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Swelling Kinetics of Poly (N-isopropylacrylamide)-based Hydrogel and its Application as Anti-diabetic Drugs Delivery System

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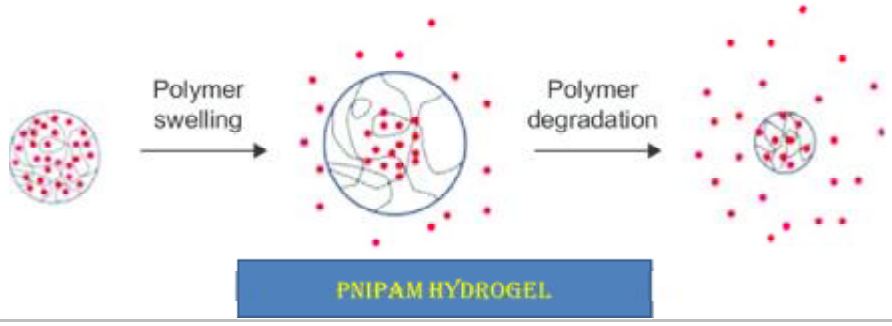
ABSTRACT

The swelling kinetics of charantin from poly (N-isopropyl acrylamide), PNIPAM–based hydrogel, is accomplished through direct weighing, before soaking the gel in fluid/drug solution at controlled temperatures inside a temperature-controlled water bath. The investigation was carried out for application as anti-diabetic drug delivery system. Charantinwas extracted from bitter melon fruit and leaf under a controlled pH of 4.5. The preparation of PNIPAM was done by varying the acrylamide between (3-12)% and it showed an increase in the lower critical solution temperature (LCST) from 32-43 ℃ indicating a direct relationship in distilled water. The effect of changing solution from distilled water, insulin,andcharantin solution, shows that the higher the polarity of the solution, the lower the LCST of the hydrogel. Charantin loaded on hydrogels N1, N2, N3 and N4 were found to be (42.51, 44.57, 43.55 and 44.61) µg/mL, respectively, when soaked in charantin solution of 52.61 µg/mL. Characterization of the hydrogels using Fourier transformed infrared (FTIR) spectroscopy shows that there is physical interaction between the hydrogel matrix and the charantin molecules. The diffusivity of the hydrogels ranged from 1.48 x 10^{-10} to 5.08 x 10^{-8} M2/s andtheir release exponentswere ≥ 0.5 indicative of non-Fickian difusional release.

GRAPHICAL ABSTRACT



Introduction

Controlled drug delivery systems can include the maintenance of drug level within the desired range, the need for smaller quantity during administrations, improved patient compliance, and ideal usage of the prescribed drug. Previousliterature alreadydescribed controlled drug delivery systems as forms of the drug that are prepared and suitedfor all kindsof administration and where the mechanism of prolonged action is inherent and completely determined by the delivery system itself. Meanwhile, the release of these drugs is accurately controlled and can be targeted to an organ or specific sites in the body. Controlled drug delivery happens when a polymer (natural or synthetic) is carefully combined with an active agent or drug in a manner that the active ingredient (agent) is released from the material in a predesigned way. The release of this active agent may be continuous over a long period; hence it could be cyclic for a long period or be triggered by external events such as the surroundings. Drug release occurs from a delivery system by diffusion, degradation or swelling followed by diffusion in a given drug delivery system; this release may occur through one or all of this mechanism.[1]The hydrogel is a permanent or chemical gel-stabilized by the covalently cross-linked network. They retain the solid properties[2] and are made up ofbiocompatible hydrophilic networks that can be constructed fromboth natural and synthetic materials. Examples include photo cross-linkable polymers, poly (amidomines), poly (L-lactide)-poly (ethylene oxide)-poly (l-lantide) (pla-peo-pla) gellan gum, hyaluronic acid, and calcium alginate.[3] Hydrogels swell due to their ability to holda large amount of water can

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also go throughchanges in shape or volume concerning physical or biological conditions such as pH, ionic concentration, temperature, or specific antigens.[4, 5] Hydrogels have great potential in the variousapplication as a result of theirinjectibility, biodegradability, relatively low cytotoxicity, and mucu-adhesiveness and turntable bio-adhesive properties. Hence the materials become more attractive for drug delivery to specific sites in the bodyand tissue regeneration.[6]

Charantin is a typical cucurbitane-type triterpenoid in medicinal chemistry and is a potential constituent with anti-diabetic properties.[7] It is a mixture of two compounds, namely, sitosterylglucoside and stigmasterylglucoside. It was demonstrated that charantin could be used in the treatment of diabetes and as a potential replace treatment.[8] Charantinhas been extracted from bitter melon fruits estimated by high-performance thin layer chromatographic method.[9] Two of the constituents found incharantin produce notable changes in blood sugar level. Thisindicates that charantin may contain some other specific components that have hypoglycemic activity in diabetes.[10] Poly (N-isopropyl acrylamide), PNIPAM-based hydrogels have been used for drug delivery in the treatment for diabetes. It has also been used to form microgels impregnated with insulin, wherein a dried polymer gel was allowed to re-hydrate in a solution of the desired material that needed to be loaded was utilized to encompass the insulin within the network of the hydrogel. It was found that this "breathing in" techniques is better at encapsulating the insulin when compared with the traditional encapsulation method in which the gelis already swelled when brought in contact with the material to be loaded.

Further, stages of insulin release were found to be functions of thermal changes, whichis important to note if the PNIPAM gel isto be used in future drug delivery systems. In a separate study, core-shellmicrogels with degradable PNIPAM as the core and non-degradable phenylboronic acid (PBA)–conjugated PNIPAM (PNIPAM-PBA) as the shell was synthesized.[11] It was reported that at room temperature, the degraded polymer segments diffused freely out of the precursor poly (N-isopropyl acrylamide-co-acrylic acid) gel shells in water.

In contrast, the PBA–modified PNIPAM- PBA nanoshells could hold most of the degraded core polymer chains under the same conditions due to its condensed structure at the collapsed state. Lowering the temperature or increasing the pH increased the swelling degree of the PNIPAM–PBA shell, which provided a method to control its permeability by temperature and pH. In this study, charantinhave been extracted from the fruits and leaves of bitter melon and loaded same into PNIPAM. We have also determined the swelling and release behaviors of the charantin-loaded hydrogels for practical applications.

Materials and Method

Sample collection

The leaves and fruits of bitter melon *(Momordicacharantin)* were collected fromSangere in Gerei Local Government Area, Adamawa State, Nigeria, and were identified by a plant taxonomist at the Department of Botany, ModibboAdama University of Technology, Yola, Adamawa State, Nigeria. The leaves and fruits were brought to the laboratory and rinsed with water to remove the soil particles.

Sample preparation

The cleaning of the leaves and fruits of the bitter melon were done using distilled water before cutting them into small pieces and then oven dried at 50 °C for 24 hours. After which the dried sample waspulverized into a fine powder using a grinding machine, the sample was then stored at a temperature of 4 °C until it was ready for use. The leave fragments were further ground using mortar and pestle. The dry weight (W/W) was obtained bydrying the subsample at a temperature of 50 °C and then reweighed.

Extraction and purification of charantin

About 100 g of the fruit samples and the leaves were soaked in water/ethanol solvent (50:50 v/v) for 72 hours. The pH of the mixtures was adjusted using a buffer solution. The extract was cooled in a coil immersed in a water bath,and then the extract was collected and then evaporated under vacuum to remove the water/ethanol solvent. The mixtures were run through a column using silica gel to separate the charantin, which was further identified using a High-Performance Liquid Chromatographywith insulin standards. The concentration level of charantinwas determined with the help of a UV-visible spectrophotometer at 734 nm.

The crude extractwas purified using the method described in the literature. 5 mL of 50:50 (v/v) methanol-water were added to the crude extract. The mixture was then sonicated for 15 min and centrifuged at 8500 rpm for 15 min to separate the supernatant from the precipitate. The precipitate was then addedto 5 mL of 70:30 (v/v) methanol-water and the mixture was again sonicated and centrifuged. The precipitates from this step were addedto 3 mL of hexane,and the steps were repeated. The precipitate from this step was re-dissolved in 200 mL of 1:1 (v/v) chloroform-methanol mixture and then adjusted to volume with methanol (to 1 mL volume for extracts obtained with pressure liquid extractor (PLE) and to 2 mL volume for that obtained with Soxhlet extraction). The purified solutions were filtered through a 0.45 m nylon membrane filter (Millipore, USA). All the precipitates were collected for analysis.

Preparation of PNIPAM-based hydrogels

Materials used in the synthesis of PNIPAM gelwere blended into powder.Free radical polymerization was used in the preparation of PNIPAM-based hydrogels were preparedby. The concentration of ammonium persulfate (APS) was 1.91 mol% while that of methylenebisacrylamide (MBA) 1.15 mol%.The equation below shows the amount of cross-linkedmethylenebisacrylamide (MBA) obtained.

$$\frac{\left(\frac{M_{MBA}}{M_{W(MBA)}}\right)}{\left(\frac{M_{NIPA}}{M_{W(NIPA)}}\right)} x\ 100\% = mol\ \% \qquad [1]$$

From the equation, $\mathbf{M_{w\ (NIPA)}}$ = molecular weight of the monomer, NIPA $\left(113\ \frac{g}{mol}\right)$, $\mathbf{M_{NIPA}}$ = the initial amount of the monomer (0.87 g), $\mathbf{M_{w\ (MBA)}}$ = the molecular weight of the

cross-linker, **MBA** $\left(154\ \frac{g}{mol}\right)$ and M_{MBA} is the unknown amount of MBA (g) to be used in the gel polymerization.

The mole% of MBA was 1.15 %. The calculated amount of the MBA based on the 0.87 g of N-isopropylacrylamide (NIPA) monomer from equation (1) was found to be 0.0136 g. Furthermore, the amount of APS was obtainedby replacing MBA in equation (1) with APS. PNIPAM-based homopolymer, denoted by gel code A, was prepared by mixing 0.87 g of NIPA, 0.0136 g of MBA, and 0.0335 g of APS. The samples were then dissolved using 7.8 mL of distilled water, before been agitated vigorously until a homogenous mixture is obtained at 4-9 ˚C. The mixing process was exothermic hence the solution was then immersed in ice and sonicated, while nitrogen gas was bubbled through for 20 minutes at 10 bars pressure to remove all dissolved oxygen. The homopolymerwas initiated with 15 µL of N, N, N′, N′-Tetramethylethylenediamine (TEMED). The solution was then gentlyswirled for 5 seconds.

The solution was later poured into 5 mm diameter cylindrical molds that were opened to terminate the free radical polymerization. The samples inside the cylindrical molds were then placed in a water bath at a temperature of 24 ˚C for 12 hours in other to strengthen the polymerized gels. The samples were then washed with deionized water 10 times in other to remove any form of chemical residue. The resulting wet gels were then cut into discs and cylindrical samples with both the diameters and heights at 5 mm. These resultant samples were then soaked in deionized water, with the deionized water changed continuouslyfor two weeks. The resultantsamples were finally removed from the deionized water, subsequently dried in a laboratory environment (29 ˚C) to remove moisture.

Co-polymer hydrogels of PNIPAM-based were also prepared using the same procedure for the fabrication of the PNIPAM homo-polymer. However, co-monomer species Acrylamide (AM) and Butyl Methylacrylate (BMA) were copolymerized with PNIPAM to form copolymer gels. Samples of PNIPAM co-polymer hydrogels which contained about 5 or 10 mol% of Butyl Methylacrylate (BMA) were initiatedwith 5 and 10 µL of Tetramethylethylenediamine (TEMED), respectively, while gels that contained 5, 10 and 15 mol% of Acrylamide (AM) were initiated with 20, 30, 40 µL of Tetramethylethylenediamine (TEMED) respectively. TEMED being added in a controlled way was to help reduce the turbidity of the hydrogels and such necessary in the production ofhydrogels transparent.

Since the transport characteristics of conventional PNIPAM-based gels are mostly dependent on the morphologies of the gels inner matrices, the gel microstructures were studied using scanning electron microscopy.

Table 1: Materials Used for the Gel Preparation and Their corresponding functions in Gel Polymerization.

Gel materials and the grade of reagent	Active functions in Polymerization of PNIPAM-based Gel
N-Isopropylacrylamide (NIPA), 97 %	Monomer
N,N,N′,N′-Tetramethylethylenediamine (TEMED), 99 %	Catalytic agent alters polymerization rate of used with APS
N,N′-Methylene bisacrylamide (MBA), 99 %	Cross-linker
Ammonium persulfate (APS), 98 %	initiating radical
Acrylamide (AM), 99.8 %	Hydrophilic Compound
Butyl Methylacrylate, 98 %	Hydrophobic Compound

Table 2. The Composition of the Gel Formed is as Presented in table 3.3.1 and it's Configuration.

Gel Code	P(NIPA) (mol%)	BMA (mol%)	AM (mol%)	APS (g)	MBA (mg)	TEMED (µL)
N1	100	-	-	0.034	0.014	5
N2	97	-	3	0.034	0.014	10
N3	94	10	6	0.034	0.014	15
N4	91	10	9	0.034	0.014	5
N5	88	5	12	0.034	0.014	10
N6	97	-	3	0.034	0.014	15
N7	94	5	6	0.034	0.014	5
N8	91	10	9	0.034	0.014	10

P(NIPA)- Isopropylacrylamide, Am- Acrylamide, BMA- Butylacrylamide, APS Ammonium persulfate, TEMED- N,N, N′,N′-tetra Methylehylenediame.

Lower critical solution temperature (LCST) determination

Modulated differential scanning calorimeter (DSC)-TA Instruments 2920 was used in obtaining the lower critical solution temperature (LCST) values of the gels.To attain equilibrium, Gel samples were first immersed in their respective media such as charantin solution and de-ionized water for a minimum of 24 hours. To simulate release processes and charantin loading, charantin-loaded gels been released and taken after 48 hours of in vitro studies, while the control gels were first immersed in de-ionized water before been transferred to phosphate buffer solution (PBS). Using deionized water heating at 38 °C/min and temperature ranging from 15 to 55 °C under a dry nitrogen atmosphere (flow rate: 40 mL/min) thermal analyses of these gels were carried.All statistical analyses were conducted using Sigma Plot for Windows Version 2.03 (SPSS). Each experiment was carried out at least two more times to attain a higher degree of precision and accuracy.

Charantin loading

The vacuum-dried gels were immersed in a charantin solution for 4 days at a temperature of4 ^{o}C and at a fixed concentration. The studies show the maximum loading of charantin within 4 days. The total charantin loaded was determined by the mass change of charantin in the solution before and after loading. Each loading experiment was carried out in triplicates. The charantin content in the loading solution was analyzed using high-performance liquid chromatography (HPLC, Waters 2690D). A Zorbax GF250 column (4.6 mm 25 cm, Agilent) was used as the separation column for carrying out theBSA analysis, with the column temperature set at 25 ^{o}C. The mobile phase flow rate (PBS, pH 7.4) was at 1.0 mL/min while the UV detection was set at 210 nm. For charantin analysis, a Symmetry 300 C_4 column (3.9 mm 15 cm, Waters) was employed. The column temperature was set at 25 ^{o}C. 0.1% trifluroacetic acid (TFA) in water with 0.1% TFA in acetonitrile. Was used in preparing the mobile phases. The separation was performed in a gradient mode, in which the volume ratio of 0.1% TFA in water to 0.1% TFA in acetonitrile was changed from 75:25 to 68:32 within 15 min. The flow rate of the mobile phase was 1.0 mL/min. The UV detection was set at 220 nm as describedabove.
The experimental protein loading was obtained from the equation below:

$$\text{Experimental Drug\%} = \frac{\text{Mass of Drug Loaded}}{\text{Mass of loaded drug} + \text{Mass of dry gel}} \quad (2)$$

From Equation (2) **t** is defined as the volume (mL) of charantin solution absorbed per mg of the dry gel during charantin loading, and C is the concentration of the charantin loading solution (mg/m).
Equation (2) was based on the following assumptions:

1. All the charantinwere dissolved in the water in the gel.
2. The charantin concentration in the gel was equal to that of the external charantin loading solution.
3. There was no charantin interaction with the polymer matrix.

Drug/fluid concentrations

Samplesof BB and Charantin were each accurately weighed to 0.1g. This 0.1 g ofcharantinwas at first dissolved with 2 mL methanol (100%) giving a stock solution of 5 mg/mL. The solutionof 5 mg/mL was thenattuned with PBS to give a final concentration of 2.5 mg/mL. BB and Charantin were prepared using the same procedures. Nevertheless, the initial dissolution of BB and Charantin were done in 2 mL each of ethanol and DMSO, while 38 mL PBS was used to adjust each solution to obtain final concentrations of 2.5 mg/mL. The ratios of DMSO:PBS, methanol:PBS, and ethanol, DW, was each be 5:95 v/v% in the final drug/sample solutions.

Filtration of sample and drug solutions wascarried out twice using filter paper to remove fragments. A final concentration of 2.5 mg/mL (Charantin and BB) and DWwas used in the characterization of the swelling properties of the hydrogels.

Transport measurementand swelling ratios

Swelling ratios of PNIPAM-based hydrogels Charantin and BB were determined using dried hydrogels while the dissolution of solvents BB and Charantin were carried out using aqueous solvents. The swelling ratios of PNIPAM-based hydrogels were determined by immersing the hydrogels into different solutionsof 5 mL volume, i. e. 2.5 mg/mL of charantin and BB or 5 mL DW, to absorb drugs/fluids. The swelling and release activities of the loaded gels were then be observed. The average swelling ratios (SR_A) were obtained by soaking the PNIPAM -based hydrogels in DW, insulin,and BB at temperatures between 28 -48 ˚C.[13]

$$SR_A = (M_t - M_o)/M_o \quad [3]$$

From equation (3), $\mathbf{M_t}$ is the mass of the gel at time ***t*** while $\mathbf{M_o}$ is the mass of the dried gel at time, $\mathbf{t = 0}$.
The fluid/drug uptake in the gels was obtained from the relative gel mass[14]

$$M_{rel} = M_t/M_s \quad [4]$$

From equation (4), $\mathbf{M_s}$ is the swollen mass at a given temperature and $\mathbf{M_t}$ is the mass of the hydrogel at the time, ***t****.The* The equilibrium volume ratio was obtained from:

$$V_{eq} = \frac{\left(\frac{\pi D^2}{4}\right)}{\left(\frac{\pi D_o^2}{4}\right)} = \frac{D^2}{D_o^2} \quad [5]$$

From equation (5), $\mathbf{D_o}$ and **D** represents diameters of the hydrogels before and after equilibrium swelling respectively.

Strain-induced in hydrogels due to swelling

Brannon-peppas and Peppashave given much explanation to the swelling dynamics of hydrogels. With resConcerningensional transport, the gel swelling is associated with the twinges in length. εdenotes strain, as it is induced in the gel as a result of swelling, is given by equation (6) to be:

$$\varepsilon = \frac{l - l_o}{l_o} \quad [6]$$

Where l denotes the length at any given time, t while l_o denotes the initial length.

Drug/fluid release from pnipa-based hydrogels

Peppas and his co-workers were able to establish an equation for drug releasemodellinmodelingich assumes dependent power law function of the form:

$$\frac{m_t}{m_i} = 4\left(\frac{D}{\pi\delta^2}\right)t^n = kt^n \quad [7]$$

From equation (7), $\frac{m_t}{m_i}$ is the fraction fluid/drug release, the geometric constant of the release systemis thus represented as ***k,*** fluid/drug release exponent as ***n***, depicting the release mechanism, is the initial mass of the swollen hydrogel prior to drug release, m_t is the mass of the hydrogel at time, **t** during drug elution δ is the thickness of the gel and D is the diffusion coefficient. Equation (7) is applicable to systems in which diffusion occurred within the polymeric networks. Constants k and n were gotten from the linear form to give;

$$ln\ (m_t/m_i) = lnk + nlnt \quad [8]$$

From the above of equation (8), the intercepts and slopes of the plots of ln (m_t/m_i) versus ln **t, k** and **n** were obtained respectively. The intercepts on theln(m_t/m_i) axis were equal to ln (k). The diffusion coefficients, D_s, were obtained from:

$$D_s = \frac{k\pi\delta^2}{4} \quad [9]$$

Where***k*** denotes the geometric constant of the release system,***π***represents the mathematical constant reflecting the ratio of a circle circumference to its diameter while ***δ*** is the thickness of the gel. The diffusion coefficients were determined at temperatures ranging from 28 to 48 ˚C. Arrheniusequationwas used in the determination of the activation energies for the different gels. This gives;

$$D = D_o\exp\left(\frac{-E_a}{RT}\right) \quad [10a]$$

and

$$\ln D = \ln D_o - \frac{E_a}{RT} \quad [10b]$$

In the two equations above, the diffusion coefficientis represented as ***D***, the diffusion constant at room temperature is denoted as D_o, the universal gas constant as ***R***, temperature ***T*** while E_a is the gel activation energy. The activation energy for each sample was obtained from the slope of a linear plot *of* ln Dversus $T^{-1}(K^{-1})$.

Results and Discussion

Extraction of charantin

The result of the extraction of charantinis presented in Table 3(a), which shows that charantin is higher in leaves sample which is 9.89% and lower in fruit of *Momordicacharantia* and has the extractable value of 4.05%. The extractability of charantin is very vital and in the preparation of diabetic drugs of different types. This is becauseintermolecular reactions occur higher temperature causes (dipole-dipole and hydrogen bonding) within the solvent to reduce, resulting in higher molecular motion and making the solute to dissolve easily in the solvent. It was reported 9.65% in leaves and 3.21% from the fruits of Momordicacharantia which is lower than the ones obtained. The higher values of the charantin can be attributed to better separations by the use of a better centrifuge at 8500 revolutions per minutes for 15 minutes which was carried out under a controlled pH of 4.5 as the results of the extraction is shown in Table 3(a).

Table 3(a): Mass of Crude Charantin Extracted from 100 g of Leaves and Fruit of Momordicacharantia.

Sample	Mass of Charantin Extracted
Fruit	4.05
Leaves	9.89

Table 3(b): the percentage of charantin extracted from 100 g of leave and fruit sample of bitter melon.

Sample	% of Charantin Extracted
Fruit	4.05
Leaves	9.89

The left sample is shown to have the highest amount of extractability of charantin using ethanol and these values of charantin are higher than previously presented and could be attributed to the centrifuge used that is responsible for total separation at 8500 revolutions per minute for 15 minutes. This extraction was done under a controlled pH of 4.5.

Purification of charantin

The high-performance liquid chromatography of charantin was carried out for both the fruit and leavea sample of charantin with the peak that grows and retains symmetry at 4.318 at a peak area of 1987. 62317 for the standard insulin where the sample is given in Table 4, the standard was used to plot a standard calibration curve to determine the concentration of charantin for both samples as given in Table 4.

Table 4: Retention time and a peak area of insulin leave and fruit sample of character from HPLC.

Sample	Retention time [Mins]	Area [MAμ*S]
Insulin	4.318	1987.6317
Leave	4.023	1475.0937
Fruit	4.291	1070. 7437

The percentage (%) concentration of charantin in the fruit and leave sample of Momordica*charantia* was found to be 56.90% and 76.73% respectively using the standard calibration curve in Figure 2.

The peak of insulin 4.205 was reported by Ansri*et al.* which agrees to the result obtained.These where the concentration of charantin in the left sample and fruit sample were found to be 74.69 μg/mL and 52.61 μg/mL respectively.

Poly (N-isopropylacrylamide) hydrogel preparation

Preparation of poly (n-isopropylacrylamide) hydrogel was synthesized from the materials and their roles in gel polymerization as given in Table 1. This was achieved by varying the concentration of acrylamide from 3% to 12%.

Characterization of poly (N-isopropylacrylamide)

Lower critical solutions temperature of PNIPAM-based hydrogel (LCSTs)

The result for the determination lower critical solution temperature and the activation energy of PNIPAM is as presented in Table 5. The result of the LCST of PNIPAM-based hydrogels susuggestshata heat trigger mechanism can be used in managing the release of drugs from the thermo-sensitive hydrogels such that the control of the LCST makes theapplication in drugs delivery systemsof PNIPAM–based hydrogels attractive.Table 4.0 summarizes the LCSTs of the P (NIPA)-based hydrogels. The LCSTs for a homopolymer soaked in distilled water was 32.8 ℃ white 32 ℃, and 34.6 ℃ was reported.[17] Withinthecrease, in the concentration of acrylamide from 3% to 12% there is an increase in lower critical solution temperature from 32–43 ℃.

It was found that when N1 was soaked in distilled water, insulin and Charantin, the LCSTs of the hydrogel was found to be 32.8 ℃, 34.6 ℃ and 36.0 ℃ in distilled water, insulin solution and charantin solution respectively.

Table 5: Activation energy and the lower critical solution temperature of P (NIPA)

Gel	Activation Energy (KJ/mole)	Lower Critical Solution Temperature (℃)
N1	120±8	32±1.9
N2	164±7	36±1.7
N3	166±7	37±1.5
N4	289±16	43±1.1
N5	118±4	30±1.5

N1 = (100 mol% P (NIPA), N2 = (93% P NIPA-3% AM), N3 = (94% P NIPA – 6%AM), N4 = (88% P NIPA – 12%AM), N5 = (91% P NIPA – 9%AM).

The LCST of PNIPAM increases as the gel changes from one solution to another. It was found to be 32.8 ℃ in distilled water whereas the gel loaded with the charantin solution was found to be 36.0 ℃ this shows that there is an interaction between the charantin and the hydrogel matrix. The value of the LCST of PNIPAM in insulin solution was found to be 34.6 ℃ which is lower than that of charantin this could be attributed their difference in molecular size and the polarity of the solution The effect of the three different solutions on LCSTs of PNIPA (N1 which is a homopolymer 100 mol % of PNIPA) is presentedin Table 6. However, charantin was found to increase the LCST of the hydrogel from 32.0 ℃ in distilled water, to 34.6 ℃ in insulin and 36.0 ℃ in charantin solution. Hence the incorporation of more hydrophobic or hydrophilic co-monomer will increase or decrease the LCSTs of the hydrogel as reported.[18] The LCST of the blank (PNIPAM) in distilled water shows agreement with those of Heskin and Guilletas well as Dhara and Chaheri.[19, 20] This can be related to the complexation or salting out of the gel with the loaded charantin increasing the hydrophilicity of the gel matrix. The result of the LCSTs of PNIPAM-based hydrogel suggests that the release of drugs from the thermo-sensitive gel is useful for application as for a drug delivery vehicle for an anti-diabetic agent. The hydrogel and the charantin interaction is responsible for the increase LCST as it is represented in Figure 3.

Table 6: Lower Critical Solution Temperature of P-NIPA Loaded in Some Solutions.

Gel	Distilled water(℃)	Insulin solution (℃)	Charantin solution (℃)
A	32.8	34.6	36.1
B	32.9	34.5	36.0
C	32.8	34.7	36.0

The fourier transformed infrared (FTIR) spectroscopy

The results of the Fourier transformed infrared (FTIR) Spectroscopy of the materials utilized in this study are presented in Figures 4 to 8.

For insulin, the broad absorption peak at 3385 cm^{-1} are due to O-H of acids with overlapping C-H stretch, and C≡C stretching vibrations, 2123.93 cm^{-1} are due to symmetry stretching vibration, while 704.87 and 920.66 cm^{-1} are due to methylene sp^3 C-H bending vibration (i.e., α carbons).

For the fruit sample of *Momordicacharantia*, the sharp peaks between 2928.86 – 2866.31 cm^{-1}can be associated with the sp^3 C-H at the methylene linkage.

The small and doublet peaks at 2367.31 cm^{-1} are due to the stretching absorption by C≡N, and the smallpeak at 1741.93 cm^{-1} is due to stretching vibrations of C=O.The peak at 1596.53 and 1655.50 cm^{-1} are due to C=C stretching vibration while the peaks between 1373.72 – 1451.93 cm^{-1}

and 1158.62–250.95 cm^{-1} are due to sp^3 C-H bending vibration and C-O stretching vibration respectively.

For leave sample of *Momordicacharantia*, the peakat 3370.57 cm^{-1} is due to O-H stretching vibration with C-H overlapping. The peaksbetween 2868.14-2934.33 cm^{-1} are due to sp3 C-H stretching vibration. The peak at 1745.64 cm^{-1} is due to C=O stretching vibration while the peaks at 1651.11, 1455.35 and 1161.54 cm^{-1} are due to C=N stretching vibration, sp^3 C-H bending vibration and C-O stretching vibration, respectively.

The broadband at 3434.10 cm^{-1} is due to O-H (alcohol) stretching vibration, while that at 3199.96 is due to sp^2 C-H stretching vibration. The peak observed at 2942.44 cm^{-1} is due to sp^3 C-H stretching vibration,and the smallest peak at 2372.07 cm^{-1} is due to the stretching vibration of C≡N. The peak observed at 1611.83 cm^{-1} is due to C=O stretching vibration, the peaks between 1330.60-1447.11 cm^{-1} are due to sp^3 C-H bending vibration and peaks between 1047.26–1097.69 cm^{-1} are due to C-O stretching vibration. The peak observed at 683.96 is due to sp^2 C-H bending vibration.

The broadband stretching at 3435.86 cm^{-1} is due to O-H stretching, and that at 3200.76 is due to sp C-H stretching. The peak at 2939.16 cm^{-1} is due to sp^3 C-H stretching vibration, while the peaks between 2105.08–2370.57 $cm^{-1}$are due to C≡C stretching vibrations. The peak at 1607.93 cm^{-1} is due to C=O stretching vibration while the peaks between 1335.34-1443.91 cm^{-1} and 1048.86-1118.28 cm^{-1} are due to sp^3 C-H bending vibration and C-O stretching vibration, respectively.The peakat 600.00 cm^{-1} is due to sp^2 C-H bending vibration when the hydrogel was soaked for 48 hours in charantin solution.

Charantin loading

The result obtained from loading the charantin on PNIPAM-based hydrogel is as presented in Table 7. It was found that the following hydrogels were loaded with the following concentration of charantin. Were loaded on the hydrogel N1, N2, N3 and N4 were found to be a total concentration of charantin concentration solution is 52.61 µg/mL.

It was found that gel N4 has the highest loading charantin. This could be due to the high concentration of the acrylamide and implies smaller pore space in the gel, the high interaction between the hydrogel matrix and the charantin molecules and it implies high adsorption and N1 has the lowest charantin loading ability. This could as a result of large pore space in the gel as a result of poor interaction leading to low adsorption of the charantin by the hydrogel. This result does not agree with Coulgh*et al.* on the earlier assumption that there is no interaction as a result obtained in this study shows that there is an interaction between the hydrogel matrix and the charantin molecules.[21]

Table 7 shows that N4 has the highest loading ability whereas N1 has the lowest drug loading ability and it can be attributed to larger pore spaces of N1. The higher loading ability is as a result of higher cross-linking of the hydrogel. Hence,the higher the acrylamide concentration,the higher the charantin loading of a hydrogel.

Table 7: Summary of Charantin Loading of Hydrogel

Gel code	The concentration of charantin solution left (µg/mL.)	Concentration loading to the gel (µg/mL.)
N1	10.10	42.51
N2	8.04	44.57
N3	9.06	43.55
N4	8.00	44.61

Release of charantin

The release kinetics at 28 ℃, 32 ℃ and 34 ℃is presented in Figure 9 (a-c) and the kinetic release of charantin from PNIPAM shows that at 28 ℃, the hydrogel for N1, N2, N3,and N4 are -0.70, -0.70, -0.49 and 0.46, respectively with N1 and N2 having the lowest whereas N4 has the highest value and the negative sign signifies that as the time increase the concentration of charantin in the hydrogel decreases. At 32 ℃, N3 has the highest value,and N1 has the value of -0.49 and -0.69, respectively, whereas with a temperature increase up to 34 ℃, N3 has the highest valueof -0.68 and lowest value of -0.84.

From the result obtained from the kinetic release, it shows that the 28 ℃, N3 and N4 < are less than –0.5 and at 32 ℃ only N3<0.5 but all other values are more significant than 0.5 which can be easily be approximated to unity.

At 28 ℃, for the hydrogel N1 between 16-24 hours, there is the high release of charantin and between 40-48 hours, the lowest release of the charantin loaded on hydrogel with the rate of change of concentration per unit time as - 0.67 $\mu gmL^{-1}S^{-1}$. The hydrogel N2 shows the rate of release at 0.742 µgmL/s and the highest release is between 16-24 hours with change in concentration From 32.23 µg/mL to 17.93 µg/mL and least releasewas observed between 40-48 hours. The gel N3 show the highest release rate between 24-32 hours unlike N1 and N2 which change in concentration was between 31.27-17.27 µg/mL and the lowest release was observed between 8-16 hours, Which corresponds to 40.43-37.67 µg/mL. N4 shows the highest release between 24-32 hours similar to that of N3 which shows changes in concentration between 22.14-11.30 µg/mL. This was reflected that at 28℃, the higher the concentration of acrylamide, the longer the time it takes for charantin to be released.

The kinetic release at 32 ℃ are shown in Figure 9 (b). The hydrogel N1 shows the highest charantinrelease between 24-32 hours with change in concentration between 25.89-7.39 µg/mL whereas the leastrelease of charantin from N1 was shown between 40-48 hours corresponding to 5.29-3.25 µg/mL change in concentration. For the gel N2, the highest release rate was between 16-24 hours and the lowest release at

this particular temperature is observed between 0-8 hours which is between 43.55-42.59 µg/mL. The hydrogel N4 shows the highest release between 16-24 hours, whereas the lowest release of charantinwas observed between 40-48 hours which correspond to 4.45-4.28 µg/mL.

The kinetic release of charantin from PNIPAM at 34 ℃ is shown in Figure 9 (c). The hydrogel N1 shows the highestrelease between 24-32 hours and lowest release between 0-8 hours corresponding to 25.89-7.39 and 42.51-40.37 µg/mL, respectively. The hydrogel N2 shows its highestrelease between 40-36-22-03 µg/mL whereas the slowest release can be observed between 0-8 hours which has a change in concentration between 44.57-43.83 µg/mL. The highest release of charantin from PNIPAM N3 is observed between 24-32 hours,andthe lowest is observed between 40-48 hours corresponding to 34.38-19.98 and 11.98-11.06 µg/mL, respectively which is unlike the one observedfor N4 which is at 16-24 hours corresponding to 35.58-24.98 µg/mL and the lowest is as the same as observed in N3 which is between 40-48 hours and its correspond to 4.45-4.28 µg/mL. The release profiles of charantin from PNIPAM gels synthesized with 3% cross linker at temperatures above and below the LCST. During the first 8 h, the release rate of charantin was the highest at 34 ℃, followed by 32 ℃. When the gel came into contact with PBS above the LCST, a temperature gradient was generated across the polymeric matrix. This resulted in the collapse of the gel surface faster than the interior of the gel matrix, forming a dense polymeric skinlayer. The skinlayer hindered the outward diffusion of the protein solution, causing a build-up of hydrostatic pressure inside the gel. This pressure would eventuallysqueezeout the water containing the protein as time passes. For charantin release at 34 ^{0}C, there was an initial burst release of surface encapsulated charantin accompanying the formation of a dense skin layer.

Diffusion coefficient of PNIPA in different fluids at 34 ℃.

The result of the diffusion coefficient of PNIPA in the different fluid is presented in Table 8. The diffusion coefficient of the hydrogels N1, N2, N3 and N4was found in distilled water, bromophenol blue and charantin solution. The diffusion coefficient value of the hydrogel N1 which is 100 PNIPAMhomopolymer was found to be 1.56x10^{-10} m^2/s in bromophenol blue and 2.50 x 10^{-10} m^2/s. The diffusion coefficient of bromophenol was found to be highest,and that in distilled water is the lowest.This result is in agreement with the result obtained by Cai*et al.*[22] The N_2 hydrogel has the diffusion coefficient values of 4.70 x 10^{-9} m^2/s, 1.10 x 10^{-8} m^2/s and 5.08 x 10^{-8} m^2/s in distilled water, bromophenol blue and charantin solutions. This respectively and it has shown to charantin in the highest and the lowest.is in distilled water.

The value obtained for hydrogel N2 was found to be in the distilled water. For the hydrogel N3 the result shows that in distilled water the diffusion coefficient is 1.60 x 10^{-10} m^2/s the value is highest in charantin which is 5.08 x 10^{-9} m^2/s. The hydrogel N3 shows the highest values of the diffusion coefficient to be 4.42 x 10^{-8} m^2/s in distilled water. The diffusion of the N4 in highest in distilled water and found to be 1.44 x 10^{-8} and 1.48 x 10^{-9} m^2/s in charantin is the lowest.

In distilled water, the diffusion is observed to be highest in the hydrogel N2 (1.44 x 10^{-8} m^2/s) and lowest in N_1 (1.56 x 10^{-10}). For the diffusion coefficient of bromophenol blue, N3 has the highest and N1 has the lowest valuesof 8.09 x 10^{-10} and 4.42 x 10^{-8} m^2/s, respectively. For the charantin diffusion coefficient, the highest is 5.08 x 10^{-8} m^2/s which was observed in N2,and the lowest diffusion coefficient can be seen in N1with the value of 2.5 x 10^{-10} m^2/s. Majority of the hydrogels used in biomedical applications have a mesh of 5nm to 100 nm size, in their swollen state. These size scales are much larger than most small molecular weight drugs that are used in pharmaceutical formulations, and, therefore, diffusion of these drugs in the swollen matrices was not hindered. The transfer of the solute was controlled by the swelling of the gel. The small hydrodynamic radii of drug molecules then allow the drugs to diffuse through the gel network. Hence, knowledge of swelling gel characteristics is crucialfor the understanding of the network structure of the gel and its capacity to function as a carrier for drug delivery purpose.

Table 8: Diffusion Coefficients for the Hydrogels in Different Fluids at 34 °C

Gel Codes	Diffusion coefficients in Distilled Water (m^2/s)	Diffusion coefficients (m^2/s) in Bromophenol blue	Diffusion coefficients (m^2/s) in Charantin
N1	1.56 x 10^{-10}	8.09 x 10^{-10}	2.50 x 10^{-10}
N2	4.70 x 10^{-9}	1.10 x 10^{-8}	5.08 x 10^{-8}
N3	1.60 x 10^{-9}	4.42 x 10^{-10}	7.90 x 10^{-9}
N4	1.44 x 10^{-8}	1.16 x 10^{-9}	1.48 x 10^{-9}

PNIPAM N-Isopropylacrylamide, Acrylamide, Gel codes, ^{N1}P (NIPA) Homopolymer (100 mol% of P (NIPA)), ^{N2}P (NIPA-co-AM) (93-3 mol%), ^{N3}P (NIPA-co-AM) (94-6 mol%), ^{N4}P (NIPA-co-AM) (88-9 mol%).

Diffusivity D of PNIPAM-loading with charantin drugs release exponent (n) and the geometric constant of PNIPAM-based hydrogels (k) at 34 ℃

The result for *n*, *k* and *D* were obtained as summarized in Table 9. The fraction release of charantinwas exponentially related to the time of drug release for hydrogels that are cylindrical n=0.5 and above to unify corresponding to Fickian diffusion.

While the release rate was then defended on t^{-05}. This represents diffusion- controlled release when *n* lies between 0.5 and 0.7 (i.e. 0.49<n<0.7), non-Fickiandiffusional release (anomalous transport) as reported that occurs, while n=0.7 corresponds to case II transport (for a cylindrical sample).

The value of ***n*** obtained isgreater than 0.49 but less than 0.71. The release exponent (*n*) provided some insights into the mechanism of the release of charantin form PNIPAM-based hydrogels withthe *n*values showing an anomalous transport mechanism at 34 ℃. This implies that as the exponential release increases, the geometric constant which contains the diffusion term decreases. The coefficient of charantin diffusion from P (NIPA) could be used to control the release of the anti-diabetic agent.

The diffusion kinetics of PNIPAM-hydrogels studies and the drug release rates were rate governed by the earlier time approximation, while the diffusion rate of drug molecules was strongly influenced by temperature. Fickianbehavior is observed in N1, N2, N3 and N4.

The small hydrodynamic radii of drug molecules allow the drug (charantin) to diffuse through the gel network. Hence, the diffusion coefficient *D* is independent of the drug concentration. The release of the drug from the polymer is fractionally and exponentially related to the release time. The release exponent*n* corresponds to the mechanism of drugs release. When n=0.5, correspond to fickian diffusion and the release rate is time depending on $t^{-0.5}$. This relation can be seen in Table 9.

Table 9: Diffusivity, D, of P (NIPA)-loaded Charantin, Drug Release Exponent (n), and Geometric Constant of P (NIPA)-Based Hydrogels (K) at 34˚C

Gel Codes	Geometric constants (K)	Release exponents (n)	Diffusion Coefficients D (m^2/s)
N1	0.01 ± 0.003	0.5 ± 0.03	2.50×10^{-10} ± 0.180
N2	0.01 ± 0.010	0.6 ± 0.035	5.08×10^{-8} ± 0.490
N3	0.01 ± 0.005	0.6 ± 0.035	7.90×10^{-9} ± 0.479
N4	0.01 ± 0.003	0.7 ± 0.04	1.48×10^{-10} ± 0.573

Swelling ratio

The result of the swelling ratio is as shown in Figure10. PNIPAM-based hydrogels were exposed to the solvents distilled water, bromophenol blue and charantin solution. The hydrogels swelled for 30 hoursinthis solvent until it equilibrates or until it swelling reach a dynamic equilibrium condition at saturation.

The swelling capacity of PNIPAM-based hydrogels was observed to be affected by temperature. The resultsfrom this show the effects of temperature on the swelling.But at equilibrium, the thermodynamically driven swelling force is countered balance by the retroactiveforce of the cross-linked structure, leading to an equilibrium state.

This swelling state allows for the widening of the gaps between the cross-links and mesh size, hence facilitating the transfer of various solutes through the hydrogels. The knowledge of hydrogels swelling characteristic enables the better understanding of the cross-linking and the network structure of the hydrogels and their capacity to function as carriers for drug delivery.

From Figure 10, it is clear that the PNIPAM hydrogels swell very well in distilled water than inbromophenol blue and charantin. The acrylamide also improved the swelling ratio. However swelling ratio is higher in copolymers,and the swelling ratio is greatest in N4 due to the amount of acrylamide in this hydrogel. For all the hydrogels, the highest swelling ratio was observed in distilled water, followed by charantin and then, bromophenol. This is attributable to the polarity of the solvents as the higher the polarity of the solvent, the higher swelling ratio of the compound in such solvent.

Conclusion

From the result of charantin extraction, it was found that there was more charantin substance in the left sample which is almost twice that of the fruit sample on the extractability is higher when ethanol is used under control at 8500 revolution rpm/min of centrifuge speed to ensure total separation the purification of charantin shows the retention time 4.318, 4.023, 4.291 for insulin leave and food samples, respectively. The concentrations on the leave sample are 74.69 µg/mL ground per/mm and 52.61 µg/mL.

The result of the preparation of PNIPAM-based hydrogels shows that at higher concentrations; there is higherCrosslinking and the higher water absorption. This will, in turn, lead to a gel with a slow release of the active ingredient. The lower critical solution temperature is increased with an increase in hydrophobicity and change with a chargeinsolvent and molecular weight or size.

Charantin loading shows slightly higher efficiency. For the FTIR results, a bond stretch later disappears after charantin loading and the swelling ratios suggest that the hydrogels swell better in distilled water that in charantin or bromophenol blue. We also observed that there mightalsobe a strong interaction between the hydrogel matrix and the charantin molecules.

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