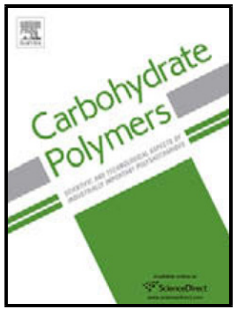

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Acetylated Cashew Gum-based Nanoparticles for Transdermal Delivery of Diclofenac Diethyl Amine

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ABSTRACT

Nanoprecipitation and dialysis methods were employed to obtain nanoparticles (NPs) of acetylated cashew gum (ACG). NPs synthesized by dialysis showed greater average size compared to those synthesized by nanoprecipitation, but they presented improved stability and yield. NPs were loaded with diclofenac diethylamine and the efficiency of the drug incorporation was over 60 % for both methods, for an ACG:NP a weight ratio of 10:1.The cytotoxicity assay demonstrated that the NPs had no significant effect on the cell viability, verifying their biocompatibility. The release profile for the diclofenac diethylamine associated with the ACG-NPs showed a more controlled release compared to the free drug and a Fickian diffusion mechanism was observed. Transdermal permeation reached 90 % penetration of the drug.

Keywords: nanoparticles; acetylated cashew gum; Diclofenac diethyl amine; cytotoxicity; transdermal delivery

Chemical compounds

acetone (CID: 180); acetonitrile (CID: 6342); acetic anhydride (CID: 7918); diclofenac diethylamine (CID:115087); dimethyl sulfoxide (CID:679); ethanol (CID:702); formamide (CID: 713); phosphoric acid (CID:1004); pyridine (CID: 1049); sodium hidroxide (CID: 14798).

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1. Introduction

In the area of pharmaceutical technology differentiated systems have been developed for targeted drug delivery. In this regard, polymeric materials have received more attention than other classes of materials in the development of drug delivery systems (Kim et al., 2009). Certain properties of polysaccharides, such as biodegradability and biocompatibility, mean that many researchers have selected these polymers for the preparation of biomaterials (Liu et al., 2008).

Cashew gum (CG) is a polysaccharide extracted from an affordable and easily available source, that is, the species *Anacardium occidentale*, which is widely distributed in northeastern Brazil. Purified cashew gum contains galactose (72-73%), glucose (11-14%), arabinose (4.6 to 5%), rhamnose (3.2-4%) and glucuronic acid (4.7 to 6.3%) in its structure (Paula and Rodrigues, 1995; Paula, de Paula, Heatley, & Budd, 1998).

In the biomedical field some potential applications of cashew gum are already known, for instance, it acts as an anti-inflammatory agent in the healing of mice (Shirato et al., 2006), shows significant antibacterial activity (Torquato, 2004; Campos,

2012), is an excellent film forming material, with potential application in nanobiomedical devices (Araújo et al., 2012), and it has demonstrated an *in vivo* anti-tumor effect (Florêncio, Melo Mota, Melo-Junior & Araújo, 2007). In the pharmaceutical area it has been reported that cashew gum can act as a gelling agent in the topical formulation of aceclofenac (Kumar, Patil, Patil, & Paschapur, 2009) and as a binder for paracetamol tablets (Gowthamarajan et al., 2011). Also, it is used to produce curcumin tablets with buccal adhesive ability and thus circumvent hepatic metabolism and improve the bioavailability of the active principle (Gowthamarajan et al., 2012).

However, there are some difficulties associated with the use of gums, such as a drop in viscosity during storage and the possibility of microbial contamination. Chemical modification not only minimizes these disadvantages but also allows more specific drug delivery (Rana et al., 2011) and it can improve the efficiency of the incorporation of the drug into the matrix (Zhang et al., 2009).

Cashew gum nanoparticles grafted with acrylic acid were obtained by radical polymerization using Ce (IV) ions as the initiator and methylene-bis-acrylamide as the crosslinker. Nanoparticles which are pH-sensitive were obtained with sizes in the range of 71-603 nm, depending on the gum/acrylic acid ratio (Silva et al. 2009). Nanoparticles based on carboxymethylated cashew gum (CMCG) and chitosan were synthesized with diameters ranging from 150 to 400 nm. Smaller particle sizes were obtained for CMCG samples with a lower degree of substitution (DS) (Silva et al., 2010).

In a previous study, acetylated cashew gum (ACG) with a DS of 2.8 was synthesized and self-assembled nanoparticles were obtained through the dialysis of an organic solution (DMSO) against a non-solvent (water). The mean diameter of the self-assembled nanoparticles obtained was 179 nm and the critical aggregation concentration

(CAC) in water was $2.1x10^{-3}$ g/L. Indomethacin (IND) was used as a hydrophobic model drug and was incorporated into the hydrophobized polysaccharide nanoparticles. A controlled drug release was observed for up to 72 h (Pitombeira et al., 2015).

Nanoparticles (NPs) as polymeric carriers of drugs have been the subject of several reviews in the literature (Liu et al., 2008; Langer & Tirrell, 2004; Uhrich, et al., 1999; Peppas, 1995; Soppimath et al., 2001; Singh & Lillard, 2009; Oh, Lee & Park, 2009; Kumari Yadav & Yadav, 2010). In this context, anti-inflammatory drugs (NSAIDs) are frequently the drug investigated, in an attempt to overcome some difficulties related to their pharmacokinetics and pharmacodynamics, as well as several adverse effects resulting from their oral and parenteral administration, such as gastric irritation and ulceration (Beck et al., 1990; Galer, et al. 2000; Jones & Rubin, 2008). Nanoencapsulation in polymeric systems protects the drug and contributes to a controlled release, thus increasing the therapeutic benefit with minimal side effects (Soppimath, et al., 2001). The transdermal route also reduces these side effects, increases patient compliance, avoids hepatic metabolism, and maintains the plasma drug concentration for a longer period (Shakeel et al., 2007; Prow et al., 2011).

In this study, different methods for the preparation of acetylated cashew gum (ACG) nanoparticles were investigated and studies on the incorporation, release and cutaneous permeation of diclofenac diethylamine were carried out, as a proof-of-concept for a transdermal drug delivery device.

2. Materials and Methods

2.1 Materials

Diclofenac diethylamine (DDA) was purchased from Henrifarma, Teresina, lot 10100025. The cashew gum (CG) was isolated from a tree of the species *Anacardium*

occidentale (M_w = 1.8x10^5 g/mol) using an adapted method previously described by Paula, Heatley and Budd (1998). The exudate was dissolved in distilled water at room temperature to give a 10% (w/v) solution. The pH was adjusted to approximately 7.0 by addition of diluted aqueous sodium hydroxide. The clear solution was successively filtered through sintered glass and the polysaccharide precipitated with ethanol at ratio of 1:3 (gum solution: ethanol). The precipitate (isolated gum) was dried in a forced air oven at 60ºC/8 h and weighed.

All other reagents were of analytical grade (Formamide, pyridine, acetic anhydride, dimethyl sulfoxide, sodium hidroxide, ethanol, acetone were purchased from Vetec and acetonitrile and phosphoric acid were purchased from Sigma-Aldrich**)**

2.2 Acetylation of cashew gum

The acetylated cashew gum (ACG) was synthesized by Motozato´s method (1986) as reported in Pitombeira et al. (2015) with a degree of substitution of 2.8. Cashew gum (1 g) was suspended in 20 ml of formamide under vigorous stirring. Pyridine (3 g) and acetic anhydride (7 g) were added and the mixture was stirred for 24h at 50°C. The ACG was obtained by precipitation with 400 mL of water. The solid was filtered, washed with water and dried in hot air.

The polysaccharide obtained was characterized by infrared spectroscopy and nuclear magnetic resonance spectroscopy. FT-IR spectra were recorded with KBr pellets on an FT-IR Shimadzu 8300 spectrophotometer in the range of 4,000 to 400 cm^1,with a resolution of 2 cm^{-1} and 15 scans. ^{1}H NMR spectra of 3% w/v solutions in DMSO-d_6 were recorded at 353 K on a Fourier transform Bruker Avance DRX 500 spectrometer with an inverse multinuclear gradient probe-head equipped with z-shielded

gradient coils and a Silicon Graphics workstation. Sodium 2,2-dimethylsilapentane-5-sulphonate (DSS) was used as the internal standard (0.00 ppm for ^{1}H).

2.3 Preparation of ACG nanoparticles

The synthesis of the nanoparticles was performed using two different methods: nanoprecipitation and dialysis (Raoa and Geckeler, 2011). Identical procedures were conducted for the synthesis of NPs containing the drug. In both methods the ACG was dissolved in 20 mL (0.1% w/w) of acetone for 15 min under magnetic stirring. Diclofenac diethylamine (DDA) is a hydrophobic drug and it was incorporated into the ACG nanoparticles at the time of their synthesis. The drug-loaded nanoparticles obtained are referred to herein as ACG-DDA-NPs. For both methods investigated three polymer/drug proportions (by weight) were studied (10:1, 10:2 and 10:5).

2.3.1 Nanoprecipitation

A solution of ACG in acetone (0.1%w/w) was dispersed in 20 mL of deionized water in a homogenizer (Ultra Turrax T25 Basic Heidolpha) at 19,000 rpm. Removal of the solvent by evaporation was carried out in a Heidolph Rzr205 rotoevaporator system at 40ºC. The material was then filtered with a 0.45 µm syringe filter and the solution centrifuged at 20,000 rpm for 2 h for purification of the polymer.

2.3.2 Dialysis

A solution of ACG in acetone (0.1%w/w) was dialyzed against deionized water using a cellulose acetate membrane (molecular weight 12,000) for 24 h. The conductivity was used to monitor the water exchange. The resulting solution was then lyophilized.

2.4 Amount of drug encapsulated and the encapsulation efficiency (%EE)

The amount of DDA in the nanoparticles was determined by UV-Vis spectroscopy at 276 nm. The analysis was performed on a Shimadzu UV-1800 equipment, coupled to a standard PC and operated via UV probe software 2:33. Quartz cells were used and readings were taken between 190 and 400 nm. The amount of encapsulated diclofenac was calculated using a calibration curve to determine the relationship between the absorbance and the concentration (R^2= 0.9998 and Y = 0.290 + 28.58X). The drug load (%) and drug efficiency (%EE) were calculated as

Drug Load (%DL)

$$\frac{\text{Mass of drug in nanoparticle} \times 100}{\text{Mass of drug + mass of nanoparticle}} \quad (1)$$

Encapsulation efficiency (%EE)

$$\frac{\text{Mass of loaded DDA} \times 100}{\text{Mass of added DDA}} \quad (2)$$

2.5 Dynamic light scattering (DLS) and zeta potential

The particle size and zeta potential were determined on a Malvern Zetasizer Nano ZS Model 3600 analyzer. The hydrodynamic diameter was measured by dynamic light scattering (DLS), using a 633 nm laser at a fixed scattering angle of 173°. The particle size was obtained considering the particle as spherical-like. Each sample was measured in triplicate and is reported as the mean ±SD (n=3).

2.6 Scanning electron Microscopy (SEM)

The scanning electron microscopy was recorded using a Jeol-6360LV field emission. To prepare the SEM sample, a drop of nanoparticles was deposited on carbon stickers on aluminum stubs, dried and coated with gold.

2.7 *In vitro* DDA release assay

The release profiles for the free DDA and the drug-loaded nanoparticles (ACG-DDA-NPs) were obtained using a dialysis system. A sample (6 mg) of the ACG-DDA NPs was introduced into cellulose acetate membrane with molecular exclusion pores of 12,000 Da and dialyzed against 50 mL of phosphate buffer solution (PBS), pH 7.4 at 37°C for 24 h.

Aliquots of 1.5 ml were withdrawn every 30 min and the drug concentration was quantified by UV-vis spectroscopy. The buffer was replenished to keep the volume constant. The measurements of the absorbance at a wavelength at 276 nm were converted into the percentage of drug released according to a previously established calibration curve for which the linearity was confirmed ($R^2 = 0.999$). The experiment was performed in triplicate and the drug concentrations were corrected considering the dilution factor. To understand the mechanism of drug release from the nanoparticles, the data were treated according to the Korsmeyer-Peppas model (Peppas, 1985) described by Equation 3.

$$\frac{Qt}{Qo} = kt^{n} \quad (3)$$

where *Qt* is the amount of drug released at time *t*, Q_0 is the amount of drug in the solution, k is a kinetic constant and *n* is the release exponent, which, according to the resulting numerical values, characterizes the mechanism of drug release. The

linearization of Equation 3 through the construction of ln Qt/ Q_0 as a function of ln*t*, provides the release exponent (*n*) and constant release (*k*).

2.8 *In vitro* permeation assay

Vertical Franz-type diffusion cells (n=5) with a diffusional area of 1.77 cm^2 were used for the permeation study. The skin used in the tests was taken from the dorsal surface of pig ears and kept refrigerated at -80°C until use. The skin was carefully placed between the donor and receiver compartment of each cell, the latter of which was filled with 7 mL of phosphate buffer solution (pH 7.4), so that the dermis was in direct contact with this medium. The temperature was maintained at 37°C with stirring at 400 rpm.

Permeation assay was prepared as follows: A volume of 200 µL of the ACG-DDA-NPs formulation in phosphate buffer (pH 7.4) at 0.5 mg/mL was placed in the donor compartment. Aliquots (1.5 mL) were withdrawn at predetermined periods and analyzed by high performance liquid chromatography (Sintov, et al (2006). For this purpose we used a MERCK HITTACHI L-7000 chromatograph with a UV detector (L-7400 LACHROM) at 276 nm and a C18 reverse phase column (250 x 4.6 mm) with a particle size of 5µm. The mobile phase consisted of a mixture of acetonitrile: water with 0.1% phosphoric acid (98:2 v/v) with a final pH of 3.5. The chromatography was performed at room temperature with a flow rate of 0.6 ml min^{-1} and automatic injection.

The same amount of buffer was added to keep the volume constant and the free DDA was also measured in order to compare the permeation profile. The data were expressed as the amount of drug permeated by the surface area of the skin (µg/cm^2).

2.9 Cytotoxicity test

The cell line used in this study was an oral squamous cell carcinoma (OSCC) obtained from the American Type Culture Collection (ATCC, Manassas, VA). Cells (5 × 10^4 cells/mL) were grown in 75 cm^2 flasks on 96-well plates and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1 % penicillin-streptomycin and 0:25% amphotericin B. The cells were treated with ACG-NPs, ACG-DDA-NPs and DDA at different concentrations for 3 h in an incubator (5% CO_2 at 37 °C, humidified atmosphere of 33%). Control cells were incubated with culture medium alone. After 24 h, DMEM without phenol red and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) were added to each well and the plates were incubated for 4 h at 37°C (5% CO_2, humidified atmosphere of 33%).The formazan crystals formed were dissolved in 2-propanol and evaluated at 560 and 690 nm using a Safire^2 multiplate reader.

2.10 Statistical analysis

Statistical analyses were performed applying one-way ANOVA and the Tukey test using Prisma software. All data reported in the tables and figures are expressed as the mean ±SEM of three independent experiments. Statistical significance for this study was considered as $p<0.05$.

3.0 Results and Discussion

The acetylated polysaccharides were characterized by infrared spectroscopy and according to the spectra in the infrared region, the intensity of vibrations at 3400 cm^{-1} present in cashew gum (CG) decrease as the acetyl groups are inserted (Fig. 1 a). The absorption bands at 1375 cm^{-1} and 1752 cm^{-1} are typical of ester groups demonstrating the acetylation of the polymer (Fig. 1 b). The acetylated polysaccharide shows yield of

65% and the same degree of substitution (2.8) observed previously by Pitombeira et al. (2015), and calculated using the ^{1}H-NMR spectra (figure not shown).

Several polysaccharides like dextran, chitosan and pululan have been chemically modified to improve their physico-chemical, mechanical or chemical-biological properties, expanding the possibilities of using it in new materials (Lemarchand, Gref, Couvreur, 2004). In the last decade there has been increased interest in developing these modified polysaccharides for the synthesis of biodegradable nanoparticles. Due to the fact that these structures showed many advantages for biomedical applications such as drug protection and the ability to control its release (Rodrigues et al., 2003; Leonard et al., 2003; Chourasia and Jain, 2004; Chourasia et al., 2006; Singh and Kim, 2007; Zhang et al., 2009).

3.1 Characterization of ACG nanoparticles

Certain variables determine the success of nanoparticle synthesis and affect the physico-chemical properties of the nanoparticles obtained. These include the conditions under which the organic phase is added to the aqueous phase and the concentration of the material involved (Rao and Geckeler 2011).

The NPs prepared by dialysis using acetone as the solvent had larger particles (302 nm) than those obtained using DMSO as solvent (179 nm). NPs synthesized by nanoprecipitation showed smaller average size (79.37 nm) compared to those synthesized by dialysis (302 nm), however a more negative zeta potential and smaller polydispersity index (PDI) values were observed for NPs synthesized by dialysis (Table 1). According to Mohanraj & Chen (2006), values lower than 0.2 for the polydispersity and above 30 mV for the zeta potential (in module) indicate good colloidal stability in solution. Thus, the particles prepared by dialysis presented better colloidal stability than those prepared by nanoprecipitation.

Under similar conditions, for NPs of dextran hydrophobically modified and with ibuprofen incorporated, Horning, Bunje & Heinze (2009) reported particle sizes of 309 nm obtained through dialysis and 77 nm for synthesis via nanoprecipitation. These results are quite similar to those observed in the study reported herein. Figure 2 shows the particle size distribution for nanoparticles with and without the drug. In Figure 3 it is possible to observe the SEM image of ACG NPs. The image shows spherical particles and the sizes determined by SEM were in the ranges of 150-280 nm and 300-450 for DDA ACG NP and ACG nanoparticles, respectively.

For both methods investigated, the addition of the drug decreases the polydispersity index. No tendency in relation to the particle size was observed on changing the nanoparticle:DDA ratio (Table 2). On applying the nanoprecipitation method, a significant increase in particle size (p<0.001) was observed for all nanoparticle:DDA ratios investigated, whereas in the case of dialysis a significant reduction in the particle size was observed for a nanoparticle:DDA ratio of 10:1, and for other ratios no statistically significant variation was observed (Table 2).

Increasing the nanoparticle:drug ratio to 10:5 promoted a decrease in the encapsulation efficiency (%EE). For the lowest drug concentration (10:1) the amount incorporated was higher than 60% for both methods (Table 2). With respect to the drug loading (DL), the highest value was obtained for a nanoparticle:drug ratio of 10:2 for both methods, although this ratio did not provide the highest encapsulation efficiency in the case of the dialysis method. Using dialysis and nanoprecipitation, Horning, Bunjes & Heinze (2009) obtained the same %EE (46.5%) for both methods. However, in other studies, lower values for the incorporation of the drug into NPs synthesized through dialysis were noted, for instance, Shi & Shoichet (2008) observed a DL of <1% and Ericco et al. (2009) reported a DL of 2.2%.

Therefore, based on the results obtained for particle size, zeta potential, yield and %EE, the nanoparticles produced by the dialysis method with a ACG-NPs:drug ratio of 10:1 were chosen for the *in vitro* release and permeation studies as well as for cell viability assays.

3.2 Cytotoxicity

Figure 4 shows the *in vitro* cytotoxicity of the ACG-NPs and ACG-DDA-NPs using the previously grown OSCC cell line as described above. Nanoparticles in different concentrations were added to the cell suspension and left for 24 h at 37°C. The cell viability was subsequently measured using the MTT assay (Fig. 4). The nanoparticles (with or without the DDA) did not show any basal toxicity up to a concentration of 150 µg/mL. Recently, similar results were obtained for polymeric modified NPs, with and without doxorubicin, under physiological conditions (Thambi et al., 2014).

Another study using 10 µg/ml of methotrexate indicated that there was no significant difference between the effects of the free drug and the drug incorporated in chitosan nanoparticles on tumor cells of the MCF-7 lineage and non-tumor cells (Nogueira et al., 2013).

However, it's possible to see that a slight reduction in cell viability in the presence of acetylated cashew gum, even if this difference is not significant, this may be due to cell line type, the dosage or the chemical composition of the gum. Sarika et al. (2015) developed Arabic gum-curcumin conjugate micelles (GA-cur), and evaluated cytotoxicity by MTT assay using on MCF-7 and HepG2 cells. At concentration of 3.125 g/mL show non cytotoxic to MCF-7, but cytotoxic was observed for HepG2 cells. Rigopoulou et al. (2012) reported that the presence of galactose moiety in the structure

of gum arabic can selectively identify asialoglyco protein receptor (ASGPR) on the surface of hepatocytes.

David et al. (2015), reported the cell viability studies when MiaPaCa2 cells were incubated with quercetin-loaded chitosan nanoparticles, blank chitosan nanoparticles and free quercetin. Blank chitosan nanoparticles did not exhibit significant changes in the cell viability. In addition, no significant difference in cell viability was observed for free quercetin between 10 and 100 μM. In comparison, quercetin-loaded chitosan nanoparticles exhibited significant reduction in the cell viability in a dose-dependent manner.

3.3 Release kinetics and *in vitro* permeation

The drug release was analyzed by diffusion through a dialysis membrane in phosphate buffer solution. Figure 5A, 5B shows the release profile for the anti-inflammatory drug (DDA) encapsulated in ACG nanoparticles and free DDA. It can be observed that the DDA was released from the ACG-NPs in a controlled manner, with a burst effect within the first 5 h followed by a more uniform release up to 24 h, after which a release of around 60% was achieved. Similar results were observed for sulfated chitosan NPs loaded with curcumin, with an average NP size of 220 nm and a maximum release rate of 70% (Anitha et al., 2011). Another study showed similar results for acetylated pullulan NPs loaded with epirubicin, with NPs of > 200 nm and controlled release rates of up to 60% (Zhang et al., 2009). Although Martins et al. (2012) reported a release of less than 20% for heparin from chitosan microparticles they obtained the release profile in two steps. The biphasic release behavior is consistent to the results obtained by Chin et al., (2014) and Ayadi at al., (2016). These authors verified that the initial fast release was due to the presence of drug adsorbed onto the nanoparticle surface or held close to it.

Liu and He (2015) reported the release of aspirin and probucol drugs from modified chitosan nanoparticles, both aspirin and probucol were released rapidly in the first 24h, and the release rate decreased significantly thereafter. The cumulative release amount of probucol was much higher than that of aspirin in the data range, which can be due the different interactions of aspirin and probucol with the modified chitosan nanoparticles matrix.

In order to investigate the mechanism through which the drug was released from the nanoparticles the Korsmeyer-Peppas equation was applied as shown in Fig. 6 A release exponent (*n*) value of 0.27 was obtained, indicating Fickian diffusion. The release of indomethacin from acetylated cashew gum also showed a Fickian diffusion mechanism (Pitombeira et al., 2015).

Similar release profiles have also been observed by using other diclofenac release systems. For instance, Liu et al. (2010) reported a slow release rate of diclofenac when encapsulated in solid lipid nanoparticles. Silva et al. (2014) confirms the capacity of bacterial cellulose (BC) membrane loaded with diclofenac to provide a sustained release, which can be successfully combined with a good biocompatibility and absorption properties.

The transdermal permeation profile for the drug incorporated into ACG-DDA-NPs compared to free DDA can be observed in Fig. 7. Both the free drug and the drug associated with the nanoparticles reached a permeation of approximately 1.5 μg/cm^2 in six hours of testing, which is equivalent to an average of 90% permeated DDA. However, the nanostructured system demonstrates a more controlled permeation profile which is maintained over time.

Sintov & Botner (2006) also confirmed the controlled and effective transdermal penetration of sodium diclofenac from a microemulsion *in vitro*. However, in contrast to

the results obtained in this study, they observed significantly higher permeation values for the microemulsion compared with the application of the drug in aqueous solution. However, it should be noted that sodium diclofenac, unlike diethylamine, does not show effective transdermal permeation due to its physico-chemical properties.

Minghetti et al. (2007) investigated the effects of three skin penetration enhancers, on four diclofenac salts, the maximal amount of diethylamine diclofenac permeated from the aqueous vehicles were 2–4 fold greater than the amounts permeated when compared with all other salts and vehicles. The possibility of diclofenac micellization in aqueous systems was considered as one of the contributors to the favorable skin penetration.

Sodium diclofenac was also incorporated into different nanoemulsions and once again it was possible to verify that nanoemulsions are effective accelerators for the permeability of the drug through the skin. The nanosize of the formulations was suggested by the authors as a permeation enhancer (Piao et al., 2007). Likewise, PLGA and chitosan were used to prepare a bilayered system of nanoparticles for the simultaneous topical delivery of two anti-inflammatory drugs and it was found that the skin permeation of the nanostructures was much higher when compared to the commercial topical gel (Shah et al., 2012).

4. Conclusions

In this study, acetylated cashew gum nanoparticles were successfully prepared via two different methods. The nanostructures were incorporated in DDA and showed great potential as carriers for controlled drug release systems as well as transdermal permeation promoters *in vitro*. The high rates of cell viability indicate that the NPs offer

good biocompatibility, with and without the drug incorporated. This means that ACG may be useful for the delivery of anti-inflammatory drugs, however comparative studies with a commercial formulation and *in vivo* assays have yet to be performed.

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Table 1: Characterization of Acetylated Cashew Gum Nanoparticles

Method	Size (nm)	ζ(mV)[a]	PDI[b]	Yield (%)
Nanoprecipitation	79.37±0.608	-20.2±1.52	0.354±0.033	24
Dialysis	302.0 ±0.971	- 35.9 ± 2.49	0.187 ± 0.025	80

a: zeta potential
b: polydispersity index

Table 2: Influence of the incorporation of the drug in nanoparticles

Method	**Nanoprecipitation**			**Dialysis**		
ACG:DDA[a]	**10:1**	**10:2**	**10:5**	**10:1**	**10:2**	**10:5**
Size[b]	90.2±1.125**	125.9±0.776**	96.48±0.752**	262.9±4.963**	304.7±5.139	291.9±6.799
ζ(mV)[c]	-18.7±1.960	-18.8±1.410	-23.7±1.040	-31.5±0.693	-32.1±1.110	-32.9±0.741
PDI[d]	0.257±0.003	0.126±0.027	0.219±0.017	0.134±0.003	0.160±0.032	0.149±0.046
DL[e] (%)	6.6	12.1	1.8	5.9	8.2	6.4
EE[f] (%)	72.6	72.6	5.4	65.5	49.2	19.4

a: acetylated cashew gum: diclofenac diethylamine ratio
b: average size of nanoparticles
c: zeta potential
d: polydispersity index
e: drug loaded
f: encapsulation efficiency
**: $p<0.001$ compared to the drug-free nanoparticles

Figure captions

Figure 1. FTIR spectra of cashew gum and ACG (A) Schemes of the acetylated cashew gum (B).

Figure 2. Nanoparticle size distribution of ACG-NPs and DDA-ACG-NPs (A) synthesized by nanoprecipitation and (B) synthesized by dialysis. (nanoparticle:drug ratio 10:1)

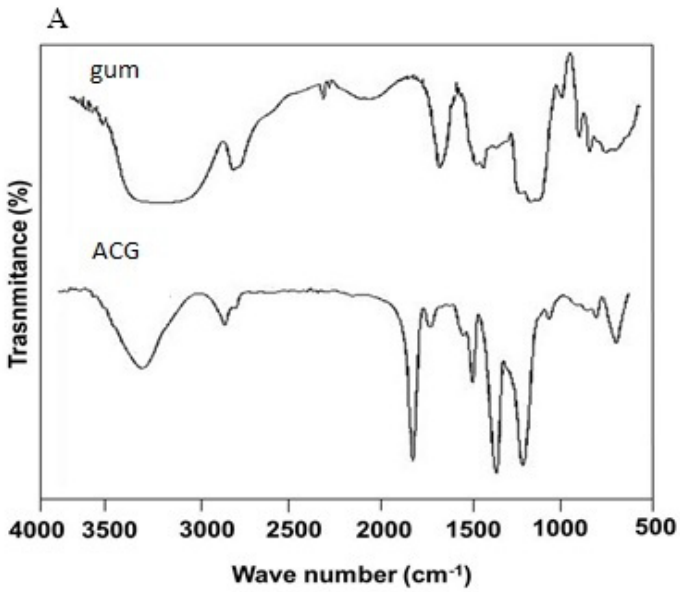
Figure 3. SEM images of ACG nanoparticles without (B) and with DDA (A).

Figure 4. Cell viability for OSCC where CT = control, ACG-NPs = acetylated cashew gum nanoparticles, DDA-ACG-NPs = acetylated cashew gum nanoparticles loaded with diclofenac diethylamine: (A) according to the polymer concentration (1 = 50 µg/ml, 2 = 75 µg/ml, and 3 = 100 µg/ml and 4 = 150 µg/ml) and (B) according to the drug concentration (n=3).

Figure 5. *In vitro* release profile for diclofenac A. DDA-ACG-NPs B free DDA (n=3)

Figure 6. Mechanism associated with *in vitro* release of diclofenac from DDA-ACG-NPs, according to the Korsmeyer-Peppas model (n =3).

Figure 7. Percutaneous penetration of DDA through pig skin after application of the drug as free DDA and in DDA-ACG-NPs (n=5).



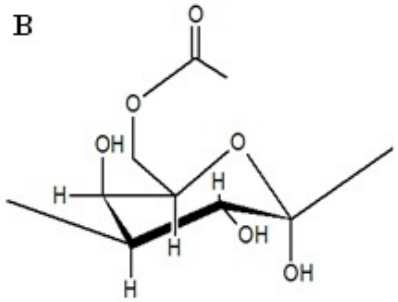


Figure 1

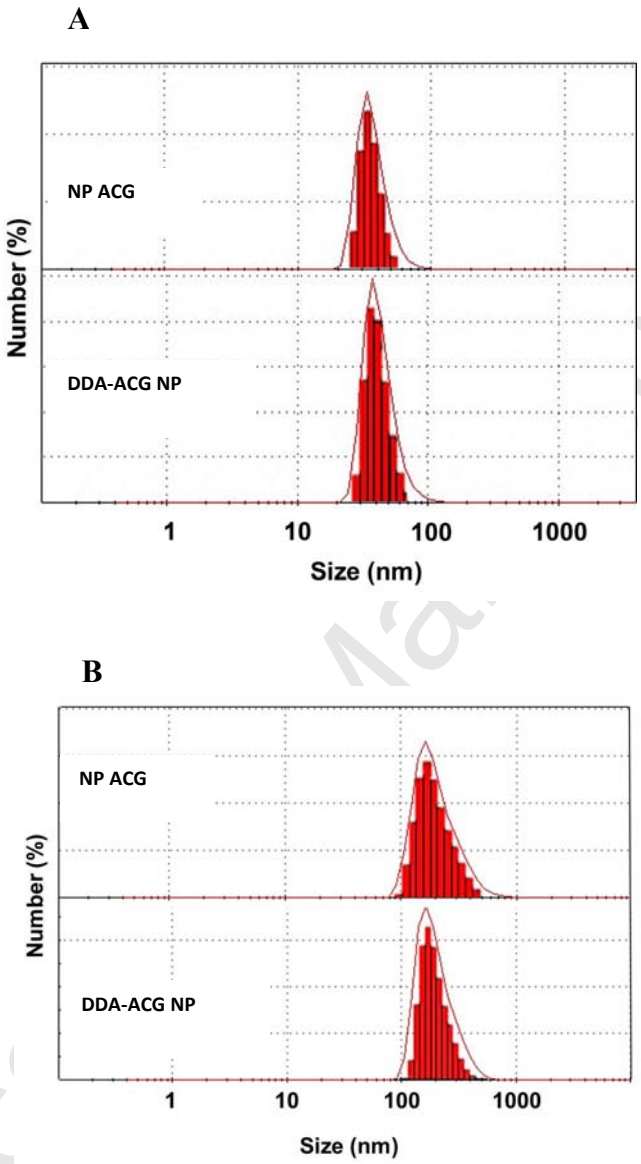
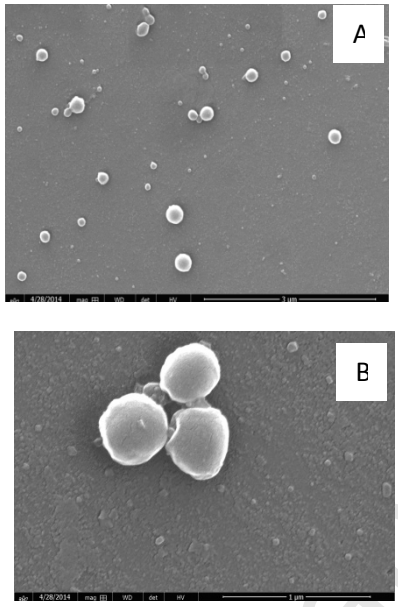


Figure 2



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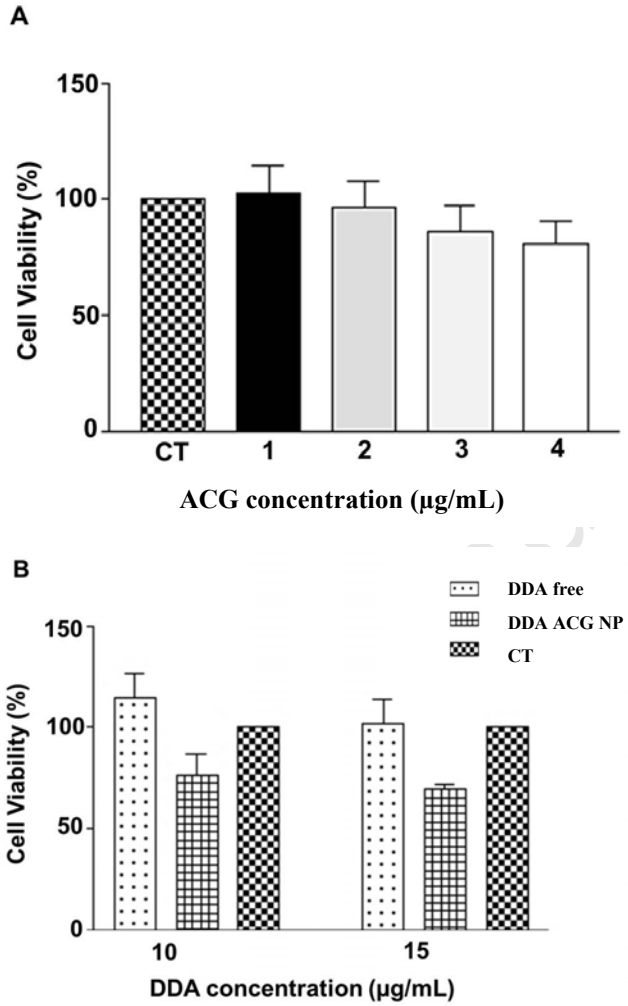

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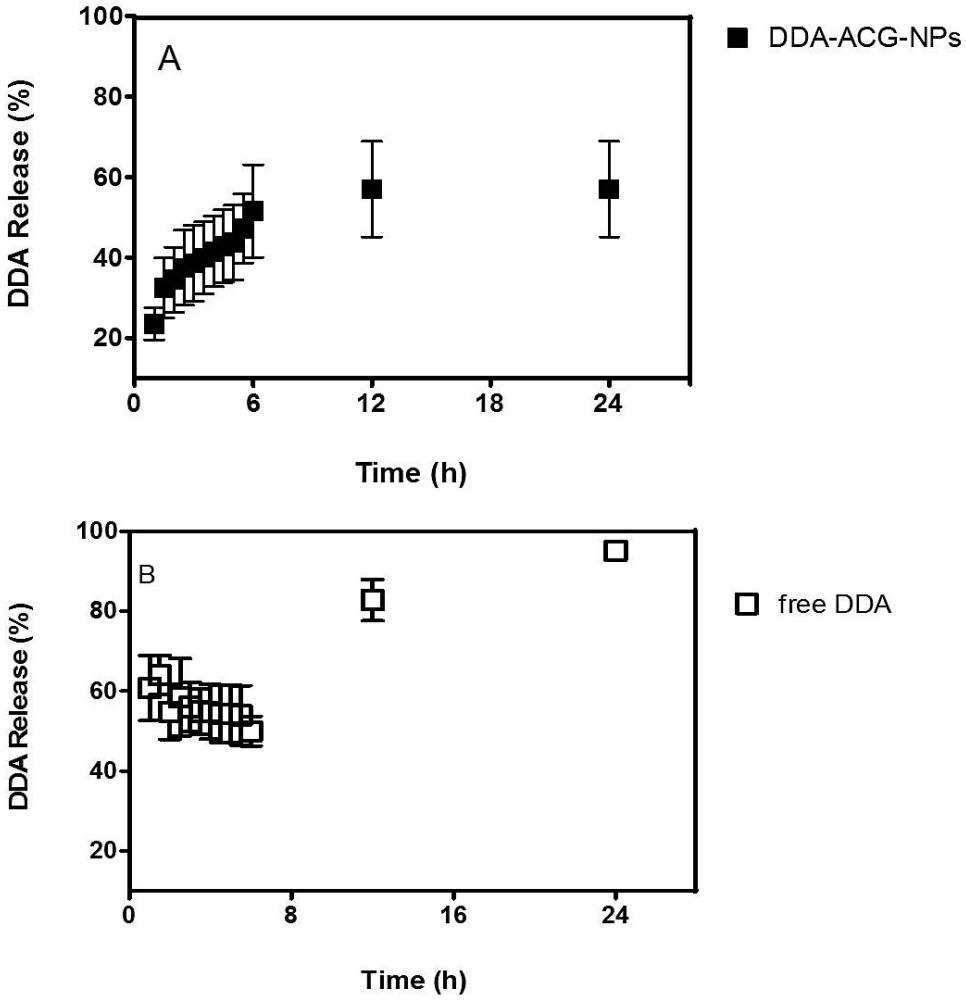

Figure 5.

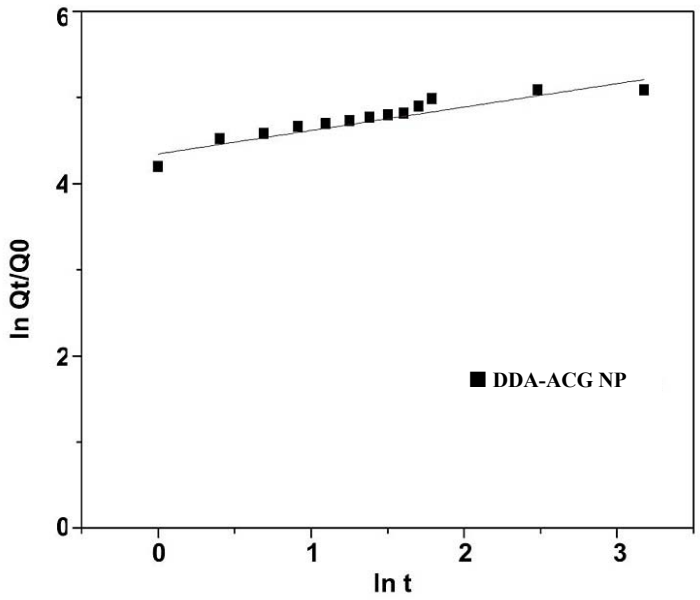

Figure 6

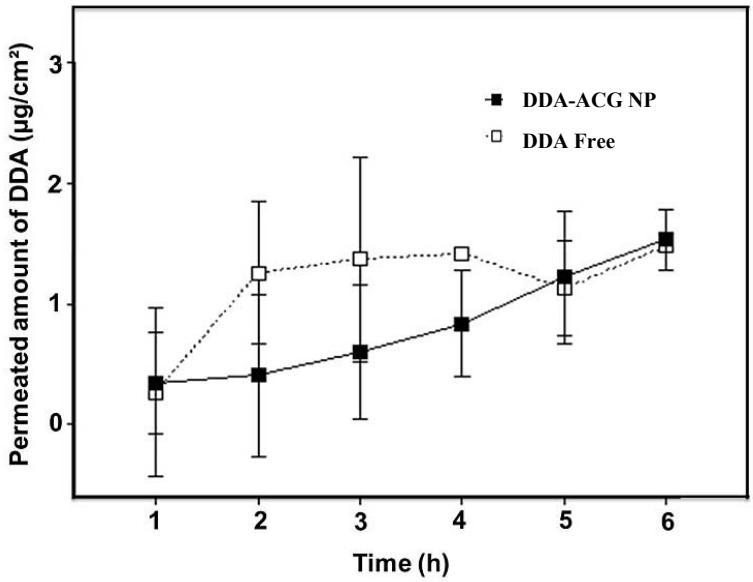

Figure 7