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Hollow polymeric (PLGA) nano capsules synthesized using solvent emulsion evaporation method for enhanced drug encapsulation and release efficiency

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Abstract
Nano-hollow polymer shells, especially those polymers which are FDA approved, have captured the attention of many researchers and scientists in the field of pharmaceutical and medical therapeutics. In the field of controlled drug/gene release, nano-capsules in colloidal solutions, i.e. particles with hollow piths, play an important role in cargo encapsulation. These nanoparticles are synthesized using a variety of procedures such as emulsion polymerization, phase separation, crosslinking of micelles, inner core etching and self-assembly. Our work proposes a novel route to prepare hollow PLGA (poly (lactic-co-glycolic) acid) nanoparticles (HNPs), which showed increased drug-encapsulation and release efficiency. The simple emulsion solvent evaporation technique was adopted to synthesize nano-hollow shells of FDA approved polymer PLGA using only one organic phase. The hollow characteristics of nanoparticles were studied using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal microscopy analysis. The particle size was analyzed by dynamic light scattering (DLS). Nanoparticles drug loading, encapsulation and release efficiency in vitro were assessed by ultraviolet spectroscopy. The developed nanoparticles were hollow and spherical in shape and approximately 80 nm in size. The drug encapsulation efficiency is 99.4% and the drug was released in a controllable manner during in vitro analysis.

Online supplementary data available from stacks.iop.org/MRX/1/045407/mmedia
Keywords: nano hollow PLGA particles, solvent emulsion evaporation method, encapsulation and release efficiency

1. Introduction

Nanotechnology is a field in science which is rapidly flourishing and encouraging the inventions and synthesis of various types of nanomaterials. These nanomaterials have a wide range of applications in the fields of medicine, electronics and pharmaceuticals [1, 2]. Nanomaterials used for therapeutic applications face some challenges, as they need to be biocompatible, biodegradable, cost effective and highly efficient in drug loading. Also, the nanoparticles have to rescue themselves from the enzymatic actions and endosome reactions within the cells before they reach the specific cellular organelles for which they were designed [3]. The development in synthesis of polymeric nanoparticles may be one of the successful attempts carried out in the field of gene, protein and drug delivery. The capacity of these nanoparticles in holding and encapsulating the cargo still remains an insurmountable barrier [4, 5].

Until now micro-hollow particles of polymers were synthesized and used for delivery of drug in cells [6, 7]. Chiang et al synthesized nanoparticles that are incorporated into polymeric micro-hollow particles for pulsatile drug release [8]. These micro particles can carry the cargo, however, there still remains the question of scalable release of specific medicinal moieties inside the cytosol [8]. A major limitation of micro-particles is that they do not have the capacity to intrude through the cytosol and reach specific targets. This barricade could be overcome by usage of highly self-modified biocompatible nanoparticles.

Polymeric nano-particles have replaced micro-particles with relatively greater efficiency in encapsulating and delivery of the drug and gene [10]. However, to date evidence for ‘endosomal escape’ has come either from cells treated with high concentrations of cargo above the therapeutic range or lack of co-localization to the pH-sensitivity [3]. To overcome this limitation many other nanoparticles such as metal nanoparticles [9], silica nanoparticles [11], liposomes using layer-by-layer technique [12], multi-functionalization technique and encapsulation technique were widely adopted. These nanoparticles do show some limitation due to their toxicity and release of cargo material instantaneously [8, 13]. Use of hollow nanoparticles may be an option for conquering these limitations and disadvantages.

Hollow nanoparticles are said to have great efficiency for gene and drug release as compared with non-hollow nanoparticles using targeted moiety due to their tailored porous structured, high cargo loading and encapsulating efficiency, zero order drug release kinetics and material reliability. Even though some hollow polymeric nanoparticles are efficient, not all polymeric nanomaterials synthesized are biodegradable and biocompatible [14].

PLGA with 50:50 molecular weights is typically used in our experiment for synthesis of hollow nanoshells. This polymer has successive monomeric units (of glycolic or lactic acid), which are linked together by ester linkages yielding a linear, amorphous aliphatic polyester product, during polymerization [15]. This polymer when subjected to acidic environment showed controllable erosion and degradation. Also, PLGA is still widely used in single solvent emulsion method giving highest yield of nanoparticles wherein physical parameters such as size, size distribution, morphology, surface modification and zeta potential were controlled successfully [16]. The material used in our experiment was PLGA (poly (lactic-co-glycolic)
acid) which is FDA approved for clinical applications and therapeutic usage. It has excellent quality of biocompatibility and biodegradability too [8].

The various methods used for synthesis of nanoparticles to date include thermal annealing method, sol–gel process, crosslinking polymerization [17], inner core etching method [18, 19] and solvent emulsion method [18]. Of the above, simple single solvent emulsion evaporation method is undeniably the most cost effective method [20]. Therefore, we used this method for the synthesis of hollow PLGA nanoparticles.

Kwon et al have reported the synthesis of PLGA nano half shells using water/oil emulsion solvent method by adding poloxamer in organic phase, which is necessary for production of nano half shells [21]. There are reports on the synthesis of polystyrene colloidal solution of half shells nanoparticles [16, 17]. Due to obvious reasons such as incomplete encapsulation of drug and untimely drug release, nano half shells do not provide the support for gene and drug delivery. The sealing and fusion of the nano half shells still remains a hurdle. Nano shells prove to be superior to nano-half shells in drug and gene delivery etc.

In this paper we report for the first time the synthesis of hollow (PLGA) nanoparticles, which release the drug in a controllable manner and may become a proficient vector to deliver gene and drug in cell organelles. The synthesis of hollow PLGA nanoparticles by single emulsion solvent method gave us an economic method of synthesis with enhanced drug encapsulation and release efficiency.

2. Experimental

2.1. Materials and methods

Poly (lactic-co-glycolic) acid (PLGA; 50:50, Mw 17 000 – 70 000) and Rhodamine 6G (R6G) were purchased from Sigma Aldrich, USA. Polyvinyl alcohol (PVA) and ethyl acetate were purchased from Kato Chemicals, Japan. Paclitaxel was purchased from Wako Ltd. Milli-Q water was used throughout the experiment. The in vitro drug release was carried out at pH 7.4 and 4.2 in phosphate-buffered solution (PBS).

2.2. Hollow nanoparticle synthesis (HNPs)

Hollow nanoparticles were prepared using the oil-in-water single solvent emulsion evaporation method, wherein the organic phase is ethyl acetate and these nanoparticles are dispersed in the PVA aqueous solution for stabilization. 50 mg of PLGA was dissolved in 1 ml of ethyl acetate and was rapidly stirred on a magnetic stirrer for complete dissolution in a beaker. This solution was vortexed for one hour. Concomitantly 5% (w/v) of 2 ml of PVA aqueous solution was prepared with the help of a magnetic stirrer keeping the stirrer temperature at 40 °C to make the solution lukewarm and avoid boiling or overheating. The above vortexed PLGA solution was prepared with the help of a magnetic stirrer keeping the stirrer temperature at 40 °C to make the solution lukewarm and avoid boiling or overheating. The above vortexed PLGA solution was added drop-wise in previously prepared 1 ml of PVA aqueous solution (the remaining 1 ml of PVA solution was cooled down to room temperature) keeping constant vortexing. After the complete addition of PLGA solution in 1 ml of lukewarm PVA solution, the cold 1 ml of PVA solution was added instantly. The vortexing was continued for 5 min, even after the whole addition of the cold PVA solution. After vortexing, this solution was sonicated with energy output of 40 W for 2 min to create a fine emulsion. This mixture was added to 50 ml of 0.05% (w/v) PVA solution to form a colloidal solution. The colloidal solution was stirred using a
magnetic stirrer for complete evaporation of the organic component. Developed nanoshells showed good stabilization when dispersed in PVA solution and preserved at room temperature.

2.3. Non-hollow nanoparticle preparation (non HNPs)

Non-hollow PLGA nanoparticles were prepared by the same emulsion solvent evaporation method using 5 ml of ethyl acetate as organic phase and dissolving 200 mg of PLGA in it. This solution was added to 3% (w/v) of PVA solution and vortexed by keeping the vortexing at maximum to obtain an emulsified solution. This solution was further dispersed in 0.01% of 20 ml of PVA solution to increase the stability of the nanoparticles. The whole colloidal solution was stirred in room temperature for two hours.

The above two colloidal solutions containing HNPs and non-HNPs prepared were centrifuged at 8000 rpm for 10 min to collect the nanoparticles. These collected HNPs and non-HNPs were washed thrice using Milli-Q water; and then dispersed in water and centrifuged at 8000 rpm for 10 min. Nanoparticles were dried and further used for characterization.

2.4. Characterization of nanoparticles

The shape and morphological characters of polymer nanoparticles were analyzed by scanning electron microscope (JSPM 7400 F, JEOL). 10 mg nanoparticles were re-suspended in 100 ml of distilled water and sonicated for 15 min. From the above solution 10 μl drop was placed on a silicon wafer and dried at room temperature to get a uniform layer of particles.

The polymer nanoparticles were also investigated by TEM (JEM 2100, JEOL) operating at 180 kV. The nanoparticles after washing were re-suspended in an equal amount of Milli-Q water and 1–2 μl were dropped on the copper grid coated with carbon film. The sample was dried at room temperature and used for observation. Also, in order to confirm the mean size of the PLGA hollow and non-hollow nanoparticle, particle size characterization based on dynamic light scattering (DLS) was performed using Zeta-sizer.

For confocal microscopy study, freshly prepared hollow nano-particles were immersed into the R6G aqueous solution having dye concentration 10−5 M. Sufficient time was allowed to adsorb the dye molecules onto the polymer nano-particles to make PLGA/R6G nano-complex. 100 μl of this solution was pipetted and dried on a confocal plate for examining the HNPs.

2.5. Drug encapsulation and loading

The drug, paclitaxel, was encapsulated in nanoparticles using emulsion solvent evaporation technique. For the synthesis of non-HNPs and HNPs we have used 200 mg and 50 mg of PLGA, respectively. The specific amount of paclitaxel ranging from 10, 20, 30 to 220 μg ml−1 keeping stages of 10 μg ml−1 in each step were added into ethyl acetate, which was suitably stirred to ensure that all materials were dissolved. The synthesis of HNPs and non-HNPs was carried out, following the same above mentioned procedures. The formed oil-in-water (o/w) emulsion was gently stirred at room temperature with the help of a magnetic stirrer for not less than four hours to evaporate the organic solvent. The colloidal suspension was sonicated for 3 mins and then the nanoparticles were separated at low speed centrifugation (3000 rpm, 5 min). The same procedure was carried out until the maximum amount of paclitaxel was encapsulated. The quantity of drug present is calculated according to UV-absorbance of paclitaxel present in supernatant at 227 nm using UV-Visible spectrometer by determining the quantity of drug [5].
The calculation of determining the concentration of drug was carried out according to the procedure described in [5].

The percentage of drug entrapped was calculated from the amount of incorporated drug in the nano-shell using the following equation:

\[
\text{Drug loading efficiency} = \frac{[\text{Amount of paclitaxel in nanoparticles}]}{[\text{Amount of paclitaxel loaded nanoparticles}]} \times 100 \frac{[\text{Paclitaxel}]}{[\text{Paclitaxel + Polymer}]} \times 100 \ (1)
\]

The drug encapsulation efficiency is calculated according to the following equation:

\[
\text{Drug encapsulation efficiency} = \frac{(\text{Amount of encapsulation paclitaxel})}{(\text{Amount of paclitaxel used for nanoparticles preparation})} \times 100 \ (2)
\]

2.6. Drug release from nanoparticles

For conducting drug release study; 150 μg ml\(^{-1}\) paclitaxel-encapsulated in hollow nanoparticles was dispersed in 0.05% PVA solution and to get nearly mono-dispersed nanoparticles; the prepared nanoparticles were filtered through a syringe using 0.8 μm pore size Whatman filter unit GmbH, Germany. The colloidal solution was filtered to obtain nano-shells that are equal to or less than 800 nm in size. In the case of non-HNPs 53 μg ml\(^{-1}\) paclitaxel-encapsulated particles were used. The above HNPs and non-HNPs were centrifuged at 3000 rpm for 10 mins and dried. The total amount of 25 mg of both HNPs and non-HNPs were collected and dispersed in 25 ml of 7.4 pH PBS buffer solutions each. This solution was further divided in 1 ml aliquots in vials and placed in an orbital shaker kept at 120 rpm min\(^{-1}\), maintained at 37 °C. The vials were taken out of the shaker and centrifuged at 8000 rpm for 10 min and readings were observed initially every two hours until the twelfth hour. After the twelfth hour, the readings were taken after the time interval of twelve hours following the same procedure as above. The experiment was performed in triplicate. The absorbance value of supernatant was taken for analysis of paclitaxel concentration using UV-visible spectroscopy at 227 nm [5]. The drug release was calculated by using the equation:

\[
\% \text{ Drug release} = \frac{[\text{Amount of paclitaxel in supernatant}]}{[\text{Amount of total paclitaxel used for preparation of drug–encapsulated nanoparticles}]} \times 100 \ (3)
\]

The same procedure as above was carried out to compare the drug release of HNPs in different in vitro environments i.e. 7.4 and 4.2 pH. We have compared percentage of drug release to time where the observation was taken till 72 hrs.

3. Results and discussion

The unique feature of selecting 50:50 PLGA polymer are (a) approval by the US FDA for drug delivery and (b) its high efficiency of biodegradability, drug biocompatibility, suitable biodegradation kinetics, brilliant mechanical properties and ease of processing. Even though the
nanoparticles of PLGA are not hollow they have large surface area. They prove to be having
great ability to conjugate with multiple diagnostic and therapeutic agents. Thus, hollow
nanoparticles proved to be more efficient than the existing non-hollow PLGA nanoparticles in
drug releasing and encapsulation activity.

The suggested mechanism for nano-hollow-shell formation may be explained as follows,
and is presented in scheme 1. The key property of PLGA is its trapping ability. It is reported
that PLGA gets diffused to the outer water phase during emulsification after trapping water into
the organic phase on account of its amphiphilic nature. Also, the hydrophilic property of PLGA
may enhance the water-trapping efficiency in the organic phase, which seems to cause fast
solidification at the surface and the creation of the hollow structure, according to the literature
[21, 22]. In our case, there is a slight modification in the procedure of emulsification: during the
synthesis of hollow shells, initially, the organic phase of PLGA and ethyl acetate was subjected
to the highest rate of aeration. The solution was vortexed for an hour, which creates air
entrainment rather than water entrainment (scheme 1(A)) [23]. The organic phase, ethyl acetate
evaporates at the later stage of the experiment (scheme 1(B)). After vortexing, at the same
instant, the surface of the emulsion solidifies quickly depending upon the air entrainment ability
owing to the rapid solvent removal phase, as mentioned in the literature [24]. In this stage, since
ethyl acetate gets evaporated and air was diffused into the inner phase through the thin solidified
film, the empty space left by ethyl acetate evaporation was replaced by thick and cold 5% PVA
until the point at which the two phases are separated and an inner emulsion is created
(scheme 1(C)) with hollow piths in the nanoparticles. Further sonication stabilizes the
nanoparticles and avoids the contact of water with nanoparticles (scheme 1(D)) [25]. This
structure is basically the same as the structure of the simple emulsion method. At this stage the

\[
\text{Scheme 1. The hypothetical representation-mechanism of formation of nano-hollow}
\text{shells.}
\]

incorporation of 0.05% of PVA stabilizes the nanoparticles leaving no passage for water to gush in [21] (scheme 1(E)). The sonication creates more stabilization and also addition of 0.05% PVA prevents particles from bursting out. Hence, instead of formation of nano-half-shells, nano-hollow-shells are created. There remains no space for water and it gets evaporated after the gentle stirring (scheme 1(F)). As the inner phase solidifies, the remaining ethyl acetate and water evaporate from outer space. And finally nano-hollow structures are generated. These HNPs were characterized further using SEM, TEM and confocal microscopy.

3.1. Scanning electron microscopy (SEM)

As seen in figure 1, the nanoparticles formed are spherical in shape. The size of the nanoparticles after the synthesis and before filtering ranged from 30–900 nm. The surface morphology of the particles seemed to be intact with smooth surfaces and the pores were absent.
on the surface, as seen in figure 1. This showed that even though the nanoparticles were washed four times and dispersed in water they were not deformed and yet remained intact with less loss of stabilization. In the images in figure 1 we can observe the presence of some hollow structures too. This was due to over-sonication carried out in water prior to the preparation of the sample for SEM. Over-sonication was carried out to crumble the hollow nanoparticles and capture the hollow characteristic of the nanoparticles.

There are adhered nanoparticles seen in figure 1, which may have happened due to the following two reasons, namely, the sample may have very high quantity of redundant emulsifier and secondly the high temperature of electron beam irradiation melts the nano-shells at a greater rate that makes the nano-hollow-shells get attached and shrink [21, 26]. However, the adhesion did not have any significant impact on dispersal in PBS solution, the nanoparticles were suspended stably in buffer solution.

Overall, nanoparticles could be filtered according to interest and used for further medical applications. Further, we successfully differentiated the characteristic hollow nature of the PLGA nanoparticles using TEM.

3.2. Transmission electron microscopy (TEM)

TEM images of HNPs are shown in Figures 2(A), (B), respectively. It could be observed that HNPs were mostly spherical. In each image nanoparticle the absence of inner cavity can be easily discerned. This hollow feature was confirmed positively in confocal study, as seen in figure 2, inset image. The ring-like structure shown in figure 2-IA depicts the hollow characteristic of nanoparticles. The nanoparticles have uptaken the dye Rhodamine 6G where there is presence of Poly (D, L-lactide-co-glycolide). Hence, it is confirmed that the particles synthesized were hollow in nature. The size of HNPs observed, in TEM, ranges from around 30–200 nm. The mean size distribution of HNPs was further confirmed with the help of DLS analysis, as seen in figure 4. It can be clearly analyzed that mean size diameter of hollow PLGA-NPs is $79 \pm 0.69$ nm. There is free dispersion of nanoparticles in PBS showing highest intensity of smaller sized particles.

Also, when compared with non-hollow PLGA nanoparticles in TEM, synthesized using oil/water emulsion solvent method, we were unable to see the hollow nature, as shown in figures 3(A) and (B). The size of the non-hollow nanoparticles was observed to be ranging from

Figure 2. The TEM images of PLGA-HNPs without inner core. Inset image IA is the confocal microscopy picture, scale bar is 0.2 $\mu$m.
20–70 nm. The DLS graph in figure 4 depicts that the mean size diameter of the non-hollow nanoparticles is $68 \pm 1$ nm.

The elemental composition of the material was determined using EDS and XPS. The material PLGA consists of carbon and oxygen, as seen in figure 5(D). The samples containing HNPs and non-HNPs were dispersed on gold-coated silicon wafer, respectively. In the graphs shown in figures 5(A)–(C), it was observed that there was the presence of carbon and oxygen. Hereby, it could be confirmed that there is the presence of carbon and oxygen in PLGA and the HNPS prepared. There was the presence of gold, as the substrate used consists of gold.

Similarly for analysis of ESCA, XPS (electron spectroscopy for chemical analysis (ESCA)), as shown in figure 6, we identified the chemical species on the surface which was utilized for the confirmation of carbon and oxygen in nanoparticles. The wide spectrum obtained shows the peaks corresponding to carbon (283.5 eV), oxygen (532 eV) and gold (82 eV). For carrying out ESCA the non-HNPs, HNPS and PLGA were spread on Au coated silicon wafer.

According to SEM, TEM and confocal microscopic analysis it was confirmed that the nanoparticles synthesized were hollow in nature and could be further used for drug encapsulation and release study.
Figure 5. The EDS analysis graphs for (A) PLGA material from company compared with the elements present in structure of PLGA given by the company (D). (B) and (C) show the EDS of HNPs and non-HNPs with presence of oxygen and carbon. The presence of Au is due to the gold-coated silicon wafer on which the material was dispersed.

Figure 6. XPS spectra of nanoparticles. XPS spectra of PLGA, PLGA HNPs and PLGA nanoparticles and peaks showing presence of carbon and oxygen.
3.3. Drug encapsulation and release efficiency

Nano-shells being hollow by nature, have the highest capacity to encapsulate drug compared to non-hollow nanoparticles. In our work we used paclitaxel as the drug and carried on the experiment for drug encapsulation and release efficiency studies.

Paclitaxel is widely used as a mitotic inhibitor in cancer chemotherapy. Direct oral incorporation of paclitaxel causes elimination of drug and has many limitations in clinical application owing to its low solubility in water and pharmaceutical solvents [27, 28]. Therefore, polymeric nanoparticles such as PLGA hollow nanoparticles prove advantageous as ideal vectors in delivering the drug safely in targeted cells.

It is reported that the drug encapsulation efficiency of PLGA nanoparticles synthesized through solvent emulsion method is lower as compared to any other method. The drug encapsulation efficiency reported ranges from 30 ∼ 70% [5, 29, 30]. The rate of encapsulation efficiency increases to 90% only if other co-polymers such as poloxamer are used conjointly [30].

As per figure 7 we see the maximum amount of drug encapsulated in nano-shells is 150 μg ml⁻¹ while 53 μg ml⁻¹ of drug gets encapsulated in non-hollow PLGA nanoparticles. Also, the material PLGA as such, used for synthesis was less in the case of nano-shells that are 50 mg. This implied that synthesis of HNPs utilizes less material leading to greater capacity of holding the drug when compared to non-HNPs.

Further, the encapsulation efficiency of the hollow nanoparticles is 99 ± 0.4% and that of non-hollow nanoparticles is 52 ± 0.2% calculated using equation (2). Also, a smaller amount of drug was attached over the surface of HNPs, which is 1 × 10⁻⁴% and 20% in the case of non-HNPs, calculated using equation (1). Therefore, we inferred that being hollow, almost all drug gets encapsulated inside the nanoparticles leaving no drug to get attached over the nanoparticle. The efficiency of drug entrapped in the nanoparticles was increased to 65% as compared to non-hollow nature of nanoparticles. Overall, this phenomenon suggested less wastage of polymer and increased drug entrapment giving enhanced safety of cargo without any damage from external forces.

The hollow nature of HNPs thus secured the drug or the cargo encapsulated. This feature could be used to encapsulate materials such as oligonucleotides, proteins etc. Further, we have done TEM observations for checking the difference in drug encapsulated HNPs, non-drug encapsulated HNPs and drug encapsulated non-HNPs if there are any. In figure 8(A) we can...
observe a shadow region in the inner core of the HNP when compared to the image in figure 2(A). This implies that the drug was encapsulated inside the particle. Also, figure 8(B) depicts the drug encapsulated in non-HNP could hardly be differentiated. However, there was the presence of shadow region in the central core of the particle, which suggested that the drug is present inside the particles.

The drug release was compared between hollow and non-hollow nanoparticles as observed in figure 9. The drug release was observed till 72 h in PBS buffer solution in 7.4 pH. The drug release of the non-hollow nanoparticles was 56% at 12 h and after 12 h it was maintained at 60%, as shown in the graph in figure 9. The drug release observed with hollow nanoparticles was 61% at 10 h and after 10 h the drug release is nearly 70%. It was observed that there is an initial burst release phase and second increased release phase. There is a 5% increase in the drug release of HNPs when compared to non-HNPs in the initial burst phase. Similarly, there was 10% increment in release of drug in the second phase till 72 h. This implied, in the case of non-HNPs, that there was the first phase of paclitaxel release that suggested the controlled dissolution of drug, accessible to the medium-used and the second phase that was after twelve hours, the release of paclitaxel entrapped in polymer. This release was totally dependent on the bulk degradation of polymer. In non-hollow PLGA nanoparticles, the drug encapsulated or here we can say that the drug embedded in the polymer nanoparticles was released with degradation of polymer [31].

In the case of hollow nanoparticles, there was quick surface degradation and maximum drug release. As the polymer PLGA chosen was of equal molecular weight of lactic to glycolic
acid, the degradation of polymer was processed as and when the surface became eroded. As a result the maximum amount of paclitaxel was released. Being hollow, the drug release was pulsatile and nearly maximum. This characteristic difference between the quantity of drug release between HNPs and non-HNPs suggested that HNPs gave quick results of drug delivery within 12 h as compared to non-HNPs.

Further, the drug release of hollow nanoparticles was studied in different *in vitro* environments. The rate of drug release was determined in different pH solutions, (PBS of pH 7.4 and 4.2). The PBS of pH 4.2 was used to mimic the acidic environment found in the endosomes of cells. The amount of drug released in acidic environment is greater than in normal pH, as seen in the graph in figure 10. The percentage of drug release was 69 ± 3% in 7.4 pH while the percentage of drug release in 4.2 pH is 84 ± 2%. The burst release observed in acidic pH is at the fourth hour, while in 7.4 pH it was at the twelfth hour of the experiment. This implied that the matrix erosion of the nanoparticles is fast in acidic medium and the degradation activity starts at the fourth hour. From figure 10 we observed that the drug release remains almost constant after twelve hours. It was found that a small amount of paclitaxel still remained

![Drug Release](image)

**Figure 10.** Paclitaxel released by hollow nanoparticles in PBS buffer solution at pH 7.4 and 4.2, respectively. The drug release in pH 4.2 is greater than in pH 7.4.

![Disintegration of HNPs](image)

**Figure 11.** The disintegration of HNPs after 72 hrs.
inside the particles from the calculations using equation (3). The SEM images showed that the particles disintegrate in buffer solution after 72 h, figure 11. The remaining paclitaxel was released eventually as the nanoparticles were subjected to complete degradation. It can be inferred from the above observation that as time increases, degradation of HNPs also increases giving complete release of drug to the surrounding medium, here in our experiment it is PBS [32].

4. Conclusion

In summary, hollow polymer PLGA colloidal nano-shells were synthesized using simple oil-in-water emulsion solvent technique, which is economic, cost effective, and leads to high yield of good quality nanoparticles. This hollow structure results from subsequent events including air entrapment, fast solidification, and phase separation with instant stabilization. PLGA nano-hollow shells have the capacity to hold the cargo material for a long time wherein the material can remain safe. The cargo material gets released with constant rate. These nano capsules were pH sensitive, inferring that these particles could be used for drug or gene delivery at proper specific time delivery. Also, as these hollow shells are nanostructures with low densities and large surface area, they may be used as carriers for drug and gene delivery for anticancer treatment.

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