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A new in vitro technique for the evaluation of drug release profile from colloidal carriers – ultrafiltration technique at low pressure

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Summary

A new in vitro technique, ultrafiltration at low pressure, is proposed for the evaluation of drug release profile from colloidal carriers such as submicron emulsions and nanoparticles. A thorough description is given after having reviewed the various available techniques for drug release evaluation from colloidal carriers. The results yielded by the new technique clearly show that miconazole release from either the submicron emulsion or a marketed micellar aqueous solution was very rapid under perfect sink conditions. This fact raised doubts and practically rules out the possibility of using submicron emulsions as a controlled-release drug delivery system for i.v. administration. The in vitro indomethacin release profile from PLA or PLGA nanoparticles was found to be biphasic with an immediate release of 40–80% of the initial drug concentration, followed by a slower exponential release which lasted a few hours. It was deduced from the overall kinetic results that the ultrafiltration method at low pressure was versatile, sensitive and capable of distinguishing between different kinetic behaviors. The technique offers the possibility of identifying the underlying release mechanism of drug from colloidal carriers.

Introduction

Colloidal drug carriers, which mainly involve submicron emulsions, nanoparticles, liposomes and lipid complexes, have been attracting increasing interest in recent years, mainly as vehicles of lipophilic drugs for i.v. administration and also as improved delivery systems for drug targeting. The progress in the field of parenteral drug delivery using submicron emulsions has been covered in numerous reviews (Singh and Ravin, 1986; Davis et al., 1987; Collins-Gold et al., 1990; Prankerd and Stella, 1990). Among the site-specific colloidal carriers, liposomes were extensively studied, and a vast number of potential applications were reported in the literature (Gregoriadis, 1988). Couvreur (1988) reviewed the potential applications of polyalkylcyanoacrylates (PACA) as colloidal drug carriers for both oral and i.v. administration. Furthermore, some success has been achieved in enhancing the efficacy and reducing the toxicity of anticancer drugs loaded in nanoparticles, as recently reported in some re-

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views (Douglas et al., 1987; Couvreur et al., 1990). The potential of the lipid complexes rather defined as mixed micelle colloidal dispersions was exploited to reduce the toxicity and to improve the efficacy of amphotericin B, a lipophilic polyene antibiotic which is the drug of choice in the treatment of disseminated fungal infections (Lasic, 1992).

Despite comprehensive biological and physicochemical characterizations of the various colloidal drug carriers, some authors have emphasized the technical difficulties encountered in thoroughly and truly examining the in vitro drug release from a colloidal dispersion (Washington, 1990; Magenheim and Benita, 1991). This should be attributed to the inability to effectively and rapidly separate the particles from the dissolved or released drug in the sink solution, owing to the very small size of the colloidal drug carriers.

The objective of this present work is to present a novel in vitro technique, the ultrafiltration technique at low pressure, able to characterize the drug release profile from at least two different colloidal drug carriers, i.e., submicron emulsions and nanoparticles.

Materials and Methods

Materials

Miconazole base was kindly supplied by Abic, Ltd, Natanya, Israel. Lecithin used was intravenous grade (Lipoid E-80 from Lipoid AG, Germany). Deoxycholic acid (DCA) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Medium-chain triglyceride (MCT) oil was obtained from the Societe des Oleagineux, St-Blangy, France. Poloxamer 188 (Pluronic F-68) was furnished by BASF (Ludwigshafen, Germany). The nanoparticle-forming polymers, poly(DL-lactide) (PLA) and poly(DL-lactide-co-glycolide, 50:50) (PLGA) were supplied by Medisorb, Ltd (Cincinnati, OH, U.S.A.). Indomethacin conformed with USP XXII and was a gift of Teva Ltd, Kfar Saba, Israel. All other ingredients used were of pharmaceutical grade.

Methods

Miconazole submicron emulsion preparation

The submicron emulsion of miconazole was prepared using the manufacturing process previously described for the preparation of a submicron emulsion of diazepam (Levy and Benita, 1989, 1990, 1991) with few modifications. The DCA and phospholipids were first dissolved in alcohol and immediately dispersed in the aqueous phase. Alcohol was removed by evaporation prior to mixing with the oil phase. The formulation (%, w/w) consisted of miconazole 1.0, MCT 20.0, Lipoid E-80 1.0, deoxycholic acid 0.5, glycerin 2.25, α-tocopherol 0.02 and water for injection to 100.

Emulsion evaluation

The evaluation of the emulsion properties (particle size distribution, zeta potential and stability assessment) was carried out using the physical methods already described elsewhere (Levy and Benita, 1989, 1990).

Miconazole content

Miconazole content was analyzed by using a HPLC system consisting of a Milton Roy HPLC (Model Constametric 3000) equipped with a variable wavelength ultraviolet detector (Spectro Monitor 3100, Milton Roy) and a Milton Roy Integrator. A Lichrosphere C-18 5 μm (Merck Co., Germany), 25 cm $\times$ 4.6 mm i.d. reverse-phase column, was used. The column was eluted with a solvent system containing methanol-aqueous solution (9 : 1). The aqueous solution was comprised of 0.05 M $NH_4H_2PO_4$. The eluent was run at a rate of 1.5 ml/min and monitored at 230 nm following injected volumes of 20 μl of miconazole standard solutions and samples. The calibration curve was found to be linear in the range of 1–40 μg/ml. In order to determine the total content of miconazole in the emulsion, the emulsion was first dissolved in isopropyl alcohol (1 : 10) and the resulting solution was then diluted with methanol (1 : 10). Each sample was analyzed in triplicate.

Indomethacin nanoparticle preparation

The indomethacin-loaded PLA nanoparticles were prepared according to a modification of the

method previously reported by Fessi and colleagues (1989). 125 mg of PLA or PLGA polymer, 7.0 mg indomethacin and 1.25 ml of methylene chloride are dissolved in 25 ml of acetone. The acetone solution is poured into 50 ml of water containing 250 mg of poloxamer 188 under moderate stirring at room temperature. The aqueous phase immediately turns milky with bluish opalescence as a result of the formation of nanoparticles effected first by rapid diffusion of acetone into the aqueous phase and finally by evaporation of methylene chloride and acetone from the colloidal suspension by rotoevaporation under reduced pressure.

Nanoparticle evaluation

Morphological examination of nanoparticles was performed using a transmission electron microscope (TEM) following negative staining with uranyl acetate or sodium phosphotungstate. Particle size distribution was measured by photon correlation spectroscopy (PCS) using a monochromatic laser ray diffusion counter (Coulter model N4SD Submicron Particle Analyzer, Luton, U.K.).

Indomethacin content

Total initial drug content was determined following complete dissolution of the nanoparticles with acetonitrile. Incorporated drug was calculated as the difference between the drug determined in the filtered nanoparticle suspension following dissolution with acetonitrile and the dissolved drug measured in the supernatant of ultracentrifuged filtered nanoparticles.

Indomethacin was assayed by the HPLC methods recommended by USP XXII (1990), performing the UV detection at 319 nm instead of 254 nm to allow better selectivity and drug identification. The C-18 column used was identical to those previously described.

In vitro release kinetic evaluation of the colloidal drug carriers

The in vitro drug release profile from the colloidal carriers was carried out immediately after preparation, using an ultrafiltration technique

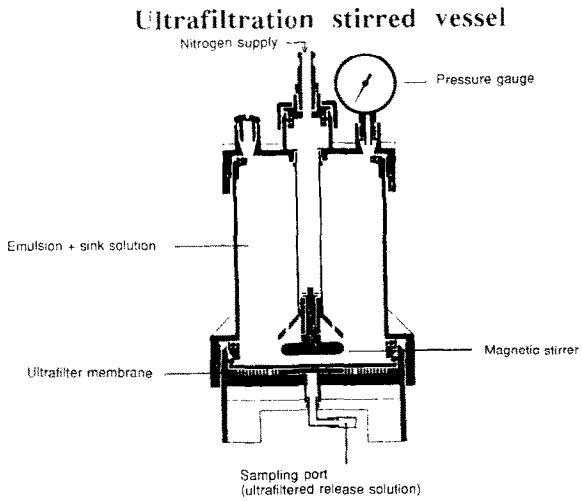

Fig. 1. Schematic illustration of the ultrafiltration technique at low pressure.

at low pressure, the equipment of which is described schematically in Fig. 1.

It should be emphasized that various ultrafiltration stirred cell models could be used, depending on the final volume of the sink solution.

Miconazole submicron emulsion

The in vitro kinetic evaluation of miconazole from the emulsion was carried out as follows: 0.2 ml of the emulsion was directly placed in a UP-110 stirred vessel (Schleicher and Schuell, Dassel, Germany) containing 500 ml of 1% HSA in Hepes buffer pH 7.4. At given time intervals, the release medium was filtered through the YM 100 ultrafiltration membrane (Amicon, Danvers, MA, U.S.A.) at low pressure (less than 0.5 bar) using nitrogen gas (Fig. 1). The membrane was selected following preliminary studies and found adequate for the present studies since it exhibited low non-specific protein binding properties. An aliquot of 0.5 ml of the clear filtrate (usually 2–3 ml) was treated and assayed for drug content using HPLC, while the remaining portion of the filtrate was recycled in the stirred vessel to maintain constant volume during the in vitro release kinetic experiments.

To 0.5 ml of filtrate, 25 μl of a methanolic solution of clotrimazole as internal standard (50 μg/ml) and 1 ml of borate buffer (pH 9) were

added and vortexed for 1 min. 10 ml of pentane : dichloromethane (1 : 1) was then added to the previous solution and again vortexed for 1 min. The sample was then centrifuged at 5000 rpm over 4 min and the upper organic phase was separated and evaporated to dryness under nitrogen flush. The drug extract was dissolved in 150 μl of methanol and a 20 μl aliquot was analyzed by the HPLC method previously described. The method was also used to evaluate the in vitro release of miconazole from a marketed i.v. micellar solution, Daktarin®.

Indomethacin nanoparticles

The in vitro release kinetic evaluation of indomethacin from PLA or PLGA nanoparticles was carried out as follows: 5 ml of indomethacin nanoparticle colloidal dispersion are placed in a stirred cell model 8200 (Amicon, Danvers, MA, U.S.A.) containing an adequate volume of sink solution (50 ml of phosphate buffer, pH 7.4, USP XXII). At given time intervals, aliquots of release medium are filtered through an ultrafiltration membrane (Amicon YM 100) using nitrogen gas at less than 0.5 bar.

The clear filtrate, collected at given time intervals and diluted with mobile phase (1 : 1), is then analyzed for indomethacin, using the USP HPLC method described in the indomethacin monograph.

Ultrafiltration technique validation

Prior to the use of the ultrafiltration technique to determine the in vitro drug release profile from the colloidal carrier, the technique required validation as already performed by Teagarden and colleagues (1988). Membrane adsorption and rejection must be accounted for in order to accurately measure aqueous concentrations of drug. The ultrafiltration membranes are specifically selected for their low nonspecific binding. The effects of membrane binding and rejection of drug are studied by ultrafiltering an aqueous solution of the respective drug at given concentrations. The recovery curves for indomethacin at two different concentrations likely to be encountered throughout the entire release process are presented in Fig. 2. The membrane appears to be

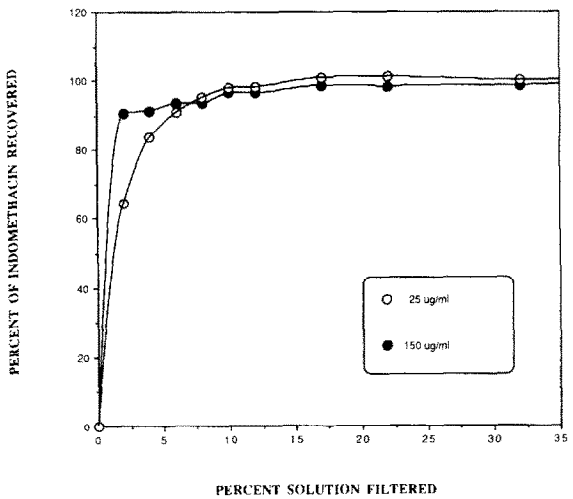

Fig. 2. Recovery curves for indomethacin at 25 and 125 μg/ml from aqueous solution using the ultrafiltration technique at low pressure.

nearly saturated after approx. 5–10% of the total volume has been filtered as evident in the leveling off of the curve. The percentage recovery was 97–98% of the observed drug concentration in phosphate buffer pH 7.4 indicating that adsorption or binding was negligible. Similar behavior was noted for the miconazole solution. Based on these results, ultrafiltration data for emulsion miconazole formulations and indomethacin nanoparticles, required only a slight correction, provided that at least 5% of the total volume was filtered to saturate the membrane.

Results and Discussion

The manufacturing process of submicron emulsion formulations yielded very fine monodisperse emulsions of miconazole with a mean droplet size of 125 $\pm$ 35 nm and a zeta potential value of -37.6 ± 8.4 mV. The emulsion was found to be both physically and chemically (miconazole content) stable over 17 months storage at 4°C.

The manufacturing method of the PLA and PLGA nanoparticles yielded homogeneous populations, the mean particle size of which was 170 $\pm$ 50 nm with an indomethacin incorporation effi-

ciency of 92.9 and 98.4% for PLA and PLGA nanoparticles, respectively. The respective observed drug contents in the nanoparticles were 5.94 and 5.81% w/w, with a final concentration of drug in the colloidal dispersion of 251 and 248 μg/ml, respectively.

Although there are well-established physicochemical methods to characterize the various drug colloidal carriers under investigation, investigators are still facing technical difficulties in properly evaluating the in vitro drug release profile from colloidal carriers. There is still a need for a method which is able to rapidly separate the disperse colloidal phase from the release solution without affecting the underlying drug release profile.

A large number of methods have been developed in order to characterize the release profiles of drugs from colloidal disperse systems. However, most of these methods suffer from some inconveniences. The membrane diffusion techniques using either diffusion cells (Hashida et al., 1980; Benita et al., 1986; Miyazaki et al., 1986; Lostritto and Silvestri, 1987) or dialysis sacs (Ammoury et al., 1989, 1990) were criticized by Washington (1989) who claimed that these kinetic experiments were not performed under perfect sink conditions, since the colloidal carrier was not directly diluted in the release medium but rather separated from the release solution by a membrane. As a result, the rate of drug appearance in the release solution does not reflect its real release profile, but rather the concentration gradient between the continuous phase of the colloidal dispersion and the release solution. To avoid the enclosure of the colloidal drug carrier in a dialysis sac, Levy and Benita (1990) attempted to establish a technique based on reverse dialysis. The colloidal carrier is directly diluted in the release medium where perfect sink conditions are diligently kept and a number of dialysis sacs have been previously suspended for the monitoring of amount of drug released. The authors reached the conclusion that the reverse dialysis sac method is not 'sensitive' enough to characterize rapid release rate of drug from the colloidal carrier. However, it can be assumed that if the drug is released over much more than one hour, then this method can be used for in vitro release profile investigation from colloidal carriers.

The in vitro kinetic methods based on dilution and separation (Tsai et al., 1986; Farah et al., 1987; Henry-Michelland et al., 1987) employ direct dilution of the carrier in the release medium. At predetermined time intervals, samples of the release dispersion solution are withdrawn, the solution is separated from the colloidal carrier – usually by filtration or centrifugation – and the amount of drug released and dissolved in the solution is determined. This method is efficient only if successful and quick separation of the diluted dispersed phase from the continuous phase is achieved. This is likely to be difficult, especially when particle size of the dispersed phase is smaller than one micron. The time required for such separation by ultracentrifugation becomes longer while release rate of the drug from the colloidal carrier can be very rapid (Washington, 1990). It should be noted that the smaller the particles the more difficult it is to separate them, while the release rate is faster due to the increased surface area available for diffusion.

Another interesting method for in vitro release profile estimation of colloidal carriers is continuous flow filtration, described by a number of researchers (Burgess et al., 1987; Koosha, et al., 1988) and reviewed by Washington and Koosha (1990) who reported that the prevailing conditions in these experiments are a compromise between opposing requirements which might damage the ability to determine the real release profile.

There are also in vitro kinetic methods where the carrier is directly diluted in the release medium, but determination of the amount of free drug released is performed in the solution without separating the carrier from the drug (Wakiyama et al., 1981; Illum, et al., 1986). This method is limited to drugs the determination of which is carried out using polarography or spectroscopy and is not altered by the presence of the colloidal carrier in the solution.

Finally, the centrifugal ultrafiltration technique has been successfully applied by Ammoury (1990) to evaluate the in vitro release profile of

indomethacin from PLA nanocapsules. Santos-Magalhaes and colleagues (1991) adopted the method to evaluate clofibride release from a submicron emulsion. However, this technique might suffer from the drawback that the marked centrifugal force needed to separate the ultrafiltrate from the nanodroplets might alter the emulsion integrity, resulting in a different drug phase distribution profile. Therefore, our laboratory attempted to adapt an ultrafiltration technique at low pressure for the purpose of evaluating drug release profiles from submicron emulsions and nanoparticles.

Ultrafiltration is accepted as an efficient method for the separation of the oily phase from the aqueous phase in an emulsion, usually in order to determine the partition of a specific compound between the different phases (Shimamoto et al., 1973; Nakagaki and Yokoyama, 1985; Teagarden et al., 1988).

The proposed kinetic method of ultrafiltration at low pressure is based on direct dilution of the emulsion or nanoparticle dispersion in the release medium (Fig. 1). The amount of drug released from the carrier is determined by sampling the filtrate which is filtered at set time intervals from the release medium. It should be emphasized that the visual appearance of the filtrate is transparent and no particles could be detected by the PCS technique, indicating that the membrane effectively removed any colloidal particles from the filtrate. It should be pointed out that the total volume of the filtrate samples used to determine the amount of drug released is negligible in comparison to the release medium volume and thus drug concentration in the filtration cell is maintained with almost no change during the course of the experiment. The small volume of sample allows filtration at a slow rate and at low pressure (less than 0.5 bar) preserving the physical integrity of the colloidal dispersion. The ultrafiltration membrane used in the experiments is characterized by low non-selective adsorption and a membrane molecular weight cut-off point of 100 000 (YM 100). In this case, the filter characteristics enable the passage of free drug released, as well as of albumin or drug-adsorbed and/or drug-bound albumin occasionally present in the release solution.

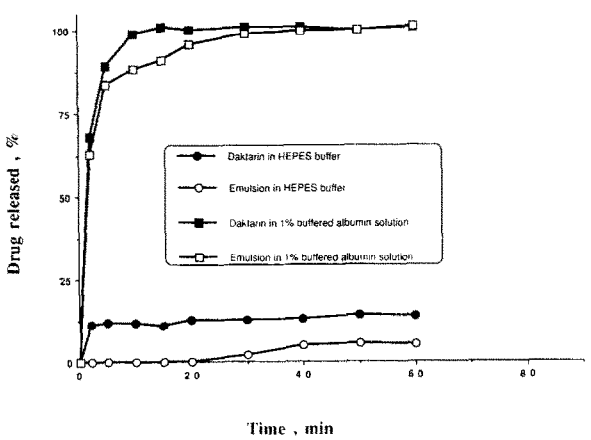

Fig. 3. Miconazole release profiles from the submicron emulsion and a marketed micellar solution (Daktarin®) using Hepes pH 7.4 buffer or 1% human albumin in Hepes pH 7.4 buffer as release solution.

Miconazole release from emulsion

It can be seen from the kinetic results exhibited in Fig. 3 that miconazole release from either the submicron emulsion or the micellar aqueous solution was very slow when the release solution consisted only of Hepes buffer, pH 7.4. This slow release rate is definitely due to the lack of sink conditions in the release solution located in the stirred vessel, as evidenced in preliminary drug solubility studies.

Replacing the release medium with a solution containing 1% human albumin in addition to Hepes buffer pH 7.4 produced a much faster release profile, either from emulsion or solution (Fig. 3). This was due to the marked solubility increase of miconazole in the human albumin solution, which was found to be 50 μg/ml, enough to ensure sink conditions thoughout the entire kinetic experiment. It can therefore be assumed that the release of drugs from a submicron emulsion takes place within a short period of time (10 s to a number of minutes) after dilution in the medium where perfect sink conditions exist. This fact is apparently surprising for lipophilic drugs, like miconazole, which partition with high preference for the oily phase in the emulsion. But it is reasonable to assume that a number of seconds

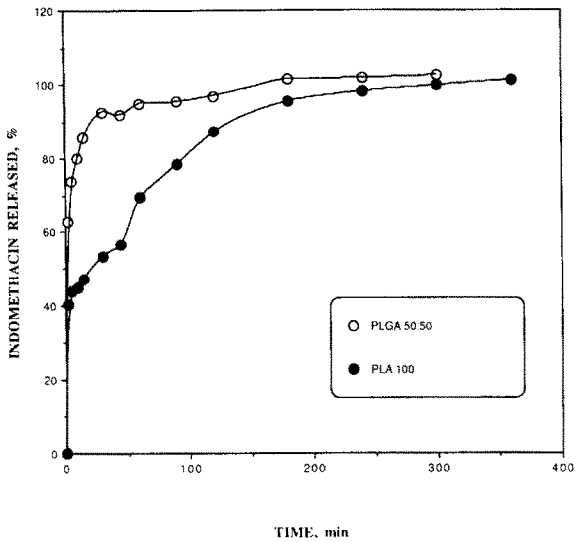

Fig. 4. Indomethacin release profiles from PLA and PLGA nanoparticles using phosphate buffer, pH 7.4, as sink solution.

after the dilution most of the drug is released from the carrier. The reason for this is most likely inherent in the vast interface area of the oil droplets which enables the passage of the drug by rapid diffusion into the environment, where perfect sink conditions prevail. After i.v. injection, the emulsion is diluted in the total blood volume, which imitates perfect sink conditions. This fact raised doubts and practically rules out the possibility that submicron emulsions can be used as a controlled release drug delivery system for i.v. injection of drugs.

Indomethacin release from nanoparticles

The in vitro release profile of indomethacin from PLA and PLGA nanoparticles under perfect sink conditions is presented in Fig. 4. The indomethacin release profile is biphasic with an immediate release ranging from 40% (PLA nanoparticles) to 80% (PLGA nanoparticles), followed by a slower exponential profile which lasts for an additional period of time ranging from 1 h (PLGA nanoparticles) to 4 h (PLA nanoparticles). The rapid initial release of indomethacin from the nanoparticles (burst effect) should be attributed to the fraction of indomethacin which is rather adsorbed or weakly bound to the large surface area generated by the formation of the nanoparticles, than to indomethacin incorporated in the polymeric network of the nanoparticles. Following dilution of the nanoparticle suspension with the release medium under perfect sink conditions, the indomethacin partitioned rapidly in favor of the release medium, accounting for the immediate release rate phase. The exponential delayed release rate phase results probably from the sink solution penetration into the nanoparticles, dissolution of the indomethacin and diffusion out of the indomethacin solution from the nanoparticles into the bulk release medium. The increase in release rate from the PLGA should be expected since it is a more hydrophilic and permeable polymer than PLA. A thorough examination of the indomethacin in vitro release from the nanoparticles under various experimental conditions will be reported in a forthcoming paper. Nevertheless, it can be deduced from the preliminary kinetic results reported in the present study that it is possible to distinguish between different kinetic behaviors using the ultrafiltration technique at low pressure. Furthermore, this method offers the possibility of analyzing the release kinetic pattern of indomethacin from the nanoparticles and to identify the underlying release mechanism which governs the in vitro release of indomethacin from the nanoparticles under perfect sink conditions.

Conclusions

The ultrafiltration technique at low pressure appears to be a very sensitive, versatile and accurate method for determining the drug release profile from colloidal carriers. The method closely approximates the conditions prevailing in vivo and is capable of differentiating between the various kinetic behaviors which might be expected from different colloidal carriers. Accordingly, the release process of drugs incorporated in colloidal carriers such as submicron emulsions or nanoparticles might last from a few minutes to a number of hours. There is no doubt that the method can be used for the characterization of the release profile of drugs from colloidal carriers intended

for i.v. administration. The difficulty of creating perfect sink conditions in the release medium can be overcome by adding human serum albumin as a 'co-solvent' to markedly increase the aqueous solubility of the lipophilic drugs under examination. The kinetic data presented in the present paper clearly show that the drug incorporated in the submicron emulsion rapidly diffuses into the environment where perfect sink conditions prevail, while under similar conditions the release of drug incorporated into nanoparticles was delayed. These kinetic results clearly exclude the use of submicron emulsions as colloidal controlled release drug delivery systems for i.v. administration while underlining the therapeutic potential of drug-loaded nanoparticles for the same application.

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