INT. J. BIOL. BIOTRCH., 17 (2): 219-226, 2020.

CARRIER ERYTHROCYTES: AN APPROACH TOWARDS EFFECTIVE DRUG DELIVERY: A REVIEW

Syeda Fatima Nadeem[1], Umar Farooq Gohar[1*], Hamid Mukhtar[1] and Muhammad Azam[2]

[1]*Institute of Industrial Biotechnology, Government College University, Lahore, Punjab, Pakistan, 54000*
Email: fatimanadeem291@gmail.com, Contact: +923324171617
Email: dr.mufgohar@gcu.edu.pk Contact: +923371444041
Email: hamidwaseer@yahoo.com Contact: +923334245581
[2]*Department of Biochemistry, Sharif City Medical and College, Lahore, Punjab, Pakistan.*
Email: Muhammad.azum22@gmail.com
*Corresponding Author's Email: dr.mufgohar@gcu.edu.pk; Contact: +923371444041

ABSTRACT

Erythrocytes are one of the most potential biocompatible carriers for various biologically active products including drugs. They can be utilized effectively as carriers for different drugs, chemicals, enzymes and proteins. At present, multiple techniques for the encapsulation of drugs within erythrocyte are there. The strategies most ordinarily utilized depend on hypotonic osmotic based methods. Erythrocytes encapsulated with different drugs permits desirable release of that drug. Incorporation of drugs within the erythrocytes primarily alters the properties pharmaco kinetically of the medications in humans as wells as in animals, improving uptake by spleen and liver and centering on reticulo-endothelial system (RES).

Key words: Drugs encapsulation, Carrier erythrocytes, Novel drug delivery, Drug release

INTRODUCTION

The delivery of the drugs indicates to the approaches, explanations, improvements and outlines for transferring the pharmaceutical substances inside the body (Tiwari *et al.*, 2012; Wang *et al.*, 2016). It might include targeting the specific site inside the body, or might contain boosting the pharmacokinetics system; irrespective, the presence of both amount and drug span is a matter of concern. For this reason, a lot of drug delivery systems have been designed which include liposomes, gels, prodrugs etc. Drug delivery systems have enhanced the pharmacokinetics, lowers the side-effects and prompted better patient consistence (Bradbury, 2003; Langer, 1990). They may likewise empower the clinical uses of synthetic compounds and biologics that were beforehand viewed as unrealistic because of high toxicity and fast leeway.

Cell based delivery of drug have huge advantages towards conventionally used methods together with prolonged release times, biocompatibility and provision of drugs to their target. Erythrocytes constitute large portion of all blood cells functions in supply of oxygen. As erythrocytes possess high concentrations of hemoglobin which is rich in iron and can be easily separated through process of centrifugation. Carrier erythrocytes have shown good potential to be developed as novel drug delivery system owing to their numerous advantages. High intracellular volume of erythrocytes enables the loading of high drug contents and provides good control over drug release (Bellad *et al.*, 2017).

Erythrocytes are viewed as a revolution in novel medication conveyance system (Foroozesh *et al.*, 2011). Erythrocytes include certain focal points that empower them to be utilized in specific circumstances as an elective drug conveyance system to other broadly utilized drug delivery systems, which can be outlined as:

1. Highly biocompatible, over that of other drug delivery carriers, which might be advanced by utilizing autologous erythrocytes, moreover, erythrocytes are biodegradable (Hamidi and Tajerzadeh, 2003; Patel *et al.*, 2009).
2. Erythrocytes empower a lot medication that is embodied in the little cells volume, with moderately elite in drug encapsulation (M Magnani *et al.*, 2002).
3. Drug loading inside the erythrocytes furnishes the medication with a fundamental leeway like the ordinary existence of red blood cells, which empowers therapeutic levels in the blood to be kept up for long time ranges, and in addition to provide a sustained release of the drug into the circulation (Alanazi *et al.*, 2011; Gasparini *et al.*, 1992).
4. The drug encapsulated in erythrocytes is shielded from untimely degradation and also protected from immune reactions (Bax *et al.*, 1996; Hu *et al.*, 2012).

5. The substance encapsulated within red blood cells do not embrace toxicological as well as its pharmacological properties till the point when comes to reticulo-endothelial system (RES). This is impressive significance on account of specific medications with massive lethality, for example, anti-neoplastic drugs (Hamidi *et al.*, 2007).
6. They go about as bioreactors, which takes into consideration embodying prodrugs that, because of the enzymatic systems that the erythrocytes have, in this manner offer ascent to the real medication itself (Magnani and DeLoach, 2012).
7. Steady state plasma concentration of drug is achieved by erythrocytes based dosage form (Pitt *et al.*, 1983a).
8. The very high sub-atomic weight peptides are encapsulated with the wide applications of biotechnology (Millan *et al.*, 2004).

Besides many advantages of carrier erythrocytes, there are some limitations as well.

1. Dose dumping may be there due to clumping of the cells (Ihler *et al.*, 1973).
2. They have limitation over the targeting of non-phagocytic tissues (Rawal, 2012).
3. Solid tumor, central nervous system and extra vascular tissue are not accessible.
4. They are less stable and sophisticated concerns related to the storage of the cells are required (Hirlekar *et al.*, 2008).

Loading Methods

Drug loading on erythrocytes is a cumbersome process, there are several processes through which drug can be loaded on to the erythrocytes.

Osmotic Based Methods

There is an exceptional potential of erythrocytes to withstand reversible change in shape or size with or without change in volume and to withstand stress related reversible deformative changes. These cell maintain their surface area to lodge enhance volume by altering their form to the spherical. This change in shape is due to increase in volume. This change is due to non-existence of excess membrane, so, surface area of cell remains fix. Gain of volume is around 25–50% (Ge *et al.*, 2018). Erythrocytes can keep their strength to tonicity of around 150 mosM/kg, membrane breaks above this limit. Above this limit, pores of size 200–500 Å are generated in the membrane. Ruptured membranes of erythrocytes reseal after isotonic conditions are restored. Erythrocytes attain their innate biconcave shape and original permeability also recovers by the administration of hypertonic saline which helps to restores isotonicity of erythrocytes upon incubation (Magnani and Rossi, 2014). A comparison of different osmotic based methods is shown in Table 1

Table I. Osmotic based methods comparison.

Loading Techniques	Loading Percentage	Pros	Cons
Dilution	20-40 %	Fast and easy especially for low molecular weight drugs	Efficiency of entrapment is less
Hypotonic Dialysis	30-45%	Better in vivo survival of erythrocytes, better structural integrity and membrane.	Time consuming. Heterogeneous size distribution of carrier erythrocytes.
Hypotonic preswelling	30-90 %	Good retention of cytoplasm and good survival *in-vivo.*	Not reported
Isotonic osmotic lysis	Not reported	Better *in-vivo* survival.	Time consuming with only the large molecules impermeability.

Chemical perturbation of the membrane

The membrane method of chemical perturbation is centered on the red blood cells when exposed to certain chemicals for example antibiotic polyene alike halothane as well as amphotericin B. There is relatively increased in the permeability of erythrocytes membranes. The drawback of this method includes the irreversible changes in the membranes of the cell, resulting in the non-popularity of the method (Gothoskar, 2004). In the year 1980, Hattori

and Kitao worked together and used the chemical perturbation of the membrane method for entrapment of duanomycin, an antineoplastic medicine, in the erythrocytes of human and mouse, and successfully succeeded (Kitao *et al.*, 1978).

Electro-Insertion or Electro Encapsulation

Electro insertion is widely known as electroporation method as well. The electro encapsulation method generates the electrical permeability induced changes with the resultant higher membrane potential differences. In the electro encapsulation method the electric shock can be fetches the changes irreversibly in the membranes of erythrocytes. Similarly, providing the incubation at 37 degree with the isotonic medium, the pores can be resealed easily. The entire procedure consists of interrupting the erythrocytes in the buffer isotonic solution consisting of chamber that is electrically discharged (Kinosita and Tsong, 1977; Sahoo *et al.*, 2012).

Electro-infusion method provides a uniform distribution of carrier erythrocytes in contrary to other osmotic based methods but special instruments are required to carry out this method and this method is complex as well. Though the drug loading percentage of electric based is around 30% and the life expectancy of the carrier erythrocytes is same as that of normal erythrocytes. Sugars, enzymes, latex particles, DNA fragment and other compounds can be encapsulated within erythrocytes through this method (Mitchell *et al.*, 1990; Mouneimne *et al.*, 1989; Zimmermann *et al.*, 1976).

Loading by electric cell fusion

In electric cell fusion technique by loading, molecules of drugs are loaded in a certain carrier erythrocytes, additionally followed by the linkages of these erythrocytes cells to the targeted ones. This electric cell fusion is further emphasized and highlighted by the electric pulse use, resulting in the discharge of the entangled molecules. For instance, the monoclonal antibody cell specific loading into the carrier erythrocyte could be considered as one of the example. A counter acting agent in contradiction of a particular protein of target cells can be cross-connected to the drug carrying cells that would guide these cells to their respective targets (Li *et al.*, 1996).

Lipid fusion technique

In this lipid fusion method, the lipid fusion vesicles comprises of erythrocytes of human along with the bioactive molecules, serves a prime in the interchange of drug molecules that are lipid entrapped. With this, the method has a low efficiency of encapsulation. The two scientists, namely the Gresonde and Nicolau tried to fuse these lipid vesicles enclosing the human erythrocytes along with the inositol hexaphosphate (Bellad *et al.*, 2016; Kumari *et al.*, 2018).

Evaluation of Resealed Erythrocytes

Blood cell count

Counting of erythrocytes is usually done through automated counting, in which the number of erythrocytes per unit volume of the entire blood are determined. Calculations of erythrocytes recovery is done by determining modifications between hematocrit and erythrocytes volume before and after encapsulation of the drug. This is done in order to reduce the loss to enhance the cell recovery during the process of encapsulation (Luque *et al.*, 1992).

Morphology

By the usage of scanning electron microscopy, the carrier erythrocytes morphology is accomplished by certainly associating the carrier erythrocytes with those of the normal untreated erythrocytes. The observation through the electron microscopy scanning might rolled out due to the progression morphologically within the red blood cells. Accordingly, rat erythrocytes are subjected to isotonic answers (300 mosM/kg) they monitor the typical morphology biconcave erythrocytes. This evolves to stomatocyte morphology (uniconcave) while they are exposed to formulas of two hundred mosM/kg, attaining spherocytic form (the most fragile of the 3) while suspension is of one hundred fifty mosM/kg (Luque *et al.*, 1992).

Osmotic fragility

Osmosis is a process in which solvent molecules tends to move from a less concentrated solution to a more concentrated solution through a semipermeable membrane. Osmotic fragility is to determine to distinguish unusual or abnormal delicacy of erythrocytes. Untreated or carrier erythrocytes are examined with the aid of exposure to hypotonic solutions, influencing them to swell, with a specific end goal to see the relative osmotic fragility of the erythrocytes (Sanz *et al.*, 1999; Talwar and Jain, 1992a).

Turbulence fragility

Turbulence fragility permits an assessment to be made on the steadiness and stability of the carrier erythrocytes in contrary to the pressure applied by the erythrocytes besides in-vivo flow disturbance (Talwar and Jain, 1992). The technique is done with the aid of the process of Deloach (Deloach *et al.*, 1977) where in erythrocytes deferment is gone a few times through a 22-measure needle.

Erythrocyte sedimentation rate.

ESR is used to distinguish between loaded and unloaded red blood cells and effect of different loading techniques on erythrocyte membrane integrity (Magnani *et al.*, 1998). Drug loaded and normal erythrocytes are washed with buffer solution and drawn in the WESTERGREN tube up to 0 mark with assistance of the rubber bulb. Tube is fixed snugly erect on the WESTREGREN standpoint. Tube is left intact for at least 1 hour and afterwards 1h ESR is measured.

Hemoglobin release

In erythrocytes, the hemoglobin amount might reduce by changes in penetrability of erythrocytes membrane amid drug loading process (Garín *et al.*, 1996; Hamidi *et al.*, 2001; Ihler *et al.*, 1973). Moreover, linking between the release of drug and the hemoglobin rate adds deducing systems engaged with drug release from erythrocytes (Hamidi and Tajerzadeh, 2003). The hemoglobin release is detected utilizing erythrocyte suspension by taking supernatant absorbance at 540 nm on a spectrophotometer (Han *et al.*, 2018; Talwar and Jain, 1992a).

***In-vitro* drug release**

Loading of numerous antibiotics on red blood cells can offer ascent to maintained release of medication having impacts pharmacokinetic conduct in vivo of carrier erythrocytes. The discharge of medication from in-vitro through carrier red blood cells is examined utilizing autologous plasma or an iso-osmotic cradle at 37 degree with a hematocrit balanced somewhere in the range of 0.5% and fifty percent. Supernatant is discarded on period durations formerly customized and supplanted via equivalent volume of autologous plasma or isotonic buffer (De Flora *et al.*, 1986; Kumar, 2011). Few researchers suggest executing in vitro the drug discharge assay from the carrier erythrocytes employing a dialysis tube (Pitt *et al.*, 1983b). The sub-atomic weight and lipid solubility of the substance constitute two factors that have a definitive pertaining to the drug release profile from the carrier erythrocytes (Eichler *et al.*, 1987). Lipid soluble medications might be discharged from the red cells via passive diffusion.

Other drugs might also come to be attached to different structure of the cell and aren't discharged with the aid of the diffusion mechanism, requiring the lysis of the cell (Villa *et al.*, 2015). Band three and glycophorin A are proteins present in high density on red blood cells extracellularly and which may additionally act as potential targets for anchoring thru covalent bond formation with distinct substances. Band 3 performs an imperative part as a carrier protein for anions (Krantz, 1997).

Route of administration of carrier erythrocytes

Intravenous is the most common route of carrier erythrocytes administration. Other than intravenous route subcutaneous, intra-peritoneal, intranasal and oral are used for the administration of carrier erythrocytes. Intra peritoneal infusion demonstrated that the survival of cells in the body were same as compared to the cells administered intravenously. According to report 25% of carrier erythrocytes survived for 14 days in the circulation. Additionally this strategy of infusing carrier erythrocytes also serves for extra vascular focusing of erythrocytes towards peritoneal macrophages. Subcutaneous administration was reported to be used for slow release of the loaded substance at the infusion site (Bourgeaux *et al.*, 2016). Some of the important applications of resealed erythrocytes are compiled in Table 3.

***In vitro* stability**

The in vitro stability of the carrier erythrocytes is evaluated by suspending the erythrocytes in autologous plasma or iso-osmotic buffer in such a way that the hematocrit value lies between 0.5% to 5%, then incubating these suspended erythrocytes at 4 and 37^{o}C (Ito *et al.*, 1989; Lizano *et al.*, 1998; Rossi *et al.*, 2001). All the characteristics of carrier erythrocytes are compiled in Table 2.

***In vitro* storage**

The released red blood cells from the in vitro storage are necessary for the success of drug delivery system. There are the suitable storage methods that are used to store the drug-loaded erythrocytes to maintain their survival

and drug contents (Villa *et al.*, 2016). Hank's balanced salt solution (Zimmermann, 1983) and acid-citrate-dextrose at 4^{o}C is a most common storage media in which cellular integrity maintains for at least 2 weeks (Alpar and Irwin, 1987). When loaded-erythrocytes are exposed to membrane stabilizers such as glutaraldehyde, dimethyl sulfoxide, toluene-2-4diisocyanate followed by lyophilization; the subsequent powder is stable for as a minimum one month (Harisa *et al.*, 2014). The storage stability can be improved by the pro drug encapsulation in which at body temperature it is converted to parent drug (Talwar and Jain, 1992b).

Table 2. Compilation of all characteristics of carrier erythrocytes (Bellad *et al.*, 2016).

Physical characterization of carrier erythrocytes	
Parameter	**Instrument/Method**
Shape and surface morphology	Transmission electron microscopy, scanning electron microscopy, phase contrast microscopy, optical microscopy
Vesicle size and size distribution	Transmission electron microscopy, optical microscopy.
Drug release	Diffusion cell, dialysis
Drug content	Deproteinization of cell membrane followed by assay of resealed drug, radio-labelling
Cellular characterization of carrier erythrocytes	
% Hb content	Deproteinization of cell membrane followed by hemoglobin assay
Cell volume	Laser light scattering
% Cell recovery	Neubaur's chamber, hematological analyzer
Osmotic fragility	Stepwise incubation with isotonic tohypotonic saline solutions and determination of drug and hemoglobin assay
Osmotic shock	Dilution with distilled water and estimation of drug and hemoglobin
Turbulent shock	Passage of cell suspension through 30-gauge hypodermic needle at 10 mL/mm flow rate and estimation of residual drug and hemoglobin, vigorous shaking followed by hemoglobin estimation
Erythrocyte sedimentation rate	Westergren tube

Table 3. Some important applications of resealed erythrocytes (Bellad *et al.*, 2016).

Disease/ Drug Category	**Drug/substance Name**	**Action/purpose**
lysosomal storage disease treatment	Lysosomal enzymes, C-glucuronidase, cells, 13-galactosidase and 6-glucosidase	Deliver lysosomal enzymes and drugs to lysosomes of the phagocytic cells
liver tumor treatment	Bleomycin Adriamycin, Carboplatin	Targeted drug delivery to treat hepatic cancer.
parasitic disease treatment	Pentamidine loaded immunoglobulin G coated erythrocytes, Glutaraldehyde treated Erythrocytes	Targeted drug delivery to parasite affected cells e.g. macrophage-affected with leishmania.
Toxicity removal	Murine loaded erythrocytes having bovine rhodanese and sodium thiosulphate	antagonize potential side effects of potassium cyanide in mice
Leukemia	L-asparaginase Aminolevulinate dehydratase	Slow release for the treatment of leukemia
Derivatives of Azidothymidine	Azidothymidinehomodinucletide encapsulated erythrocytes	slow delivery of antiviral drug
Derivatives of Deoxycytidine	Antiviral nucleotide analogue	Target macrophages
Derivatives of Azathioprene and AcycIovir	Heterodinucleotide of azidothymidine and AcycIovir	Protects against HIV virus.

Conclusion

The erythrocytes are suitable as natural transporters for therapeutic agents including various drugs, peptides, enzymes and genes. Carrier erythrocytes are safe and viable for maintained and targeted drug delivery as this novel

drug delivery system has no toxic influences. For pharmaceutical sciences drug loaded erythrocytes have turned into a diversion evolving innovation. The biodegradation of erythrocytes delivers no toxicity. Additionally targeting to the organ of the reticulo-endothelial system is conceivable with carrier erythrocytes and they take ideal zero-order kinetics of drug release. The advancement of creative and new dosage form utilizing this idea of drug conveyance will open another skyline in pharmaceutical industry.

REFERENCES

Alanazi, F.K., G.E.-D.I. Harisa, A. Maqboul, M. Abdel-Hamid, S.H. Neau and I.A. Alsarra (2011). Biochemically altered human erythrocytes as a carrier for targeted delivery of primaquine: an in vitro study. *Archives of pharmacal research*, 34: 563-571.

Alpar, H., and W. Irwin (1987). Some unique applications of erythrocytes as carrier systems. *adv. Biosci.*, 67: 1-9.

Bax, B., P. Talbot, M. Bain, E. Parker-Williams and R. Chalmers (1996). *In vivo survival of human carrier erythrocytes*, edited, Portland Press Limited.

Bellad, K.A., B.K. Nanjwade, M.S. Kamble, and T. Srichana (2017). Resealed erythrocytes based drug delivery system. World journal of pharmacy and pharmaceutical sciences. 6:2.446-482.

Bourgeaux, V., J.M. Lanao, B.E. Bax and Y. Godfrin (2016). Drug-loaded erythrocytes: on the road toward marketing approval. *Drug design, development and therapy*, 10: 665.

Bradbury, J. (2003). Beyond pills and jabs. *The Lancet*, 362: 1984-1985.

De Flora, A., U. Benatti, L. Guida and E. Zocchi (1986). Encapsulation of adriamycin in human erythrocytes. *Proceedings of the National Academy of Sciences*, 83: 7029-7033.

Deloach, J., S. Peters, O. Pinkard, R. Glew and G. Hiler (1977). Effect of glutaraldehyde treatment on enzyme-loaded erythrocytes. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 496: 507-515.

Eichler, H., S. Gasic, B. Daum, S. Bacher and G. Steger (1987). In vitro drug release from human carrier erythrocytes. *Adv. Biosci.*, 67: 11-15.

Foroozesh, M., M. Hamidi, A. Zarrin, S. Mohammadi-Samani and H. Montaseri (2011). Preparation and in-vitro characterization of tramadol-loaded carrier erythrocytes for long-term intravenous delivery. *Journal of Pharmacy and Pharmacology*, 63: 322-332.

Garín, M.-I., R.-M. López, S. Sanz, M. Pinilla and J. Luque (1996). Erythrocytes as carriers for recombinant human erythropoietin. *Pharmaceutical research*, 13: 869-874.

Gasparini, A., M. Tonetti, B. Astroff, L. Rowe, W. Satterfield, R. Schmidt and J.R. DeLoach (1992), Pharmacokinetics of doxorubicin loaded and glutaraldehyde treated erythrocytes in healthy and lymphoma bearing dogs. In: *The Use of Resealed Erythrocytes as Carriers and Bioreactors*, edited, pp. 299-304, Springer.

Ge, D., L. Zou, C. Li, S. Liu, S. Li, S. Sun and W. Ding (2018). Simulation of the osmosis-based drug encapsulation in erythrocytes. *European Biophysics Journal*, 47: 261-270.

Gothoskar, A.V. (2004). Resealed erythrocytes: a review. *Pharmaceutical Technology*, 28: 140-155.

Hamidi, M. and H. Tajerzadeh (2003). Carrier erythrocytes: an overview. *Drug delivery*, 10: 9-20.

Hamidi, M., A. Zarrin, M. Foroozesh and S. Mohammadi-Samani (2007). Applications of carrier erythrocytes in delivery of biopharmaceuticals. *Journal of controlled release*, 118: 145-160.

Hamidi, M., H. Tajerzadeh, A.-R. Dehpour, M.-R. Rouini and S. Ejtemaee-Mehr (2001). In vitro characterization of human intact erythrocytes loaded by enalaprilat. *Drug Delivery*, 8: 223-230.

Han, X., C. Wang and Z. Liu (2018). Red blood cells as smart delivery systems. *Bioconjugate chemistry*, 29: 852-860.

Harisa, G.I., M.F. Ibrahim, F. Alanazi and G.A. Shazly (2014). Engineering erythrocytes as a novel carrier for the targeted delivery of the anticancer drug paclitaxel. *Saudi Pharmaceutical Journal*, 22: 223-230.

Hirlekar, R., P. Patel, N. Dand and V. Kadam (2008). Drug loaded erythrocytes: as novel drug delivery system. *Current pharmaceutical design*, 14: 63-70.

Hu, C.M.J., R.H. Fang and L. Zhang (2012). Erythrocyte-Inspired Delivery Systems. *Advanced healthcare materials*, 1: 537-547.

Ihler, G.M., R.H. Glew and F.W. Schnure (1973). Enzyme loading of erythrocytes. *Proceedings of the National Academy of Sciences*, 70: 2663-2666.

Ito, Y., T. Ogiso, M. Iwaki, I. Yoneda and Y. Okuda (1989). In vitro stability of insulin-loaded erythrocytes after freezing storage. *Journal of pharmacobio-dynamics*, 12: 201-207.

Kinosita, K. and T. Tsong (1977). Hemolysis of human erythrocytes by transient electric field. *Proceedings of the National Academy of Sciences*, 74: 1923-1927.

Kitao, T., K. Hattori and M. Takeshita (1978). Agglutination of leukemic cells and daunomycin entrapped erythrocytes with lectin in vitro and in vivo. *Experientia*, 34: 94-95.

Krantz, A. (1997). Red cell-mediated therapy: opportunities and challenges. *Blood Cells, Molecules, and Diseases, 23*: 58-68.

Kumar, R., S. Jha, M. Kumar, P.S. Chauhan, S.K. Singh and B.P.S. Sagar (2012). Encapsulated erythrocytes for novel drug delivery sysytem. *Journal of Drug Delivery and Therapeutics.* 2(4): 61-67.

Kumar, M. (2011). Resealed erythrocytes as a carrier for drug targeting: A review. *International Journal of Pharmaceutical & Biological Archive*, 2: 1357-1373.

Kumari, R., P. Panda, R. Singh, D. Vishwakarma, J. Mishra and N.K. Verma (2018). Resealed Erythrocyte: an Approach to Targeted Drug Delivery. *To Chemistry Journal*, 1: 1-11.

Langer, R. (1990). New methods of drug delivery. *Science*, 249: 1527-1533.

Li, L., M. Hensen, Y. Zhao and S. Hui (1996). Electrofusion between heterogeneous-sized mammalian cells in a pellet: potential applications in drug delivery and hybridoma formation. *Biophysical journal*, 71: 479-486.

Lizano, C., S. Sanz, J. Luque and M. Pinilla (1998). In vitro study of alcohol dehydrogenase and acetaldehyde dehydrogenase encapsulated into human erythrocytes by an electroporation procedure. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1425: 328-336.

Luque, J., M.I. Garín, S. Sanz, P. Ropero and M. Pinilla (1992). Properties of hypotonized, crosslinked and crosslinked-permeabilized rat erythrocytes as potential carrier systems. In: *The Use of Resealed Erythrocytes as Carriers and Bioreactors*. edited, pp. 81-89, Springer.

Magnani, M. and J.R. DeLoach (2012). *The use of resealed erythrocytes as carriers and bioreactors*. Springer Science & Business Media.

Magnani, M. and L. Rossi (2014). Approaches to erythrocyte-mediated drug delivery. *Expert opinion on drug delivery*, 11: 677-687.

Magnani, M., L. Rossi, M. D'ascenzo, I. Panzani, L. Bigi and A. Zanella (1998). Erythrocyte engineering for drug delivery and targeting. *Biotechnology and applied biochemistry*, 28: 1-6.

Magnani, M., L. Rossi, A. Fraternale, M. Bianchi, A. Antonelli, R. Crinelli and L. Chiarantini (2002). Erythrocyte-mediated delivery of drugs, peptides and modified oligonucleotides. *Gene therapy*, 9: 749.

Millan, C.G., M.a.L.S. Marinero, A.Z. Castaneda and J.M. Lanao (2004). Drug, enzyme and peptide delivery using erythrocytes as carriers. *Journal of controlled release*, 95: 27-49.

Mitchell, D., G. James and C. Kruse (1990). Bioactivity of electric field-pulsed human recombinant interleukin-2 and its encapsulation into erythrocyte carriers. *Biotechnology and applied biochemistry*, 12: 264-275.

Mouneimne, Y., P.-F. Tosi, Y. Gazitt and C. Nicolau (1989). Electro-insertion of xeno-glycophorin into the red blood cell membrane. *Biochemical and biophysical research communications*, 159: 34-40.

Patel, R., M. Patel and N. Patel (2009). An overview of resealed erythrocyte drug delivery. *Journal of Pharmacy Research*, 2 (6): 1008. 1012.

Pitt, E., D.A. Lewis and R.E. Offord (1983a). The use of corticosteroids encapsulated in erythrocytes in the treatment of adjuvant induced arthritis in the rat. *Biochemical pharmacology*, 32: 3355-3358.

Pitt, E., C.M. Johnson, D.A. Lewis, D.A. Jenner and R.E. Offord (1983b). Encapsulation of drugs in intact erythrocytes: an intravenous delivery system. *Biochemical pharmacology*, 32: 3359-3368.

Rossi, L., S. Serafini, L. Cappellacci, E. Balestra, G. Brandi, G.F. Schiavano, P. Franchetti, M. Grifantini, C.-F. Perno and M. Magnani (2001). Erythrocyte-mediated delivery of a new homodinucleotide active against human immunodeficiency virus and herpes simplex virus. *Journal of Antimicrobial Chemotherapy*, 47: 819-827.

Sahoo, C.K., G. Vennela, T.K. Sahoo and A.K. Moharana (2012). An overview on resealed erythrocytes. A novel approach to drug delivery. *Int J Pharm Pharm Sci.*, 4: 71-6.

Sanz, S., C. Lizano, J. Luque and M. Pinilla (1999). *In vitro* and *in vivo* study of glutamate dehydrogenase encapsulated into mouse erythrocytes by a hypotonic dialysis procedure. *Life sciences*, 65: 2781-2789.

Talwar, N. and N. Jain (1992a). Erythrocytes as carriers of metronidazole: in-vitro characterization. *Drug development and industrial pharmacy*, 18: 1799-1812.

Talwar, N. and N.K. Jain (1992). Erythrocytes as carriers of metronidazole: In-vitro characterization. *Drug Development and Industrial Pharmacy*, 18: 1799-1812.

Talwar, N. and N. Jain (1992b). Erythrocytes as carriers of primaquine-preparation: characterization and evaluation. *Journal of controlled release*, 20: 133-141.

Tiwari, G., R. Tiwari, B. Sriwastawa, L. Bhati, S. Pandey, P. Pandey and S.K. Bannerjee (2012). Drug delivery systems: An updated review. *International journal of pharmaceutical investigation*, 2: 2.

Villa, C.H., A.C. Anselmo, S. Mitragotri and V. Muzykantov (2016). Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems. *Advanced drug delivery reviews*, 106: 88-103.

Villa, C.H., D.C. Pan, S. Zaitsev, D.B. Cines, D.L. Siegel and V.R. Muzykantov (2015). Delivery of drugs bound to erythrocytes: new avenues for an old intravascular carrier. *Therapeutic delivery*, 6: 795-826.

Wang, B., L. Hu and T.J. Siahaan (2016). *Drug delivery: principles and applications*. John Wiley & Sons.

Zimmermann, U. (1983). Cellular drug-carrier systems and their possible targeting. In: *Targeted drugs* (Goldberg E.P. Ed.). New York: John Wiley & Sons, pp.153-200.

Zimmermann, U., F. Riemann and G. Pilwat (1976). Enzyme loading of electrically homogeneous human red blood cell ghosts prepared by dielectric breakdown. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 436: 460-474.

(*Accepted for publication February 2020*)