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SOLID COLLOIDAL DRUG DELIVERY SYSTEMS: NANOPARTICLES

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SUMMARY

After outlining the criteria of an ideal solid colloidal drug delivery system, a review is made under 5 headings of the various systems described in the literature. None of these systems are ideal. The problems of maximizing the drug content of the solid system are discussed. Viruses and vaccines, diagnostic agents, cytotoxics, flukicides and antiarthritics are given as examples of the drugs that may be incorporated into these so-called nanoparticles. The biggest barrier to successful exploitation of nanoparticles to minimize rapid hepatic clearance.

INTRODUCTION

Much of the recent effort aimed at maximizing the efficiency of drug therapy has utilized the biopharmaceutics route. Within the confines of traditional drug delivery systems little else could be done. However, at an increasing rate over the last 10 years or so, reports have appeared in which the pharmaceutics route has been taken to solve this problem. New drug delivery systems have been developed. General reviews of advances across all systems are available (e.g. Robinson, 1978). Special types of systems are continually being reviewed (Marty and Oppenheim, 1977; Juliano, 1980; Widder et al., 1979).

Considerable effort has been put into colloidal drug delivery systems which are useful for general systemic use. These essentially submicron systems are typified by liposomes. An enormous literature exists for these fluid or semifluid systems. Examples of the wide applications of liposomes are given in reports and reviews by Pagano and Weinstein (1978); Szoka and Papahadjopoulos (1980); and Papahadjopoulos (1978).

Another group of submicron-sized delivery systems are solid particles. By analogy with microparticles and microencapsulation procedures, these solid submicron particles can be termed nanoparticles. As it is not likely that any of these nanometer-sized systems are 'capsule-like' or have a discrete shell, the word nanocapsule is not an appropriate description. Recently Allan et al. (1980) have introduced another term, nanovesicles, to describe

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the 60 nm vesicles produced during ionophore A 23187-induced budding of human erythrocytes. These non-solid vesicles have been excluded from this review.

Recently Groves (1980) outlined some of the problems in determining the size characteristics of submicron particulate systems. He concluded that care is required in the interpretation and application of the results from different sizing procedures.

The term nanoparticle has so far only been applied to a few examples of such solid particles but it is already clear that they represent a whole new class within drug delivery systems. Many of the applications to which liposomes have been put await investigation using nanoparticles.

Irrespective of the type of nanoparticle used, they all attempt to deliver drugs to specific cells or organs. Although fashionable terms such as zip-coded drugs and magic bullets can be used for this specific delivery, the targeting of drugs in this way means: (1) the circulating levels of free and protein bound drugs are reduced drastically; (2) the total amount of drug used is reduced; and (3) the cost of the delivery increases due to the technology of making the delivery system.

Provided that the increased manufacturing costs are not as great as the savings achieved in reduced drug dosage and repair of adverse and toxic reactions to the drug, the system could be economically viable.

Classes of drugs which require activation by hepatic microsomal enzymes for activity at other sites in the body would normally be excluded from consideration as candidates for incorporation into nanoparticles.

REQUIREMENTS OF A NANOPARTICLE SYSTEM

Before reviewing the advantages and disadvantages of individual types of nanoparticles, it is essential to establish criteria of usefulness for such a system.

The ideal nanoparticle drug delivery system, made up of a carrier material and the payload of drug, would meet the following behavioural criteria: (1) it must accumulate or remain at its desired site of action; (2) it must release the drug at a suitable rate at the desired site of action; (3) it must be pharmaceutically acceptable with regard to stability and ease of administration; (4) if parenteral use is envisaged, it must be able to be sterilized; and (5) the carrier material, when made up as the delivery system, must be non-toxic and biodegradeable.

In the clinical usage of particulate delivery systems, embolism of relatively large particles in a defined capillary bed can sometimes be achieved by local perfusion procedures. However, such embolism, followed by drug release from degrading particles, does not generally occur unless the particles have a diameter of at least about that of erythrocytes. Merland et al. (1978) found that particles at least 10 μm diameter were needed to cause embolism in some tumour capillaries. Kanke et al. (1980) have administered radiolabelled polystyrene divinylbenzene microspheres to beagle dogs and followed the clearance. The smallest of the size ranges chosen (around 3 μm) cleared the lung capillary bed and were located in the phagocytic cells of the liver, spleen and bone marrow. The largest particles (around 12 μm diameter) were mechanically filtered by lung capillaries. They concluded that the lower size limit for embolism of such inert particles was somewhere between 7 and 12 μm. When clinical procedures leading to local embolism are not possible or desira-

ble, it would be necessary to restrict the particle size to the non-embolic nanometer range to avoid indiscriminate embolism following general systemic administration. In this situation, the nanoparticles must accumulate or remain at the desired site of action by a mechanism other than embolism. Solid nanoparticles are unable to fuse with cell walls analogously to some liposomal preparations. The efficiency of most of the solid colloidal systems depends on their ability to be taken up or phagocytosed by the cells of interest. Walters and Papadimitriou (1978) have reviewed many aspects of phagocytosis. The intracellular enzymes, particularly the lysosomal system are therefore the real target of many of the nanoparticle systems. Couvreur et al. (1980a) have used the term lysosomotropic carrier to describe this feature of these drug delivery systems.

However, when colloidal particles are administered to the body, the normal reticulo-endothelial defence mechanism is activated. Unless specific delivery to, for example, the Kupffer cells of the liver is required, the greatest barrier to effective use of nanoparticles is the ability to target them elsewhere. Liposomes also suffer from this problem. Hepatic blockade by the prior administration of drug-free liposomes (Gregoriadis et al., 1977) or methyl palmitate (Tanake et al., 1975) has shown some promise.

Although it is possible to also achieve blockade with dextran sulphate (Bradfield and Wagner, 1977) or with other particulate systems such blank nanoparticles or colloidal carbon, most workers usually administer their nanoparticle products without blockade and investigate the resultant cell and organ distribution or product efficacy. However, as discussed below, there is a growing appreciation of the inefficiency of this approach and a number of procedures have been developed for specific delivery systems in an attempt to overcome the hepatic clearance problem.

It should be remembered that when determining the efficacy of any drug delivery system, little is known regarding the specific organ or tissue drug levels necessary for therapeutic effects. Hence the ideal nanoparticle system has to make its payload of drug available at an appropriate rate when it gets to its desired site of action. Firstly, if the drug is associated, in an available state, with the surface of the nanoparticle, the rate of availability would be the rate of delivery to the site of action. However, despite of relatively large specific surface area of colloidal particles, the total amount of drug so available would be limited.

Secondly, the drug could desorb from the surface of pores in the carrier matrix and diffuse out of the pores. The extent of porosity and tortuosity and the rate of diffusion would be important parameters in determining drug release. Whilst the development of nanoparticle systems is in its infancy, it would be unfortunate if any individual system was discarded simply because the rate-limiting step in drug availability had not been properly determined.

The ideal nanoparticle system has to have a long enough shelf-life for the pharmaceutical industry to consider manufacture. One of the biggest disadvantages of the liposomal delivery system is the difficulty in storing a physical stable product for more than a few months. Unlike liposomes which cannot be freeze-dried, solid systems are readily stored as reconstitutable lyophilized powders. The colloidal nature of nanoparticles means that when reconstituted, they do not sediment rapidly, they can be readily protected against flocculation and coagulation, and they are unlikely to occlude hypodermic needles or blood capillaries.

Filtration cannot be used to achieve sterility since the nanoparticles have a similar size to the contaminant. The systems developed so far have paid insufficient attention to this behavioural criterion. It is possible that the manufacturing procedure can be assumed to minimize contamination. For example, the addition of glutaraldehyde and gel chromatography purification procedures assists in ensuring no bacterial contamination is present in protein-based nanoparticles (Oppenheim and Stewart, 1979).

Since almost every system is expected to be used parenterally, any reference to other routes of administration has not been included in this review. It is, however, possible that membranes lining various body organs such as the nose and gut may allow the passage of colloidal particles. Hence these other routes will need to be investigated in detail in the future. For these routes, sterility of the administered product is not so important.

The problem of biodegradability has to be kept in proportion. If the product is only going to be used once as a diagnostic agent, as, for example, colloidal sulphur tagged with ^{99m}Tc, or as an inoculation procedure (e.g. influenza virus on polymethylmethacrylate nanoparticles) biodegradeability is of lesser importance than if the product is to be administered repeatedly. Long-term accumulation of particulate material must be considered with gold, used in arthritis (Vernon-Roberts et al., 1976), magnetic microspheres used to deliver cytotoxics (Widder et al., 1979) or any of the other poorly degradeable products considered below. Conversely, biodegradation must not be too rapid, otherwise the nanoparticle will have degraded before it reaches its desired site of action.

The removal of the toxic residual monomer in some of the emulsion-polymerized particles would unrealistically increase the cost of the delivery system. Any system incorporating natural macromolecules would have to be screened for antigenic response.

The various types of nanoparticles described in the literature need to be evaluated against this discussion of the ideal solid colloidal drug delivery system. Although problems with maximizing the payload of drug in each total delivery system are inherently involved with the manufacturing procedure utilized, a number of the systems have similar problems. Hence these problems will be discussed after comments have been made on the method of manufacture of the more important systems.

TYPES OF NANOPARTICLES

(a) Non-biodegradeable acrylate particles

One of the original colloidal systems was developed by Speiser and his group (Birrenbach, 1973; Birrenbach and Speiser, 1976; Kreuter and Speiser, 1976). Individual monomers such as N,N′-methylenebisacrylamide or methylmethacrylate are solubilized in a hexane solution. Polymerization is induced by exposure to gamma irradiation or ultraviolet light. The result of this emulsion polymerization procedure was originally termed nanocapsules (Speiser, 1976) but more recently the nanoparticle term has been used (Kreuter, 1978). The 80–250 nm particles are stored as a freeze-dried powder. Although a comparatively simple manufacturing procedure is used, there are 3 main problems associated with this type of nanoparticle.

Residual monomers of methylmethacrylate and acrylamide have been blamed for toxicity of prosthetic materials (Dillingham et al., 1975). Dangerous levels are difficult to define and hence to ensure the safety of these particles for in vivo use, it is imperative

that all the monomer must be used up during the polymerization step or the final product must be washed free of residual monomer. Kopf et al. (1976) has discussed this residual monomer problem for polyacrylamide nanoparticles and concluded that simple washing of the final product was sufficient to reduce the monomer level to well below any potentially dangerous level. Kreuter and Zehnder (1978) found that methylmethacrylate nanoparticles contained no more than 1% of extraneous material such as the methacrylate monomer.

Secondly, no evidence has been put forward to show that polymethylmethacrylate particles would be biodegraded. Indeed if these polymers were degraded to any significant extent, their use in prosthetics would be limited. The auto-catalysed hydrolysis of acrylate polymer colloids as described by Fitch et al. (1979) requires an acidity not possible in body fluids.

Finally the use of radiation to induce polymerization may lead to unwanted changes in drugs and biological materials. Although knowledge of these possible changes is incomplete, some information can be gained from the reviews by Schulman (1973), Trutnau et al. (1978 and 1979) and Fielitz et al. (1979).

Ekman and Sjöholm (Ekman et al., 1976; Ekman and Sjöholm, 1975) have modified this non-biodegradeable system by incorporating proteins within a polymethylmethacrylate particle. The protein is mixed with the monomer solution and the polymerization induced by the addition of ammonium peroxodisulphate and N,N,N′,N′-tetramethylethylenediamine. The polymeric network trapping the proteins is porous and this allows easy penetration of the particles by other molecules seeking to interact with the entrapped protein. The entrapped proteins have significantly increased stability against heat denaturation or proteolytic enzyme degradation (Ekman et al., 1976).

(b) Other non-biodegradeable polymeric particles

Colloidal sulfur is commonly labelled with ^{99m}Tc and administered to apparently healthy and diseased humans and animals as a diagnostic agent. The experimental techniques and methodology developed for such a system can readily be transferred to other drug delivery systems. Colloidal sulfur is commonly protected against aggregation by the addition of gelatin.

Colloidal gold as ^{198}Au is used for a variety of diagnostic and therapeutic purposes (Wade, 1977). It is supplied as a sterile suspension stabilized by gelatin and sodium citrate. The particles have a size between 2 and 60 nm although narrower ranges can be demanded. Colloidal gold and gold salts are retained in the body for a considerable time, although not necessarily at the desired site of action. Vernon-Roberts et al. (1976) describe patients with detectable gold levels up to 23 years after having stopped therapy.

Over the years Banker and his group has developed a porous micron-sized system based on macromolecules such as ethyl cellulose or cellulose acetate phthalate. The active drug is molecularly entrapped within the pores of the polymeric latex. Although the main application appears to be oral administration of a stabilized flocculated aggregate of particles (Boylan and Banker, 1973), there appears to be some potential for administration of deflocculated discrete particles by other routes (Gurny et al., 1979).

Perhaps the most exciting development within the area of nanoparticles with residual biodegradability problems is that of the magnetically responsive microspheres. These

colloidal particles have been described by Widder et al. (1979). 10–20 nm particles of Fe_3O_4 are emulsified with an aqueous solution of serum albumin in cotton seed oil. After homogenization to produce submicron droplets, the albumin is cross-linked by heat denaturation or by reaction with formaldehyde or 2,3-butanedione.

The particles are then localized at various sites in the body by the application of magnetic fields. In this way one of the major problems of bypassing the Kuppfer cells is overcome. Peterson and Kreuger (1977) have discussed the reversible field-induced agglomeration of magnetic colloids and the work of Smith and Bruce (1979) raises the potential problem of sheer flocculation of the magnetic microspheres after administration.

Appreciating that the polyacrylamide and polymethacrylate nanoparticles had biodegradeability problems, other European groups began to develop delivery systems based on other monomers.

(c) Biodegradeable polymeric systems

200 nm diameter spherical particles are formed by the polymerization of an alkylcyanoacrylate in an aqueous acidic medium in the presence of a surfactant (Couvreur et al., 1979a). Polymers based on these monomers have been used as surgical sutures for some years (Pani et al., 1968). The particles are degraded by hydrolysis of the carbon chain forming formaldehyde and an alkylcyanoacetate. The rate of degradation is a function of the alkyl chain-length with the shorter methyl and ethyl chains degrading faster than the longer butyl chains (Pani et al., 1968; Couvreur et al., 1979b). The butyl ester with its slower degradation rate is well tolerated in vivo (Leonard, 1970).

The solid particles are quite porous but without any continuous limiting envelope surrounding the particle (Couvreur et al., 1979a). The extent of this porosity does not seem to be varied in different batches of nanoparticles.

(d) Mixed polymer/macromolecule systems

Another approach was taken by the Edman et al. (1980) to improve biodegradeability. They argued that if dextran comprised part of the hydrocarbon chain in the polyacrylamide, the polymer should be metabolized more readily. They incorporated proteins such as albumin and enzymes such as carbonic anhydrase into the porous polyacryldextran. They claim that the duration of enzymatic effects in vivo can be controlled adequately without unwanted intracellular accumulation of polymers. The authors do not specify a particle size but it is presumed to be submicron by analogy with their earlier work on polyacrylamides (Ekman and Sjöholm, 1978).

(e) Systems using natural macromolecules

The macromolecules used are generally proteins. The protein molecules are aggregated by a variety of techniques and then the aggregates are stabilized either by heat denaturation or chemical cross-linking. Since many drugs specifically bind to proteins, the payload of the delivery system can be higher than for those systems in which there is a nonspecific incorporation.

An early colloidal system was based on the heat-denatured macroaggregated albumin (MAA). Such systems are commonly used in nuclear medicine. An water-in-oil emulsion is made with the protein contained in the droplets of water. If the emulsion is made fine

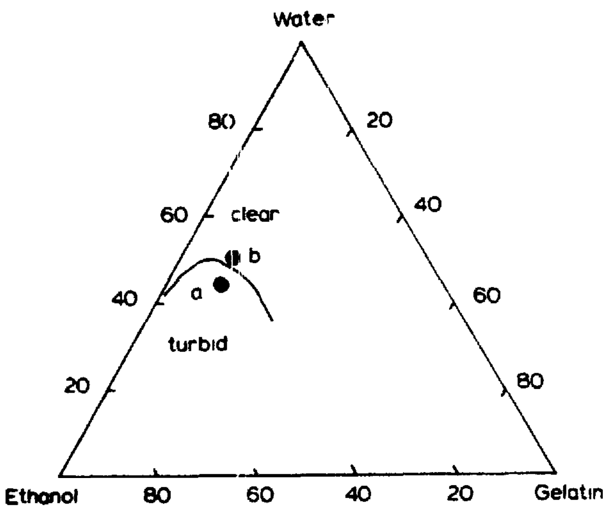
enough, submicron aggregates of protein are formed after the emulsion is heated to drive off the water. The colloidal particulate system developed by Kramer (1974) is now used in a variety of areas such as delivery of cytotoxics. The method of manufacture can limit the payload. The presence of the oil means that drugs with lipophilic character may preferentially reside in that phase and hence have limited interaction with the protein.

The major concern with administration of proteinaceous material is antigenic response. Clinical experience with the larger MAA systems has indicated very few problems. The only serious situations are those reported by Atkins et al. (1972) when a fatal reaction occurred immediately after injection with [^{131}I]MAA and Littenberg (1975) of an anaphylactoid response to MAA used in a lung scan. Hence systems using this general technology of production are unlikely to suffer antigenity problems. However, the use of albumin or other proteins in different configurations or aggregate formation procedures may give different antigenic problems. Each system would have to be evaluated. As a general rule this has not been done, presumably because until a definite commercial product looks possible, no organization is prepared to pay the costs of a trial. Animal studies can only provide a guideline.

Another method of making nanoparticles based on natural macromolecules has been developed by our group in Australia. The impetus behind the work was a desire to increase payloads of colloidal drug delivery systems, to make a biodegradeable system and a scientific curiosity as to what happened to a particular aqueous mixture of proteins when aldehydes were added.

The initial experiments were done in Zurich during early 1974. Speiser had a big group working on the poorly biodegradeable acrylate particles. In Australia we were starting work on a microencapsulation of aspirin project (D'Onofrio et al., 1979). In general, the coacervation method of microencapsulation of a core material by gelatin involves desolvating the gelatin so that it forms a coacervate phase (point a on Fig. 1). The coacervate droplets deposit on the core material. As this desolvation proceeds, the system is seen to go cloudy as the phase boundary is crossed and the system separates into the two phases. The desolvation process can be regarded as a gradual removal of the solvent molecules from around the macromolecule and the macromolecule 'rolling up' in the 'less friendly' environment. We argued that at a point such as point b on Fig. 1, the protein macromolecule would be a discrete, poorly solvated, rolled up molecule. If we could maintain that conformation we would be able to produce colloidal particles. The microencapsulation tanning method of addition of aldehydes like formaldehyde or glutaraldehyde seemed hopeful. The key to produce nanoparticles rather than aggregates of hardened coacervate droplets was an adequate monitoring of the desolvation process.

Initially a Nepho-Colorimeter was used. It was recently replaced by the simpler to use and simpler to maintain Turner Nephelometer. A typical desolvation profile is given in Fig. 2. The slow initial decrease in light scattering that occurs as the desolvating agent is added is due to the dilution of the protein solution. When the protein solution is around point b in Fig. 1, the amount of light scattered starts to rise rapidly. If too much desolvating agent is added, the phase boundary is crossed and a visibly turbid coacervate system results. This overshoot can be corrected by the addition of resolvating agents. Fig. 3 shows that different resolvating agents give different plots. Isopropanol is often used on a routine basis.



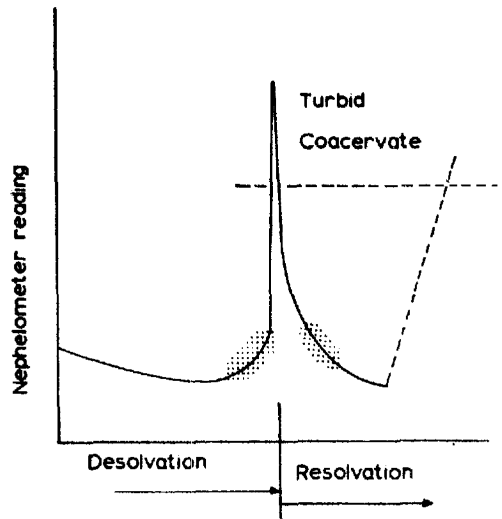


Fig. 1. Three component (gelatin–water–ethanol) diagram.

Fig. 2. Schematic representation of Nephelometer readings as first desolvation and then resolvation of a protein solution occurs. The hatched parts correspond to the conditions preferred for nanoparticle hardening.

The preferred conditions to make nanoparticles correspond to that part of the light scattering plot which starts to rise rapidly. If excessive desolvation has occurred and a coacervate forms, these preferred conditions can be re-achieved by adding an appropriate resolvating agent. The parts of the light scattering profile corresponding to these preferred conditions are hatched in Fig. 2.

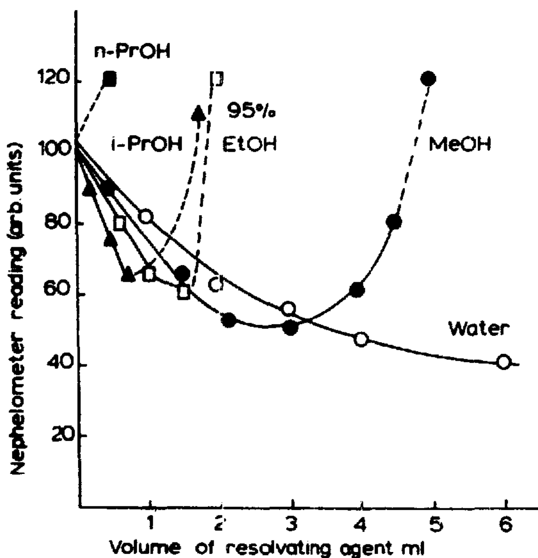


Fig. 3. Resolvating effect of water and various simple alcohols on serum albumin desolvated with ammonium sulphate solution.

During the hardening steps it is essential to keep the system well stirred. Magnetic stirring beads and simple paddle stirrers do not provide sufficient shear to stop particle–particle aggregation. It was necessary to use a laboratory homogenizer to provide sufficient energy to the system to minimize particle–particle association. It is necessary to stir continuously rather than intermittently to ensure a reproducable particle size. Excessive aggregation, leading to sheets and blocks up to 100 μm in size, occurs in the part of the desolvation profile before the preferred conditions are reached. If the protein is excessively desolvated, i.e. in a state very close to the phase boundary, discrete particle formation is hard to obtain, instead gels and massive lumps form during the hardening.

Glutaraldehyde has been found to be a better cross-linking agent than formaldehyde. This may be due to the dialdehyde group or due to a more reproducable polymeric form of the aldehyde available from stored commercial samples (Richards and Knowles, 1968). It has been shown that the amount of protein denaturation caused by glutaraldehyde is small (Hopwood et al., 1970). This is particularly important if the base molecule used in the production of nanoparticles has an action of its own. Enzymes may retain some of their activity when they are used to form nanoparticles (Oppenheim, unpublished results).

Normally an excess amount of glutaraldehyde is used. This is done to obtain rapid and reproducible hardening. The pH at which the hardening is done is important. If the pH is above about 8, then the reaction is very rapid and excessive cross-linking occurs. The resultant product is normally coloured yellow and either made up of particles greater than 5 μm in diameter or of massive aggregates or 'sheets'. If the hardening is carried out below about pH 4, the rate of reaction is too slow and a poor yield of particles is obtained. pH control is also needed to ensure an adequate payload of many drugs.

If the excess amount of glutaraldehyde was allowed to react to completion, the resultant product is massively cross-linked and not in discrete particles. The hardening reaction is terminated by the addition of sodium metabisulphite. This does two things. The pH is lowered from say 7 to 5 and this decreases the rate of the hardening considerably. Secondly, and more importantly, the bisulphite reacts with the glutaraldehyde. Vigourous stirring is maintained during this killing reaction so that any cross-linking that does occur does not cause large particles or aggregates to form.

A surfactant is included in the formulation to act as a solubilizing agent for water-insoluble active ingredients and also to act as a wetting agent to facilitate dispersion of the final product in water. Originally, polysorbate 80 was used but it was found that its cloud point was too high and it would desolvate in preference to the protein (Marty, 1977). It was found that 0.5% (w/v) polysorbate 20 was suitable for wetting and dispersion of the product, but higher concentrations could be used to achieve adequate payloads of drugs.

The gel chromatography clean up, using, for example, Sephadex G50m, not only separates the void volume nanoparticles from the lower molecular weight impurities and unincorporated drug but can also be used to separate most of the polysorbate 20 from the nanoparticles. The basic method of production of this type of nanoparticle was the subject of a suite of patents (The Pharmaceutical Society of Victoria; and Speiser, 1974). Upon the repeated injection of albumin and gelatin nanoparticles into rodents and dogs, no gross antigenic response has been observed.

PAYLOAD CONSIDERATIONS

There is often argument about the extent of incorporation of a particular drug in one or another delivery system. Claims are made about the percentage incorporation (e.g. Couvreur et al., 1980b). Whilst this is interesting, the more important issue is how much, i.e. what payload of, drug can be delivered per unit weight of nanoparticles or unit volume of reconstituted delivery system.

The drug payload of the nanoparticles needs to be maximized so that minimum carrier is required. Reduced overall weight of the delivery system per unit dose delivered will mean a reduced volume of vehicle is required to disperse the dose. A number of factors could potentially influence the payload.

Drug stability

If the drug degrades in an aqueous environment, the time of contact with water will influence the amount of pure drug incorporated into the nanoparticle. Since most such drugs have a pH-dependent degradation profile, pH needs to be closely controlled. The manufacturing procedure has to minimize the time over which degradation may occur. A number of cytotoxic drugs are light-sensitive (e.g. cyclophosphamide). Hence during the manufacturing procedure, exposure to light should be minimized. However, since the final product usually consists of the drug incorporated within the bulk of a solid particle, light-induced degradation of the delivery system should be less of a problem.

Drug interaction with carrier

The greater the extent of drug binding to the macromolecule, the larger the potential payload. The type of binding is important. If there is an irreversible chemical bond (as, for example, with the alkylating cytotoxic agents to proteins), any incorporated drug may be essentially unavailable. On the other hand equilibrium processes such as protein binding have to maintain a high extent of interaction throughout the manufacturing process. If water is removed from the environment of the protein, the drug may partition off the protein and into the water and hence be lost from the nanoparticle. In this case both the extent of protein binding and the water solubility would be important determinants.

Couvreur et al. (1979b) found that the percentage of methotrexate incorporated into polyethylcyanoacrylate particles decreased from about 40% to 15% as the total drug concentration increased from about 10 μg/ml to 200 μg/ml. Converted to payloads it means that the payload increased from 4 μg/ml of starting solution to 30 μg/ml of starting solution. We have also found that the payload of a wide range of drugs (e.g. salicylate, 5-fluorouracil) increases as the drug/protein ratio is increased even though the percentage incorporation decreases.

In our system we have found that increasing the Polysorbate 20 from 2% to 5% increased the payload of an acidic water-insoluble experimental flukicide, 10645, from about 1.4% to about 4% (Boag, 1979). It was suggested that at the higher surfactant concentration additional drug could be co-solubilized with the albumin. There was no evidence of porosity being induced by the increase in surfactant concentration.

Many drugs have a low solubility in water. A low number of molecules in the vehicle will mean a low number of potential interactions with the carrier molecule and a low

eventual payload. By changing the pH, the solubility can be increased. The number of interactions can then be increased. For another experimental acidic water-insoluble flukicide, 30000, a similar increase in payload was achieved by increasing the pH to around 9, obtaining the interaction with the albumin and then reducing the pH of the clear system to 7 for production of the nanoparticle. Without this pH cycling, the system remained turbid and microcapsules of 30000 were formed (Boag, 1979).

Particle-making procedures

Each of the 3 – chemical, irradiation and thermal – methods of converting the individual carrier molecules into a particulate aggregate can influence the payload. If a chemical cross-linking procedure using agents such as glutaraldehyde is used, then amine groups on either the drug or the carrier molecule can be involved in the reaction. This would then result in less pure drug being able to be incorporated in the nanoparticle. Methotrexate will react with glutaraldehyde and this reaction will interfere with its incorporation into proteinaceous nanoparticles (Oppenheim and Stewart, unpublished results).

The problems of radiation causing drug degradation have been discussed earlier. When heat is used to denature proteins or to accelerate polymerization reactions, degradation reactions such as oxidation are also accelerated.

Particle porosity

If the particle contains entrapped protein or enzyme, porosity is needed to allow materials access to the proteins or enzymes. However, protein can leak from the porous particle (Edman et al., 1980). These authors found that the yield of entrapped protein in polyacrylate particles may be increased by the addition of dextran to make a polyacryl-dextran particle. The addition of dextran decreases the gel pore radius and secondly it sterically retains the protein inside the gel during polymerization. This means that the fraction of protein immobilized in the polymeric threads is decreased. Hence the fraction of enzyme resisting thermal treatment is reduced and the extent of leakage is increased. In this case an increased payload has resulted in an increased leakage rate.

If the drug can readily debind and be released from the nanoparticle by passage down the pores, the same drug payload should result if the nanoparticle is made with the drug present or if the nanoparticle is preformed and the drug adsorbed later. Couvreur et al. (1980b) showed that polyalkylcyanoacrylate nanoparticles do not have this equivalence of payload and postulate an inner surface of carrier inaccessible for adsorption. Extrapolation of their data suggests that between 20 and 30% of the added dactinomycin is well incorporated and the easily adsorbed (and hence desorbed) material could be seen as poorly incorporated.

EXAMPLES OF ACTIVE MATERIALS INCORPORATED

(a) Viruses and vaccines

The early work by Birrenbach and Speiser (1976) used human IgG and tetanus toxoid adsorbed on polymethylmethacrylate nanoparticles. The adjuvant effect was comparable

to the traditional Freund's adjuvant system. Kreuter and Liehl (1978) extended this work using influenza virus as the associated material. The adjuvant effect observed in mice and guinea pigs was equal or better than that of aluminium hydroxide. In the case of subunit vaccines, it was considerably better than aluminium hydroxide. Preliminary tests could find no tissue damage at the site of injection one year after administration. Kreuter and Haenzel (1978) found that the best effect occurred if the nanoparticles were between 100 and 200 nm in diameter; if they were greater than 500 nm very little adjuvant effect was observed.

Although not strictly delivering active material to a specific organ or cell, the association of virus and vaccination material with nanoparticles to stimulate various immune systems within the body needs further exploration.

(b) Diagnostic agents

Excluding the well-known colloidal sulfur used extensively in nuclear medicine when tagged with ^{99m}Tc, the tagging or labelling of nanoparticles with radioisotopes has been used to investigate the distribution, clearance and excretion of the nanoparticles. Oppenheim et al. (1978) showed that following intravenous injection, at least 50% of the dose of cross-linked gelatin nanoparticles surface conjugated with ^{99m}Tc accumulated in the liver of rats within 15 min. This disposition is comparable to the behaviour of other colloids of similar size. The lower efficiency of nanoparticle uptake by the liver, compared with [^{99m}Tc]sulfur colloid (about 75%) is probably because the nanoparticle preparation was not homogeneous. Since these experiments were performed, a Bio-Gel A-5m purification step has been developed which yields a much more uniform product which should improve liver uptake. Following intramuscular and intraperitoneal administration, about 5% and 3% of the radioactivity was found in the liver, respectively, 30 min after administration (Oppenheim, 1980).

Kreuter et al. (1979) found liver accumulation of radioactivity following intravenous administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles in rats and mice. There was no movement from the injection site even after 70 days following intramuscular injection.

Fluorescein and its derivatives are often used to monitor cellular processes. Couvreur et al. (1977) showed that fluorescein entrapped in polyacrylamide nanoparticles could be taken up by some cultured rat fibroblasts. Couvreur et al. (1979a) found that fluorescein could be sorbed into the porous biodegradeable alkylcyanoacrylate nanoparticles if the pH was between 2 and 8. They found that the non-ionized fluorescein sorbed onto the particle pore surface and the payload was greater for ethyl compared to methylcyanoacrylate particle. Upon storage as a suspension, these particles degrade, the methyl derivative being less stable. The amount of fluorescein sorbed decreases both with length of storage and a decrease in pH. Hence any diagnostic system using these particles would need to have a restricted reconstituted shelf-life.

Oppenheim and Stewart (1979) found that fluorescein isothiocyanate (FITC) could be conjugated to the surface of gelatin and albumin nanoparticles. In some preliminary work, some EMT-6, WEHI-3 and SP-1 tumour cells were separately incubated with some FITC–gelatin nanoparticles. All 3 showed uptake of the nanoparticles. This work shows that this type of nanoparticle has free surface amino groups which might be useful for

drug or prodrug binding. It also indicates that this type of nanoparticle can be phagocytosed by some tumour lines and hence the protein-based nanoparticle might be useful in cancer chemotherapy.

Ljungstedt et al. (1978) used proteins immobilized in polyacrylamide nanoparticles which also contained FITC-labelled dextran or labelled inactive proteins to detect and separate lymphocytes with specific surface receptors.

(c) Cytotoxics

When reviewing biophysical targeting of antitumour agents, Widder et al. (1979a) identified 3 stages of targeting. First-order targeting involves restricted distribution of a delivery system to the capillary bed of the target site. Second-order targeting refers to the selective direction of carrier or drug to tumour cells versus normal cells. Finally in third-order targeting there is carrier-directed release of the drug at selected intracellular sites. This infers the delivery system entering the target cell by either phagocytosis or cell fusion. It is vital that second-order targeting be successful. Widder et al. are pessimistic since they point out most colloidal carriers are rapidly cleared by the reticuloendothelial system, and intralesional macrophages consume the endocytizable carriers thereby interfering with one of the important local host defences that retards tumour growth and metastasis.

With these problems in mind, Widder et al. (1979b) have incorporated doxorubicin into magnetic microspheres with payloads of about 9%. First-order targeting within the capillary beds in the desired area is accomplished, in principle, by the application of a magnetic field. As the particle degrades, the drug should then have immediate access to the tumour cells.

Widder et al. (1979a) also briefly reviews the use of antibody cytostatic drug conjugates. With wide-spread or highly metastasized tumours it would appear logical to try this approach. There appear to be few detailed attempts to put antibodies onto solid nanoparticle carrier systems. Rowland et al. (1975) showed that if *p*-phenylenediamine mustard was linked to poly-L-α-glutamic acid and then that system conjugated with a rabbit antitumour immunoglobulin, survival times and rates of mice with EL4 lymphoma markedly increased.

Couvreur et al. (1979b, 1980b) have shown that polyalkylcyanoacrylate nanoparticles can incorporate dactinomycin, vinblastine and methotrexate. In normal animals there appears to be some improved drug delivery to some organs by the nanoparticles compared to the free drug. The action of the system on tumour-bearing animals and selective uptake by different cell types has yet to be investigated.

Hashida et al. (1977) found that in the rat the intragastric administration of 5-fluorouracil entrapped within 1.6 μm gelled gelatin microspheres presented as a gel-in-oil dispersion, resulted in an increased amount of drug in the regional lymph nodes and an increased amount transported into the thoracic lymph when compared to aqueous solutions of the drug. Although the results of a number of investigations on the physical in vitro behaviour of the system have been published, few experiments appear to have been done to date to determine if there is a corresponding increase in efficacy in tumour-bearing animals. Sugibayashi et al. (1979) showed that such a system did increase the life-span of mice with an Ehrlich Ascites carcinoma.

Ryser and Shen (1980) showed that methotrexate could be covalently conjugated to poly(L-lysine) of molecular weights ranging from 2700 to 130,000. The cellular uptake of conjugated drug was about 200 times that of free drug when tested on a cultured, Chinese hamster, ovary cell line known to be drug resistant because of a deficient methotrexate transport. They postulate that intralysosomal hydrolysis of the polymeric backbone enabled an active form of methotrexate to be released.

We have been able to make cross-linked albumin nanoparticles containing 5-fluorouracil with payloads around 6–8%. Lower payload figures have been obtained for the alkylating agents, chlorambucil, melphalan and methotrexate. Cell culture and animal studies are underway.

(d) Flukicides

The usual problem in nanoparticle therapy is to stop hepatic clearance of the dose form. However, for helminth infestations of the liver, nanoparticle delivery seems ideal. Liposomes seem to offer some advantages of the treatment of *Trypanosoma brucei* (Greunberg et al., 1979) and *Leishmania donovani* (New et al., 1978). Human infestation is a major global disease but one that is economically unattractive to the pharmaceutical industry. Paradoxically, eradication of the infestation of sheep and cattle appears to be more economically viable.

We recently completed what appears to be the first attempt to incorporate flukicides into nanoparticles. Marty (1977) had shown that the flukicide, Nitroxynil, could be incorporated in nanoparticles with a final payload of around 1.5%. We worked on two experimental flukicides, 9335 and 10645. Like Nitroxynil, 9335 is a halogenated nitrated phenol derivative. 10645, on the other hand, was a halogenated trinuclear phenol with a much lower solubility at pH 7. 10645 has activity against both the mature and immature form of *Fasciola hepatica,* whereas the commercial formulation of 9335 is only active against the mature form. These types of compounds are generally assumed to be de-couplers of oxidative phosphorylation. Their therapeutic indices are low.

Nanoparticles with a payload of around 4–5% were made. Since 1 g of such nanoparticles will disperse in about 3 ml of normal saline, the effective solubility of either compound is now around 1.6% (w/v). This represents something like 2 or 3 orders of magnitude increase in solubility for the drug.

The 10645 product was tested (Boag et al., 1980) against mature and immature flukes in experimental rats. Four-week-old male and female Wistar rats were infected with metacercaria of *Fasciola hepatica.* Six days after administration of the nanoparticles by either the intravenous or subcutaneous routes, the number of immature flukes in the livers of the rats was determined post-mortem. A solution of 10645 in a suitable solvent system was used as control. The results showed that the nanoparticle formulation could be used at about twice the dosage of the control formulation before toxic reactions appeared. Hence, incorporation of the flukicide into nanoparticles has not impaired its efficacy but has doubled its therapeutic index.

This approach to helmenth infestation needs much greater exploration.

(e) Antiarthritics

If a joint is inflammed, it is inefficient to used intramuscular or oral administration of

gold salts or corticosteroids. Realizing this problem, a number of groups (e.g. Cleland, 1979) have tried a liposomal preparation intra-articularly. Colloidal gold has lost much of its traditional appeal because of poor efficacy. However, the work of Astorri et al. (1979) showing that Levamisole strongly inhibits the phagocytosis of radiogold colloidal particles by the hepatic reticuloendothelial system, whereas its optical isomer significantly increases the rate, opens the possibility of co-administration of appropriate materials directly into the inflammed joint.

Using the general method outlined earlier we have been able to produce albumin-based nanoparticles containing prednisolone and separately gold sodium thiomalate with payloads of around 3% and 7%, respectively. In vivo testing is currently underway. As one of the more common debilitating diseases, arthritis needs an increased amount of work to minimize its effects.

THE FUTURE

Greater effort needs to be put into maximizing payloads in all types of nanoparticles. The current swing towards biodegradeable nanoparticles needs to be maintained. For effective use of this type of drug delivery system, careful attention must be paid to the method and the rate of drug release from the nanoparticle. A wide range approach needs to be taken when considering the types of diseases and conditions which may be suitable for nanoparticle therapy. For example, nanoparticles may form the basis of a sprayable weevilicide product.

However, the biggest hurdle for human use of this type of drug delivery is to develop an effective technique to stop unwanted hepatic clearance. The range of procedures described earlier in this review needs to be tried on all types of nanoparticles. Other procedures also need to be developed and investigated.

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