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Preparation of Nanomaterials for Drug Delivery System via Microemulsion Polymerisation Method

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Abstract

Microemulsion polymerisation method was employed in an initial attempt to prepare poly (alkylcyanoacrylate) nanomaterials. In this work,a region of water-in-oil microemulsion stabilised by non-ionic surfactant was identified and used as a templatefor the preparation of poly (alkylcyanoacrylate) nanomaterial. The non-ionic surfactants used were polyoxyetehylene (20) sorbitan monooleate (Tween 80) and Sorbitan monolaurate (Span 20). Monomers were added into the microemulsion template in order to obtain nanomaterial. The key physical properties of the materials were characterized employing standard techniques such as HPLC, FTIR, DLS, SEM and the *in vitro* release of insulin by dissolution method.Results from the FTIR showed that the poly (alkylcyanoacrylate) nanomaterials were successfully prepared and that the polymerisation of poly (alkylcyanoacrylate) nanomaterial was completed. The morphologies observed using SEM showed a porous honeycomb structure indicative of a bicontinuous type microemulsion. The size of the microemulsion template were found to be more than 300 nm which in turn resulted in the production of nanomaterials with particle size of more than 140 nm. Finally, from the dissolution studies, the nanomaterials was able to entrap a good amount of insulin but were unstable at the pH studied which mimic the gastrointestinal (GI) tract condition.

Keywords: microemulsion; nanomaterials, polymerisation; drug delivery; insulin

INTRODUCTION

Materials that are downsized into smaller components exhibit different properties from their bulk properties. A new group of materials or particles is encountered when the sizes are in the range of nano-scale. The interesting mesoscopic properties of this group of material have opened up a new frontier in science and especially in the field of nanomaterials. Nanoparticles formed the largest part of the nanomaterials group and can be made from many available materials with at least one dimension below 100 nm. The potential applications of nanomaterials of nanoparticles are enormous. They include in the fields of electronic, biomedical, health care and agriculture (Tsuzuki, 2009). Various approaches have been developedto prepare them such as via high-temperature decomposition (Jeong*et al.*, 2007; Redl *et al.*, 2004; Sun *et al.*, 2002; 2004), sonochemical method (Dai *et al.*, 2013; Vijayakumar*et al.*, 2000) bulk co-precipitation (Massart, 1981; Kim *et al.*, 2001; and Harris *et al.*, 2003). The chemical co-precipitation method beingthemost conventional method(Massart, 1981; and Massart *et al.*, 1995).A recent green approach to synthesise magnetic nanocrystals has also been reported (Chiu et al., 2014). Another development also showed that the preparation of nanoparticles by using a compartmentalised surfactant systems namely water-in-oil microemulsions exhibited promising results and especially whereby the particle size can be easily controlled (Liu et al, 2004).

Microemulsions are colloidal dispersions of eitherwater-in-oil and oil-in-water stabilised by amphiphiles (surfactants). They are transparent, homogenous, and isotropic dispersions. Microemulsions were brought to the attention of the scientific community beginning in the late 1940's by Schulman and a series of collaborators (Hoar & Schulman, 1943; Schulman *et al*., 1948, 1949). Since then numerous attempts have been made to investigate various aspects of microemulsions from the treatment of microemulsions as colloidal systems (Adamson, 1969; Ahmad *et al.*, 1974) to more theoretical contributions (Reiss, 1975). They can be usedto carry out chemical reactions and, in particular, tosynthesise nanomaterials. One such example is microemulsion polymerisation. Due to their small anduniform droplet size, theycan form uniform nanomaterialsvia polymerisation processes (Watnasirichaikulet al 2000).

With that note, our previous work reported the phase behaviour of a stable W/O microemulsion regions formed by non-ionic surfactants (Laili & Hamdan, 2015). Now, we like to prepare poly (alkycynoacrylate) nanomaterialsvia microemulsion polymerisation using the reported biocompatible non-ionic surfactants systems. Our main interest isnot only in its potential to incorporate biological moleculesinto the aqueous phase but also its retention behaviour. It is also well known that non-ionic

surfactants with the right combination of water and oil produce microemulsion regions without the need of using co-surfactant in some cases which would probably be beneficial to minimise protein denaturation in such systems compared to many ionic surfactant that requires cosurfactant. Thusmain objective of this work is to prepare poly (alkycynoacrylate) nanoparticlesusing microemulsion as a template. Secondly, to incorporate of a model protein drug (insulin) into the system and assess its encapsulation and release behaviour.

MATERIAL AND METHODS

Chemicals

The non-ionic surfactants, polyoxyetehylene (20) sorbitan monooleate (Tween 80) (95%) (HLB 15.0); Sorbitan monolaurate (Span 20)(>95%) (HLB 8.5) were purchased from Sigma-Aldrich. 1-butanol, ethyl oleate and insulin were purchased from Sigma-Aldrich. All components were used as received without further purification. The water used throughout the study was double distilled.

Preparation of poly (ethyl 2-cyanoacrylate) PECA nanomaterialsby interfacialW/O microemulsion

The PECA nanomaterials were prepared following the method used by Watnasirichaikul and co-workers (2000). 200 mg of ethyl 2-cyanoacrylate monomer is dissolved in 600 mg of chloroform. The mixture was then mixed with 10 ml of selected microemulsion template and mechanically stirred at 700 rpm overnight at 4 ^{o}Cfor the polymerisation process to take place. The nanomaterials were then isolated from the microemulsion medium by repeated washing in ethanol. The nanomaterials were centrifugated to remove residual oil and surfactant and were then freeze-dried for 48 hours. The mechanism referring to the polymerisation is shown in Figure 1(Yordanov and Bedzhova, 2011).

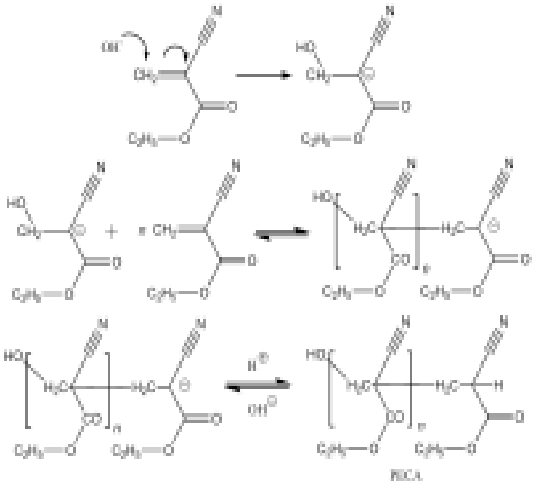

Figure 1: The mechanism of poly (ethyl 2-cyanoacrylate) PECA polymerisation (Yordanov and Bedzhova, 2011).

Preparation of Poly (ethyl 2-cyanoacrylate) Nanomaterials Containing Insulin

In this work, the nanomaterials containing insulin were prepared following the same method as above but, for this work, the aqueous component of the microemulsion template was replaced with an aqueous solution of insulin having a concentration 100 units/ml and a pH 7.4.

Dynamic Light Scattering Measurements

2 ml of selected microemulsion samples were prepared and kept in the water bath at 25^{o}C overnight to equilibrate. A Zetasizer Nano Series (Malvern Instruments, Worcestershire, UK) set at 25^{o}C were used to measure the droplet size. The measurement were made in triplicate.

Scanning Electron Microscope

All the samples, after being properly cleaned, rinsed, dehydrated, and dried were coated with gold (thickness of 15nm) by a Polaran SC7640 sputter gold coater (Quorum Technologies) at an accelerating voltage of 2.2 kV prior to imaging. Care was taken by using disposable gloves and tweezers to prevent unwanted deposits. The sample wassprinkled on a carbon tape mounted on an aluminium SEM stub. After coating, the sample specimens were imaged in the high vacuum sample chamber equipped with electron optic column and electronics console. The SEM instrument employed was a JEOL JSM - 5900LV Scanning Electron Microscope (JEOL Ltd, Japan) fitted with a Tungsten filament.

Assessing Level of Insulin Entrapment

A dispersion consisting of 1.6 g of the polymerised insulin microemulsion was diluted with 10 ml of water. The pH is adjusted to pH 2.5 using hydrochloric acid. 300 µl of this dispersion were mixed with 300 µL of solution containing methanol and water at 80:20 (v:v) at pH 2.5. The mixture was then centrifuged. The supernatant was then injected into the HPLC equipment using a C18 column (Luna 5µm C18 (2), 150 mm x 3.0 mm; Phenomenex). The mobile phase used contained a mixture of acetonitrile and sodium dihydrogen phosphate at a weight ratio of 23.5 % by weight. The pH was adjusted to 2.5 using orthophosphoric acid. The column was maintained at 50^{o}C and the flow rate at 0.5 ml/min. The eluent was monitored at a wavelength of 214 nm. The percent entrapment of insulin was calculated from the difference between the total amount of insulin added to the polymerisation template and the untrapped amount measured in the supernatant.

In Vitro Release Study

A previouslyreported method to determine*in vitro* release of insulin was adopted in this work (Watnasirichaikul *et al.*, 2002). The release of insulin from the polymerised material was carried out by diluting 63.0 mg of dry polymer with encapsulated

insulin to 20 ml with PBS (pH 6.8) which was subsequently stirred at 50 rev/min in a water bath (37^{o}C). Samples of 200 ul were removed at various times and released insulin was analysed by HPLC as described above for the determination of encapsulated insulin. The release was monitored for 8 h at this pH. Release was also studied as described above at pH 1.2 (mimicking the GI tract condition) for 2 hours.

RESULTS AND DISCUSSSION

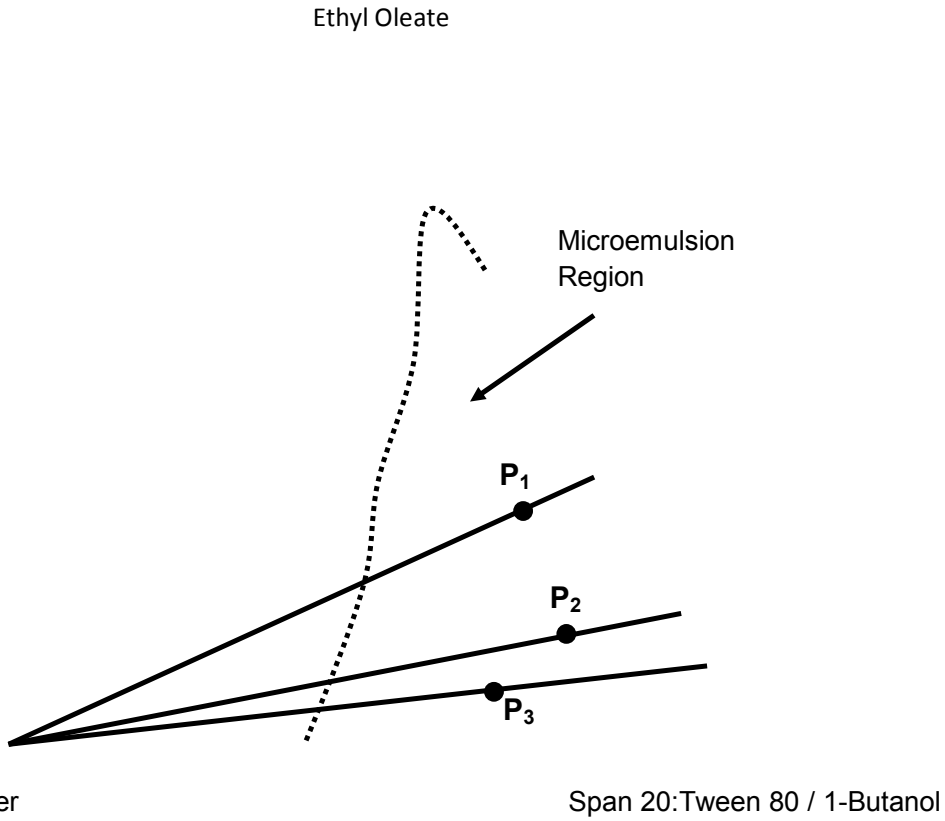

Figure 2 The points P_1, P_2, and P_3 selected as templates for interfacial cyanoacrylate polymerisation.

Figure 2 shows the location of the W/O microemulsion as reported earlier (Laili & Hamdan, 2015). The compositions of the microemulsion chosen as templates are shown in Table 1.

Table 1 The compositions of selected points P_1, P_2 and P_3.

	Percent by Weight		
	Water	Mix Surfactant:1-Butanol	Ethyl Oleate
P_1	13	52	35
P_2	16	66	18
P_3	30	62	8

Figure 3 shows the FTIR spectrum of the prepared poly (ethyl 2-cyanoacrylate) nanocapsules after washing and freeze drying.

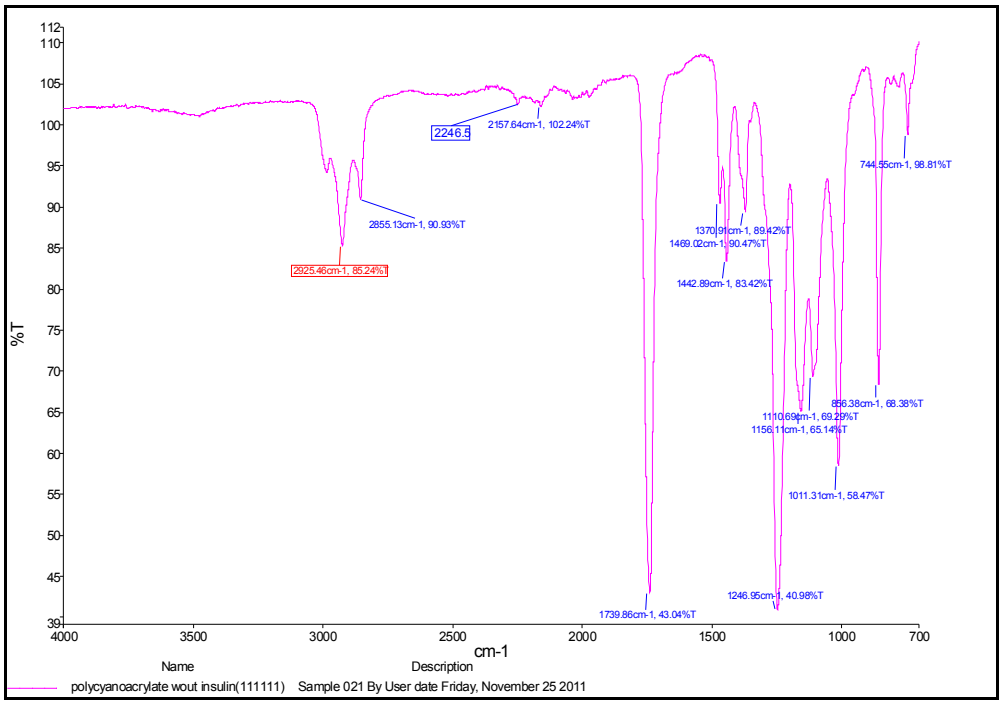

Figure 3 FTIR spectrum of poly (ethyl 2-cyanoacrylate).

The observed FTIR spectrum is similar to those reported in the literature for this material (Ariasa *et al.*, 2001; Han *et al.*, 2008). It shows the typical absorption peaks for the carbonyl C=O ester (1739 cm^{-1}), C≡N groups (weak, 2246 cm^{-1}) and C-H (2855-2988 cm^{-1}). The rest of the peaks observed at lower wavenumbers were due to C-H bending (1370-1669 cm^{-1}) and the stretching and bending of the C-CO-C group (1110-1246 cm^{-1}). One striking observation was the absence of prominent absorption peaks at around 1600-1650 cm^{-1}. These peaks would be due to the presence of C=C functional group. This is satisfying because this functional group is only observed in the ethyl 2-cyanoacrylate monomer and disappears in the poly (ethyl - 2-cyanoacrylate) due to the formation of polymer chain as reported by Han and co-workers (2008).From the FTIR results, it may therefore be concluded that the polymerisation of ethyl - 2-cyanoacrylate was completed.

Figure 4 shows the morphologies of the prepared nanomaterials at points P_1, P_2, and P_3. All of the three compositions showed a porous honeycomb structure indicative of a bicontinuous type of microemulsion rather than a high yield of discrete nanocapsules, although some nanoscale product was also evident. The size presented by intensity percent for the microemulsion template and the prepared nanomaterials at different compositions P_1, P_2 and P_3 is tabulated as shown in Table 2.

From the table, the size of the microemulsion template measured by DLS was found to be more than 300 nm. This again suggested that a bicontinuous type of microemulsions was formed. The size of the prepared nanocapsules was found to be large (> 140 nm). The value of 140 nm is close to the reported value of 150 nm (Watnasirichaikul *et al.*, 2000) using Crillet 4 and Crill 4 as the non-ionic surfactants. The observed value is however smaller compared to the reported average value of 250 nm (Krauel *et al.*, 2005) using Crill 1 and Crillet 4 super. This may be due to different non-ionic surfactants used namely Crill 1, Crill 4, Crillet 4 and Crillet 4 super. In this work, Span 20 and Tween 80 were used. It is known that non-ionic surfactants are sensitive to temperature. Therefore, the differences may be attributed to a difference in temperature at which the samples were measured.

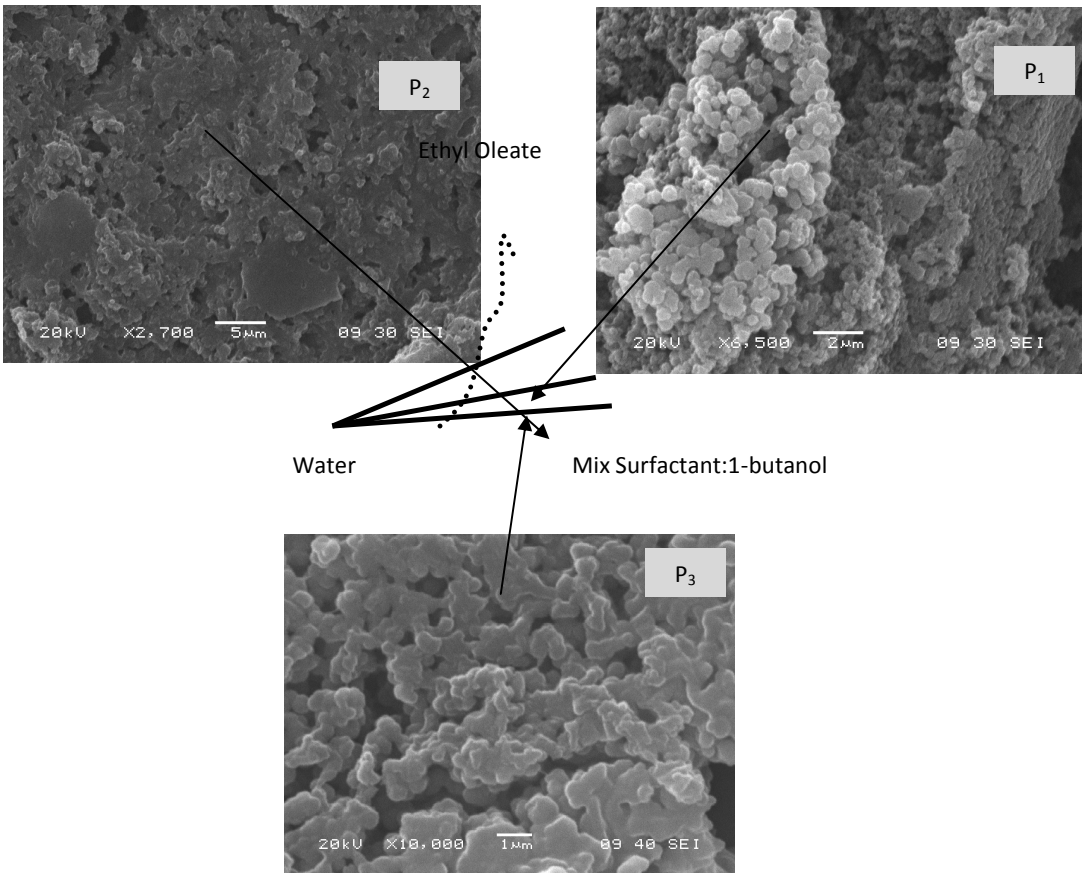

Figure 4 SEM images of nanocapsules at compositions P_1, P_2 and P_3 showing porous structures at magnifications 6500, 2700 and 10000x, respectively.

Table 2 The mean diameter, in nm, of the templates and nanomaterials from DLS measurement extracted from the scattering data using intensity calculation from the Malvern software. S.D. = standard deviation.

Compositions	Microemulsion Size (nm)	S.D.	Nanocapsules Size (nm)	S.D.
P1	393	5.4	147	4.2
P2	381	0.5	190	0.9
P3	336	2.3	178	2.7

Characterisation of poly (ethyl 2-cyanoacrylate) nanomaterials with insulin

The same composition at point P_1 (Figure 2) was chosen to prepare nanomaterials with insulin. From the DLS measurement, the size of the nanomaterials with insulin was found to be 178 nm. To determine the amount of insulin entrapped in the nanomaterials, reversed phase HPLC was carried out on the combined washings from the synthesis. Figure 5 shows the chromatogram for insulin in the supernatant (free drug). From the HPLC study, the concentration of insulin in the supernatant was found from the area of the peak at the retention time 4.33 min.

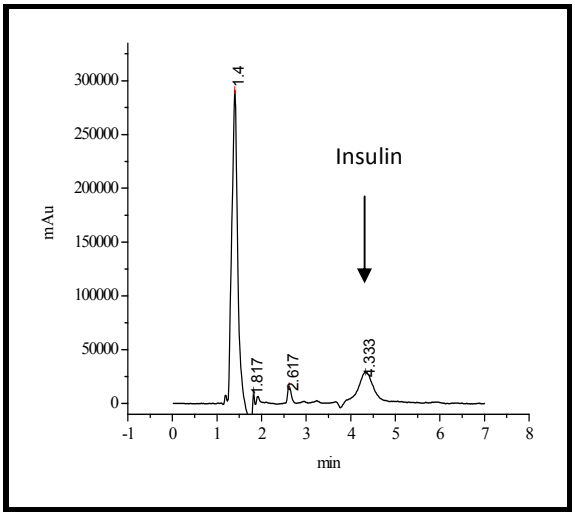

Figure 5 HPLC chromatogram for insulin in the supernatant separated by C18(2) column at an elution rate of 0.5 ml/min, 50°C, 214 nm.

Using the calibration graph for pure insulin (Figure 6), by using this formula:

$$\% \text{ Insulin Entrapped} = \frac{(\text{Total Drug} - \text{Free Drug})}{\text{Total Drug}} \times 100\%$$

it was found that more than 85 % of the insulin was entrapped in the polymer during synthesis.

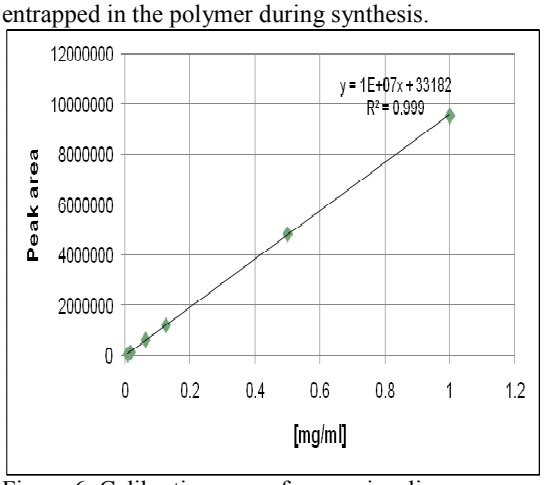

Figure 6: Calibration curve for pure insulin

Figure 7 a andb shows the percent drug release of insulin entrapped in the cyanoacrylate polymer at pH values of 1.2 and 6.8, respectively. The result showed that the percent of drug release reached more than 50 % after 30 min elapsed in the pH 1.2 medium, while a release of more than 80 % entrapped insulin in less than 60 min for the pH 6.8 medium.

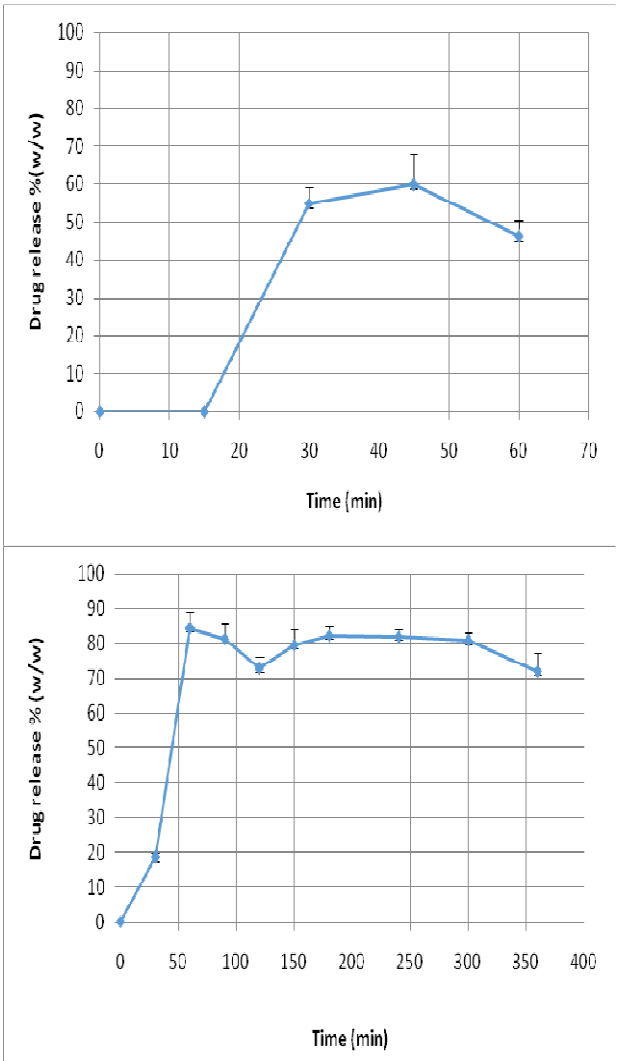

Figure 7 The drug release curve for insulin entrapped in the cyanoacrylate polymer measured at 37 °Cin (a) pH 1.2 and (b) PBS buffer (pH 6.8). The values represent mean ± S.D. in triplicate measurements.

CONCLUSION

As a summary, poly (alkylcyanoacrylate) nanomaterial were successfully prepared, with the production being of large size and a large amount of nanostructured honeycomb-type material. The results also suggested that while the polymer was able to entrap a good amount of insulin, they were not able to prolong the release of drug for long at both pHs studied that mimic the GI conditions. The polymer was quite unstable, even under these mild conditions, and degraded to liberate the insulin. It is likely that this was greatly assisted by the honeycomb nanostructuring of materials produced. This might have some future interest for drug delivery applications, even though it was not the discrete nanocapsule structure sought in this work.

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