9.0 SUMMARY

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. Drugs may be administered in suppository form for either local or systemic effects. Conventional suppositories have advantages 1. They exert local action on rectum 2. To Promote Evacuation of the Bowel 3. To Provide a Systemic Effect.

In this work a new drug delivery system “suppositories loaded nanoparticles” were developed, present work explain the mechanism of nanoparticles release from the suppositories in five steps 1. Delivery of suppositories to rectal cavity 2. Dissolution (release of nanoparticles) by the rectal fluids 3. Nanoparticles entry 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.

The recent investigations revealing the importance of ASA 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 of 80 to 325 mg daily is effective in secondary prevention stage.

Compatibility study carried to examine compatibility issue if any via examining the mixture of ASA and excipients by differential scanning calorimeter (DSC) and fourier transform infra red spectroscopy (FTIR).
Initially ASA-nanoparticles prepared employing ionicgelation as method for the preparation of nanoparticles and the effect of process parameter on ASA-nanoparticles (chitosan and STPP) was evaluated. The prepared nanoparticles were evaluated for Percentage of drug entrapment (%P.D.E.), Surface texture and particle size of aspirin-chitosan nanoparticles using SEM and TEM & in-vitro Drug release.

ASA-Suppositories were prepared using hydrophilic base like glycerogelatin base has good diffusion efficiency compared to other bases, to select the best base composition percentage glycerin (57% to 80%): gelatin (6% to 25%) ratio was varied. Suppositories were prepared employing fusion method. ASA-Suppositories were evaluated for various evaluation tests including Appearance and physical integrity of suppositories, weight variation, mechanical strength, content uniformity, disintegration, in-vitro drug release.

Suppositories loaded ASA-nanoparticles were prepared by fusion method. Based on the previously performed in-vitro characterization studies for aspirin nanoparticles and aspirin suppositories, suitable ASA-nanoparticles and base compositions were identified for the preparation suppositories loaded of ASA-nanoparticles. Therapeutic equivalent quantities of lyophilized ASA-nanoparticles were added in to the molten glycerogelatin base and suppositories loaded ASA-nanoparticles were prepared. The prepared suppositories were evaluated for appearance and physical integrity, weight variation, mechanical strength, content uniformity, disintegration, in-vitro drug release.

A comparative in-vivo evaluation study and in-vivo safety study performed for ASA-suppositories and suppositories loaded ASA-nanoparticles, new land rabbits were chosen as a model for in-vivo absorption study. A simple high performance liquid chromatographic method was developed and validated for ASA in blood samples, the linearity (10-100) µg/ml ($r^2 = 0.999$). The retention time was observed 5.3 minutes was
observed by using buffer pH 2.5, acetonitrile, and isopropyl alcohol in (85:1:14). The validated method was used for the determination of pharmacokinetic profiles like Area under curve (AUC_{\text{0-t}}), Area under zero infinity (AUC_{0-\text{infinite}}), C_{\text{max}}, t_{\text{max}}, half life, elimination rate constant, Mean residence time (MRT). Safety study was carried after continuous use of ASA-suppositories and suppositories loaded ASA-nanoparticles after four weeks. Histological examination was performed. At the end of each week animal was scarified and rectal tissue was isolated for histological examination.

ASA-nanoparticle prepared successfully based on ionic gelation method with increase in the chitosan concentration 0.4 mg/mL to 2 mg/mL Z - average diameter ranged from (116 ± 0.5 nm to 381 ± 1.7 nm), zeta potential (24.73 ± 0.01 m.v to 47.99 ± 0.06 m.v), Poly Dispersability Index ranges from (0.16 ± 0.01 to 0.44 ± 0.01) and pH value of ASA-nanoparticle increase form (2.3 to 5.5). Drug entrapment efficiency (24.4 ± 0.45 to 90.4 ± 0.5) and In-vitro drug release carried out at the end of 24 h the drug release was ranges from (25.1±1.01 to 88.3±1.1).

Nanoparticles prepared with variable concentration of STPP (0.25 mg/mL - 2.25 mg/mL) and to investigate the effect on nanoparticles Z - average diameter ranged from (166.3±5.86 nm to 801.3±1.52 nm), zeta potential value (22.3±1.52 m.v to 23.3±1.52 m.v), Poly Dispersability Index (0.103±0.02 to 0.53±0.02) pH value (3 to 4), Percentage yield (18.3±1.52 to 60.6±1.52) and Percentage drug entrapment (32±1 to 73±1.52). In-vitro drug release percentage of drug at the end of 24hr (32±1 to 75±1). From the evaluation tests it was proved that the Fa9, Fa18 75±1%, 88.3±1.1 % of drug release and highest drug entrapment of73±1.52, 90.4 ± 0.5 was identified for the incorporation in glycerogelatin base.
ASA-Suppositories prepared based on fusion method evaluated for various evaluated to investigate the best base composition. Weight variation study of all the suppositories were exhibited good uniformity (1.004 ± 0.001 to 1.05 ± 0.06). Mechanical strength of suppositories (0.82 ± 0.01 to 2.11 ± 0.01), Content uniformity of the suppositories (93.5 ± 0.5 to 98.56 ± 0.5) disintegration time (8 ± 0.7 to 10 ± 1.2) and liquefaction temperature of the suppositories was ranged from (29 °C to 31°C) 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. The drug release and dissolution behavior were compared between Fs1-Fs24. It was conclude that with increase in the gelatin composition the drug release was extended sustained without any immediate release.

When in-vitro dissolution profiles were fitted to various kinetic models, the Korsmayer’s 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. Fs2, Fs4, Fs9, Fs11, Fs13, Fs15, Fs16, Fs20, Fs23 and Fs24 were proved to be best base composition for the incorporation of nanoparticles.

Suppositories loaded ASA-nanoparticles prepared using the identified nanoparticles and base compositions. The prepared suppositories loaded nanoparticles were evaluated similar evaluation tests. Results indicates weight variation (1.02 ± 0.004 to 1.05 ± 0.2), 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 ASA nanoparticles loaded suppositories slightly decreased compared to aspirin suppositories earlier prepared the values ranges from (7.2 ± 0.3 to 8.5 ± 2.3). *In-vitro* dissolution study carried out for a period of 24hr 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. Fas2 (82.3 ± 1.52), Fas4 (71 ± 0.85), Fas9 (86.4 ± 1.63) and Fas11 (74.06 ± 0.9) Fas23 (63.83±1.25), Fas24 (63.83±1.25), Fas20 (56±1) was release successively from the formulations. When the dissolution data fitted to *in-vitro* kinetics the results shoes the influence of unknown mechanism on drug release behavior follows zero order with diffusion type of release.

Comparative pharmacokinetic study carried out in rabbit’s indicates no interaction of with the rabbit plasma, from the results it was observed that $t_{\text{max}}$ was ranges from (2±0.02 to 2±0.75) in ASA-suppositories formulations Fs1, Fs3, Fs4, Fs8, Fs11, and Fs12 it was extended to (8±1.2 hr). The MRT was extended from (2.61±0.15 hr) to (7.78±0.65 hr) in the optimized Fas11 formulation. Histological examination conducted for four weeks represent the control rectal tissue illustrate that epithelium was normal with goblet cells arranged in series can be seen clearly. This may be due to the continuous localization of ASA-suppositories on rectal wall in addition to the mechanical irritation caused by during insertion of suppositories, there is no much severe changes observed in case of suppositories loaded ASA-nanoparticles as observed when compared to ASA-suppositories.
CONCLUSION AND RECOMMENDATIONS

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. Suppositories are semisolid dosage form; they induce local effect after insertion into the body cavities. Suppositories offers many advantages a) the portal circulation is bypassed prevent first pass metabolism b) drug irritating in gastric mucosa can be given in this manner c) the influence of gastric pH and enzymatic juices can be avoided d) it can administered to patients who cannot or will not swallow e) absorption of rectum will be more rapid and more regular than from stomach or intestine f) duration of action can be prolonged, with few of disadvantages a) patient acceptability b) limited knowledge mechanism

Aspirin chemically acetyl salicylic acid (ASA) is in wide use for its anti thrombolytic, antipyretic and anti-inflammatory by irreversible inhibition of platelet cyclo-oxygenase thus inhibiting generation of thromboxane A2 and new finding revealed the importance of ASA for its anti carcinoma effect. ASA is the approved as an agent to prevent transient ischemic attacks and heart stroke beyond its anti-inflammatory action. Present investigation recommending the use of ASA for moderate risk in cardiovascular events. The continuous use of ASA for 5 years sown to be effective in preventing the colorectal carcinoma proved in randomized clinical trials. Doses required for preventing the effect of colorectal carcinoma relatively high (300 to 500mg), recent studies indicates the effective use ASA at low dose (75-300mg) for long term use reduce the colorectal carcinoma. These finding provide a new interest on ASA molecule which was 100 years old.
The present investigative work “Conception and evaluation of suppositories loaded nanoparticles as a drug delivery system”, 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.

In this work suppositories loaded nanoparticles of ASA prepared using chitosan. Chitosan is a modified natural prepared by N-deacetylation from natural biopolymer. Ionicgelation method based on ionic interaction based on electrostatic interaction between amine group of chitosan and polyanion of such as calcium chloride and sodium Tripolypophosphate. The effect of independent variables on ASA nanoparticles (chitosan and STPP concentration) on particle size (nm), % Percentage of drug entrapment, Poly Dispersability index (P.D.I), pH, and zeta potential (m.v). Ideal formulations were isolated for incorporation, ASA-glycerogelatin suppositories were prepared to select suitable base composition. Ideal base composition thus selected as a carrier for the nanoparticles.

Suppositories loaded ASA-nanoparticles can facilitate the entry of the nanoparticles in to the systemic circulation through the rectal fenestrated capillaries.

The present aim of the work aims to prepare, evaluate in-vivo performance and safety study of ASA.

The preformulation study of ASA and excipients used during the formulations were investigated via examining the mixture of ASA and excipients by DSC and FTIR
spectroscopy, the same for ASA also. From the preformulation study it was concluded that the excipients tested can be effectively used for formulation. Based on the results FTIR and DSC analysis, no changes in structural of drug were evident.

The optimized formulation of nanoparticles loaded suppositories following anomalous type of transport. In-vivo study was randomly designed to evaluating suppositories loaded nanoparticles containing ASA, rabbits were chosen as animal models for in-vivo absorption study. Analytical method used was for pharmacokinetic profile of suppositories loaded ASA nanoparticles and ASA-suppositories. The pharmacokinetic parameters $t_{\text{max}}$, $t_{1/2}$, AUC, MRT were increase significantly compared with conventional suppositories except $C_{\text{max}}$ and $K_{\text{el}}$. Histological study was carried at periodical intervals for four weeks. The animals are made to receive each one ASA-suppositories and suppositories loaded ASA-nanoparticles per day at the end of the first week first group of animals were scarified fallowed by successive groups in first, second, third and fourth week. The scarified animal rectum were isolated, rinsed, with saline solution and stored in 10% carbonated formalin, The rectal tissues were embedded in paraffin wax and cut in 3µm thickness. The satins were stained with eosin and examined under binocular light microscope. Histological examination conducted for four weeks represents the rectal tissue with necrosis (damages cells) can be seen after one week of continuous administration. The histological examination of successive in second week third week and fourth in week tissue with ulceration was observed which may be severe necrosis were as normal goblet cells were observed when nanoparticles loaded suppositories used for four weeks hence the present developed delivery systems provides a safe and convenient means for long term usage of drugs with suppositories and provides new trend in effective delivery of drugs.