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Research Article

Design and *In Vitro* Haemolytic Evaluation of Cryptolepine Hydrochloride-Loaded Gelatine Nanoparticles as a Novel Approach for the Treatment of Malaria

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Abstract. Cryptolepine hydrochloride-loaded gelatine nanoparticles were developed and characterised as a means of exploring formulation techniques to improve the pharmaceutic profile of the compound. Cryptolepine hydrochloride-loaded gelatine-type (A) nanoparticles were developed base on the double desolvation approach. After optimisation of formulation parameters including temperature, stirring rate, incubation time polymer and cross-linker (glutaraldehyde) concentrations, the rest of the study was conducted at two different formulation pH values (2.5 and 11.0) and by two different approaches to drug loading. Three cryoprotectants—sucrose, glucose and mannitol—were investigated for possible use for the preparation of freeze-dried samples. Nanoparticles with desired size mostly less than 350 nm and zeta potential above ±20 were obtained when formulation pH was between 2.5 and 5 and above 9. Entrapment efficiency was higher at pH 11.0 than pH 2.5 and for products formulated when drug was loaded during the second desolvation stage compared to when drug was loaded onto pre-formed nanoparticles. Further investigation of pH effect showed a new isoelectric point of 6.23–6.27 at which the zeta potential of nanoparticles was zero. Sucrose and glucose were effective in low concentrations as cryoprotectants. The best formulation produced an EC_{50} value of 227.4 μM as a haemolytic agent compared to 51.61 μM by the free compound which is an indication of reduction in haemolytic side effect. There was sustained released of the compound from all formulation types over a period of 192 h. Stability data indicated that the nanosuspension and freeze-dried samples were stable at 4 and 25°C, respectively, over a 52-week period, but the former was less stable at room temperature. In conclusion, cryptolepine hydrochloride-loaded gelatine nanoparticles exhibited reduced haemolytic effect compared to the pure compound and can be developed further for parenteral delivery.

KEY WORDS: cryptolepine hydrochloride; gelatine type (A); haemolysis; malaria; nanoparticles.

INTRODUCTION

Malaria is one of many diseases in the tropics which mankind have so far failed to eradicate. It is caused by the plasmodium parasites of which one species, the *Plasmodium falciparum* is considered deadly. The main approach to malaria management is chemotherapy with antimalarial drugs most of which have become ineffective (1), prompting the need for newer agents as well as enhancing the efficacy of existing ones through formulation techniques.

Cryptolepine hydrochloride (5-methyl, 10*H*-indolo [3, 2-*b*] quinoline hydrochloride) (Fig. 1), an alkaloid derived from *Cryptolepis sanguinolenta* (Lindl), is established to have antimalarial activity and is being investigated as a potential for the management of many other conditions (2–4). The antimalarial activity of the compound has been found to be similar to other quinoline antimalarial compounds such as chloroquine and act within the acidic food vacuole of the parasite where it interferes with β-haematin activity (5,6) and this interference inhibits the conversion of the toxic by-product of haemoglobin digestion into the harmless pigment hemozoin, resulting in cell lyses and death. Activity of cryptolepine had been associated with the basic nitrogen (N-5) on its molecule (2,5–7). The presence of basicity has long been known to influence the ability of the quinolines to accumulate in the acidic food vacuole of the plasmodium parasite where they exert their activity (8,9). Though the antimalarial activity of cryptolepine hydrochloride is not in doubt, it has been reported to be potentially cytotoxic (6,10,11). A number of synthetic strategies have been carried out in an attempt to improve the antimalarial capability of cryptolepine and reduce its DNA intercalation property (5,12,13), but there are limited reports on the use of formulation strategies to improve the profile of the compound (14). The main objectives of any formulation strategy are to deliver a bioactive compound(s) in a form that ensures efficacy, safety, acceptability, ease of administration, stability and relative affordability. The efficacy and safety of the bioactive compound(s) are enhanced by formulations that achieve target delivery to the affected tissues and cells, limiting general systemic distribution, avoiding uptake by the reticuloendothelial system and through sustained release. Targeted delivery is particularly important in cancer chemotherapy due to the

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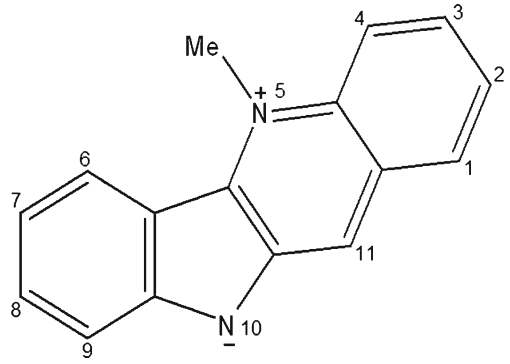

Fig. 1. Chemical structure of cryptolepine

adverse effect of non-selective anticancer agents on normal cells (15,16). Targeted delivery is also important for the treatment of intracellular infections including malaria (17). Many of the targets such as apicoplast, responsible for the exoerythrocytic development of the malaria parasite, and biosynthetic pathways including vitamin and folate metabolism amongst others (18–21) for antimalarial drug activities can be located within the parasitophorous vacuole of infected erythrocytes. Formulations which are able to ensure prolonged blood residence time of drugs as well as being able to deliver the drug across the erythrocytes membrane will greatly enhance the antimalarial activity of those drugs which act on the erythocytic forms of the parasites which are largely responsible for the many observed clinical symptoms of malarial. These symptoms include fever, chills anorexia, headache, vomiting, diarrhoea, perspiration and malaise and those of severe complicated malaria including impaired consciousness, prostration, respiratory distress, multiple convulsions, circulatory collapse, haemoglobinuria, abdominal bleeding and pulmonary oedema (22–24). For those drugs such as the quinolines to which cryptolepine hydrochloride belonged, deliveries into the food vacuole will greatly enhanced activity.

Many of the problems associated with conventional dosage forms and delivery systems such as poor bioavailability, non-specificity, rapid metabolism and excretion amongst others can be solved through pharmaceutical nanotechnology. The technology is particularly useful for poorly soluble drugs and drugs which are rapidly extracted by the liver during first pass metabolism. Site-specific delivery can be achieved with nanoparticles and limit the systemic distribution of drugs and hence reduce unwanted side effects. Improved pharmacokinetic properties (25,26) of nanoparticulate dosage forms lead to improve therapeutic outcomes such as reduced immunogenicity, reduced uptake by organs and the reticuloendothelial system (27–29). Sustained release is achievable with nanoformulations, a feature which is particularly important in the treatment of parasitic diseases such as malaria due to the increased residence time of the drug in plasma and hence increased contact time with erythrocytes (30,31).

Based on evidence that drugs formulated into nanoparticles have advantages such as improved efficacy and safety over conventional dosage forms (25–27) and evidence that some antimalarial compounds have shown tremendous improvement in their efficacy and safety when formulated into nanoparticles (31–35), this study was undertaken to explore pharmaceutical nanotechnology as a formulation strategy to improve the pharmaceutics profile of cryptolepine hydrochloride for parenteral administration. The aminoquinoline antimalarial compounds to which cryptolepine belong act mainly within the food vacuole of the parasite (5,36), where they inhibit hemozoin formation leading to parasite death. The pH within the vacuole is acidic and accumulation of the quinoline compounds is largely due to their weakly basic nature (8) with the basic nitrogenous group being important (9). It is important that when these compounds are encapsulated, embedded or linked to any nanocarrier, the ability of the compound to accumulate within the food vacuole should not be lost. Thus, the choice of appropriate carrier is very important. Apart from being compatible, biodegradable, nontoxic and ease of fabrication, possession of basic nitrogenous groups by the carrier will be an added advantage for the ability of the active compound to be delivered to its site of action which is the parasite's food vacuole.

Gelatine is a natural polymer which has already been approved for use in parenteral products (37). It is nontoxic, biodegradable and biocompatible and possesses basic nitrogenous groups which make it attractive as a carrier for the quinoline compounds. The use of pharmaceutical excipients of animal origin such as gelatine carries with it the risk of contamination of those products with transmissible spongiform encephalopathies (TSEs) such as bovine spongiform encephalopathy. In the case of gelatine however, the rigorous manufacturing processes such as acid, alkaline and heat treatments inactivate TSE agents and minimise TSE risk in drug products (38–40). There are several approaches to preparation of gelatine nanoparticles (41–45); however, the double desolvation approach (46) is the most widely used method. This approach is relatively inexpensive, easy to follow and reproducible and hence was considered suitable for the current study. The formulations were characterised and evaluated for their *in vitro* haemolytic activity.

MATERIALS AND METHOD

Materials

Gelatine type A from porcine skin (Bloom 175) was used as the carrier polymer; glutaraldehyde solution grade II (25 %, *w/v*), glucose anhydrous, mannitol, sucrose and trypsin from bovine pancreas were all purchased from Sigma-Aldrich. Acetone analytical grade (99.5 %) and formic acid were products of Scharlau. Acetonitrile (high-performance liquid chromatography (HPLC) grade) and formic acid were from Merck. Cryptolepine hydrochloride (>98 %) was isolated from *C. sanguinolenta*. Milli-Q water (conductivity 18.2 mΩ) was obtained with Milli-Q system. Cellulose ester dialysis membranes Floate-A-Lyzer G2® (volume 5 ml, mwco 3.5–5 kDa) were products of Spectrum laboratories.

Equipment

The equipment used in the study are Torrey Pines Scientific hot plate equipped with magnetic stirrer; Thermoscientific Sorval ultracentrifuge (wx ultra series), rotor type 70T1 series number 366; Labconco freeze dryer system (model 7806020); GLS aqua 18 plus water bath; Seven Easy pH meter (mettle Toledo) supplied by Global Science; Labnet vortex mixer; Eppendorf MiniSpin centrifuge (F-45-12-11); Zeta sizer nanoseries by Malvern instruments; Buchi rotary evaporator Rotavapor® R-215 equipped with heating bath B-491,

vacuum pump V-700 and vacuum controller V-850; a 2-ml capacity liquid–liquid extraction apparatus MIXXOR® purchased from Sigma-Aldrich and Binder stability chamber, model APT.LineKBF-ICH 240, USA.

The HPLC System consisted of an Agilent Series 1100 liquid chromatography system equipped with a quaternary pump (part no. G1311A), a degasser (part no. G1322A) and an electrochemical photodiode array detector (part no. G1315B) (Agilent, Waldbronn, Germany). The chromatographic column used was Gemini 5 μ C18 110 Å 250, 250×2.00 mm equipped with a column guard and a cartridge. Both the analytical and guard columns were purchased from Phenomenex (Auckland, New Zealand). Data acquisition was by the ChemStation™ software version A.10.02 Build 1757 (Agilent Technologies, Cheshire, UK). Other equipment include A sonicator (Bandelin sonorex® RK 510) produced by Bandelin electronics (Germany), Thermo Scientific vacuum pump model 420-1902 and analytical balance CP225D produced by Sartorius Ag (Germany). Other general laboratory glassware were used.

Methods

Preparation of Gelatine Nanoparticles

Gelatine nanoparticles were formulated according to the double desolvation approach by Coester *et al.* (46) with modifications. Briefly, gelatine was dissolved in Milli-Q water with the application of heat. Acetone was added to precipitate the high molecular weight fraction followed by removal of low molecular weight fraction by decantation. The high molecular weight fraction was re-dissolved in water and desolvated a second time by drop-wise addition of acetone under magnetic stirring. Glutaraldehyde was added to crosslink the particles and to stabilise the formulation. The product was incubated over a period of time. The acetone was removed in vacuo using a Buchi rotary evaporator, and the product was purified by double centrifugation and re-dispersed in Milli-Q water.

Characterisation of Nanoparticles

Particle size, zeta potential and polydispersity index (PDI) were determined by dynamic light scattering technique using the nanoseries zeta sizer. Samples were equilibrated for 120 s and measured at 25°C at a back scattering angle of 90°. Three measurements were made per sample. Particle morphology was established by transmission electron microscopy (TEM) using Tecnai™ G^2 spirit twin transmission electron microscope 125 kV.

Optimisation of Formulation Parameters

A number of important formulation parameters including effect of glutaraldehyde concentration, cross-linking time, formulation pH and pH of re-dispersion medium and gelatine concentration were investigated. After the initial screening, two levels of gelatine (2.5 and 5 % (*w*/*v*), based on volume of product) and glutaraldehyde solution (1.2 and 1.6 % (*v*/*v*), based on volume of product) were further investigated by using all possible combinations. The formulation was carried out at the following optimized conditions: temperature, 50°C; stirring rate, 350 rpm; incubation time, 12 h and 1,640 g/min for 15 min ultracentrifuge conditions. Two levels of pH (2.5 and 11.0) were also investigated further at fixed gelatine (5 %, *w*/*v*) and glutaraldehyde solution (1.6 %, *v*/*v*) concentrations. The effect of pH on nanoparticles parameters was also investigated by first suspending purified formulation at buffed pH and secondly by titrating a batch of purified nanosuspension over the pH range 3 to 10, whilst monitoring particle parameters at pH points corresponding to that of the first investigation.

Formulation of Cryptolepine-Loaded Gelatine Nanoparticles

Two types of gelatine formulations from the above study were selected for drug loading: 5 % (*w*/*v*) gelatine, pH 2.5 and 5 % (*w*/*v*) gelatine, pH 11.0 and constant glutaraldehyde concentration of 1.6 % (*v*/*v*). Two levels of drug concentrations were used, 1 % and 2 % (*w*/*v*). Drug loading was carried out using two different methods. In the first approach, the required volume of stock drug solution was added to desolvated gelatine solution, and the pH of the mixture was adjusted to either 2.5 or 11.0. A second desolvation was then carried out by drop-wise addition of acetone. The preparation was stabilised with 1.6 % (*v*/*v*) glutaraldehyde and incubated for 12 h under magnetic stirring at room temperature to yield products designated as 2.5ds and 11.0ds for formulations at pH 2.5 and 11.0, respectively. In the second approach, the drug solution was added to pre-formed purified nanoparticles suspension and incubated for a further 12 h under magnetic stirring to yield products designated as 2.5ad and 11.0ad for products formulated at pH 2.5 and 11.0, respectively. All products were purified by double centrifugation as described above, re-suspended in Milli-Q water and stored at 4°C until analysed.

Determination of Yield, Entrapment Efficiency and Drug Content

Yield and entrapment efficiency were determined based on earlier reports (43). Content determination was done in line with other studies (37,43,47) with modification. Nanoparticles were digested with trypsin from bovine pancreas in a water bath at 37°C until solution becomes clear. One millilitre of clear solution was then treated with few drops of 0.01 M NaOH solution and extracted with 1 ml chloroform using a 2-ml capacity liquid–liquid extractor, MIXXOR®. Prior to use, the efficiency of the extraction device and procedure was investigated by determining recovery of samples spiked with known concentrations of cryptolepine.

Stability Studies

Stability study was carried out on nanoformulations in accordance to the International Conference on Harmonization (ICH) guidelines for drug products intended for storage in a refrigerator (48). Freshly prepared and characterised samples in sealed glass vials were stored at 25°C/60 % relative humidity in a Binder stability chamber and 4°C in a refrigerator. Samples were withdrawn at predetermined times and characterised for size, zeta potential and polydispersity index. These parameters were compared to those prior to storage. Stability of lyophilised samples was investigated at 25°C/60 % relative humidity in a Binder stability chamber over 52 weeks.

Lyophilisation

Three cryoprotectants (glucose, sucrose and mannitol) were investigated within the concentration range 3:1 to 1:3 cryoprotectant/nanoparticles ratio. Prior to lyophilisation, the exact weight of nanoparticles per mill was established by drying 1 ml portions of nanosuspension without cryoprotectants. The weight of recovered particle was used as the basis for determining the weight of cryoprotectant required for each weight ratio. Portions of nanosuspension (1.5 ml) in 2 ml capacity cryo-vials were seeded with the required weight of cryoprotectant and mixed by gentle vortex. Samples were frozen at −40°C and dried using the cycle described by Zillies *et al.* with modification (45). In brief, primary drying was carried out at −50°C at a chamber pressure of 0.1 mbar for 10 h followed by secondary drying at 20°C for a further 8 h. Lyophilised samples were re-suspended in Milli-Q water and characterised by size, zeta potential and polydispersity index. These parameters were compared to those before lyophilisation.

In Vitro Release Study of the Formulations

In vitro release characteristics of the formulations were investigated using two methods. In the first instance (37), nanoparticles containing the equivalent of 4.5 mg of cryptolepine were suspended in 150 ml of phosphate buffed saline (PBS; pH 7.4) and incubated at 37°C in a horizontally shaking water bath (100 rpm). One-millilitre samples were removed into 2 ml cryo-vials and centrifuged at 13,500 rpm for 5 min using an Eppendorf MiniSpin centrifuge (F-45-12-11). The supernatant was carefully removed and analysed using a previously developed HPLC method (49). The sediment was returned to the release vessel in 1 ml release medium.

In the second method (43), nanoparticles containing the equivalent of 4.5 mg cryptolepine was hydrated with 5 ml release medium and introduced into a cylindrical cellulose ester dialysis membrane: Floate-A-Lyzer® G_2. The membrane was suspended vertically in 150 ml PBS in specially designed release vessels (Fig. 2). The temperature of the set up was maintained at 37°C and under magnetic stirring. One-millilitre samples were taken at predetermined times and analysed as before. Both methods were carried out under sink condition.

Table I. Particle Parameters for Plain Gelatine (A) Nanoparticles Formulated at Different pH Values

pH	Particle size (nm)	Zeta potential (mV)	PDI
2.5	243.00±67.98	22.63±2.57	0.04±0.03
3.0	216.60±42.06	20.53±1.96	0.06±0.05
3.5	287.20±17.90	16.90±0.30	0.08±0.01
4.0	356.93±59.27	16.47±0.49	0.11±0.02
4.5	522.37±121.25	16.23±0.55	0.17±0.05
5.0	NA	14.80±0.35	0.59±0.38
10.0	607.73±267.93	−27.77±6.62	0.49±0.19
11.0	233.50±39.89	−39.37±0.68	0.13±0.05

The pH was set before the second desolvation step
NA not applicable and is used to designate particles size above 6,000 nm (n=3)

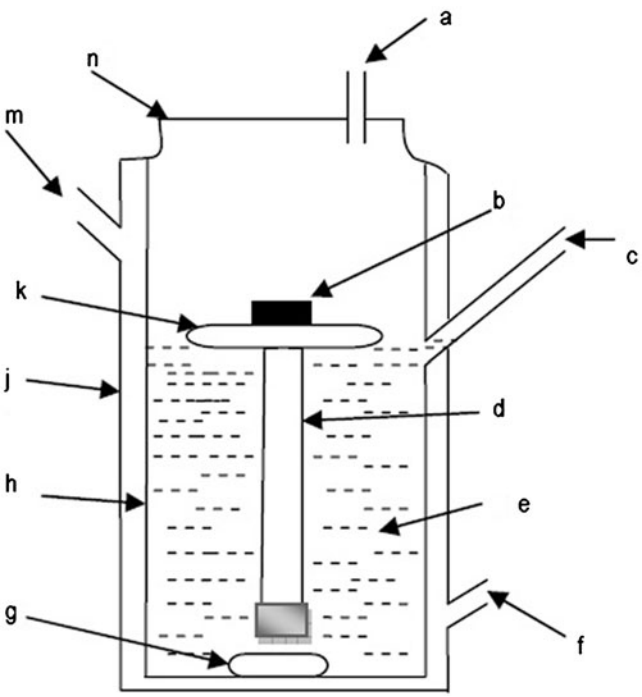

Fig. 2. Two-dimensional diagram of release vessel used for drug release study by dialysis. **a** Thermometer inlet; **b** membrane cup; **c** sampling tube; **d** cylindrical dialysis membrane (Float-A-Lyzer® G_2) containing nanoformulation; **e** release medium; **f** constant temperature fluid inlet; **g** magnetic stirrer; **h** inner vessel containing release medium; **j** outer jacket containing constant temperature fluid; **k** circular floatation ring designed to keep membrane afloat in a vertical position; **m** constant temperature circulating fluid outlet; **n** removable lid

In Vitro Haemolytic Studies

The study incorporates some aspects of the approach by Salauze and Decouvelaere (50). Fresh rat blood collected in a vacuteiner tube was centrifuged at 1,000 rpm for 5 min to remove white blood cells and other cell debris. The red blood

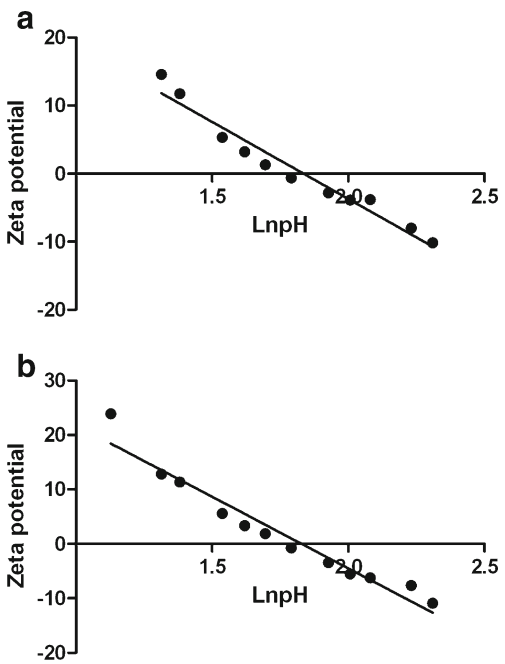

Fig. 3. Relation between Ln pH and the zeta potential of nanoparticles suspended in buffers at different pH **a** and by titration **b**

Table II. Particle Parameters for a Two-Level Combination of Gelatine and Glutaraldehyde Concentrations

Gelatine/glutaraldehyde concentration	Particle size (nm)	Zeta potential (mV)	PDI	%Yield
2.5:1.2 %	117.17±16.91	24.13±3.08	0.18±0.03	23.42±3.21
5:1.2 %	257.47±64.56	20.93±1.40	0.09±0.01	25.46±2.60
2.5:1.6 %	120.83±20.49	23.57±1.36	0.10±0.01	33.59±4.14
5:1.6 %	275.93±19.11	19.77±0.15	0.09±0.01	26.88±2.96

Formulation was carried out at pH 2.5 (n=3)

cells (RBCs) were washed three times with normal saline and suspended in PBS (pH 7.4) and stored at 4°C until used. Four different formulations (2.5ds, 2.5ad, 11.0ds and 11.0ad) and solution of free drug were tested in the concentration range 1–16,400 μM, with concentrations equidistantly spaced on a log scale. Test samples (1 ml) were added to 100 μl aliquots of RBC suspension and incubated at 37°C for 1 h. Intact RBCs were removed by centrifugation at 1,000 rpm for 5 min at the end of the incubation period. Aliquots of the supernatant (100 μl) was dissolved in 2 ml of an ethanol (99 %)–HCL (37 %) mixture (39:1). Absorbance of the resulting solutions was measured at 398 nm by spectrophotometry against appropriate blanks. The absorbance of Triton X-100 (a powerful haemolytic agent) treated samples were equated to 100 % haemolysis. The haemolysis produced by each test sample was expressed as a percentage of that produced by Triton X-100. The blank for each test sample was the sample treated exactly the same way as the test sample but without addition of RBCs.

RESULTS

Formulation of Gelatine Nanoparticles and Optimization of Formulation Parameters

Several key formulation parameters were investigated and optimized. Table I gives a summary of the particle parameters for products formulated at different pH values. It was realized that both particle size and PDI increased with pH from pH 2.5 to 5.0. Between pH 5 and 9, it was difficult to obtain stable products as the gelatine precipitated at the point of desolvation. However, above pH 9, it was again possible to produce stable formulations with reduced particle size. It was also observed that the zeta potential fell significantly towards the pH of unstable products but surged above pH 9 and assumed a negative value. To further investigate the effect of pH, nanoparticles formulated at pH between 2.5 and 3.5 were re-suspended in buffer solutions at different pH (pH range 3–10) after the last centrifugation stage. In another investigation, the pH of another batch was varied by titration, and the particle parameters were assessed in both instances. It was found that the relation between pH and zeta potential was nonlinear; however, a plot of Ln pH against the zeta potential (Fig. 3a, b) was fairly linear with R^2 value of 0.95 for both graphs. The equations of the linear regressions were:

$$ZP = -22.62LnpH + 41.53 \quad (1)$$

from Fig. 3a and

$$ZP = -26.3LnpH + 48.10 \quad (2)$$

from Fig. 3b.

These two equations allowed for the calculation of the pH at which the zeta potential was zero and below or above which it was positive or negative, respectively. That pH by analogous was the new isoelectric point of the gelatine in the nanoparticulate form. Alternatively, this isoelectric point can also be calculated by finding the antLn of the intersection (1.836 and 1.829 for Fig. 3a, b, respectively) on the LnpH axis. These

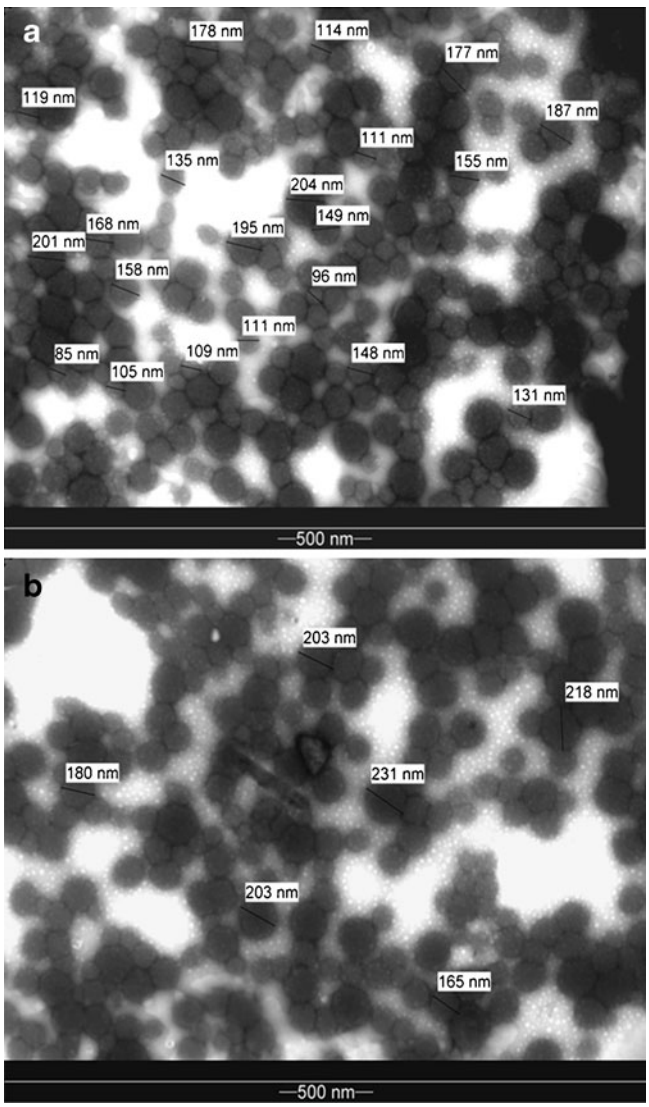

Fig. 4. Transmission electron microscope images of the selected formulation: **a** is the image of plain particles and **b** is that of cryptolepine-loaded nanoparticles. Formulation was carried out at pH 2.5, and drug was loaded during second desolvation

calculations yielded pH values of 6.27 and 6.23 for Fig. 3a, b, respectively.

Based on results of the preliminary study, two concentrations of gelatine and glutaraldehyde were further studied using all possible combinations. The results (Table II) indicated that any of those combinations will lead to particles of desired characteristics.

Formulation of Cryptolepine Hydrochloride-Loaded Gelatine Nanoparticles

The TEM images (Fig. 4a, b) of plain and cryptolepine hydrochloride-loaded gelatine nanoparticles showed particles of good size distribution and morphology. Drug loading was carried out at two levels each of pH, drug concentration and method of loading (see "Methods"). The results (Table III) showed the formulations at two different pH values with drug loaded during the second desolvation step or loaded onto pre-formed nanoparticles. Drug loading during second desolvation resulted in relatively larger particles compared to those obtained when drug was loaded onto pre-formed nanoparticles. There was also an intra-method variation in terms of particle size between formulations at pH 2.5 and 11.0 with the former resulting in smaller particles.

The yield of nanoparticles was determined by freeze drying 1 ml of nanosuspension. The recovered weight was expressed as a percentage of the total components per millilitre of the suspension. Entrapment efficiency is the percentage of drug loaded onto nanoparticles. This was derived by expressing the difference between total drug used and total drug detected in the supernatant after centrifugation as a percentage of the total drug used. Drug content was determined by the complete digestion of a known weight of nanoparticles with trypsin followed by extraction and quantification of the drug content which was expressed as a percentage of nanoparticles digested. The %recovery of the extraction device for the determination of drug content was found to be 108.24±12.14 of spiked concentration (n=6). Entrapment efficiency values showed that there was a higher drug loading at pH 11.0 compared to pH 2.5 for both loading methods.

Stability Studies

Short- and long-term stability assessment results (Tables IV and V) indicated that all the aqueous formulations were stable at 4°C over the storage period recommended by the ICH (48), on the bases of particle size, zeta potential and PDI. Products stored at room temperature showed significant particle growth and reduced zeta potential after just 2 weeks indicating a reduced stability. Thus, for long-term storage at room temperature, it may be appropriate to store samples in a freeze-dried state.

Lyophilisation

The bases for this study was that the nanosuspension exhibited reduced stability on storage at room temperature as indicated by the results of the stability studies shown in Table V. Glucose sucrose and mannitol as cryoprotectants were investigated for use in the preparation of dried cryptolepine-loaded gelatine nanoparticles. The focus was to get a cryoprotectant which will be efficient in low concentration. From the results (Table VI), on the scale of 3:1 to 1:3 cryoprotectant-to-nanoparticles ratio, sucrose and glucose were found to be efficient compared to mannitol as indicated by particle parameters before and after drying. Based on this, further samples were prepared and dried with sucrose (1:1) and investigated for stability at room temperature. The results of that study (Table VII) showed the products exhibited stability under the study conditions. The stability study of the lyophilized samples was limited to room temperature because of the predicted outcome of such studies at 40°C/75 % relative humidity due to the low melting temperature of gelatine.

In Vitro Release Studies

Drug release study was carried out by two different methods: beaker method, in which nanoparticles were introduced directly into the release medium; and by dialysis in which the formulation was separated from the release medium by a dialysis membrane. The choice of float-A-Lyzer as the dialysis device was based on earlier reports which indicated the device was efficient for drug release studies (51–54). Both methods (beaker and dialysis) showed that there was a higher initial release (burst release) when drug was loaded onto pre-formed nanoparticles (formulations 2.5ad and 11.0ad) compared to formulations in which drug was loaded during the second desolvation stage (Figs. 5 and 6). This initial released was followed by a gradual and sustained release over a long period of time. The dialysis approach was further used to study the

Table III. Particle Parameters for Cryptolepine-Loaded Gelatine Nanoparticles Formulated at pH 2.5 and 11.0 Using 5 % (w/v) and 1.6 % (v/v) Gelatine and Glutaraldehyde Concentrations, Respectively

Formulation type	%Drug used	Particle Size (nm)	Zeta potential (mV)	PDI	%Yield	EE (%)	%Drug Content
2.5ad	1	162.69±2.34	27.43±0.95	0.04±0.01	24.05±5.03	72.58±1.67	1.46±0.32
11.0ad	1	285.60±10.15	−31.27±0.25	0.25±0.03	38.19±4.53	88.58±10.65	1.08±0.08
2.5ad	2	198.43±45.01	22.87±0.49	0.04±0.03	27.15±2.00	70.42±1.81	1.19±0.07
11.0ad	2	298.93±2.68	−30.30±0.82	0.25±0.05	36.98±5.10	87.96.0±8.80	1.20±0.22
2.5ds	1	236.20±19.19	22.67±0.32	0.05±0.01	32.15±2.53	86.27±1.71	2.52±0.23
11.0ds	1	556.50±5.70	−35.47±0.50	0.18±0.06	38.52±2.08	95.45±1.07	2.28±0.13
2.5ds	2	248.17±2.12	21.28±1.56	0.06±0.01	27.19±18.2	77.88±3.50	2.71±0.01
11.0ds	2	481.10±69.90	−35.20±0.14	0.13±0.04	49.20±13.17	93.40±1.40	3.62±0.91

Drug was loaded onto pre-formed nanoparticles (2.5ad and 11.0ad) or during second desolvation (2.5ds and 11.0ds) (n=3)
PDI polydispersity index, *EE* entrapment efficiency

Table IV. Stability of Nanoparticles (Aqueous Suspension) Assessed Based on Changes in Size, Zeta Potential and PDI on Storage at 4°C Over a 52-Week Period

		Time (weeks)								
Batch	Parameter	0	1	2	3	4	8	12	24	52
2.5 ds	Size (nm)	215.44	223.22±31.20	221.03±33.36	219.80±25.21	232.37±31.05	233.29±26.33	228.67±28.36	229.11±25.41	278.22±36.23
	ZP (mV)	24.31	25.13±3.10	23.45±4.12	23.00±2.11	22.36±1.85	21.77±2.84	22.14±2.81	21.45±4.21	21.91±3.13
	PDI	0.05	0.03±0.01	0.01±0.00	0.01±0.00	0.10±0.03	0.12±0.02	0.12±0.04	0.13±0.05	0.13±0.08
2.5 ad	Size (nm)	223.58	224.28±43.05	227.13±41.02	233.88±39.22	234.78±46.51	245.65±45.32	239.70±44.7	241.33±48.31	244.18±58.81
	ZP (mV)	24.22	25.30±3.33	25.37±2.85	22.31±4.01	24.53±2.52	24.24±3.31	25.41±6.20	24.61±2.12	24.56±1.63
	PDI	0.08	0.08±0.02	0.09±0.03	0.08±0.02	0.07±0.01	0.09±0.01	0.11±0.04	0.11±0.03	0.12±0.08
11.0 ds	Size (nm)	284.56	294.29±55.53	301.77±67.37	299.56±45.50	309.12±77.33	321.42±51.23	322.09±73.21	335.1.8±73.33	328.40±72.61
	ZP (mV)	−33.52	−33.18±4.21	−32.70±1.55	−29.76±3.33	−27.55±2.32	−28.11±2.22	−28.29±4.44	−25.9±4.21	−26.83±2.10
	PDI	0.15	0.17±0.02	0.167±0.03	0.19±0.02	0.16±0.03	0.17±0.02	0.18±0.07	0.19±0.08	0.20±0.09
11.0 ad	Size (nm)	312.50	309.33±82.60	322.84±86.08	356.07±84.99	354.80±88.21	366.06±95.32	348.65±121.22	351.30±97.65	356.75±102.21
	ZP (mV)	−32.43	−32.81±2.88	−32.17±2.55	−28.63±2.47	−27.68±1.01	−26.7±3.38	−25.08±3.21	−25.2±0.54	−24.84±4.61
	PDI	0.18	0.17±0.02	0.17±0.06	0.19±0.06	0.14±0.08	0.16±0.12	0.17±0.08	0.211±0.11	0.22±0.10

No significant changes in the parameters were observed (n=3)
ZP zeta potential, *PDI* polydispersity index

Table V. Stability of Nanoparticles (Aqueous Suspension) Assessed Based on Changes in Size, Zeta Potential and PDI on Storage at Room Temperature (25°C/60 % Relative Humidity) Over a 24-Week Period (n=3)

		Time (weeks)							
Batch	Parameter	0	1	2	3	4	8	12	24
2.5 ds	Size (nm)	215.44	241.24±	233.14±63..50	387.49±79.55	643.92±210.33	876.20±322.90	1,001.94±621.82	1,542.33±601.21
	ZP (mV)	24.31	26.33±4.10	22.80±2.21	19.1±2.41	18.46±2.88	17.93±2.09	18.88±1.71	14.16±0.54
	PDI	0.05	0.08±0.02	0.123±0.04	0.19±0.03	0.25±0.10	0.29±0.12	0.31±0.20	0.51±0.32
2.5 ad	Size (nm)	223.58	226.22±63.55	355.26±78.68	401.67±81.05	772.32±313.38	911.08±544.53	1,103.82±701.61	NA
	ZP (mV)	24.22	23.80±3.00	22.42±3.26	18.53±3.53	16.44±1.24	15.24±3.66	15.21±3.12	NA
	PDI	0.08	0.09±0.06	0.109±0.10	0.18±0.07	0.20±0.08	0.27±0.13	0.334±0.11	NA
11.0 ds	Size (nm)	284.56	375.66±144.81	559.27±202.55	908.45±215.88	1,606.4±662.3	NA	NA	NA
	ZP (mV)	−33.52	−30.91±5.01	−22.60±1.08	−14.86±0.05	−14.5±0.90	NA	NA	NA
	PDI	0.15	0.16±0.05	0.24±0.13	0.48±0.21	0.43±0.27	NA	NA	NA
11.0 ad	Size (nm)	312.50	343.39±105.37	449.65±154.84	764.30±157.41	819.80±421.2	975.45±388.62	NA	NA
	ZP (mV)	−32.43	−31.24±2.11	−23.77±3.01	−18.65±2.22	−17.42±0.25	−16.06±0.24	NA	NA
	PDI	0.18	0.18±0.12	0.18±0.05	0..20±0.06	0.29±0.10	0.33±0.20	NA	NA

ZP zeta potential, *PDI* polydispersity index, *NA* not applicable and applied to particles above 6,000 nm in size

released profile of aqueous stability samples stored at 4°C over 52 weeks (Fig. 7), lyophilized samples (Fig. 8) and drug release from lyophilized stability samples stored at 25°C over 52 weeks (Fig. 9). The areas under the peaks (Table VIII) of fresh samples and corresponding stability samples were compared statistically by using paired Student's *t* test. These statistical analyses showed the differences were statistically insignificant.

In Vitro Haemolytic Studies

The haemolytic tendencies of the four formulations under study were investigated and compared to solutions of the pure drug. Figure 10a–e represent the relations between the logarithm of the molar concentrations and the percentage haemolysis of the formulations and the pure compound. The plots were obtained with Graphpad prisms statistical software with a build-in modified four parameter Hill's equation (55). The haemolysis produced by each concentration was expressed as a percentage of the haemolysis produced by Triton X-100 which is a potent haemolytic agent. For ease of analysis, the range obtained with each agent ('*x*–*y*')% was transformed to 0–100 % by using the relation:

$$\mathrm{nv} = \frac{(\mathrm{ov} - \mathrm{om}) \times \mathrm{nr}}{\hat{\mathrm{o}}\mathrm{r}} + \mathrm{nm} \qquad (3)$$

where nv, ov, om, nr, ôr and nm are the new value, old value, old minimum, new range, old range and new minimum, respectively. The statistical summary of the results (Table IX) showed that the compound is largely a mild haemolytic agent with the minimum concentration producing 50 % haemolysis (EC_{50}) of 51.6 μM. All the four formulations were less haemolytic compared to the pure compound, with the best formulation in terms of EC_{50} values being formulation 2.5ds which has an EC_{50} value about five times higher than that of the pure compound and has the potential for further development.

DISCUSSION

Formulation of Gelatine Nanoparticles and Optimization of Formulation Parameters

A number of formulation parameters do affect the formation and characteristics of gelatine nanoparticles. Such parameters include the pH, cross-linker concentration, gelatine concentration, incubation time and the temperature. Though most of these parameters have been investigated in earlier works by other researchers (46), it was important that when the procedure was applied to a new drug entity, the parameters be re-assessed and optimized to suit the setup. Gelatine type (A) from porcine skin is obtained by pre-treatment of collagen with acids which leaves amide groups largely unaffected resulting in a positively charged gelatine, having an isoelectric point of about 9 (56,57). At low pH, the electrical characteristics of the gelatine remained largely unaffected and particles exhibited positive zeta potential. As the pH was raised, the positive groups were neutralized bringing the ratio between positively charged amide groups and negatively charged hydroxyl groups towards unity. At the same time, the zeta potential dropped, becoming zero at the point of neutrality. A further rise in pH above the isoelectric point resulted in the hydroxyl groups becoming dominant and the particles assumed a negative zeta potential as was observed in the results of Table II. Stability of dispersed systems is affected by the zeta potential of particles as the latter defines the degree of attraction or repulsion between particles. The larger the zeta potential, the greater the repulsion and hence the stability as the particles will have less tendency to coalesce. From the results of the regression analysis of the relation between pH and zeta potential of the particles as shown in Fig. 3a, b and Eqs. 1 and 2, it is important that for the long-term storage of gelatine (A) nanoparticles, the pH of the dispersed phase must be further away from pH 6.2. From those results, it was also observed that re-suspending purified nanoparticles in buffer solutions reduced the zeta potential at all pH values compared to when nanoparticles were

Table VI. Comparison of Particle Parameters Before and After Freeze Drying with Cryoprotectants at Different Weight Ratios (n=3)

Cryoprotectant	Weight ratio	psbd	psad	zpbd	zpad	PDIbd	PDIad
Sucrose	3:1	136.64	136.00±1.56	22.32	23.57±1.80	0.17	0.16±0.03
Sucrose	2:1	109.08	107.033±1.08	27.73	24.47±0.67	0.15	0.13±0.05
Sucrose	1:1	105.65	122.57±15.72	22.39	25.33±1.25	0.21	0.20±0.07
Sucrose	1:2	233.44	300.79±54.03	20.84	20.77±2.93	0.11	0.59±0.35
Sucrose	1:3	208.41	935.93±100.15	22.40	24.63±6.47	0.08	0.70±0.34
Glucose	3:1	330.59	335.93±4.26	19.55	18.33±0.95	0.09	0.05±0.00
Glucose	2:1	110.88	117.47±0.67	24.38	23.67±0.35	0.10	0.10±0.02
Glucose	1:1	144.35	146.63±1.27	22.01	23.17±1.10	0.11	0.09±0.03
Glucose	1:2	107.22	135.50±17.84	24.33	24.97±1.45	0.09	0.19±0.02
Glucose	1:3	261.14	1,141.27±820.13	19.64	15.23±3.61	0.08	0.86±0.17
Mannitol	3:1	269.17	496.67±4.04	19.84	15.10±1.18	0.10	0.77±0.02
Mannitol	2:1	275.86	NA	19.86	NA	0.09	NA±
Mannitol	1:1	191.43	NA	22.15	NA	0.09	NA
Mannitol	1:2	194.47	NA	27.27	NA	0.13	NA
Mannitol	1:3	209.04	NA	20.23	NA	0.15	NA

psbd particle size before drying, *psad* particle size after drying, *zpbd* zeta potential before drying, *zpad* zeta potential after drying, *PDIbd* polydispersity index before drying, *PDIad* polydispersity index after drying, *NA* not applicable and applied to particles above 6,000 nm in size

Table VII. Stability of freeze-dried nanoparticles assessed based on changes in size, zeta potential and PDI on storage at 25°C/60 % relative humidity over a 52 week period

Batch	Parameter	Time (weeks)							
		0	1	2	3	4	8	12	24	52
2.5 ds	size	121.75	117.09±27.54	130.86±34.12	128.97±32.42	135.48±23.77	132.81±37.44	137.90±44.40	136.26±41.41	167.06±33.04
	ZP	23.78	24.15±3.01	23.21±2.50	22.34±0.84	23.29±2.56	25.67±0.28	23.33±2.45	21.12±90	22.30±1.43
	PDI	0.11	0.12±0.02	0.12±0.09	0.13±0.02	0.11±0.06	0.15±0.08	0.18±0.10	0.17±0.06	0.19±0.07
2.5 ad	size	234.23	241.88±52.01	239.20±39.01	244.76±71.49	242.65±40.02	237.08±47.33	253.67±15.00	258.12±34.28	253.37±43.74
	ZP	25.05	24.22±2.22	24.62±1.98	25.01±2.05	25.29±1.25	23.56±2.16	24.42±0.89	23.45±2.33	22.19±2.08
	PDI	0.11	0.09±0.01	0.14±0.03	0.16±0.02	0.19±0.02	0.14±0.05	0.20±0.08	0.21±0.07	0.28±0.10
11.0 ds	size	294.51	295.22±86.21	301.35±50.35	317.04±30.20	307.00±32.20	288.43±38.03	312.92±27.39	304.39±45.80	322.67±05
	ZP	−28.77	−26.32±4.45	−26.65±4.05	−29.01±4.87	−27.88±3.54	−27.37±4.44	−27.44±4.08	24.90±3.89	22.56±2.43
	PDI	0.13	0.14±0.05	0.13±0.02	0.14±0.01	0.16±0.07	0.21±0.11	0.24±0.10	0.28±0.09	0.26±0.12
11.0 ad	size	321.09	302.02±17.56	300.55±23.22	333.08±21.28	337.11±32.70	339.39±47.82	336.18±28.28	342.17±40.30	349.04±37.09
	ZP	−30.55	−30.03±2.23	−28.00±2.16	−25.76±4.01	−24.54±4.45	−24.50±3.68	−23.55±1.36	−23.21±2.00	−22.24±3.07
	PDI	0.14	0.14±0.03	0.17±0.08	0.20±0.07	0.22±0.06	0.24±0.03	0.25±0.01	0.29±0.10	0.28±0.13

Measured parameters did not differ significantly from those of freshly dried sample ($n=3$)
ZP zeta potential, *PDI* polydispersity index samples were dried with sucrose (1:1)

Fig. 5. Drug released from freshly prepared aqueous nanosuspension established with the beaker method. *Black down-pointing triangle* 11.0ds, sample formulated at pH 11.0 with drug loading during second desolvation; *black up-pointing triangle* 11.0ad, samples formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles; *black circle* 2.5ad, sample formulated at pH 2.5 with drug loaded onto pre-formed nanoparticles; *black square* 2.5ds, samples formulated at pH 2.5 with drug loaded during second desolvation

suspended in Milli-Q water. This probably could be due to ionic interaction between buffer salts and nanoparticles which may have reduced the electrical repulsion. The differences in drug entrapment observed at the two different formulation pH values (2.5 and 11.0) as seen in Table III may be due to the fact that at a higher pH the polarity of gelatine (A) reversed from positive to negative as indicated by the negative zeta potential. Cryptolepine being a basic nitrogenous compound was more attracted by electrostatic force to the negatively charged gelatine than it was to positively charged species of the polymer leading to increased drug loading. There was also a higher yield at pH 11.0 possibly due to the higher entrapment efficiency.

Lyophilisation

The reason behind the preparation of lyophilised samples was the apparent instability exhibited by the aqueous nanoformulations at room temperature as depicted by the results in Table V. From this investigation, we found glucose and

Fig. 6. Drug release from freshly prepared aqueous nanosuspension established with dialysis method. *Black down-pointing triangle* 11.0ds, sample formulated at pH 11.0 with drug loading during second desolvation; *black up-pointing triangle* 11.0ad, samples formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles; *black circle* 2.5ad, sample formulated at pH 2.5 with drug loaded onto pre-formed nanoparticles; *black square* 2.5ds, samples formulated at pH 2.5 with drug loaded during second desolvation

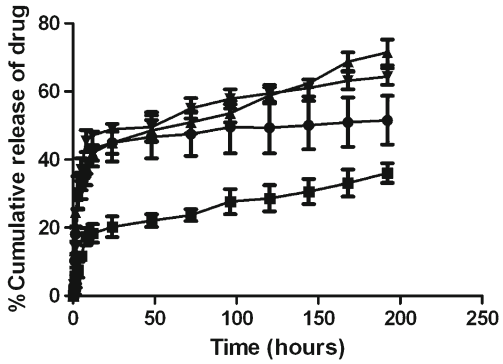

Fig. 7. Drug release from stability samples of aqueous nanosuspension established with dialysis method. *Black down-pointing triangle* 11.0ds, sample formulated at pH 11.0 with drug loading during second desolvation; *black up-pointing triangle* 11.0ad, samples formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles; *black circle* 2.5ad, sample formulated at pH 2.5 with drug loaded onto pre-formed nanoparticles; *black square* 2.5ds, samples formulated at pH 2.5 with drug loaded during second desolvation

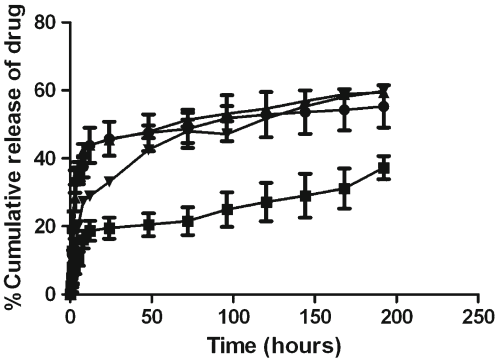

Fig. 9. Drug release from lyophilised stability samples of cryptolepine hydrochloride-loaded gelatine nanoparticles established with the dialysis method. *Black down-pointing triangle* 11.0ds, sample formulated at pH 11.0 with drug loading during second desolvation; *black up-pointing triangle*11.0ad, samples formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles; *black circle* 2.5ad, sample formulated at pH 2.5 with drug loaded onto pre-formed nanoparticles; *black square* 2.5ds, samples formulated at pH 2.5 with drug loaded during second desolvation

sucrose to be effective in low concentrations compared to mannitol as shown in Table VI, though a similar study had shown that all the cryoprotectants investigated in our study were effective (45). It must be noted, however, that very high concentrations of cryoprotectants were used in that study. The lyophilised samples were stable at 25°C/60 % relative humidity; this stability was depicted by the maintenance of products characteristics including particle size, zeta potential, PDI as shown in Table VII and drug release characteristics evidenced in Fig. 9.

In Vitro Release Studies

Drug release from batches produced by loading drug onto pre-formed nanoparticles (2.5ad and 11.0ad) were higher and more rapid compared to release from those formulations prepared by loading drug during the second desolvation stage (2.5ds and 11.0ds) as shown in Figs. 5 and 6. The possible explanation of the observed release patterns is that either the drug molecules were less efficiently attached to the pre-formed nanoparticles or a fraction of the drug was encapsulated into nanoparticles core when loading was done during the second desolvation. The initial higher released from batches produced at pH 11.0 may also be due to the fact that during formulation when pH was 11.0, there was a reversal of the electrical properties of gelatine (A) with negatively charged hydroxyl groups becoming dominant. Thus, a fraction of the drug may have been loaded as a result of electrostatic attraction between the basic nitrogenous group of cryptolepine and hydroxyl groups of gelatine. At the pH of release medium (pH 7.4), amide groups of gelatine became dominant conferring a positive charge on the polymer and a loss of electrostatic attraction between polymer and drug, a situation which may have resulted in the immediate released of the fraction of drug loaded as a results of electrostatic attraction. The sustained release characteristics of the formulations was important in the context of malaria chemotherapy as contact time between drug and infected RBCs will be enhanced (17) and also allows for reduction of dosing frequency. Of the four formulations test, formulation 2.5ds prepared at pH 2.5 with

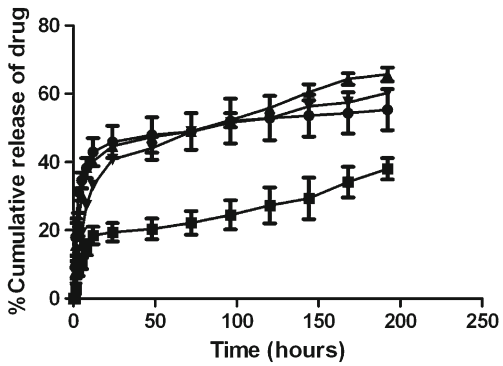

Fig. 8. Drug release from freshly prepared lyophilised samples of cryptolepine hydrochloride-loaded gelatine nanoparticles established with the dialysis method. *Black down-pointing triangle* 11.0ds, sample formulated at pH 11.0 with drug loading during second desolvation; *black up-pointing triangle* 11.0ad, samples formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles; *black circle* 2.5ad, sample formulated at pH 2.5 with drug loaded onto pre-formed nanoparticles; *black square* 2.5ds, samples formulated at pH 2.5 with drug loaded during second desolvation

Table VIII. Areas Under the Drug Release Curves of Freshly Prepared Samples (Aqueous Suspension and Freeze Dried) and Corresponding Stability Samples

	Area under drug released curve			
Sample status	2.5ad	2.5ds	11.0ad	11.0ds
Fresh aqueous sample	9,133	4,357	10,186	9,418
Aqueous stability sample	9,115	5,035	10,535	10,661
Fresh lyophilised sample	9,569	4,836	10,090	9,366
Lyophilised stability sample	9,555	4,758	9,932	8,998

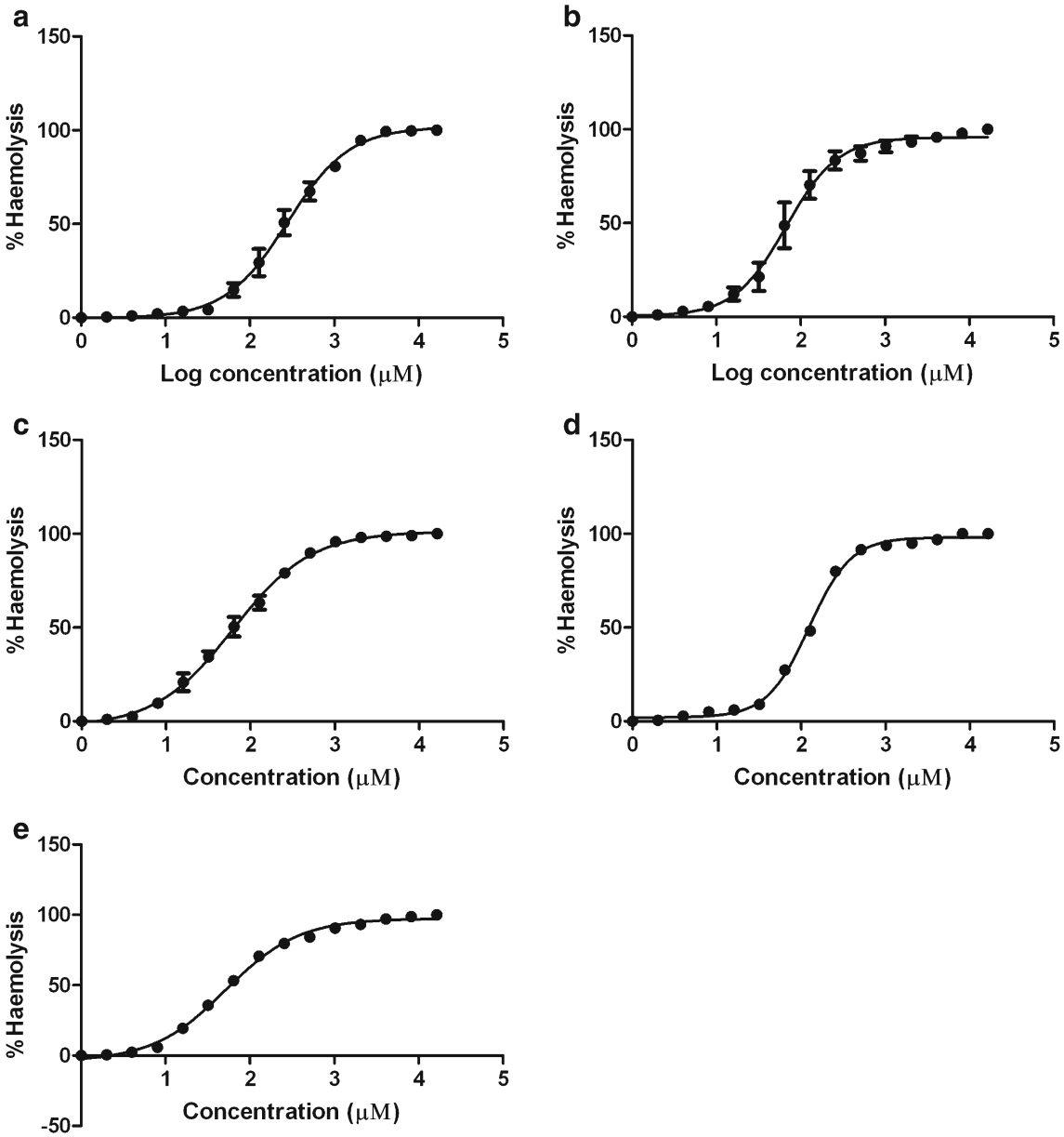

Fig. 10. Concentration *versus* %haemolyses of the four different formulations and the pure compound. **a** Formulation 2.5ds prepared at pH 2.5 with drug loaded during second desolvation, **b** formulation 2.5ad prepared at pH 2.5 with drug loaded onto pre-formed nanoparticles, **c** formulation 11.0ad formulated at pH 11.0 with drug loaded onto pre-formed nanoparticles, **d** formulation 11.0ds prepared at pH 11.0 with drug loading during second desolvation and **e** the pure drug solution. The ranges of percentage haemolysis were normalized to the range 0 to 100 % for each test sample using Eq. 3

drug loaded during the second desolvation was considered the best as drug released was more gradually compared to the other formulations.

A comparison between the two release methods showed no significant observable differences in the released pattern other than the time lag between drug release and drug detection in the receptor compartment observed with the dialysis method. This observation was largely due to the time taken for the released drug to diffuse through dialysis membrane. Use of float-A-Lyzer dialysis membrane has a number of advantages over the beaker method such as ease of sampling and non-contamination, and no centrifugation of samples is required. The release vessel presented in Fig. 2 is novel in its design and can easily be adapted for large-scale dissolution studies by dialysis for dosage forms meant for parenteral administration. Thus, in all subsequent release studies such as the release of drug from aqueous stability samples, freeze-dried samples and freeze-dried stability samples were done using the dialysis approached. Drug released characteristics of the aqueous stability samples as well as those of the lyophilised samples shown in Figs. 7, 8 and 9 were all statistically similar to the pattern of release from freshly prepared samples based on a paired Student's *t* test statistical analysis of the areas under the release curves as captured in Table VIII. It can thus be said that storage or lyophilisation did not adversely affect the drug release.

Table IX. Statistical Summary of the Parameters of the Hill's Plot

Statistical parameter	Agent				
	2.5ds	2.5ad	11.0ad	11.0ds	Cryptolepine solution
$LogEC_{50}$	2.443	1.826	2.093	1.794	1.713
Hill slope	1.175	1.414	1.748	0.9386	0.9995
EC_{50}	277.4	66.93	123.8	62.20	51.61
Standard error					
$LogEC_{50}$	0.03254	0.04394	0.01496	0.03120	0.02799
Hill slope	0.09446	0.1805	0.09249	0.06062	0.06014
95 % CI					
$LogEC_{50}$	2.377 to 2.509	1.737 to 1.914	2.063 to 2.123	1.731 to 1.857	1.656 to 1.769
Hill slope	0.9843 to 1.366	1.049 to 1.778	1.561 to 1.935	0.8162 to 1.061	0.8780 to 1.121
EC_{50}	238.5 to 322.8	54.56 to 82.11	115.5 to 132.7	53.80 to 71.92	45.31 to 58.79
R square	0.9873	0.9700	0.9953	0.9919	0.9929

2.5ds, 2.5ad, 11.0ad and 11.0ds have the same meanings as defined in the text. The concentration of all agents tested was 1–16,400 μM
EC_{50} the concentration of the agent corresponding to 50 % haemolysis, *95 % CI* the 95 % confidence interval of the values

In Vitro Haemolytic Studies

One of the side effects of parenteral dosage forms is haemolysis at the site of injection due to destruction of corpuscles of cells or tissues (58). It is thus important that any dosage form targeting the parenteral route be investigated for this particular effect.

The results in this study presented in Table IX and Fig. 10a–e showed that the extent of reduction in haemolysis was depended on the method of drug loading, with formulations prepared by loading drug during the second desolvation stage (formulation 2.5ds and 11.0ds) showing greater reduction in haemolytic tendency compared to those formulated by loading drug onto pre-formed nanoparticles (2.5ad and 11.0ad). The reason for that observation is not readily known but could be due to the fact that when drug was loaded onto pre-formed nanoparticles, mechanism of loading may be that of an association between drug molecules and surface groups of the nanoparticles. Thus, when drug-loaded nanoparticles were mixed with erythrocyte suspension, there was a greater contact between drug on nanoparticulate surface and erythrocytes. On the other hand, drug loading during the second desolvation may have resulted in drug being largely encapsulated within the core of the particles thereby reducing contact between drug molecules and erythrocytes

CONCLUSIONS

Cryptolepine-loaded gelatine nanoparticles have been successfully formulated based on the double desolvation approach. There was good entrapment efficiency with particles having desired characteristics. Sucrose and glucose were efficient in low concentrations as cryoprotectant. Nanoformulations of cryptolepine exhibited reduce haemolytic side effects, making the preparation suitable for parenteral use on that basis. The sustained and gradual release of the drug from the formulations is a desirable feature of a good antimalarial drug for the clearance of parasites over a prolonged period. The aqueous formulations showed good stability under refrigeration condition and at room temperature in lyophilised form.

Conflict of interest There is no conflict of interest to disclose.

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