of drug(s) and carrier matrix in a specially designed closed container. Sprayer or atomizer is used for spraying. Drying of sprayed droplets is achieved with simultaneous application of hot air. Suitable for getting microparticles with rapidity, and with low production cost. It is an elementary uninterrupted process having reproducibility. It is having industrial applicability and high scale-up potentiality. The process is unsuitable for thermolabile drugs. This limitation can be overcome by inclusion of protectants like whey protein and resistant starch, N-Tack starch in the formulation [14-16].

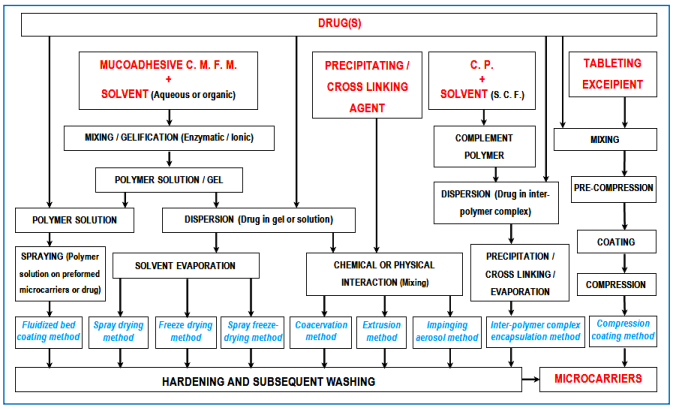

Fig. 1- Method of evolution of mucoadhesive microcarriers.
C. M. F. M. : Carrier Matrix Forming Material (Polymer); C. P.: Complement Polymers; S. C. F: Super Critical Carbon Dioxide Fluid.

Quality of microcarriers can be improved by the addition of plasticizers, that promoting coalescence of polymer on drug particles. Plasticizer promotes formation of spherical and smooth surfaced microcarriers. Microcarrier shape, size, and size distribution can be controlled by manipulating feed rate and rate of spraying, nozzle size and architecture, and drying temperature. This technique is independent on solubility characteristics of drug and polymer [5,16].

Spray freeze drying possesses processing steps parallel to freeze drying and spray drying. This requires atomization of aqueous solution or dispersion, of drug and polymer, into a cold vapor phase of a cryogenic liquid (e.g. liquid nitrogen) to get dispersion of frozen droplets. Resulted droplets are subsequently freeze dried. The process results CER microparticles with controlled size and larger specific surface area, but utilizes high energy and long processing time, and increases production cost by 30-50 times, comparing spray-dried one [14-16].

Hydrogel methods is an 'all-aqueous' system that eliminates residual solvents in microcarriers. Microcarriers were formed from dispersion of watery polymeric gel and drug. The mixture is transformed into micro-droplets, with suitable process. Resultant micro-droplets were hardened or cross-linked to get microcarriers [5,14-16].

Dispersion and ionic gelification method uses hydrocolloids of alginate, carrageenan, or pectin as polymer. Hydrocolloid is affected by ionic interaction. Surfactant is included in the formulation to stabilize the dispersion of drug in hydrocolloid. Upon stabilizing the system, cross-linking or hardening agent added to get microparticles. This method results smaller sized particles of diverse shapes having wider range of size distribution, but is easy to scale-up [5,14-16].

Dispersion and enzymatic gelification method uses milk proteins as polymer, and enzymes as gelling agent. Possesses alleviated feasibility for controlling particle size and produces water insoluble microparticles with improved aesthetic property. It is unsuitable for large-scale production [5,14-16].

Extrusion method is alternately known as pulsation or vibrational jet nozzle method or cold gelation method. Here dispersion of drug in hydrocolloids of alginate and carrageenan is passed through a nozzle at high pressure, as droplet, into the solution of cross-linking or hardening agent to form microcarriers. Droplets are prepared preferably with pulsation or vibration of the jet nozzle. Alternately, coaxial flow or electrostatic field can be used. Prilling uses atomizer for preparing the droplet. It is a simple and cheap method but is unsuitable for large scale productions associated with difficulties in scale-up [5,14-16,18-20].

Impinging aerosol method is a novel technique that involves impinges interaction of aerosols of gelling polymer and aerosols of cross-linking or hardening agent, from opposite directions in a chamber. The atomized droplet of alginate solution gels-out as they encounters with calcium chloride aerosols and fall as microbeads and are collected from the bottom of the chamber. Drug is mixed with alginate solution prior to atomization or aerosol formation. The technique possesses continuous processing capability and higher potentiality for scale-up, and is suitable for thermolabile drugs [5,14-16,21,22].

Complex coacervation refers to phase separation of a liquid precipitate, or phase. Its synonym is dispersion and interfacial polymerization. Phase separation takes place when solutions of two hydrophilic colloids are mixed under suitable conditions. The drug is dispersed in a solution of core material (polymer in liquid manufacturing vehicle phase). The coating material phase is prepared by dissolving immiscible polymer in a suitable vehicle [5-8,14-16].

Physical mixing of the coating material phase and the core material phase is done under stirring. Microencapsulation is achieved following any of methods: (i) by changing temperature of the polymer solution, (ii) by adding a salt or a non-solvent or an incompatible polymer to the polymer solution, or (iii) by inducing a polymer-polymer interaction. Subsequently coating is hardened by thermal, cross linking or desolvation techniques, to stabilize microcarriers [23].

Alternately phase separation can be achieved following solvent evaporation/removal method or phase inversion method. Solvent evaporation method is a non-aqueous method and uses organic solvent. Solvent removal method follows vacuum drying and is used when aqueous or non-volatile solvent is used [5-8].

ICESCF is a patented method of microencapsulation, which employ supercritical CO_2 fluid (SCF), as medium for processing. This involves dissolving each of complementary polymers at once in same SCF, or one by one to form separate complementary polymer solutions followed by mixing separate solutions together to interact contained complementary polymers, resulting inter-polymer complex solution in SCF. At least two complementary polymers, having ability to interact in solution phase, is desirable, to form an inter-polymer complex [17].

Drug is scattered in complementary solutions before or after dissolving the associated polymer before interaction or mixing step. Otherwise, they scattered in the inter-polymer complex solution before or after dissolving inter-polymer complex, but before precipitating inter-polymer complex. Precipitation could be achievable by either changing the pressure and or temperature of SCF or adding a non-solvent constituent. Otherwise, resultant dispersion is concentrated by spray drying the same after its atomization. Low molecular weight alcohol and or poloxamer and or ethylene oxide-propylene oxide tri-block copolymer used as solubilizer for improving solubility of complementary polymers and or of inter-polymer complex in SCF [5,15-17,24].

Hot melt microencapsulation method involves melting of polymer (copolymer of poly [bis(p-carboxy phenoxy) propane anhydride] and sebacic acid) followed by incorporating drug (particles size less than 50 μm) and continued mixing. The mixture is suspended in a non-miscible solvent (viz. silicone oil) with stirring. Under stirring condition the dispersion is heated at a temperature (5°C above the melting point of polymer). The stabilized dispersion is cooled to solidify polymer particles followed by decantation to separate microcarriers. Resulting microcarriers are washed with petroleum ether. Moderate operational temperature is the only disadvantage of this method [5].

Direct compression encapsulation involves compression of drug powder and excipients blend firstly into a pellet and subsequent coating in fluidized bed processor with mucoadhesive polymer. The process is cheaper and suitable for commercial-scale production, and is designed to improve shelf-life of product [5,15,16,25,26].

Fluidized bed coating and agglomeration involves spraying of coating solution onto the bed of drug under fluidized condition in a specially designed vessel called fluidized bed processor. Said process is having high reproducibility, can be adapted to give multilayer coatings, suitable for commercial-scale production, and can be easily scaled-up. The process is suitable for giving mucoadhesive

polymer coating to the pre-formed microparticles, and is costly and difficult to master [20,27].

Pan coating methods uses conventional pans for coating**.** The process is suitable for giving mucoadhesive polymer coating to the preformed microparticles. It is cheap and have high scale-up potentiality but difficult to master [14-16].

Evaluation of MMs is done to achieve reproducibility of process, assess performances and control quality [2,28,29]. They be evaluating for various parameters namely particle shape, size and size distribution; flow properties; surface charge and morphology; swelling or water uptake study; swelling index; drug loading and content uniformity; *in vitro* release and release kinetic; *in vivo*, *ex vivo* and *in vitro* mucoadhesion; and so on [2,3]. Physico-chemical interactions of drug(s) and excipients should be studied with Fourier transforms infrared spectroscopy, powder X-ray diffraction, differential scanning calorimetry, and so on. *In vivo* performance study, gastroretentivity study, ^{1}H- and ^{19}F-MRI studies, x-ray diffraction studies, *in vivo -in vitro* correlation study, and stability study has to done and results be statistically evaluated [2-8,15,16,30-33].

Adhesive strength of microcarriers can be measured following *in vitro*, *in vivo* and, *ex vivo* techniques. Commonly used *in vitro* procedure are falling liquid film method, novel electromagnetic force transducer method, Wilhelmy plate technique, shear stress measurement, everted sac technique, adhesion number method, and so on. *Ex vivo* methods include tensile strength measurement, shear strength measurement, and chip based systems. Important *in vivo* techniques are gamma scintigraphy technique, gastrointestinal transit time measurement, measurement of the residence time, and so on [3,4].

For better *in vitro* and *in vivo* correlation, studies on the variability of biological substrate should be confirmed by examining properties like permeability, electro physiology, or histology, etc.

An increase in polymer concentration increases mucoadhesive strength in solid dosage form. Along with molecular weight or chain length the spatial or helical conformation polymer chain plays important role in mucoadhesion. The mucoadhesive force of polymer increases with an increase in its molecular weight up to 100,000, beyond this there will be insignificant effect. As the cross linking density of water-soluble polymer increases the mucoadhesive strength decreases. Grafting of polymers onto the preformed network and inclusion of adhesion promoters in the formulation (free polymer) enhances mucoadhesion of cross-linked polymers.

The pH of the medium is important for the degree of hydration of cross linked polymers and dissociation of functional groups on carbohydrate moiety and amino acids of polypeptide, as well as certain ionisable mucoadhesive polymers. Protonated carboxyl groups efficiently react with mucin molecules comparing ionised carboxyl groups.

An increase in initial contact time of microspheres increases mucoadhesive strength. An increase in mucin turnover decrease mucoadhesion. The mucoadhesive effectiveness needs to be evaluated in diseased state like common cold, gastric ulcers, cystic fibrosis, ulcerative colitis, fungal and bacterial infections of female reproductive tract, and inflammatory conditions of eye.

Conclusion

MM system had potentiality to get oral CER dosage forms. Basing upon the potential site of application/absorption, biocompatibility, and safety, suitable mucoadhesive polymer should be selected. Method for devising microcarriers should be adopted, basing upon the physicochemical properties of the polymers, and wished properties of the microcarriers. Targeting of site-specific MMs can be done by grafting appropriate ligands on their surface. However, much more work is needed for eliciting clinical utility of MMs based oral CER formulations.

Conflicts of Interest: None Declared.

References

[1] Shaikh R., Raj Singh T.R., Garland M.J., Woolfson A.D. and Donnelly R.F. (2011) *Journal of Pharmacy and Bioallied Sciences*, 3(1), 89-100.

[2] Alli S.M.A., Samanta A., Mukherjee B., Ali S.M.A., Dehury G. and Kanungo S. (2010) *International Journal of Pharmaceutical Science and Technology*, 5(2), 40-55.

[3] Saikh M.A.A. (2013) *World Journal of Pharmaceutical Research*, 2(3), 297-307.

[4] Saikh M.A.A. (2013) *World Journal of Pharmaceutical Research*, 2(6), 2793-2804.

[5] Alli S.M.A., Ali S.M.A., Fatmah K. and Fatmah N.Y. (2011) *International Journal of Life Science & Pharma Research*, 1(1), L41-L59.

[6] Alli S.M. (2011) *International Journal of Nanomedicine*, 6, 619-629.

[7] Alli S.M. (2011) *International Journal of Nanomedicine*, 6, 1699-1707.

[8] Alli S.M., Ali S.M. and Samanta A. (2011) *Drug Development and Industrial Pharmacy*, 37(11), 1329-1338.

[9] Asane G.S., Nirmal S.A., Rasal K.B., Naik A.A., Mahadik M.S. and Rao Y.M. (2008) *Drug Development and Industrial Pharmacy*, 34(11), 1246-1266.

[10] Roy S., Pal K., Anis A., Pramanik K. and Prabhakar B. (2009) *Designed Monomers and Polymers*, 12(6), 483-495.

[11] Kunisawa J., Okudaira A., Tsutusmi Y., Takahashi I., Nakanishi T., Kiyono H. and Mayumi T. (2000) *Vaccine*, 19(4-5), 589-594.

[12] Rajput G., Majmudar F., Patel J., Thakor R., Rajgor N.B. (2010) *Systematic Reviews in Pharmacy*, 1(1), 70-78.

[13] Leung S.H.S. and Robinson J.R. (1990) *Journal of Controlled Release*, 12(3), 187-194.

[14] Shivanand P. (2010) *International Journal of Pharma and Bio Sciences*, 1(2), 1-7.

[15] Saikh M.A.A. (2013) *Journal of Drug Delivery and Therapeutics*, 3(5), 121-131.

[16] Saikh M.A.A. (2013) *International Research Journal of Pharmacy*, 4(8), 232-236.

[17] Thantsha M.S., Cloete T.E., Moolman F.S. and Labuschagne P.W. (2009) *International Journal of Food Microbiology*, 129(1), 88-92.

[18] Graff S., Hussain S., Chaumeil J.C. and Charrueau C. (2008) *Pharmaceutical Research*, 25(6), 1290-1296.

[19] Hébrard G., Hoffart V., Beyssac E., Cardot J.M., Alric M. and Subirade M. (2010) *Journal of Microencapsulation*, 27(4), 292-302.

[20]de Vos P., Faas M.M., Spasojevic M. and Sikkema J. (2010) *International Dairy Journal*, 20(4), 292-302.

[21]Sohail A., Turner M.S., Coombes A., Bostrom T. and Bhandari B. (2011) *International Journal of Food Microbiology*, 145(1), 162-168.

[22]Sohail A., Turner M.S., Prabawati E.K., Coombes A.G. and Bhandari B. (2012) *International Journal of Food Microbiology*, 157(2), 162-166.

[23]Zhang L., Liu Y., Wu Z. and Chen H. (2009) *Drug Development and Industrial Pharmacy*, 35(3), 369-378.

[24]Moolman F.S., Rolfes H. and Van Der Merwe T.L. (2010) *United States Patent,* 7, 641,917.

[25]Graff S., Chaumeil J.C., Boy P., Lai-Kuen R., Charrueau C. (2008) *Biological & Pharmaceutical Bulletin*, 31(2), 266-272.

[26]Chan E.S. and Zhang Z. (2002) *Food and Bioproducts Processing*, 80(2), 78-82.

[27]Champagne C.P. and Fustier P. (2007) *Current Opinion in Biotechnology*, 18(2), 184-190.

[28]Parikh D.C. and Amin A.F. (2008) *Expert Opinion on Drug Delivery*, 5(9), 951-965.

[29]Christmann V., Rosenberg J., Seega J. and Lehr C.M. (1997) *Pharmaceutical Research*, 14(8), 1066-1072.

[30]Pund S., Joshi A., Vasu K., Nivsarkar M. and Shishoo C. (2011) *International Journal of Pharmaceutics*, 411(1-2), 106-112.

[31]Karande A.D. and Yeole P.G. (2006) *Dissolution Technologies*, 13(1), 20-23.

[32]Corá L.A., Andreis U., Romeiro F.G., Américo M.F., Oliveira R.B., Baffa O. and Miranda J.R. (2005) *Physics in Medicine and Biology*, 50(23), 5523-5534.

[33]Weitschies W., Blume H. and Mönnikes H. (2010) *European Journal of Pharmaceutics and Biopharmaceutics*, 74(1), 93-101.

[34]Lavelle E.C. (2001) *Critical Reviews in Therapeutic Drug Carrier Systems*, 18(4), 341-386.

[35]Park K. (2009) *Journal of Controlled Release*, 134(1), 1.

[36]Poiroux G., Pitié M., Culerrier R., Ségui B., Van Damme E.J., Peumans W.J., Bernadou J., Levade T., Rougé P., Barre A. and Benoist H. (2011) *Photochemistry and Photobiology*, 87(2), 370 -377.

[37]Jepson M.A., Clark M.A. and Hirst B.H. (2004) *Advanced Drug Delivery Reviews*, 56(4), 511-525.

[38]Roth-Walter F., Schöll I., Untersmayr E., Fuchs R., Boltz-Nitulescu G., Weissenböck A., Scheiner O., Gabor F. and Jensen-Jarolim E. (2004) *Journal of Allergy and Clinical Immunology*, 114(6), 1362-1368.

[39]Lehr C.M., Bouwstra J.A., Kok W., Noach A.B., de Boer A.G. and Junginger H.E. (1992) *Pharmaceutical Research*, 9(4), 547-553.

[40]Woodley J.F. (2000) *Journal of Drug Targeting*, 7(5), 325-333.

[41]Liu Q., Shao X., Chen J., Shen Y., Feng C., Gao X., Zhao Y., Li J., Zhang Q. and Jiang X. (2010) *Toxicology and Applied Pharmacology*, 251(1), 79-84.

[42]Gabor F., Wirth M., Jurkovich B., Haberl I., Theyer G., Walcher G. and Hamilton G. (1997) *Journal of Controlled Release*, 49(1), 27-37.

[43]Pinto V.P., Debray H., Dus D., Teixeira E.H., de Oliveira T.M., Carneiro V.A., Teixeira A.H., Filho G.C., Nagano C.S., Nascimento K.S., Sampaio A.H. and Cavada B.S. (2009) *Advances in Pharmacological Sciences*, 862162.

[44]Wang S.Y., Yu Q.J., Bao J.K. and Liu B. (2011) *Biochemical and Biophysical Research Communications*, 406(4), 497-500.

[45]Gercken J. and Renwrantz L. (1994) *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 108(4), 449-461.

[46]Goldstein I.J., Blake D.A., Ebisu S., Williams T.J. and Murphy L.A. (1981) *Journal of Biological Chemistry*, 256(8), 3890-3893.

[47]Kruszewska D., Kiela P., Ljungh Å., Erlwanger K.H., Weström B.R., Linderoth A. and Pierzynowski S.G. (2003) *Biology of the Neonate*, 84, 152-158.

[48]Avichezer D. and Arnon R. (1996) *FEBS Letters*, 395(2-3), 103-108.