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Research Article

Formulation and *in vitro* investigation of aspirin nanoparticles loaded suppositories as a drug delivery system for colorectal carcinoma

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ABSTRACT

Aspirin suppositories are in commercial use suffer with side effects such as irritation, burning sensation, rectal hemorrhage. The aim of the present work is to formulate aspirin nanoparticles loaded suppositories and to perform *In vitro* investigation for the prepared suppositories. Initially aspirin-chitosan nanoparticles were prepared by ionic gelation method and the nanoparticles evaluated for different *In vitro* evaluation studies; based on results the best formulation was selected and in order to know the diffusion efficiency, different compositions of aspirin glycerogelatin suppositories were prepared and subjected to various *In vitro* evaluation studies and best composition was selected. From the previously performed evaluation studies best formulation from aspirin nanoparticles incorporated in to selected glycerol gelatin bases and evaluated for *In vitro* characteristics. The results indicates that formulation Fa9 Aspirin nanoparticles were proved to be best formulation with 88.3±1.1 % of drug release at the end of 24hr, with zero drug release. *In vitro* characterization performed for aspirin suppositories indicates that Fs2, Fs4, Fs9 and Fa11 was proved to be best composition with highest percentage of drug release at the end of 60 minutes with 98.06±1, 99.3±0.45, 97.6±1.8 and 97±1 drug release and other characteristic studies performed indicates that all formulation are ideal characteristics. Previously selected bases composition used for the loading of nanoparticles based on displacement value results indicates that drug release appears with a lag phase initially and controlled for a period of 24hr.

Keywords: Aspirin; Chitosan; Nanoparticles; Aspirin Suppositories; Colorectal Carcinoma; Glycerogelatin Suppositories

INTRODUCTION

Suppositories which are designed to melt at the physiological temperature compare to oral administration the rectal or vaginal drug delivery has gain importance therapeutically by avoiding the first pass metabolism (T.W. Hermann 1995). The bioavailability of polycarbophil with polyethylene bases has shown the improvement in bioavailability. Non steroidal anti inflammatory drugs like acetaminophen, ibuprofen, indomethacin, salicylic acid, aspirin and some steroidal anti inflammatory drugs like dexamethasone, betamethasone were widely used in the form of suppositories to increase their bioavailability (Christian De Muynck 1992, Kirsti Gjellan 1994). The absorption of drugs from the suppositories bases will greatly influenced by the following four parameters 1. Melting time of base 2. Dissolution rate of the drug 3. pH of the rectal fluid and 4. Amount of fluid in rectum. The solvent system must be organic and non polar (Werner Lowenthal 1965). The fat chemical composition in the indomethacin suppositories will influence the drug release behavior, added monoglyceride melting point to the triglyceride and fatty acids, methyl esters will also influence the drug release behavior. In the design of sustained release suppositories of indomethacin it was observed that addition of polycarbophil to the polyethylene glycol base has improved the bioavailability from 5% to 8% (Ehab A. Hosny 1995). Comparative study of two dissolution methods 1. Continuous flow through cells 2. Rotating paddle apparatus from fatty bases and water soluble bases conclude that the drug release from water soluble bases will be faster compared to fatty bases. The release rate of Indomethacin increases significantly with surface active agents (A. Archondikis 1989), based on the solubility of drug in fat (slowly dissolving in water, rapidly dissolving in water, soluble in lipids) plays very vital role. It is the driving force drug diffuses based on water solubility. Drugs like paracetamol which is insoluble in fat will be influenced by the concentration and micronisation of aspirin will have good rapid absorption from rectum (A.J.M. Schoonen 1979). Ibuprofen suppositories formulated with cocoa butter, witepsol E_{75}, and polyethylene gly-

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col (PEG) bases, in which cocoa butter based formulations are more efficient, compared to polyethylene glycol bases and reported that suppositories are more stable formulation compared to oral solutions (S.A. Ibrahim 1990). The attempts were made to evaluate the efficiency of prepared *in-situ* gelling and mucoadhesive acetaminophen liquid suppository on human subjects has proven to be better in analgesic and antipyretic activity (Chong-Kook Kim 1998). Layered suppositories were formulated with different compositions of excipients, which have revealed changing the excipient ratio will alter the release kinetics, (Nicola Realdon 1997) the effect of suppository bases on release behavior of potent antimicrobial agents revealed the importance of selection of base. Controlled phase release suppositories was first explained by *E. Allemann et al.,* (E. Allemann 1992).

The present investigative work, explains the release of nanoparticles from the suppositories will be five phase release mechanism 1. Delivery of suppositories to rectal cavity 2. Dissolution (release of nanoparticles) by the rectal fluids 3. The nanoparticles will enter in fenestrated capillaries 4. Diffusion of drug from nanoparticles. 5. Nanoparticles whose size is restricted to the entry of fenestrated capillaries due to the mucoadhesive character nanoparticles will adhere to mucosa of rectum for a prolonged period of time and release the drug in controlled manner.

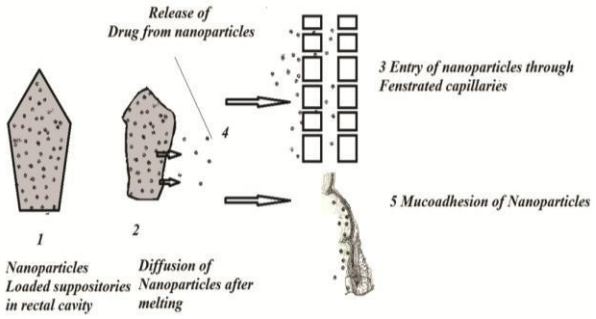

Figure 1: Schematic representation mechanism involved in nanoparticles

Ionic gelation was selected as the method of preparation of nanoparticles and chitosan as the polymer for effective controlled release. Chitosan which is a (β-1,4)-linked D-glucosamine, *N*-deacetylated derivative of chitin, the most abundant natural polymer after cellulose, constituting the exoskeleton of arthropods and cell walls of fungi and yeast, (A.K. Anal 2003) which is generally represented as a homopolymer. However, the deacetylation process is rarely complete, and most commercial products tend to be a copolymer of *N*-acetylglucosamine (NAG) and *N*-glucosamine repeat units (A.K. Anal 2005, J.W. Lee 1999, Illum. L. 1998). The novel mechanism which involved in the formation of nanoparticles first explained as the complexation between positively charged chitosan or macromolecules (polysaccharides) and poly anionic molecules such as sodium tripolyphosphate (STPP) or calcium chloride (Yao 1995, X.Z. Shu 2002). Anionic behavior of STPP, molecular weight of chitosan and pH plays important role in the ionically crosslink with chitosan (Pilar Clavo 1997). Due to their sub-cellular and submicron size, can penetrate deep into tissues through fine capillaries, cross the fenestration present in the epithelial lining (e.g., liver and lungs) (Wei Sun 2008). Aspirin and probucol combination loaded chitosan nanoparticles has revealed that encapsulation efficiency and loading capacity of aspirin and probucol are affected greatly by, pH value, STPP concentration and nature of drugs (Wan Ajun 2009). The chitosan- alginate nanoparticles containing gatifloxacin reservoir type nanoparticles has shown the efficiency of sustaining the drug for a period of 24 h by non-fickan type of diffusion (Sanjay K. Motwani 2008). Measures are taken to find out the relationship between entrapment efficiency and the amount of drugs (insulin, diclofenac sodium, and salicylic acid) inside the particles, the effect of zeta potential and surface charge of the produced micro/nanoparticles, and could not found any correlation between entrapment efficiency and zeta potential respective surface charge (Ahmad 2008). But by the modulation of surface charge, particle size and morphological properties of chitosan–STPP nanoparticles has shown a simple linear relationship with increasing chitosan to STPP weight ratio, but the zeta potential at fixed chitosan to STPP ratio showed a linear decrease with increasing chitosan concentration (Yaowalak Boonsongrit 2006). An investigative work of preparation of acetyl salicylic acid-caffeine complexes for rectal administration revealed the clinical importance. The rectal administration will be safe and convenient for long term use, of patients who are suffering with rectal carcinoma (Quan Gana 2010). The recent investigations revealing the importance of aspirin and salicylates, for the rectal carcinoma and other types of carcinomas. Low dose of 100 mg a day can keep the cancer away, it was proved with number of randomized clinical trials. The dietary supplement of aspirin reduces the risk of cancer. At a dose 81 to 325 mg daily is effective in secondary prevention stage (Ehab A. Fouad 2010). Colorectal cancer is the second most common cancer around 5% of the total population around the world, who are suffering with the disease. Clinical trials conducted with 2 x 2 factorial designs and the study has reported the reduction of risk with 75 mg aspirin daily and 40 to 50% reduction in proximal colon and adenomas with aspirin and no reduction in distal adenomas (Peter C Elwood 2009).

Aspirin was chosen as the drugs of choice since the aspirin suppositories are available official from many years. The usage of these suppositories directly when the drug come in contact to mucosal tissue cause the irritation, burning sensation of the rectal area. In addition, bleeding, difficulties hearing, ringing in the ears, change in the amount of urine, persistent or severe nausea/vomiting and unexplained tiredness can be

Table 1: Composition of chitosan-aspirin Nanoparticles

Formulation code	Fa1	Fa2	Fa3	Fa4	Fa5	Fa6	Fa7	Fa8	Fa9
Chitosan (mg/mL)	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
Aspirin (mg/mL)	1	1	1	1	1	1	1	1	1
(STPP) (mg/mL)	1	1	1	1	1	1	1	1	1

Table 2: Composition of aspirin-glycerogelatin suppositories for six suppositories

Formulation code	Fs1	Fs2	Fs3	F4	Fs5	Fs6	Fs7	Fs8	Fs9	Fs10	Fs11	F12
Gelatin (gm)	0.663	0.828	0.994	1.16	1.326	1.65	2.33	1.99	1.83	1.66	1.5	1.33
Glycerin(gm)	5.96	5.80	5.636	5.2	5.31	4.98	4.30	4.64	4.80	4.97	5.13	5.30
Water(gm)	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Aspirin(mg)	600	600	600	600	600	600	600	600	600	600	600	600

Table 3: Composition of aspirin nanoparticles loaded glycerogelatin suppositories

Formulation code	Fas2	Fas4	Fas9	Fas11
Gelatin (gm)	0.828	1.16	1.83	1.5
Glycerin (gm)	5.80	5.2	4.80	5.13
Water (gm)	Q.S	Q.S	Q.S	Q.S
Aspirin nanoparticles (mg)	600	600	600	600

*Formula for six suppositories

expected as major side effects (Peter M Rothwell 2010). The objective of the present work was to prepare aspirin nanoparticles loaded suppositories which will deliver the therapeutic quantity of drug by reducing their side effects and improves the bioavailability thereby reducing the patient complaint.

Experimental

Materials

Chitosan (degree of deacetylation of 88.40% with viscosity of 175cps) was provided as gift sample from Indian see foods, Ltd, Cochin, Kerala, India. Sodium tripolyphosphate (anhydrous) and glycerinated gelatin was purchased from LOBA Chemie, (Pvt) Ltd., India. Aspirin was obtained as a gift sample from Hetro pharmaceuticals (Pvt) Ltd., India. De-ionized water was procured from Millipore, India. All the chemicals and other reagents were received as analytical grade were used.

Preparation of Chitosan-Aspirin Loaded Nanoparticles

Aspirin loaded nanoparticles were prepared by the previously discussed method the chitosan solutions of different concentrations were prepared 0.4%, 0.6%, 0.8%, 1%, 1.2%, 1.4%, 1.6%, 1.8%, and 2% (mg/mL) were prepared as shown in (tab. 1) by dissolving chitosan in (1%w/w) acidified water which was previously prepared by dissolving in acetic acid at room temperature at overnight stirring. Aspirin was added to the previously prepared chitosan solutions. STPP was dissolved in deionized water to prepare 1 mg/mL concentration. The chitosan solution now added drop by drop in equal volume in to the prepared STPP solution. A nanoparticle starts forming spontaneously by ionic gelation mechanism. The nanoparticulate suspension appears as turbid in nature allowed for stirring for more than 2 h at room temperature subjected for further analysis (Bardia A 2007). The 10mL of the prepared nanoparticles suspension was transferred to centrifugal tubes isolated by centrifugation 16,000 X g, 15ºC and resuspended in de-ionized water. The nanoparticles suspension was freeze-dried keeping the centrifugal tube in tilted position for a period of 24 h without glycerol bed the percentage yield was calculated as follows and the results were displayed in tab. 4.

$$Percentage\ Yield = \frac{Weight\ of\ drug\ loaded\ nanoparticles}{Total\ weight\ of\ components} \times 100$$

Preparation aspirin glycerinated gelatin suppositories

Aspirin containing glycerinated gelatin suppository formulations were optimized to get best composition. The concentration of gelatin (10%, 12.5%, 15%, 17.5% 20%, 25%) and glycerin (65%, 70%, 72.5%, 75%, 77.5%, 80%) varied from every formulation were prepared (Fs1-Fs12) by fusion method. The amount of drug and base required was calculated based on the displacement value shown in tab. 2 (Flossman E 2007). Glycerin, gelatin and water melted to 60 :C, over water bath followed by the addition of micronized aspirin under stirring to have content uniformity. The mixture was cooled to 50 :C and poured in to the pre calibrated metallic mould and placed into the refrigerator for solidification.

Incorporation of Aspirin nanoparticles in gelatin suppositories

Aspirin nanoparticles incorporated in the suppositories as discussed in the literature (Eskandar Moghimipour 2009). Glycerinated gelatin was selected as the base for loading nanoparticles. Based on literature drug diffusion efficiency was comparatively more in glycerinated gelatin (Ivo Setnikar 1962). Based on the previously performed characterization studies for the nanoparticles and aspirin suppositories, best formulations were selected, and based on displacement value 100

mg of aspirin nanoparticles were added to the molten base and suppositories were prepared by fusion process in metallic mould. The base which was fused at 60:C, then the nanoparticles was added and stirred with glass rod to have content uniformity and poured in to the one gram metallic mould and solidified in refrigerator by keeping overnight. Composition of aspirin-chitosan nanoparticles loaded suppositories was shown in (tab. 3).

Characterization of aspirin nanoparticles

Determination of Percentage of drug entrapment (%P.D.E.)

The nanoparticles were evaluated for percentage of drug entrapment (%P.D.E) as follows. The nanoparticles Percentage drug entrapment was calculated by observing the percentage of drug unentrapped present freely in the supernatant nanoparticulate suspension after centrifuging using a Remi cold centrifuge (Remi Pvt.Ltd, India). The suspension for 30 min at 16,000 X g, 15 :C and amount of drug entrapped was determined by using the formula (Haidy Abass 2010). The results were present in (tab. 4).

$$\%P.D.E = \frac{Total\ amount\ of\ drug - Free\ drug\ present\ in\ supernatant}{Total\ amount\ of\ Aspirin} \times 100$$

Surface texture and particle size analysis of aspirin-chitosan nanoparticles

Measurement of particle size, zeta potential and poly dispersibility (Size distribution) were found for the prepared nanoparticulate suspension by using Zeta sizer® 3000-HS (Malvern Instruments, U.K). Particle morphology and surface characters has been observed by using (SEM, Hitachi SU-70 Pleasanton, CA) High resolution Scanning Electron Microscope Microscopy. (SEM) (T.A. Adegboye 2008) The pH of the nanoparticles suspension was adjusted to neutral and one drop of the suspension placed on aluminum pan and adhered to carbon tapes and they are placed on specimen stubs coated less than (10 µm) the SEM image reported in results.

***In vitro* Drug release aspirin nanoparticles**

In vitro drug release of aspirin nanoparticles were performed as discussed in literature (Sanae Kaewnopparat 2009) in phosphate buffer at pH 7.2. The nanoparticulate suspension is resuspended in pH 7.2 phosphate buffer and placed in dialysis bag (molecular weight was cutoff 10 kDa). 5 mL of dissolution medium was added in to the dialysis bag and both ends of the bag was sealed and bag was placed in receptor compartment containing 50 mL of pH 7.2 phosphate buffer at 37 ± 5°C over a magnetic stirrer with 50 rpm the assemblage was shown in (fig. 4). At periodical time interval, the samples were withdrawn from the receptor compartment the same in quantity of fresh buffer was replaced in order to maintain sink conditions and the samples were analyzed using U.V. spectrophotometry.

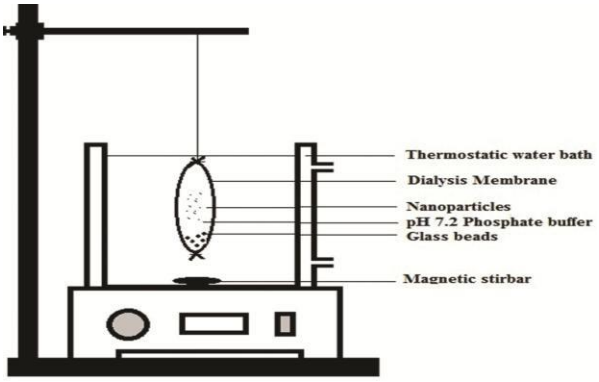

Figure 2: Diagrammatic representation of nanoparticles *In vitro* drug release assemblage

***In vitro* characterization of aspirin suppositories and aspirin nanoparticles loaded suppositories**

Appearance and physical integrity of suppositories

Appearance of the prepared suppositories (Sanjay K. Motwani 2008) was evaluated with naked eye physical integrity of the suppositories was evaluated by slicing the suppositories with stainless steel blade in to two half's and observed to find out the presence of fissure and pits.

Weight variation

Twenty suppositories were weighed and average weight was calculated. Each suppository was then individually weighed by using digital balance (Sanjay K. Motwani 2008). Not more than two of the individual masses deviate from the average mass by more than 5% and non deviate by more than twice.

Mechanical Strength

The breaking strength or crushing strength was determined for measuring fragility or brittleness of suppositories, which assess whether the suppositories will be able to withstand the hazards of packing, transporting and normal handling or not. Suppositories were randomly selected and subjected for crushing using (Erweka hardness tester) (F.A. Oyarzun 2009).

Content uniformity

Content uniformity test was determined by spectrophotometric method. The suppository was individually melted, dissolved in 100 ml of phosphate buffer pH 7.2 in separate volume flask and the solution was filtered using 0.45 µ membrane. After suitable dilution the also measured using U.V. spectrometry at a wave length of 225 nm (Akhlesh Kumar Jain 2008).

Disintegration Test

The disintegration time is a critical factor in the determination of the release rate of the active ingredient (s) from the suppository (Noha Nafee 2009). During this test, the time taken for the suppository to melt or disperse is measured when immersed in a water bath maintained at constant temperature ($37^0C + 1^0C$). The

time required for the suppository to melt or disperse in the surrounding water was noted.

Liquefaction time

Liquefaction time of the suppositories was determined based on the procedure discussed in literature (Tamer Guner 2004). Glass cylinder with external diameter 50 mm, internal diameter of 20 mm and length 30 mm, two ends of the cylinder was fixed with circulating pump; temperature of circulating medium was maintained at 37 ºC. Inside the cylinder cellophane dialyzer tubing was used to hold the suppository and then the rubber tubing from water circulation pump was attached to one end of the glass cylinder separate provision is provided in to the glass cylinder for thermometer arrangement.

Stability studies

Satiability studies were conducted based on the procedure discussed in literature (S. Calis 1994), to assess the stability of nanoparticles, suppositories and suppositories loaded nanoparticles the samples are stores at room temperature (22 :C – 27 :C) and at refrigerator conditions 5 :C for three months, sampling was done at 1, 2, 3 months and evaluated for FT - IR (Fourier Transform - Infra red) and DSC (Differential Scanning Calorimetery) to find out the changes during storage.

***In vitro* dissolution for aspirin suppositories and chitosan aspirin nanoparticles loaded suppositories**

In vitro drug release has performed by placing the suppository in a dialysis bag whose molecular weight was cut 10 kDa and 5 mL of dissolution medium pH 7.2 phosphate buffer was added in to the bag and sealed and the bag was placed in the receptor compartment containing 50 mL of phosphate buffer at 37 ± 5ºC over a magnetic stirring at 50 rpm samples were withdrawn at regular intervals and replaced with equal amount of buffer for a period of 24 h (Han-Gon Choi 2009). The assemblage was designed as shown in the represented in fig. 3.

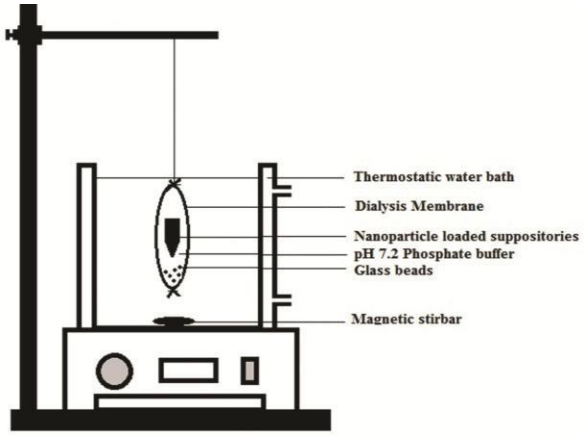

Figure 3: Diagrammatic representation of suppositories and nanoparticles loaded suppositories *In vitro* drug release assemblage

Drug release Kinetics

The data obtained from the *In vitro* drug release study of aspirin nanoparticles, aspirin suppositories and aspirin nanoparticles loaded suppositories was fitted to various kinetic equations (Martin Siewert 2003).

Statistical analysis

All the tests and results were performed in multiple of three and expressed as a mean S.D. (standard deviation) the results were subjected to if p - Value less than 0.05 it was considered to be significant.

RESULT AND DISCUSSION

The Study of aspirin nanoparticles, which are formed due to ionic interaction between cationic polymer chitosan and STPP the processing parameters such as effect of concentration of chitosan, pH, zeta potential and stability studies were performed as discussed and reported below. Aspirin suppositories made by fusion method evaluated to weight variation, mechanical strength, content uniformity, different composition and liquefaction time measured and *In vitro* release discussed in detail. Aspirin nanoparticles loaded suppositories prepared with fusion method evaluated as discussed earlier for aspirin suppositories.

DSC and FT - IR study

The Infrared spectroscopy results of aspirin loaded nanoparticles revealed the presence of aspirin with strong vibrations at 1176 cm^{-1} (C=O stretch), 1561.44 cm^{-1} (OCO with anti symmetric stretch, 1429 cm^{-1} (OCO symmetric stretch) and 1227 cm^{-1}, 1297 cm^{-1} and 1198 cm^{-1} (C-O and C-C stretching modes) shown the similar peaks in pure aspirin shown in fig. 4a. The characteristic peaks of chitosan 1540 cm^{-1} (amino group) and 1650 cm^{-1} (carbonyl group) amines which are bases the corresponding conjugates and derivatives showing a strong absorption peak between 2250 and 3071.7 cm^{-1} the aldehyde groups from covalently amine bonds with amino group, the amino group starching vibration at 3500 cm^{-1} and hydroxyl group at 3400 was clearly seen. Also there is a -NH_2 (scissoring) and N-H (wagging) and amie group of chitosan at 1550 cm^{-1} and 1650 cm^{-1} and 900 cm^{-1} – 600 cm^{-1}. The bonds at 1100-1000 cm^{-1} appears to polysaccharide structure fig. 4b. represent the aspirin loaded chitosan nanoparticles gelatin being a protein, the IR spectrum, shown in exhibits the characteristic amide absorption bands at 1660 cm^{-1} and 1550 cm^{-1} and the FTIR of chitosan nanoparticles loaded glycerol gelatin suppositories shown in Fig. 4c. showing the characteristic peaks of aspirin and chitosan.

Differential scanning calorimeter (Thermal analysis) performed for chitosan thermogram of chitosan showing at endothermic peak fig. 5a. between 50 - 120:C which may be due to the evaporation of residual water left in chitosan and exothermic decomposition was seen between 208°C to 308°C. When thermogram of aspirin loaded chitosan nanoparticles was observed

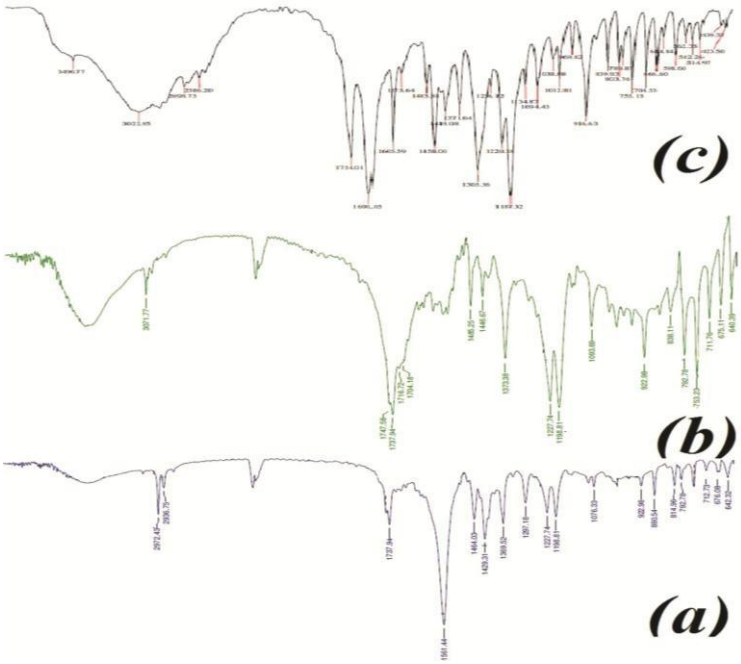

Figure 4: FTIR spectra's of a) Pure Aspirin b) Chitosan-Aspirin nanoparticles c) Chitosan-Aspirin nanoparticles loaded gelatin suppositories

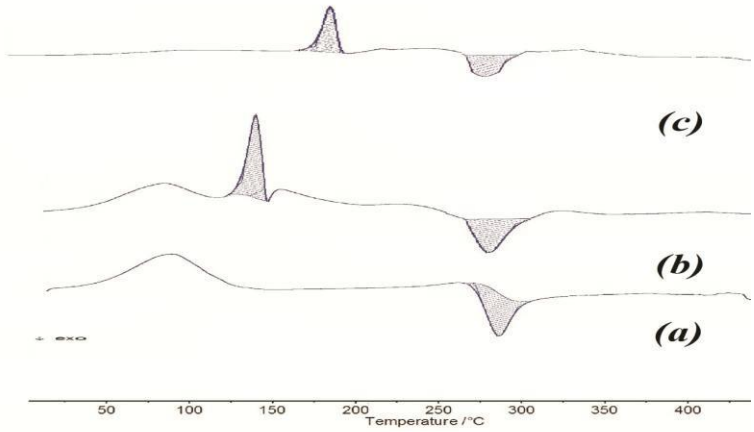

Figure 5: Thermograms of a) Pure chitosan b) Chitosan-Aspirin nanoparticles c) Chitosan-Aspirin nanoparticles loaded gelatin suppositories

there was a strong endothermic peak was observed 134°C to 147°C which was shown in fig. 5b. indicating the presence of aspirin in nanoparticles. When nanoparticles loaded suppositories were evaluated using thermal analysis a strong endothermic peak between 134:C to 145:C was observed indicating the presence of aspirin in suppositories represented in results fig. 5c.

***In vitro* characterization of Aspirin-chitosan nanoparticles**

Percentage yield of the lyophilized nanoparticles for a period of 24 h was found from the earlier discussed procedure tabulated in tab. 4 for the formulations Fa1 to Fa9 (63 ± 0.5 to 97 ± 0.2). Spherical and irregular shaped nanoparticles were formed spontaneously up on contact with the STPP solution under magnetic stirring the lyophilized was observed under SEM particle analysis performed shown in fig. 6.

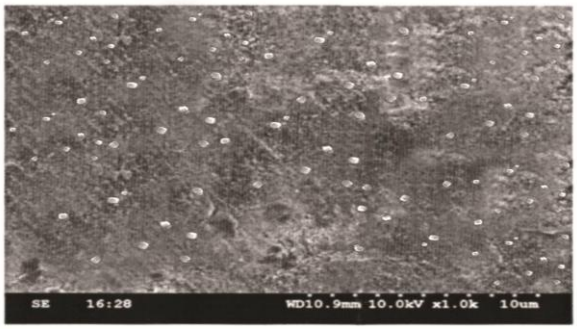

Figure 6: SEM image of aspirin-chitosan nanoparticles

The z - average diameter ranged from (116 ± 0.5 nm to 381 ± 1.7 nm) the results has shown in represented in tab. 4 and the fig. 7 showing a good correlation in the case of chitosan-Aspirin nanoparticles. With increase in the concentration of chitosan from 0.4 mg/mL to 2 mg/ml (Fa1-Fa9) there is a considerable increase in the

Table 4: Particle Size, Zeta potential, pH, Poly Dispersability Index (PDI), Percentage yield (% P.Y), Percentage drug entrapment (% P.D.E)

Formulation Code	Concentration (mg/mL)	Size of nanoparticles (d.nm)	Zeta potential (mV)	pH	% P.Y	PDI	% P.D.E
Fa1	0.4	116 ± 0.5	24.73 ± 0.01	2.3	63 ± 0.5	0.16 ± 0.01	24.4 ± 0.45
Fa2	0.6	142 ± 1.5	27.43 ± 0.02	2.7	68 ± 0.5	0.23 ± 0.015	36.4 ± 0.37
Fa3	0.8	162 ± 1.5	31.6± 0.02	3.2	71 ±2.0	0.3 ± 0.02	46.5 ± 0.43
Fa4	1	202 ± 0.5	35.2 ± 0.01	3.8	75 ± 0.3	0.31 ± 0.01	54.4 ± 0.2
Fa5	1.2	236 ± 1	37.3 ± 0.2	3.9	82 ±0.04	0.32 ± 0.01	65.2 ± 0.1
Fa6	1.4	271 ± 1	40.2 ± 0.001	4.2	88 ±0.01	0.36 ± 0.01	75.2 ± 0.2
Fa7	1.6	306 ± 1	42.1 ± 0.01	4.6	93 ± 0.5	0.41 ± 0.01	83.2 ± 0.2
Fa8	1.8	350 ± 1.15	45.39± 0.01	5.4	95 ± 0.3	0.42 ± 0.01	88.4 ± 0.2
Fa9	2	381 ± 1.7	47.99 ± 0.06	5.5	97 ± 0.2	0.44 ± 0.01	90.4 ± 0.5

particle size and zeta potential represented in tab. 4. represented values ranges from (24.73 ± 0.01 to 47.99 ± 0.06).

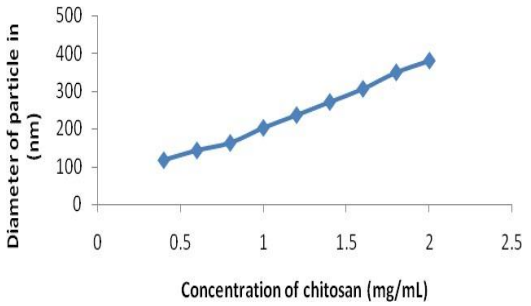

Figure 7: Mean particle size vs. concentration of chitosan (mg/mL)

Since the chitosan is a cationic polymer the zeta potential value appears in terms of (+) positive values. The Poly Dispersability Index ranges from (0.16 ± 0.01 to 0.44 ± 0.01) graph plotted shown in fig. 8. To know the effect of concentration vs. particle size vs. zeta potential representing the graph is linear showing the correlation increasing in concentration with increase in zeta potential and simultaneously there is increase in poly dispersibility. pH of the nanoparticles suspension increasing with increase in concentration of chitosan the values ranges from (2.3 to 5.5).

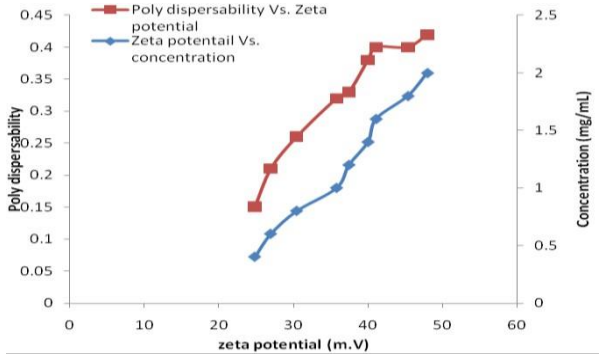

Figure 8: Poly dispersability vs. zeta potential vs. concentration of chitosan (mg/mL)

Percentage drug entrapment performed as discussed above and the results have a correlation with increase in the particle size there is increase in the percentage drug entrapment of aspirin nanoparticles represented in tab. 4. the value ranges from (24.4 ± 0.45 to 90.4 ± 0.5) there is correlation observed with increase in the concentration (mg/mL) with increase in the percentage drug entrapment.

***In vitro* drug release of Aspirin-chitosan nanoparticles**

The results of *In vitro* dissolution study performed for the formulations Fa1 to Fa9 in 7.2 pH phosphate buffer. The comparative dissolution profile of nanoparticles for a period of 24 h, was displayed in tab. 5 and fig. 9.

Initially the formulations (Fa1 to Fa9) shown a fast release pattern may be due to the adsorbed drug present over the nanoparticles (25.1±1.01 to 88.3±1.1). With (88.3±1.1) of drug release and (90.4 ± 0.5) of drug entrapment Fa9 formulation was proved to best formulation selected for the incorporation in glycerogelatin base.

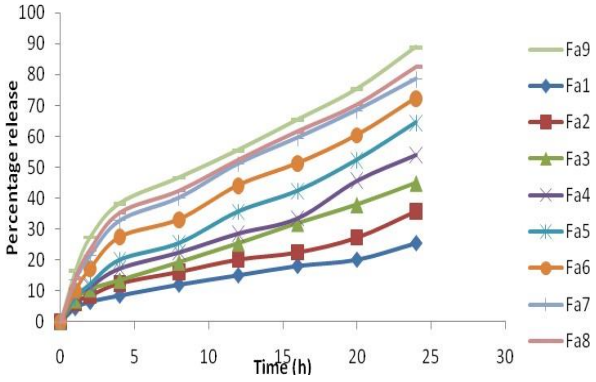

Figure 9: Graph representing Fa1 to Fa9 aspirin-chitosan nanoparticles formulations

The drug release profiles were fitted to drug release kinetics and the results were displayed in (table 6). The drug release profile was best fitted to Peppas equation, the n value of nanoparticles were present between (0.4815 to 0.6251) which is near 0.5 indicates the drug release follows non-fickian diffusion model. When the values are fitted to zero order the regression value (0.9926 to 0.990) is nearer to 1 indicating that they are following zero order release.

Table 5: *In vitro* evaluation tests of aspirin suppositories (Fs1-Fs12)

Formulation code	Weight variation (gm± S.D)	Mechanical strength (kg ± S.D)	Content uniformity (% content ±S.D)	Disintegration time (minutes ± S.D)	Liquefaction Temperature (ºC)
Fs1	1.01 ± 0.012	1.71 ± 0.01	95.06 ± 1.4	8.9 ± 1.7	29 :C
Fs2	1.01 ± 0.013	0.88 ± 0.01	95.33 ± 1.1	8 ± 0.7	29 :C
Fs3	1.004 ± 0.001	1.107 ±0.01	96.4 ± 0.5	8.5 ± 0.8	30 :C
Fs4	1.06 ± 0.01	1.31 ± 0.01	96.2 ± 1	8.4 ± 1	29 :C
Fs5	1.01 ± 0.01	2.11 ± 0.01	96.16 ± 1.04	10 ± 1.2	31 :C
Fs6	1.02 ± 0.005	1.91 ± 0.03	93.5 ± 0.5	9 ± 1.1	31:C
Fs7	1.02 ± 0.005	1.53 ± 0.01	96.66 ± 2.3	8.9 ± 0.1	30 :C
Fs8	1.02 ± 0.001	1.61 ± 0.02	97.66 ± 0.5	8.8 ± 0.8	30 :C
Fs9	1.05 ± 0.06	0.82 ± 0.01	98.56 ± 0.5	8.2 ± 0.6	29 :C
Fs10	1.01 ± 0.006	1.9 ± 0.01	97.1 ± 1	9.5 ± 1	30 :C
Fs11	1.003 ± 0.007	1.61 ± 0.01	94.2 ± 1	8.7 ± 1.2	29 :C
Fs12	1.002 ± 0.006	1.53 ± 0.02	95.16 ± 1.6	8.4 ± 0.3	29 :C

Table 6: *In vitro* evaluation tests for nanoparticles loaded suppositories

Formulation code	Weight variation (gm± S.D)	Mechanical strength (kg ± S.D)	Content uniformity (% content ±S.D)	Disintegration Time (minutes ± S.D)	Liquefaction Temperature (ºC)
Fas2	1.02 ± 0.008	0.78 ± 0.4	82.3 ± 0.5	7.2 ± 0.3	29 :C
Fas4	1.02 ± 0.004	1.10 ± 0.1	76 ± 1.1	8.1 ± 2	30 :C
Fas9	1.05 ± 0.2	0.81 ± 1.2	88.9 ± 1.8	7.8 ± 1.2	29 :C
Fas11	1.01 ± 0.4	1.21 ± 1.4	74.2 ± 0.8	8.5 ± 2.3	30 :C

***In vitro* characterization of aspirin suppositories**

Characterization of aspirin suppositories performed initially the suppositories were transacted and observed there is no display of fissure and pits present. In weight variation study all the suppositories were exhibited good uniformity (1.004 ± 0.001 to 1.05 ± 0.06). The mechanical strength is directly proportional to the concentration of gelatin, where as the concentration of glycerin and water is inversely proportional in respect to mechanical strength of suppositories (0.82 ± 0.01 to 2.11 ± 0.01), Content uniformity of the suppositories performed as discussed earlier all the formulation shown good content uniformity in all the formulation (93.5 ± 0.5 to 98.56 ± 0.5), disintegration time indicates that with increase in the percentage of water and glycerin there is considerable decrease in time and with increase in gelatin concentration there is increase in time for disintegration (8 ± 0.7 to 10 ± 1.2) and liquefaction temperature of the suppositories was ranged from (29 :C to 31:C) formulation containing highest gelatin concentration has shown a considerable increase in liquefaction time at the same formulation containing high concentration of water shown a decrease in liquefaction time all the results were displayed in shown in tab. 7.

Dissolution study performed to the prepared suppositories for a period of 60 minutes with periodic sampling. The drug release and dissolution behavior were compared between Fs1-Fs12 when compared it was conclude that with increase in the gelatin composition the drug release was extended sustained without any immediate release. When formulations containing highest water and glycerin composition was observed the drug release was immediate. Formulations Fs2, Fs4, Fs9 and Fs11 formulations were suited to be the best for the immediate release drug release behavior of aspirin suppositories was shown in tab. 8 and fig. 10.

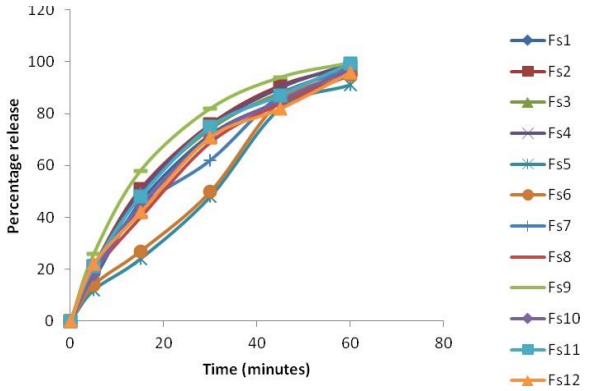

Figure 10: Graph representing Fs1 to Fs12 aspirin suppositories

When *In vitro* dissolution profiles were fitted to various kinetic models. The Peppas equation reveals n value between (0.548 to 0.852) first order model regression value was between (0.899 to 0.986) and the zero order model regression values lies between (0.465 to 0.969) the values stated indicates that the drug release was non-fickian type of diffusion and fallowing first order kinetics. The results were displayed in tab. 8.

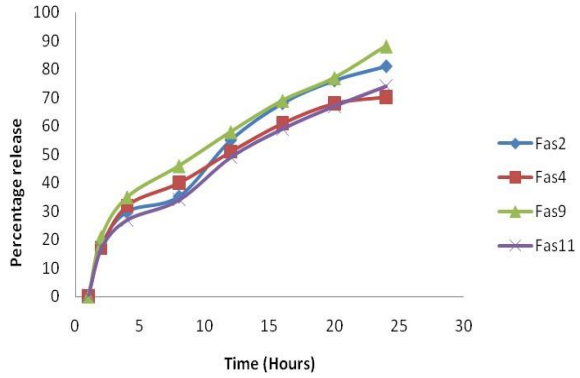

Figure 11: Graph representing Fas2, Fas4, Fa9 and Fas11 nanoparticles loaded suppositories

***In vitro* characteristic of aspirin nanoparticles loaded suppositories**

The nanoparticles loaded suppositories were prepared by taking therapeutically equal 100 mg of aspirin lyophilized nanoparticles of fa9 formulation containing highest percentage of encapsulation efficiency and incorporated in selected based on displacement value glycerogelatin base composition. Based on the previously performed evaluation studies for the aspirin suppositories Fs1 to Fs12 it was proved that Fs2, Fs4, Fs9 and Fs11 suppositories containing formulations are having good diffusion efficiency. When the aspirin nanoparticles loaded suppositories were subjected to different *In vitro* evaluation studies includes weight variation (1.02 ± 0.004 to 1.05 ± 0.2) of the all the suppositories are ideal, mechanical strength (0.78 ± 0.4 to 1.21 ± 1.4) of the suppositories slightly decrease may be due to the incorporation of nanoparticles the internal integrity of the suppository might be decreased, content uniformity of the suppository value ranges from (74.2 ± 0.8 to 88.9 ± 1.8), when disintegration results were observed, comparatively it was concluded the disintegration time of aspirin nanoparticles loaded suppositories slightly decreased compared to aspirin suppositories earlier prepared the values ranges from (7.2 ± 0.3 to 8.5 ± 2.3), liquefaction does not shown any considerable difference in the melting time results were displayed in tab. 10.

In vitro dissolution study was performed for a period of 24 h with periodical sampling the results displayed in tab. 11. and fig. 11. it was observed from the results that a peculiar behavior was observed at the initial time of drug release all the formulation are showing a lag phase with lowest amount of drug release. This kind of mechanism may be due to the time taken for the release of nanoparticles from the suppositories at the end of 24h Fas2 (82.3 ± 1.52), Fas4 (71 ± 0.85), Fas9 (86.4 ± 1.63) and Fas11 (74.06 ± 0.9) was release successively from the formulations and Fas9 formulation stands best with highest percentage of drug release was observed at the end of 24 h results displayed in tab.11.

In vitro drug release profile was fitted to various kinetic models the results indicates that the Peppas equation displays that the n value ranges from (0.301 to 0.477) which clearly indicates that the drug release was fickian type of diffusion and regression value of zero order kinetic indicates that the value (0.800 to 0.899) the drug release was near to zero order detailed values was shown in fig. 11.

CONCLUSION

Aspirin nanoparticles were formulated and evaluated for various parameters. The highest percentage entrapment of 90.4 ± 0.5 was found in formulation Fa9. The highest zeta potential of 47.99 ± 0.06 was found in formulation Fa9. The drug release profile of Fa9 formulation was extended for a period of 24 h and follows zero order drug release pattern. The release exponent value obtained from Peppa's model for all the formulations shows non-fickian type of drug release which was proved to be best formulation. Aspirin suppositories were prepared with varying compositions of gelatin, glycerin, and water from various characterization studies it was concluded that with increase in concentration of gelatin the hardness, liquefaction temperature, disintegration time was extend. Similarly with increase in concentration of glycerin and water there a considerable decrease in liquefaction temperature, hardness, and disintegration time was decreased. *In vitro* drug release performed for the prepared (Fs1-Fs12) formulation indicates that Fs2, Fs4, Fs9, Fs11 best formulations with highest drug release at the end of 60 minutes. When the drug release profiles fitted to kinetic models revealed that the drug release was following first order kinetics, peppas equation indicates the drug release was non-fickian type. Based on the above observations includes *in- vitro* aspirin nanoparticles and aspirin suppositories best compositions was selected and loaded with nanoparticles of Fa9 formulation and evaluated for different evaluation studies. The evaluation studies proved that all the formulation is following fickian type of diffusion and Fas9 proved to be best formulation with faster liquefaction with (86.4 ± 1.63 %) of drug release at the end of 24 h. Thus the prepared formulation may reduce the risk of utilization of aspirin through rectal rout, and increase the bioavailability and may improve patient complain.

AUTHOR'S STATEMENT

No conflict of interest

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