International Journal of Pharmaceutics, 22 (1984) 137–146
Elsevier

IJP 00760

Review Article

Therapeutic possibilities of drugs encapsulated in erythrocytes

David A. Lewis and H. Oya Alpar

Department of Pharmaceutical Sciences, University of Aston in Birmingham, Birmingham B4 7ET (U.K.)

(Received May 10th, 1984)
(Modified version received July 31st, 1984)
(Accepted August 13th, 1984)

Summary

The use of erythrocytes as carriers for drugs has been reviewed. Methods for encapsulating drugs into erythrocytes has been discussed with experimental detail. Rigorous loading conditions used in the past have resulted in the formation of vesicles with poor survival times in vivo. More recent loading conditions such as preswelling or dialysis techniques have largely overcome survival problems in vivo. Under clinical conditions erythrocytes have been loaded with drugs by employing standard blood bags as an encapsulating vessel. Electrical and antibiotic loading methods have also been discussed. The limited use of drug- and enzyme-loaded erythrocytes under clinical conditions have shown that resealed erythrocytes can be safely infused into humans. In animal experiments the successful use of encapsulated materials largely depended on the integrity of the cells in vivo. Mild loading conditions have given excellent results in experiments involving the depletion of endogenous substrates by encapsulated enzymes and in where experiments where the circulating cells have acted as a slow release system for drugs. Reference has been made to other slow delivery systems such as liposomes or synthetic micro-vesicles and nano-particles.

The possibility of fusing erythrocytes with other cells with the subsequent transfer of encapsulated material by Sendai virus or polyethylene glycol is also discussed (39 references).

Correspondence: D.A. Lewis, Department of Pharmaceutical Sciences, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, U.K.

0378-5173/84/$03.00 © 1984 Elsevier Science Publishers B.V.

Introduction

The encapsulation of pharmacologically active compounds in microvesicles offers attractive possibilities in future therapy. These include the use of such vesicles in vivo where an encapsulated drug may be protected from premature degradation, inactivation and excretion. Encapsulated drugs protect the host from unwanted immunological effects and circulating vesicles may, by acting as a slow-release system, sustain drug levels in the blood at higher levels and for longer periods than drugs administered by orthodox systemic routes. Other possibilities include the targeting of drugs directly to a site of action such as a tumour or a diseased tissue or organ. A naturally occurring cell with possibilities as an encapsulating vesicle is the erythrocyte. Since the cells are in common clinical usage in transfusions much is known about the techniques for collecting and storing cells. Erythrocytes are completely degradable and provided that compatible cells are used in patients there is no possibility of a provoked immunological response. It is the purpose of this article to review the present position of the erythrocyte as an encapsulating vesicle.

Encapsulation techniques

The majority of encapsulation techniques described involving erythrocytes employ hypotonic lysis of the cells followed by restoration of tonicity which reseals the cells. When drugs were included in the hypotonic solutions they were, in part, encapsulated by the reformed cells. Early systems (e.g. Ihler et al., 1973) used water to lyze erythrocytes. Under hypotonic conditions the cells swelled rapidly and large holes or tears opened in the membrane admitting the drug to be encapsulated. The cells were reformed by restoring tonicity and stabilized by annealling for some time at 37°C. Most of the cytoplasmic constituents were lost in these rapid swelling techniques and the reformed cells contained only a few percent of the drug in solution. If the loaded cells were returned to the circulation they were rapidly removed by the recticuloendothelial system.

Today, three methods are employed which give good survival characteristics when the cells are returned to the circulation. These methods may be summarized as: (a) preswell dilution techniques; (b) dialysis techniques; or (c) dielectric breakdown of the erythrocytes.

Preswell dilution techniques

This technique was first employed by Rechsteiner (1975). The principle of the method consists of first swelling the erythrocytes without lysis by placing them in slightly hypotonic solution. The swollen cells are then recovered by centrifugation at low *g* and the cells taken to the point of lysis by the addition of relatively small volumes of water. The gentle swelling of the cells results in a good retention of cytoplasmic constituents and good survival times in vivo (Humphreys and Ihler, 1981).

We have recently modified this technique and obtained excellent survival times

for drug-loaded erythrocytes (haematocrit value 76) in rats, mice, guinea pigs and rabbits (Pitt et al., 1983a). Our procedure consisted of centrifuging blood to obtain the packed cells and preswelling the cells by the addition of 4 vols. of a modified Hanks balanced salt solution (HBSS) where the sodium and potassium ion concentrations in the HBSS were reversed (reversed HBSS). We also adjusted the tonicity of the reversed HBSS to 0.67 of the normal value. The swollen cells were recovered by centrifugation and then covered with a layer of haemolyzed erythrocytes prepared by adding 1 vol. of water to 1 ml of packed cells. The amount of haemolysate layered on top of the swollen cells consisted of 100 μl of haemolysate for each ml of packed cells. The drug to be encapsulated was dissolved in water or hypotonic buffer solution and layered in 100 μl portions on top of the haemolysate layer. For each ml of packed cells 200 μl of drug solution was added. The drug solution and the packed cells were gently mixed together by inversion before centrifuging. After centrifuging the supernatant was retained to act as a new barrier between the cells and a new portion of drug solution which was layered on top of the supernatant prior to mixing with the cells followed by centrifugation. Drug solution was added until the cells reached the point of lysis. This point may be calculated but was more conveniently observed. At the point of lysis old fragile cells lysed and were observed as a thin layer of white ghosts on top of the packed cells following centrifugation. When the presence of these ghosts was observed the calculated amount of 10 times isotonic HBSS was added to the cells to restore normal tonicity and the cells were washed with normal HBSS to remove free haemoglobin and free drug. We have encapsulated up to 25% of the drug or enzyme in solution (Alpar et al., 1984; Pitt et al., 1983a).

Incubation of the loaded erythrocytes in HBSS saturated with fluorescein isothiocyanate (FITC) was carried out when the cells were returned to the circulation as the fluorescein-labelled cells were distinguished from normal cells by examining a blood film under an ultraviolet microscope. The haemolysate layer employed in the encapsulation procedure gave osmotic protection when the drug solution was added to the packed cells and also supplied cytoplasmic constituents which entered the swollen cells at the same time as the drug.

Dialysis techniques

Dialysis was first employed by Klibensky (1957). In this technique the packed cells were placed in dialysis tubes which are immersed in hypotonic media. The use of dialysis tubes resulted in the retention of cytoplasmic constituents in the vicinity of the erythrocytes reducing the loss of cytoplasmic constituents when the cells were resealed (DeLoach and Ihler, 1977). In the procedure employed by DeLoach and Ihler (1977) human erythrocytes at haematocrit values of 70–80 were dialyzed against 1–2 litres of distilled water for 2 h at room temperature. At 5–10-min intervals the dialysis tubes were removed and their contents gently mixed by massage.

After 2 h the cells were resealed by restoring tonicity. Substances to be encapsulated were added to the dialysing medium and encapsulation loadings of 30–45% of the materials added have been reported (Ihler, 1980). DeLoach et al. (1980)

developed an encapsulation dialyzer based on this procedure which could load up to 150 ml of packed cells in one run.

Dielectric breakdown

Many cells including erythrocytes when subjected to intense electric fields form pores in their membranes prior to complete lysis. Dielectric breakdown was first employed by Kinosita and Tsong (1978) as a means to load erythrocytes. Using mouse or human erythrocytes the cells were subjected to electric pulses in [^{14}C]sucrose solutions. It was found that the cells incorporated sucrose when the pulse duration exceeded 20 μs at voltages of 1–2 $kV \cdot cm^{-1}$. Excellent survival times for the loaded cells were found in vivo (mice) provided that the pulse duration did not exceed 160 μs. In this method the pores remained open at low temperatures but closed completely when the cells were annealed at 37°C. Stachyose was added to the solution as it was found that this sugar prevented haemolysis (Kinosita and Tsong, 1978). High molecular weight substances such as urease have been encapsulated by this method (Zimmermann et al., 1976). A major disadvantage of this method was that expensive equipment was required and the method had no obvious advantages over the simple dialysis of preswelling techniques.

Antibiotics

Polyene antibiotics such as amphotericin-B damage micro-organisms by increasing the permeability of their membranes to metabolites and ions. This property was exploited with erythrocytes when amphotericin-B was used to load erythrocytes with the anti-leukaemic drug daunomycin (Deuticke et al., 1973). The preparation was used with marginal success to treat leukaemic mice. We have tested this method by using the antibiotic to encapsulate L-Lys-L-Phe, but have found that the survival times of the resealed cells in vivo is poor (Desai and Lewis, unpublished results).

Storage

Very few reports have commented on the storage of materials encapsulated in erythrocytes. Most authors appear to have encapsulated their preparations immediately before returning them to the circulation. Ihler (1983) comments that the well-known techniques available for the storage of erythrocytes used for transfusions are equally applicable to erythrocytes loaded as carriers with drugs or enzymes. We have found that encapsulated preparations store without loss of integrity in vivo when suspended in HBSS at 4°C for 2 weeks. We have also achieved similiar results by suspending the cells after encapsulation in oxygenated HBSS containing 1% soft bloom gelatin (Sigma Chemicals, Poole, Dorset). The tubes containing the packed cells were gently rotated at 4°C in the presence of 4 vols. of the HBSS solution. When the gels had set the tubes were stored in a refrigerator for 2 weeks. When required the tubes were placed in a waterbath at 37°C to liquefy the gel. The cells were recovered by gentle centrifugaton and washed in HBSS prior to use. An advantage of storing the cells in a gel was that the cells dispersed easily when the gel liquefied and avoided the 'clumping' that occasionally occurred when the cells were stored in liquid media (Alpar et al., 1984). Under clinical conditions standard blood

bags may be used for both encapsulation and storage. When Green et al. (1980) used desferrioxamine encapsulated in erythrocyte ghosts to treat patients with excess iron stores, blood was taken from patients directly into blood bags. After centrifugation the plasma was squeezed from the bags leaving packed cells with a haematocrit value of approximately 80. Additions of solutions including desferrioxamine were made directly to the bags and the solutions mixed by massage. Finally the encapsulated desferrioxamine was infused i.v. into the patients over 1 h. The whole procedure including removal of blood and encapsulation took 4–5 h. By using the patients own blood and carrying out the encapsulation in the bag compatability and sterility problems were minimal. A small sample of the encapsulated desferrioxamine was retained for batch analysis and on the results of this analysis the number of cells infused was adjusted to give the required dose.

An alternative procedure would be to use Group O (universal donor) cells and by using the preswelling or dialysis techniques a batch of the preparation could be prepared in advance and stored under conditions used for the normal storage of erythrocytes used in transfusions. A batch analysis is necessary to establish the exact amount of drug encapsulated.

After storage cells were always tested to see if their integrity had been retained before returning then to the circulation. We tested the cells for integrity in vivo by mixing a small sample of the preparation with the donors phagocytic leucocytes (e.g. from a blood sample). If the cells had lost their integrity, they were rapidly attacked by the phagocytic leukocytes and this was observed with a microscope. We also subjected the stored cells to deoxygenation and re-oxygenation. Resealed living cells behaved exactly as freshly isolated normal erythrocytes whereas dead cells did not oxygenate and rapidly discoloured owing to the formation of methaemoglobin. In practice this was an all-or-none phenomenon as preparations that were past their useful life went brown in a matter of hours. Our results with a large number of stored preparations have shown that we can store cells with confidence for up to 2 weeks at 4°C.

Toxicity

No toxic effects have been reported in animal studies directly due to the use of erythrocytes as circulating blood carriers. In human studies Green et al. (1980) reported that no untoward effects were found in his patients receiving desferrioxamine in erythrocyte ghosts. Patients gave normal values when tested for liver function and coagulation values. In another clinical trial where patients were infused with glucocerebrosidase encapsulated in erythrocytes as a replacement therapy in Gaucher disease no side-effects were observed and the author (Beutler et al., 1977) commented that the experiments showed that resealed erythrocytes could be safely infused into patients and if 'loaded' at a high haematocrit value (e.g. as packed cells) circulated in vivo for a prolonged time. Side-effects directly related to the drug are reduced since the encapsulation process effectively reduces the levels of free or protein bound drug in the circulation.

Changes in erythrocyte shape

The natural shape of the erythrocyte is responsible in part for the volume changes that assist the encapsulation of drugs to hypotonic lysis. The normal shape is a biconcave disc. This shape is maintained by a contractile protein, 'elinin' (Nakao et al., 1960). When cells are suspended in media of varying tonicity the volumes of the cells change owing to the passage of water through the membrane. The cell acts as an osmometer (Guest, 1948) for in hypertonic media they shrink and in hypotonic media they swell. On swelling the cell changes from a biconcave disc to a sphere (spherocyte) which has a lower ratio of surface area-to-surface volume; a volume increase of 154–175% over the biconcave disc depending on the species of cell (Hoffman, 1958). At this stage there is little stretching of the membrane but further reduction of tonicity will elevate internal pressure and finally lead to a rupture of the outer membrane. Rapid hypotonic procedures lead to the complete rupture of cells which reform in isotonic media to form 'white ghosts'. It is less certain what happens at the point of lysis when less rigorous techniques are used to preserve cytoplasmic constituents (red ghosts). At the point of lysis numerous 'holes' appear but whether these apertures are open pores or tears is not known. We have found that the maximum amount of swelling of the cells prior to lysis is necessary to encapsulate drugs. Using a fluorescein derivative of methotrexate (F-MTX) we found that over 80% of the cells took up F-MTX at the point of lysis compared with only 1–2% at the preswelling stage (Pitt et al., 1983a).

Applications of encapsulated drugs in erythrocytes

Early attempts to encapsulate materials into erythrocytes was made by Straub (cited by Gardos, 1953) who encapsulated ATP in erythrocyte ghosts. Marsden and Ostling (1959) extended this work by demonstrating that high molecular weight compounds could be encapsulated in erythrocyte ghosts by using dextrans over a molecular weight range from 10,000 to 250,000 as the encapsulated material. An early application of the technique to pharmacology was in enzyme replacement. Ihler et al. (1973) encapsulated rat kidney β-galactosidase and β-glucosidase into human erythrocytes and showed that the encapsulated enzymes retained their activity inside the cell. Some success has been obtained with encapsulated enzymes under clinical conditions. A single transfusion of encapsulated enzyme was given to a 5-week-old boy with galactose-1-phosphate uridyl transferase deficiency. The boy was injected with 10 ml of packed cells per kg and his blood level of galactose phosphate fell to reach normal values 5 days after the transfusion. Although he was a true homozygote in that his enzyme deficiency persisted, his sugar levels were successfully controlled by a galactose-free diet (Harris, 1977). Attempts have been made to treat Gaucher disease with glucocerebroside encapsulated in erythrocytes (Beutler et al., 1977). The experiments were inconclusive but the loaded cells were found to survive for up to 10 days in treated patients. No untoward reactions were found towards the cells or encapsulated enzyme found in the patients and their blood counts, blood

pressure and renal function remained normal throughout the treatment. No evidence of intravascular activation of the clotting mechanism was observed. In other experiments desferrioxamine encapsulated in erythrocytes has been used to treat patients with excess stores of iron (Green et al., 1980). This drug is rapidly cleared from the circulation and the substance was loaded into erythrocytes in an attempt to slow down its clearance. The effectiveness of the encapsulated drug was compared with that of an infusion of the free drug and it was found that the encapsulated drug significantly increased the rate of clearance of iron compared with the free drug. Cells from patients with familial hyperargininemia (i.e. arginine-deficient erythrocytes) were used to encapsulate bovine liver arginase by hypotonic lysis and the cells were resealed by annealing at 37°C after isotonicity was restored (Adriaenssens et al., 1976). The cells were maintained at 4°C in vitro in acid citrate glucose medium for 12 days and throughout that period they maintained urea production. Non-treated arginase-deficient cells from the same patients produced only traces of urea.

As might be expected, much of the work reported has been with animals. The rationale underlying the treatment of Gaucher disease by enzymes encapsulated in liposomes was investigated in animals (Beutler et al., 1977). In the disease phagocytic cells accumulate glucocerebroside which cannot be degraded owing to the deficiency of glucocerebrosidase. DeLoach and Barton (1981) found that macrophages grown in culture were able to take up glutaraldehyde-treated erythrocytes loaded with β-galactosidase, or alkaline phosphatase, into phagolysosomes and that the enzymes remained active for up to 30 h in culture. When returned to the circulation erythrocytes treated with the minimum of glutaraldehyde were removed by the spleen, but erythrocytes treated with higher concentrations of glutaraldehyde were removed by the liver. This allows some degree of targeting of drugs to the liver or spleen. Asparaginase encapsulated in erythrocytes is a possible anti-leukaemic therapy. This has been tested in several animal species including monkeys (Updike et al., 1976) where plasma levels were significantly depressed. Using the same system in mice Alpar and Lewis (1983) completely removed asparagine from plasma in vivo over a 14-day period. Subsequently we have found that the encapsulated enzyme was able to 'cure' mice implanted with the 6C3HED tumour whereas the same amount of free enzyme did not. The encapsulation of low molecular weight material has produced variable results. Encapsulated methotrexate was found to escape rapidly from canine erythrocytes (DeLoach and Barton, 1981). However, Pitt et al. (1983a) encapsulated corticosteroid phosphate esters which were unable to escape from the cells. However, the action of endogenous phosphatases in the erythrocyte hydrolyzed the ester allowing the free steroid to diffuse through the membrane and maintain useful therapeutic blood levels of steroid for at least 10 days. Encapsulated corticosteroid phosphate esters were found to be superior to free steroid phosphates administered i.v. in the treatment of adjuvant induced arthritis in the rat (Pitt et al., 1983b).

Targeting

Apart from the uptake of erythrocyte ghosts by the recticuloendothelial system, very few attempts have been made to target drugs elsewhere in vivo. Kitao et al. (1978) encapsulated daunomycin in erythrocytes and treated the L1210 tumour in mice. The mice treated with the free drug survived for an average of 10 days but with the encapsulated preparation the average was 12.5 days whilst untreated animals survived for only 7.1 days. When the mice were treated with wheat germ agglutinin (a lectin) this treatment increased the survival time of the mice treated with encapsulated daunomycin to 17.9 days. The lectin probably combined with both the membrane of the erythrocyte and the membrane of the tumour cell bringing both into close contact by aggregation. Lectin treatment alone did not prolong the survival times of the mice. Some interesting work has been carried out using fusion techniques. The Sendai virus induces fusion of erythrocytes with other cells without loss of cytoplasmic contents (Zakai et al., 1974; Furusawa et al., 1974). Erythrocyte ghosts have been loaded with soluble macromolecules or with ferritin or latex spheres (0.1 μm diameter) and fused with other cells by the Sendai virus, allowing transfer of the encapsulated material to take place (Loyter et al., 1975; Wille and Willecke, 1976). Fusion can also be achieved by the use of polyethylene glycol (PEG) and successful fusions have involved the transfer of functional tRNA and mRNA (Capecchi et al., 1977) .

Other carrier systems

Other drug delivery systems have been developed in addition to erythrocytes. These include liposomes (Gregoriadis, 1976), nanocapsules prepared by the micellar polymerisation of acrylamide or methylacrylamide (Birrenbach and Speiser, 1976), microcapsules prepared from polylactic acid which are biodegradable (Masson et al., 1976) or from synthetic materials such as nylon (Chang, 1972). Blood proteins such as albumin have been used as microsphere or nanosphere carriers (Fiume et al., 1979). Some attempts have been made to target liposomes and other carriers to specific sites. One novel system tested with success in vitro was to incorporate magnetite into albumin microspheres and directing the magnetic microspheres by an externally applied magnetic field to a specific target (Widder et al., 1976). The incorporation of magnetic material into erythrocytes for the same purpose has been proposed by Zimmermann et al. (1978). Other systems must be regarded as alternatives to erythrocytes for carrying drugs but their main disadvantage is that they do not survive for long when administered in vivo.

Conclusions

The use of erythrocytes as drug carriers is still in the experimental stage but the use of microcapsules in some form appears likely in the near future. Erythrocytes

have some advantages over other systems in that by the use of gentle loading techniques, such as preswelling or dialysis the loaded cells can survive for long periods in vivo. Cells can be separated on gradients into fractions dependent on the age of the cells (Rennie et al., 1979). The use of loaded young cells separated from older cells may well prolong survival times in vivo. Another advantage of erythrocytes is that they are non-toxic and on clinical evidence to date safe to use in patients. Liposomes offer two advantages over erythrocytes in that they can hold greater amounts of drug than erythrocytes and they are more stable. However, the poor survival times of liposomes in vivo is a serious disadvantage. The effective targetting of erythrocytes or liposomes is still some way off but the fusion of drug-loaded erythrocytes with other cells offers some exciting possibilities in molecular engineering in the future.

References

Adriaenssens, K., Karcher, D., Lowenthal, A. and Terheggen, H.E., Use of enzyme loaded erythrocytes in the correction of arginase-deficient erythrocytes in familial hyperargininemia. Clin. Chem., 22 (1976) 323–326.

Alpar, H.O. and Lewis, D.A., Circulating asparaginase encapsulated in erythrocytes depletes plasma asparagine and glutamine levels in vivo. J. Pharm. Pharmacol., 35 (1983) 48P.

Alpar, H.O., Desai, J. and Lewis, D.A., Pharmaceutical possibilities of intact erythrocytes as drug carriers. J. Pharm. Pharmacol., (1984) in press.

Beutler, E., Dale, G.L., Guinto, E. and Kuhl, W., Enzyme replacement therapy in Gauchers disease. Preliminary clinical trial of a new enzyme preparation. Proc. Natl. Acad. Sci. U.S.A., 74 (1977) 4620–4623.

Birrenbach, G. and Speiser, P.P., Polymerized micelles and their use as adjuvants in immunology. J. Pharm. Sci., 65 (1976) 1763–1766.

Capecchi, M.R., Vunder-Haar, R., Capecchi, N.E. and Sveda, M.M., The isolation of a suppressible nonsence mutant in mammalian cells. Cell, 12 (1977) 371–381.

Chang, T.M.S., In Artificial Cells, Charles C. Thomas, Springfield, IL, 1972, p. 64.

DeLoach, J.R. and Ihler, G.M., A dialysis procedure for loading erythrocytes with enzymes and lipids. Biochem. Biophys. Acta, 496 (1977) 136–145.

DeLoach, J.R., Harris, R.L. and Ihler, G.M., An erythrocyte encapsulator dialyser used in preparing large quantities of erythrocyte ghosts and encapsulation of a pesticide in erythrocyte ghosts. Anal. Biochem., 102 (1980) 220–227.

DeLoach, J.R. and Barton, C., Glutaraldehyde treated carrier erythrocytes for organ targeting of methotrexate in dogs. Am. J. Vet. Res., 42 (1981) 1971–1974.

Deuticke, B., Kim, M. and Zolinev, C., The influence of amphotericin-B on the permeability of mammalian erythrocytes to non-electrolytes, anions and cations. Biochim. Biophys. Acta, 318 (1973) 345–359.

Fiume, L., Mattioli, A., Balboni, P.G. and Barbanti-Brodano, G., Albumin conjugates of fungal toxins and inhibitors of DNA synthesis. In Gregoriadis, G. (Ed.), Drug Carriers in Biology and Medicine, Academic Press, New York, 1979, pp. 3–22.

Furusawa, M., Nishimura, T., Yamaizumi, M. and Okada, Y., Injection of substances into single cells by cell fusion. Nature (Lond.), 249 (1974) 449–450.

Gardos, G.,, Akkumulation der Kalium onen durch menschliche Blutkorperchen. Acta Physiol. Acad. Sci. Hung., 6 (1953) 191–196.

Green, R., Lamon, J. and Curran, D., Clinical trial of desferrioxamine entrapped in red cell ghosts. Lancet, 1 (1980) 327–330.

Gregoriadis, G., The carrier potential of liposomes in biology and medicine. New Engl. J. Med., 295 (1976) 704–710 and 765–770.
Guest, G.M., Osmometric behavior of normal and abnormal human erythrocytes. Blood, 3 (1984) 541–555.
Hoffman, J.F., Physiological characteristics of human red blood cell ghosts. J. Gen. Physiol., 42, (1958) 9–28.
Harris, R.C., Enzyme replacement in red cells. New Engl. J. Med., 296 (1977) 942–943.
Humphreys, J.D. and Ihler, G.M., In H. Bundgaard (Ed.), Alfred Benzon Symposium 17: Optimization of Drug Delivery, Munksguaard, Copenhagen, 1981, pp. 270–284.
Ihler, G.M., Glew, R.H. and Schnure, F.W., Enzyme loading of erythrocytes. Proc. Natl. Acad. Sci. U.S.A., 70 (1973) 2663–2666.
Ihler, G.M., Erythrocyte carriers. Pharmacol. Ther., 20 (1983) 151–157.
Kinosita, K. and Tsong, T.W., Survival of sucrose-loaded erythrocytes in the circulation. Nature (Lond.), 272 (1978) 258–260.
Kitao, T., Hattori, K. and Takeshita, M., Agglutinaton of leukaemic cells and daunomycin entrapped erythrocytes with lectin in vitro and in vivo. Experimentia, 341 (1978) 94–95.
Klibensky, C., Ph.D. Thesis, Hebrew University Jerusalem, 1957.
Loyter, A., Zakai, N. and Kulka, R.G., Ultramicroinjection of macromolecules or small particles into animal cells. J. Cell Biol., 66 (1975) 292–305.
Marsden, N.V.B. and Ostling, S.G., Accumulation of dextran in human red blood cells after haemolysis. Nature (Lond.), 184 (1959) 723–724.
Mason, N., Thies, C. and Cicero, T.J., In vivo and in vitro evaluation of a microencapsulated narcotic antagonist. J. Pharm. Sci., 65 (1976) 847–850.
Nakao, M., Nakao, T. and Yamazoe, S., Adenosine triphosphate and maintenance of shape of the human red blood cell. Nature (Lond.), 187 (1960) 945–946.
Pitt, E., Johnson, C.M., Lewis, D.A., Jenner, D.A. and Offord, R.E., Encapsulation of drugs in intact erythrocytes: An intravenous delivery system. Biochem. Pharmacol., 32 (1983a) 3359–3368.
Pitt, E., Lewis, D.A. and Offord, R.E., The use of corticosteroids encapsulated in erythrocytes in the treatment of adjuvant induced arthritis in the rat. Biochem. Pharmacol., 32 (1983b) 3353–3358.
Rechsteiner, M.C., Uptake of protein by red cells. Exp. Cell Res., 93 (1975) 487–492.
Rennie, C.M., Thompson, S., Parker, A.C. and Maddy, Human erythrocyte fractionation in Percoll density gradients. Clin. Chim. Acta, 98 (1979) 119–125.
Updike, S.J., Wakamiya, R.T. and Lightfoot, E.N. Jr., Asparaginase entrapped in red blood cells: action and survival. Science, 193 (1976) 681–683.
Widder, K., Fluoret, G. and Senyei, A. Magnetic micro-spheres: synthesis of a novel parenteral drug carrier. J. Pharm Sci., 68 (1976) 79–82.
Wille, W. and Willecke, K., Retention of purified proteins in resealed human erythrocyte ghosts and transfer by fusion into cultured murine cells. FEBS Lett., 65 (1976) 59–62.
Zakai, M.R., Kulka, G. and Loyter, A., Prevention of haemolysis by bivalent metal ions during virus-induced fusion of erythrocytes with Ehrlich ascites tumour cells. FEBS Lett., 40 (1974) 331–333.
Zimmermann, U., Riemann, F. and Pilwat, G., Enzyme loading of electrically homogenous human red blood cells ghosts prepared by dielectric breakdown. Biochem. Biophys. Acta, 436 (1976) 460–474.
Zimmermann, U., Pilwat, G. and Esser, S., The effect of encapsulation in red blood cells on the distribution of methotrexate in mice. J. Clin. Chem. Clin. Biochem., 16 (1978) 135–144.