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# Development of a rectal nicotine delivery system for the treatment of ulcerative colitis

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**Abstract**

The aims of this investigation were: i. to develop a rectal nicotine delivery system with bioadhesives for the treatment of ulcerative colitis and ii. to evaluate nicotine transport and cytotoxicity of the delivery system using Caco-2 cell culture systems. Rectal nicotine suppository formulations were prepared in semi-synthetic glyceride bases (Suppocire AM and AI, Gattefosse Inc.) by fusion method. The in vitro release of nicotine was carried out in modified USP dissolution apparatus 1. Differential scanning calorimetry (DSC) and powder X-ray diffraction were used to study the polymorphic changes if any in the formulations. An LC method was used for the assay of nicotine. The effect of bioadhesives (glyceryl monooleate (GMO), and Carbopol) on the nicotine flux was evaluated using Caco-2 cell permeability studies and Caco-2 cell viability was determined using the MTT toxicity assay. In vitro release studies indicated that the low melting AI base was superior to that of the AM base. Presence of GMO in the formulation enhanced the release of nicotine whereas Carbopol showed an opposite effect. The enhanced release of nicotine in the presence of GMO was found to be partly due to the melting point lowering effect of this compound. Caco-2 cell absorption studies showed that there was a decrease in the flux of nicotine in the presence of both the bioadhesives. The flux of the fluorescein marker which is used to study the integrity of the cell monolayers was found to be slightly higher only in the presence of 10% (w/w) Carbopol. Nicotine, Carbopol, and GMO do not have any cytotoxic effect on these cell monolayers within the concentration range used in the formulations. Rectal nicotine formulations containing bioadhesives were developed and characterized. Both in vitro release and cell culture studies have indicated that one can manipulate the nicotine release from these rectal delivery systems by incorporation of various bioadhesives or the use of different bases in the formulation. Nicotine concentration below 2% (w/v) and bioadhesive concentration below 10% (w/w) do not have any cytotoxic effect on Caco-2 cells. © 1999 Published by Elsevier Science B.V. All rights reserved.

*Keywords:* Ulcerative colitis; Nicotine suppository; Bioadhesive; Carbopol; Glycerylmonooleate; Caco-2 cell

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## 1. Introduction

Ulcerative colitis (UC) is one of the most common and difficult gastrointestinal diseases to treat effectively. UC is limited to the large intestine. Starting in the rectum, UC may extend proximally to affect a variable length of the colon (Malatjalian, 1987). For decades, sulfasalazine (a conjugate of 5-aminosalicylic acid and sulfapyridine, linked by an azo bond), was the drug of choice for the treatment of this disease. However, its use has been limited because of the side effects (nausea and vomiting, headache, fever and rash) of the sulfonamide portion of the drug (Segars and Gales, 1992). On the other hand, the 5-ASA portion of sulfasalazine is known to be the active part of this drug (Azad Khan et al., 1977). The mechanism of action of 5-ASA is thought to be topical rather than systemic (Peskar et al., 1987). This drug is marketed under the trade name of Mesalamine and is available as tablet, enema and suppository formulations. The problem with orally administered Mesalamine is the rapid absorption of the drug in the upper gastrointestinal tract (75%) as compared to only 19% in the colon (Segars and Gales, 1992). Therefore, only a small amount of the active agent comes in contact with the distal colonic mucosa. A second problem encountered during the oral administration of 5-ASA tablets is that when this dosage form reaches the colon or rectum, it is covered with solid stool and is expelled rapidly from the colon (Bruckstein, 1990). In contrast, topical application of 5-ASA as enema and suppository formulations were found to be highly effective. 5-ASA enemas are best suited for patients with left-sided colitis whereas suppositories are effective in all patients suffering from UC. 5-ASA suppository formulations were found to be effective in 83–89% of the treated patients (Campieri et al., 1986, 1989).

Harris et al. (1982), first documented that inverse relationship between non-smokers and the development of UC. Calkins's study (Calkins, 1988) also supported the claim of Harris and co-workers. Treatment of UC with transdermal nicotine patches (Pullan et al., 1994), and nicotine gum (Lashner, et al., 1990) have demonstrated that symptoms of UC dissipate significantly with the use of nicotine delivery systems. The use of nicotine in the treatment of ulcerative colitis has been studied extensively and reported elsewhere (Rhodes and Thomas, 1995; Kennedy, 1996; Cicero et al., 1997 and Thomas et al., 1998). Because the 15 mg of the transdermal nicotine causes substantial side effects in up to two thirds of the patients (Pullan, 1994; Thomas et al., 1995), formulations that are more effective and better tolerated have been developed for the local delivery of nicotine in the treatment of UC. Local delivery of nicotine as an enema and its effectiveness in UC have also been reported (Zins et al., 1996; Green et al., 1997). Green and his co-workers (1997) have utilized a bioadhesive polymer (Carbomer) in the enema. This polymer was thought to interact with the adherent mucus layer on the surface of the large bowel mucosa and therefore could bring the drug molecules into immediate contact with the inflamed mucosa. Pharmacokinetic analysis from this study also revealed that the relatively small rise in serum nicotine and the extended biological half-life may partly be caused by slower absorption of nicotine from this dosage form.

With our previous success with rectal delivery of 5-ASA along with bioadhesive (Haney and Dash, 1994), we propose that nicotine can ideally be delivered by this rectal route. The goal of this study is to develop a novel drug delivery system for the effective and local delivery of nicotine to the distal portion of the intestine. We hypothesize that bioadhesion will enhance the therapeutic efficacy of the dosage form by increasing contact time and intimacy of the contact at the site of action. Therefore, it is expected that a prolonged local effect of the drug may be achieved. This sustained release formulation will reduce the frequency of administration and increase patient compliance. This will also make it possible to administer the required dose of the drug at the site of the disease with minimal systemic concentration, which will limit side effects.

Study of the transport of drugs across membranes have been greatly facilitated by the availability of tissue culture systems permitting access to basolateral and apical cell surfaces. Caco-2 cell culture systems have created an interest in using these systems as in vitro models to investigate drug transport at the cellular level (Borchardt, 1995; Tamura et al., 1996; Gan and Thakker, 1997). Caco-2 cell monolayers which are widely used as models of intestinal epithelial monolayers have also been utilized to evaluate the rectal absorption of macromolecules from suppository formulations (Utoguchi et al., 1998). We have used Caco-2 cell culture method to study nicotine transport through epithelial cells in the presence and absence of various additives. Examination of the integrity of the cell monolayers and potential cytotoxic effects of the drug and the additives were also performed with the Caco-2 cells.

## 2. Materials and methods

### 2.1. Suppository base

Suppocire AI (melting range: 32.5–36.5°C, monoglyceride content $< 3\%$) and Suppocire AM (melting range: 34–36°C, monoglyceride content $< 1\%$) (Gattefosse Inc., Saint-Priest, France) are semi-synthetic glycerides of sturated fatty acids from C12 to C18 bases and are used in this study.

### 2.2. Bioadhesives

Carbopol-934 and Carbopol-974 (BFGoodrich, Cleavland, OH, USA), crosslinked acrylic acid polymers and Glyceryl monooleate (melting point 19–23°C, monoglyceride content $< 94\%$) (Eastman Chemical AG, Switzerland) were used as bioadhesives. Carbopol-934 is a traditional Carbopol resin polymerized in benzene and contains less than 100 ppm benzene (residual solvent). Carbopol-974 on the other hand is polymerized in toxicologically preferred solvent (ethylacetate) and contains less than 0.7% of ethylacetate as a residual solvent. Carbopol-934 has viscosity profiles similar to Carbopol-974. A 0.5% of either polymer at a pH of 7.4 has a viscosity of 29 400–39 400 cps (measured by Brookfield viscometer at 20 rpm). Additionally, the 974 grade is potassium neutralized about 3% (of acid group) for process reasons. The amount of bioadhesive incorporated into each suppository varied from 0–2% (w/w) in the case of Carbopol and 0–50% (w/w) in the case of GMO.

### 2.3. Assay of nicotine

Nicotine (MW 162.2) used in this study was obtained from Sigma Chemical Company, St. Louis, MO, USA and was available as an oily liquid (has a specific gravity of 1.0097 at 20°C refereed to water at 4°C) with 98–100% purity. Nicotine is miscible with water below 60°C and very soluble in alcohol, chloroform, and oils (Budavari, 1989). HPLC method developed in our laboratory (Dash and Wong, 1996) was used to determine the nicotine content in the formulation and in the release medium. The chromatographic separation was achieved on a reversed phase C-18 column with UV detection at 260 nm. This isocratic system was operated at ambient temperature and used citrate buffer:methanol (85:15%, v/v) with an apparent pH 2.4 as the mobile phase. The flow rate was maintained at 0.7 ml/min.

### 2.4. Formulation of suppositories

Suppositories were prepared by fusion method. Physical mixture of the drug and the bioadhesives were added to the molten base (at $40 \pm 1.0$°C) in a casserole with constant stirring using a glass rod. The melt was poured into 2 ml disposable plastic molds and allowed to cool at an approximate rate of 1°C/min. The suppositories were harden at $10 \pm 2.0$°C in an ice bath over a period of 1 h. The drug load in the suppositories was kept at 2% (w/w).

### 2.5. Characterization of polymorphism and melting behavior in the formulation

Polymorphism is the capability of a substance to crystallize into two or more different crystalline forms. Suppositories have been reported to show

polymorphic changes (Haleblian and McRone, 1969). The polymorphic behavior in the formulation if any, was studied using differential scanning calorimetry (DSC) (Model DSC-50, Shimadzu, Kyoto, Japan) and X-ray diffractometry (Model D5005, Siemens) methods (Dash and Suryanarayanan, 1991). Suppository samples were filled in a zero-background quartz holder and exposed to CuKα radiation (45 kV × 40 mA) in a wide angle X-ray diffractometer. The instrument was operated in the step-scan mode, in increments of 0.05°2Θ. The angular range was 1 to 40° 2Θ and counts were accumulated for 1 s at each step. The date collection program used was JADE 3.0. Polymorphic changes during storage could be detected by comparing the X-ray diffraction patterns and DSC thermograms of the samples before and after storage. DSC was also utilized to test the melting characteristics of the suppositories in the presence and absence of other additives and to determine the physical state of the drug in the formulation.

### 2.6. In vitro release of nicotine from formulations

In vitro release of nicotine from suppositories was carried out in USP dissolution apparatus 1 with slight modification (The United States Pharmacopeia, 1985). Suppositories were placed in a wire mesh basket specially designed for suppository formulations (Hansen Research Inc., Chatsworth, CA) and Sorensen's phosphate buffer (900 ml), pH 7.4 at 37 ± 0.5°C was used as the release medium. The conventional USP stainless steel basket was replaced with polyurethane basket with the same external dimensions as the USP basket and has twelve linear slots of 0.25 mm allowing for a porosity of 52% (Palmieri, 1981). The buffer was prepared by mixing 197 ml of $KH_2PO_4$ solution (9.1 g/l) to 803 ml of $Na_2HPO_4$ solution (9.48 g/l). At predetermined time intervals (0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, and 420 min), 1 ml of the release medium was collected through a sample filter set (Hanson Research, Chatsworth, CA, USA) and replaced with fresh buffer. The nicotine content in the release medium was determined by the HPLC assay method described earlier. The solubility of the nicotine salt in buffer at various pH has been reported (Santi et al., 1991). It was reported to be 0.288 g/ml at a pH of 7.0 and at 37°C. The maximum amount of nicotine present in a 2 g suppository used in this study was 0.4 g. Therefore, dissolution studies were carried out under sink condition.

### 2.7. Effects of storage on the in vitro release of nicotine from formulations

Suppositories were made using both AI and AM bases. All these formulations contain 2% (w/w) Nicotine and 2% (w/w) Carbopol 974. One batch was stored at room temperature (23 ± 1.0°C), the other was stored at refrigerated temperature (4 ± 1.0°C) over a period of 4 months. The in vitro release characteristics of nicotine from suppositories stored under both these conditions were then evaluated.

### 2.8. Stability of nicotine in the formulation

The drug content in the suppositories were studied at time zero (immediately after preparation) and after 4 months from their date of manufacture. The suppositories tested were AI, AM, AI base containing 10% (w/w) GMO, AM base containing 10% (w/w) GMO, AI base containing 2% (w/w) Carbopol 974, AM containing 2% (w/w) Carbopol 974. All of these formulations contained 2% (w/w) nicotine and were stored at room temperature (23 ± 1°C). Known amount of suppository was dissolved in 1 ml of methylene chloride and volume was adjusted to 100 ml with the mobile phase. Nicotine content was determined using HPLC.

### 2.9. Caco-2 absorption studies

The absorption of nicotine alone, and in the presence of the mucoadhesives (2% w/w Carbopol 934) (Robinson and Park, 1984; Park and Robinson, 1985; Robert et al., 1988), and the cell specific bioadhesive (10–20% w/w of GMO) (David and Dorschel, 1992; Engstrom et al., 1995) was determined using side-by-side diffusion apparatus (Miller et al., 1995). Confluent cell mono-

layers grown on collagen-coated polycarbonate filters were removed from their plastic insert supports, and placed in the diffusion chamber. After 30 min of pre-incubation in Hannk's Balanced Salt Solution (HBSS) with 0.1% BSA, 3 ml of drug solution (10 μg/ml) in HBSS solution was placed in the donor compartment. The receiver compartment consisted of exactly the same solutions in the donor compartment without the drug. Samples (200 μl) were removed from the receiver side at 5, 15, 30, 45, and 60 min and replaced with fresh buffer. Samples were also collected from the donor side at the start of the experiment (time 0) and again at the end of the experiment (time 60 min). At the end of the absorption studies, monolayers were removed from the side-by-side diffusion chamber, washed three times with ice-cold HBSS and solubilized with 0.1% Triton X-100. Samples from the donor, receiver, and cell solutions were analyzed for nicotine content using a HPLC method.

### *2.10. Caco-2 cell monolayers integrity study*

The integrity of the Caco-2 cell monolayers was determined by examining the permeability of the monolayers to the fluorescent marker, fluorescein. The confluent Caco-2 monolayers were placed in side-by-side diffusion chambers and 3 ml of assay buffer was placed in the donor and receiver compartments of the chambers. The assay buffer that was added to the donor compartment contained the various treatments and formulation products and 10 μM fluorescein. Samples (300 μl) were removed from the donor compartment at the start and the end of the permeability experiment. Samples (300 μl) were also removed from the receiver compartment at 0, 5, 10, 15, 30, 45, and 60 min. All samples were replaced with equal volumes of assay buffer. The fluorescein in the samples was measured using a Shimadzu RF5000 spectrofluorometer at 488 nm excitation and 510 nm emmission. The amount of fluorescein in the receiver compartment was expressed as a percentage of the amount added to the donor compartment at the start of the experiment.

### *2.11. Caco-2 viability studies*

The effects of Nicotine, GMO, Carbopol 934 on Caco-2 cell viability was determined using the MTT toxicity assay (Hansen et al., 1989). The assay is based on the ability of living cells to metabolize the tetrazolium salt, MTT (3-(4,5-dimethylthizaol-2-yl)-2,5-diphenyl tetrazolium bromide), to a chromophore whose absorption can be measured spectrophotometrically. For the present study, Caco-2 cells were removed from the membrane inserts with a non-enzymatic cell dissolution solution (Sigma Chemical; St. Louis, MO) and seeded at a density of 15000 cells/well in 96-well plates. The cells were then incubated at 37°C for a period of 60 min with, either nicotine–solution, nicotine–Base, nicotine–GMO, or nicotine–Carbopol 974. Aliquots of MTT (25 μl; 5 mg/ml) were added to each well and the cells were incubated for an additional 2 h at 37°C. The cells were then solubilized with the MTT solvent (20% sodium dodecyl sulfate in a 50:50 volume ration of DFM (*N*,*N*-dimethyl formimide) and water) and the absorbance of the wells were measured at 570 nm using a microtiter plate reader.

## 3. Results and discussion

### *3.1. Characterization of polymorphism in the formulation*

Polymorphism is the capability of a substance to crystallize into two or more different crystalline forms. Any polymorphic changes in the suppository formulation may change its melting point, release kinetics and bioavailability. Polymorphic behavior in the formulation and during storage (over a period of 4 months) was studied by DSC and X-ray diffraction methods. The DSC thermograms of suppositories prepared from AI and AM bases are shown in Fig. 1. Heating and cooling cycles as well as long term storage did not have any effect on the thermograms of these suppositories. The powder X ray diffraction patterns of the suppositories prepared from AM and AI bases are shown in Fig. 2. The X-ray diffraction patterns of the samples are identical with

respect to peak positions. The patterns revealed an intense peak at a very low angel ( $\approx 2.6°2\Theta$) followed by an amorphous halo with a few characteristic peaks. The intensity of the peaks vary from sample to sample, since the samples were not filled in the holders in a manner suitable for quantitative analysis, no conclusions about crystallinity of the samples can be drawn from the differences in the intensities of the peaks. The XRD patterns of the samples are virtually identical with respect to the peak positions. On the basis of these scans, no differences in solid-state were observed on re-heating, or after long term storage or type of base used. Therefore in conclusion, no change in the powder diffraction patterns and DSC curves were found in these samples, indicating no polymorphic changes occurred during formulation or storage. These studies further revealed the physical state of the drug in the formulations (Dash, 1997). Nicotine base is a viscous liquid, miscible with water below 60°C, and very soluble in oils (Budavari, 1989). As expected, the drug was present in a molecularly dispersed state in the suppository formulations.

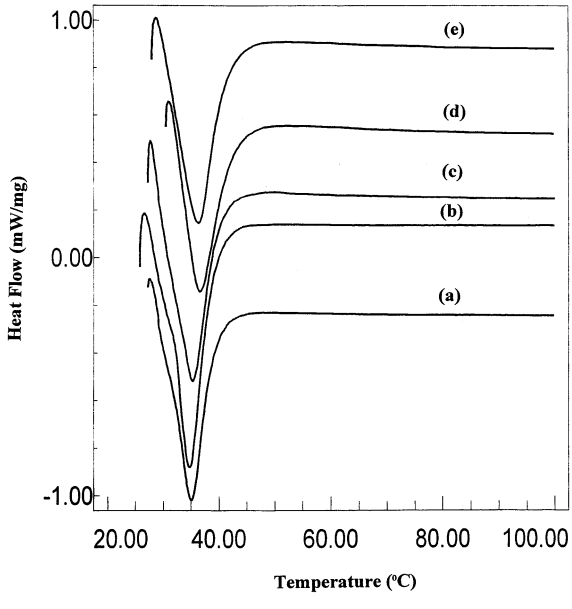


Fig. 1. Differential scanning calorimetric curves of: (a) AI base suppositories with 2% (w/w) nicotine; (b) AI base suppositories with 2% (w/w) nicotine after cooling and re-heating; (c) AI base suppositories with 2% (w/w) nicotine after long term storage (at 4°C for 4 months); (d) AM base suppositories with 2% (w/w) nicotine; and (e) AM base suppositories with 2% (w/w) nicotine after cooling and re-heating.

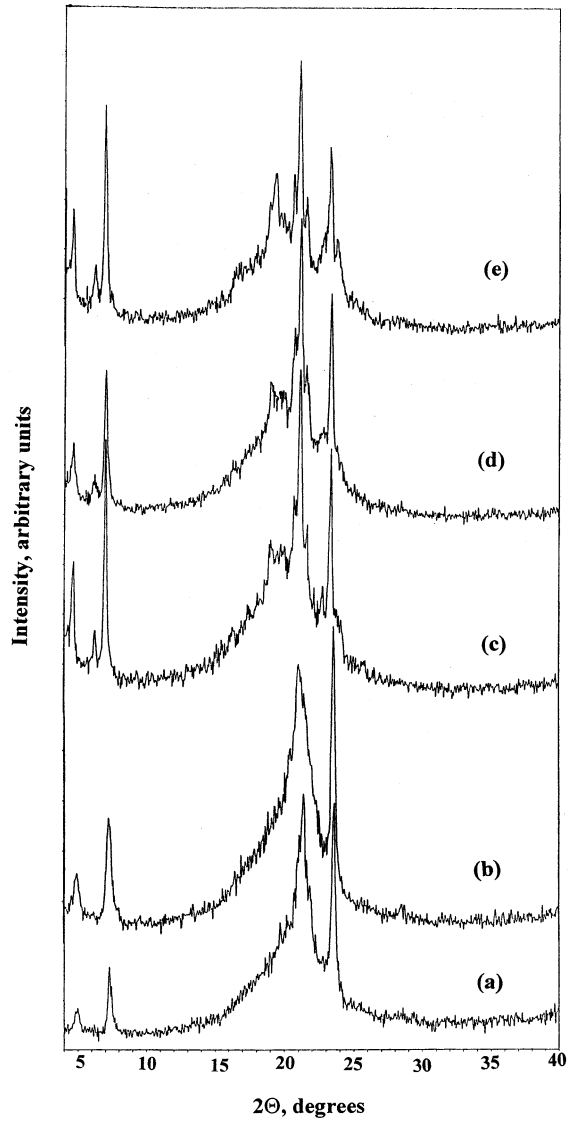


Fig. 2. Powder X-ray diffraction patterns of: (a) AM base suppositories with 2% (w/w) nicotine; (b) AM base suppositories with 2% (w/w) nicotine after cooling and re-heating; (c) AI base suppositories with 2% (w/w); (d) AI base suppositories with 2% (w/w) nicotine after cooling and re-heating; (e) AI base suppositories with 2% (w/w) nicotine after long term storage (at 4°C for 4 months).

### 3.2. Effects of different bases on the nicotine release

From our previous experiences with bioadhesive suppository formulations for 5-aminosalicylic acid, it was evident that when Carbopol was used as the bioadhesive, low melting point Suppocire bases provided better in vitro release characteristic (Haney and Dash, 1994). Therefore, in this investigation, Suppocire AI (melting range 32.5–36.5°C) and AM (melting range 34–36°C) bases were used. The rate and extent of nicotine release from AI and AM base Suppositories containing 2% (w/w) nicotine and 2% (w/w) of Carbopol 974 is shown in Fig. 3. Suppositories prepared from AM base showed a slower release of the drug as compared to the AI base suppository. The faster release of nicotine from the AI base could be due to the low melting point of the base, which melts the suppository faster as compared to the AM base. This observation was also visually confirmed during the in vitro dissolution studies. The melting peak of these two suppositories were then determined from the DSC curves. The melting peak of the AI base suppositories ($34.16 \pm 0.21$°C; $n = 3$) was found to be lower than the AM base ($35.90 \pm 0.30$°C; $n = 3$). Secondly, the release of drug from both the suppositories can also be affected by the solubility of the drug in the bases. However, in this study, nicotine is present in the dissolved state in both the bases. Therefore, this factor did not significantly contribute to the differences in the release rate from both these suppositories.

### 3.3. Effects of bioadhesives on the nicotine release

We are also interested in determining the effect of various Carbopols (Carbopol 934 and Carbopol 974) and their load on the release of nicotine from the suppositories. The drug load in all cases was kept at 2% (w/w). Fig. 4 represents the effects of bioadhesive (Carbopol-974) load on the in vitro release of the nicotine from AM suppository base. The presence of the bioadhesive substantially reduced the rate of nicotine release from suppositories. Suppositories prepared from AM base but containing different loads of Carbopol-934 also showed a similar effect as shown in Fig. 5. The results from these studies (Figs. 4 and 5) indicated that there was no significance difference in the in vitro release rate of nicotine from sup-

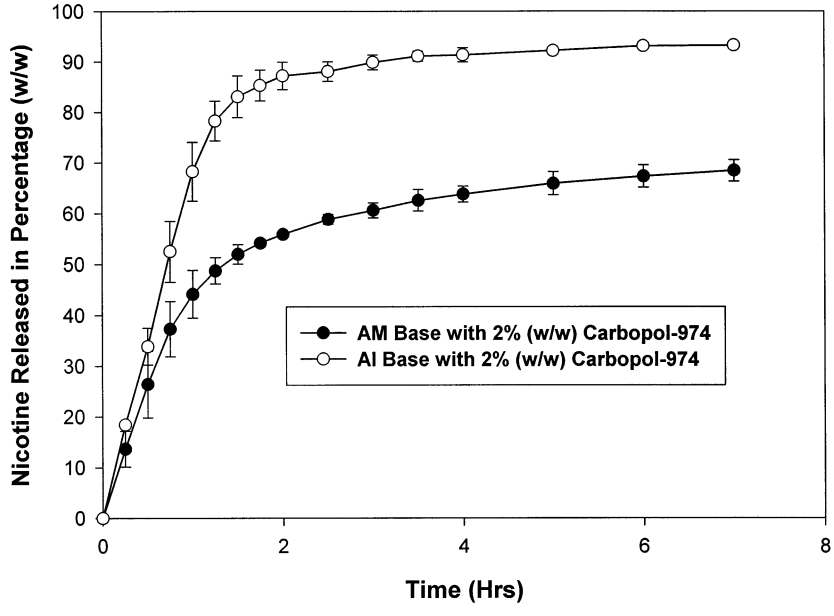


Fig. 3. Effect of types of base used (AM or AI) on the release of nicotine from suppositories.

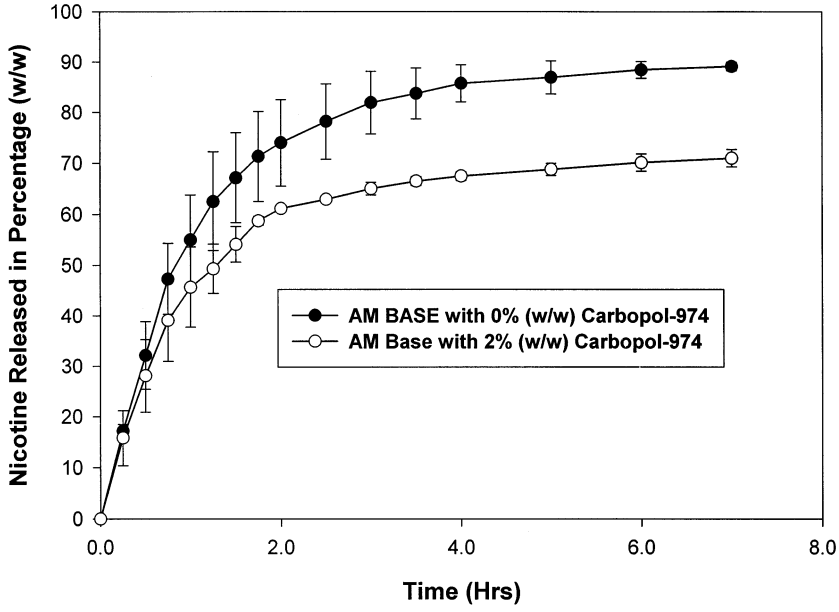


Fig. 4. Effect of Carbopol-974 load on the release of nicotine from suppositories prepared from Suppocire AM base.

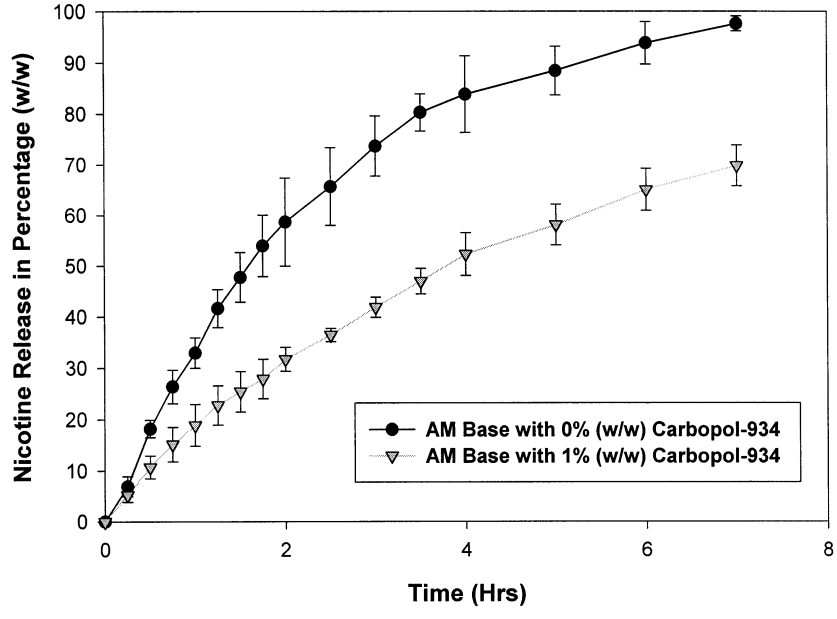


Fig. 5. Effect of Carbopol-934 load on the release of nicotine from suppositories prepared from Suppocire AM base.

positories containing the two different types of Carbopol. The effect of Carbopol load on the in vitro release of nicotine from suppositories prepared from AI base was found to be similar to that of the AM base. DSC studies also confirmed that presence of 2% (w/w) Carbopol in the formulation raised the melting point by at least 1°C. However, comparison of nicotine release from

AM and AI bases with 2% w/w of Carbopol revealed that in the former case the release is distinctly bi-phasic where as this phenomena was not seen in the later case (Fig. 3). The bi-phasic release in this case of AM base could be explained by the fact the suppositories made from this base melt slowly in the dissolution medium as compared to the AI base. During the initial period, the release of the drug to the dissolution medium occurs by a diffusion controlled mechanism. In the case of the AI base suppositories, since the melting is rapid in the dissolution medium, one should expect erosion controlled release rather than diffusion controlled release.

Glycerylmonooleate (GMO) has been reported to act as a bioadhesive but its mechanism of action is quite different from Carbopol (Engstrom et al., 1995). Carbopol is believed to act as a bioadhesive by attaching to mucin. However, GMO is considered as a bioadhesive which needs only water but not mucin for its bioadhesion properties. GMO is a water-insoluble but water-swelling lipid which forms different types of lyotropic liquid crystals in the presence of water. Reversed micellar phase is formed when the water content is 0–5% (w/w), followed by the lamellar phase (5–20% w/w of water) and finally the fully swelled cubic phase when the water content is 35% (w/w). The bioadhesion force determined on pig tongue mucosa was reported to be twice as large for the lamellar phase compared to the cubic phase. The effect of GMO load on the release of nicotine from rectal suppository formulation was then carried out. Fig. 6 depicts the in vitro release characteristics of nicotine from AM base containing various proportions GMO. Interestingly, the data revealed that incorporation of 10% (w/w) and above GMO in the formulation significantly increase nicotine release within a short period of time (60 min). The quick release of nicotine from this base was thought to be due to the melting point lowering effect of GMO in the formulation. The effects of GMO on melting point were confirmed with DSC studies. The temperature of the melting peak from the melting endotherms of AI suppositories containing 0, 5, and 10% (w/w) of GMO was then determined in triplicate in a DSC. This was found to be $34.16 \pm 0.21$, $33.30 \pm 0.11$ and $32.90 \pm 0.11$°C, respectively. Moreover incorporation of 10% and more of GMO in the suppositories showed similar release characteristics as compared to 25 and 50% GMO. Therefore, for

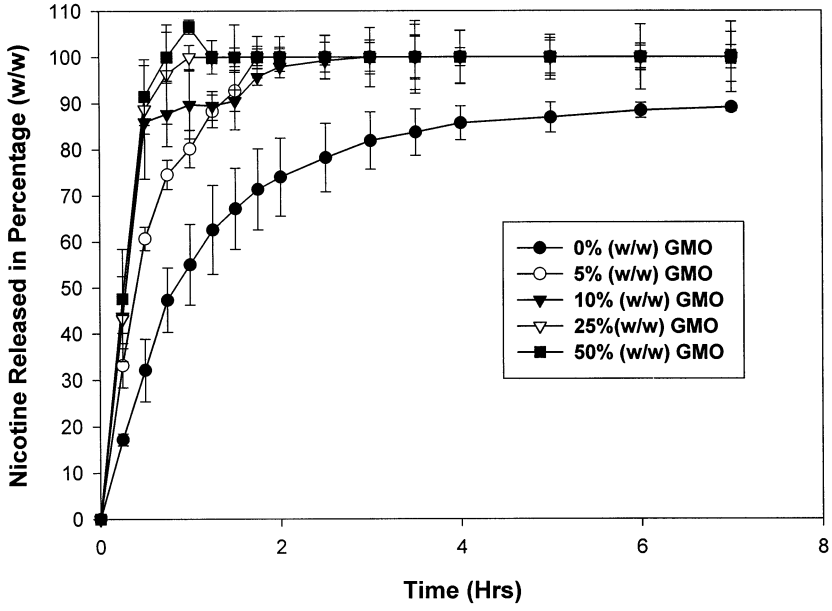


Fig. 6. Effect of GMO on the release of nicotine from suppositories prepared from Suppocire AM base.

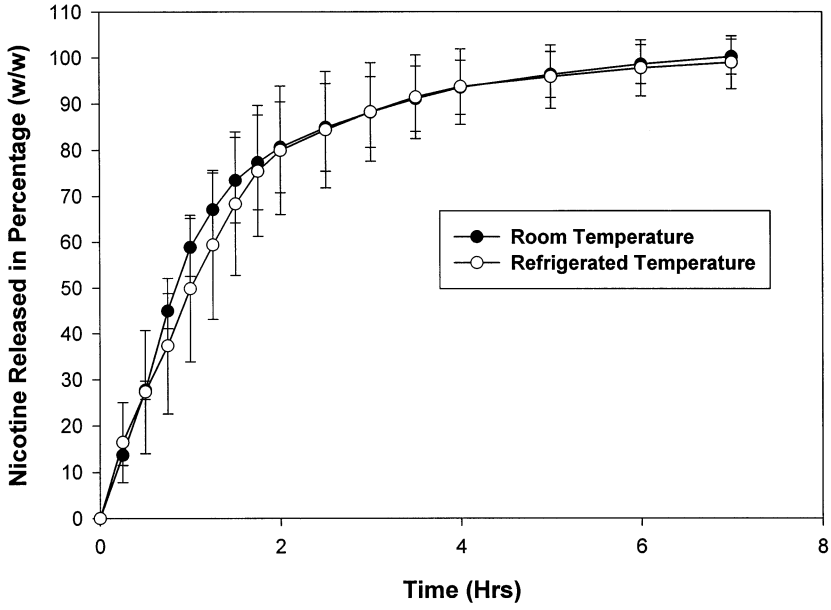


Fig. 7. Effect of storage conditions on the in vitro release of nicotine from suppositories prepared from Suppocire AI base.

cost effective purposes, 10% (w/w) GMO was considered as the optimum level in the formulation. However, in the case of Carbopol, more than 2% (w/w) of bioadhesive in the formulation did not produce an elegant suppository due to improper mixing of Carbopol during congealing of the molten base. Therefore, 2% (w/w) of Carbopol was found to be the maximum concentration that can be used in this dosage form.

### *3.4. Effects of storage on the release kinetics of nicotine from AM and AI bases*

Both 2% (w/w) nicotine and 2% (w/w) Carbopol 974 were incorporated, respectively into the AI and AM base and stored at both room temperature (23°C) and refrigerated temperature ($4 \pm 0.5$°C). The in vitro release of nicotine from AI suppositories after a 2 months of storage under two different conditions was then carried out. The results are shown in Fig. 7. No significance difference was noticed in the release characteristics for both the formulations. The AM base suppositories also showed similar results.

### *3.5. Stability of nicotine in the formulation*

The nicotine contents in both AI and AM suppositories were studied at the time of preparation and after storage for 4 months at room temperature (23°C). The nicotine content was determined by the LC method. The drug load in the formulations was found to be with in 99.1–99.7%. Result from this study indicated that there was no significant degradation of the drug in the formulation during the 4 months of storage at room temperature.

### *3.6. In vitro Caco-2 cell culture studies*

The absorption of nicotine alone and with bioadhesives was carried out in our laboratory using the Caco-2 cell culture model. These absorption studies indicated that there was a decrease in the flux of nicotine across Caco-2 monolayers in presence of both the bioadhesives (Fig. 8). This decrease in nicotine flux through the monolayers could be explained by the fact that adhesion to the monolayer could produce an additional barrier for diffusion, thereby reducibly the net flux of nicotine. A similar study in Caco-2

cells also indicated that flux of nicotine through these cell monolayers can be affected by the concentration of bioadhesive used (Fig. 9). These two studies clearly indicated that one could manipulate the flux of nicotine through cell monolayers by the use of various formulation additives and their concentration. However, more than 2% (w/w) of Carbopol as bioadhesive could not be used in these suppository formulations. Therefore, this 2% (w/w) preparation was thought to be optimal for these formulations. One interesting observation could be drawn from the in vitro release and Caco-2 cell absorption studies for suppositories containing GMO. Incorporation of GMO in the suppositories was shown to decrease the melting

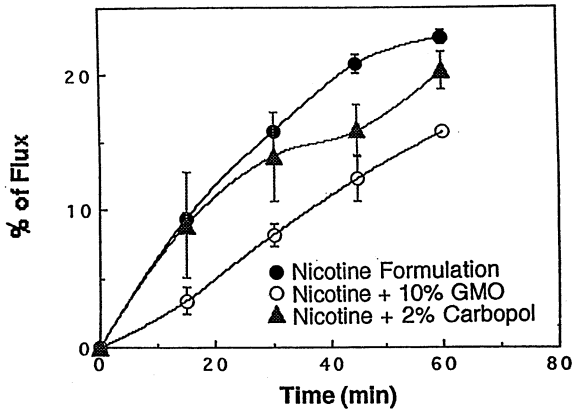


Fig. 8. Effect of bioadhesives (Carbopol-974 and GMO) on the flux of nicotine through Caco-2 cell monolayers.

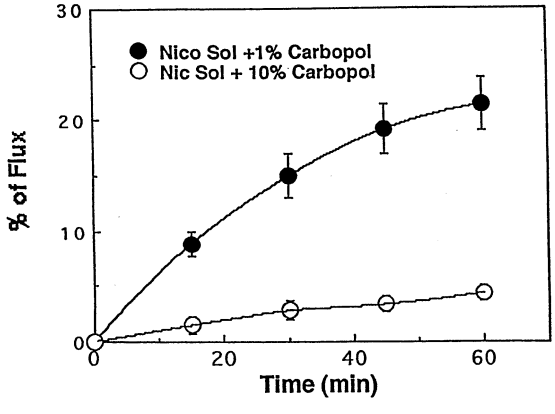


Fig. 9. Effect of Carbopol-974 concentration on the flux of nicotine through Caco-2 cell monolayers.

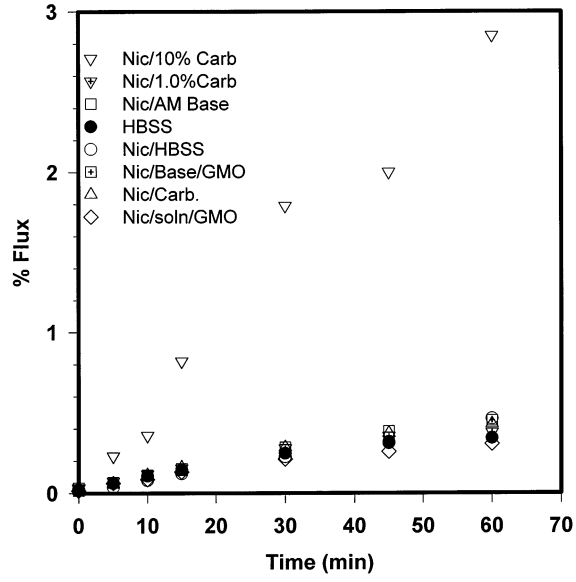


Fig. 10. Fluorescein flux through Caco-2 cell monolayers exposed to: (▽) nicotine and 10% (w/v) of Carbopol 974; (▽) nicotine and 1% (w/v) of Carbopol 974; (□) nicotine and AM base; (●) HBSS buffer; (○) nicotine in HBSS buffer; (□) nicotine in AI base and 20% (w/w) GMO; (△) nicotine and Carbopol 974; and (◇) nicotine solution and 20% (w/w) GMO.

point of the base and thereby cause a quick release of the drug into the release medium. However, the nicotine flux was found to be lower in the case of formulation containing GMO as compared to nicotine solution. This difference can be explained by the fact that adhesion of GMO to the cell monolayer could increase additional barriers to diffusion and therefore, reduces its flux through the barrier.

The integrity of the cell monolayers were then determined by examining the permeability of the monolayers to the fluorescent marker, fluorescein. The flux of the fluorescein marker through the monolayers was found to be significantly higher in the presence of nicotine with 10% (w/v) Carbopol (Fig. 10). The flux of the fluorescein marker through collagen-coated polycarbonate membranes without cells was then studied as a control. The fluorescein flux in the blank membrane is shown in Fig. 11. No significant difference in the flux across the blank membranes was noticed among the various formulations studied. Since the

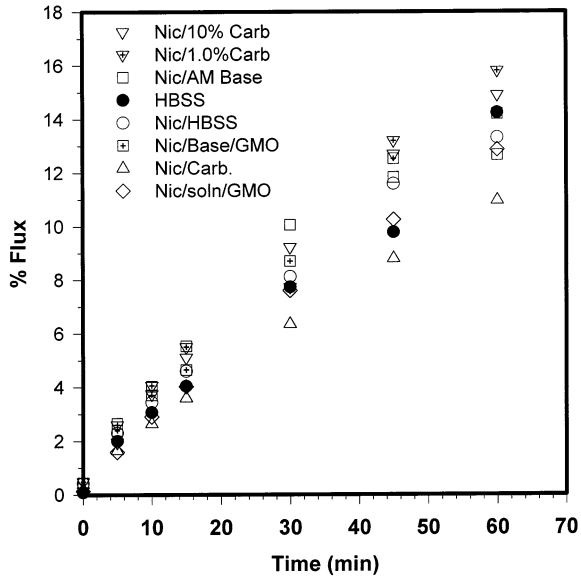


Fig. 11. Fluorescein flux through collagen-coated polycarbonate membranes without cells exposed to: (▽) nicotine and 10% (w/v) of Carbopol 974; (▽) nicotine and 1% (w/v) of Carbopol 974; (□) nicotine and AM base; (●) HBSS buffer; (○) nicotine in HBSS buffer; (□) nicotine in AI base and 20% (w/w) GMO; (△) nicotine and Carbopol 974; and (◇) nicotine solution and 20% GMO.

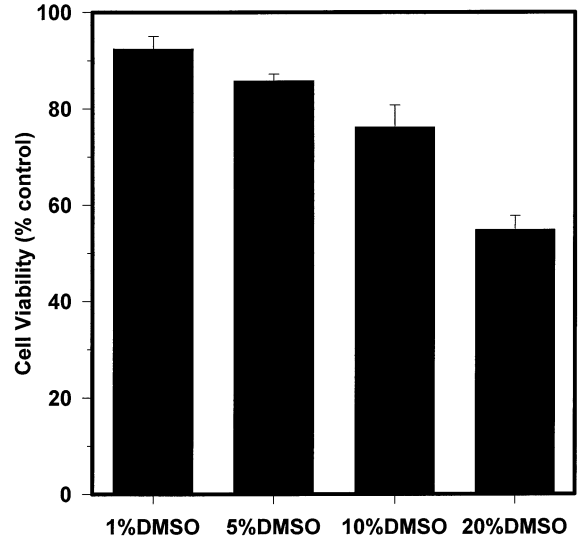


Fig. 12. MTT toxicity studies in Caco-2 cells exposed to various concentrations of DMSO.

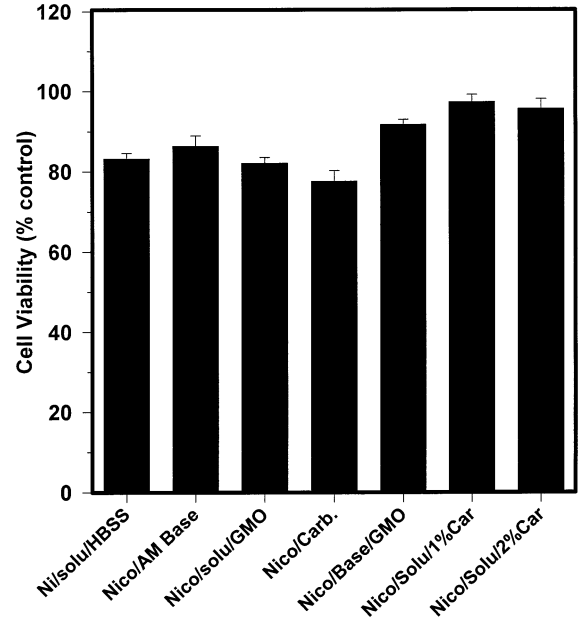


Fig. 13. MTT toxicity studies in Caco-2 cells exposed to various nicotine formulations.

fluorescein flux through Caco-2 monolayer was found to be higher in the presence of nicotine with 10% (w/w) Carbopol (Fig. 10), cytotoxicity if any, of nicotine and other bioadhesives (Carbopol and GMO) was determined using MTT toxicity assay. The results of these studies are depicted in Figs. 12 and 13. Fig. 12 shows the MTT toxicity studies in Caco-2 cells following exposure to various concentrations of Dimethyl Sulfoxide (DMSO). As expected, the viability of cells decreased when DMSO concentration was increased. MTT toxicity studies in Caco-2 cells following exposure to nicotine in various formulations is shown in Fig. 13. The GMO base formulation, as well as the lower concentrations of Carbapol (1 and 2%), had minimal cytotoxic effects on Caco-2. The remaining formulations examined had cytotoxicities in Caco-2 cells that were similar to that associated with low concentrations of DMSO (1–5%). However, with the possible exception of the 10% Carbapol formulation (represented as Nic/Carb in Fig. 13), the cytotoxicity was insufficient to produce changes in the permeability on nicotine in

the Caco-2 cell monolayers. These preliminary in vitro studies examining release, permeability and cytotoxicity, indicate the feasibility of delivering nicotine across the epithelial cell membranes.

## 4. Conclusions

Nicotine suppositories in two semi-synthetic glyceride bases (AI and AM) were developed. Two bioadhesive (Carbopol and GMO) can be successfully incorporated into these suppositories. GMO was found to give a faster release of the drug as compared to Carbopol. This was believed to be due to the melting point lowering effect of GMO. GMO with high monoglyceride content is more hydrophilic than Suppocire (with low monoglyceride content $< 1\%$ and high triglyceride content). The addition of GMO into the Suppocire based formulation will increase hydrophilicity of the matrix, thereby enhancing nicotine release and possibly changing the mechanism of release of nicotine from diffusion to erosion controlled mechanisms. The optimal concentration of Carbopol and GMO in these suppository formulations were, 2% and 10% (w/w), respectively. Caco-2 cell monolayers studies indicated that the presence of both the bioadhesives affected the nicotine flux through the monolayers. Future studies are aimed at evaluating these delivery systems in an in vivo animal model.

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