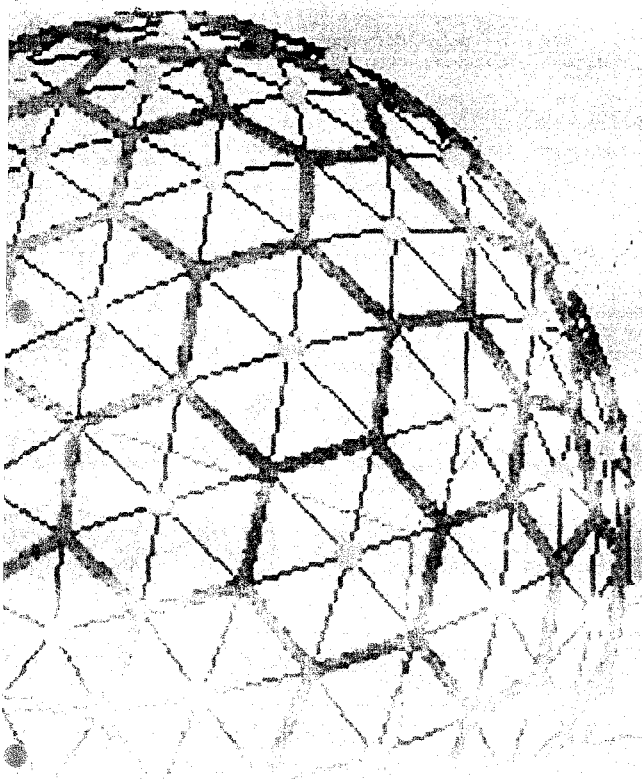
8.0 APPENDIX - I

ষোড়শ পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেস

(২৮ ফেব্রুয়ারি-১ মার্চ ২০০৯)
বর্ধমান বিশ্ববিদ্যালয়, বর্ধমান



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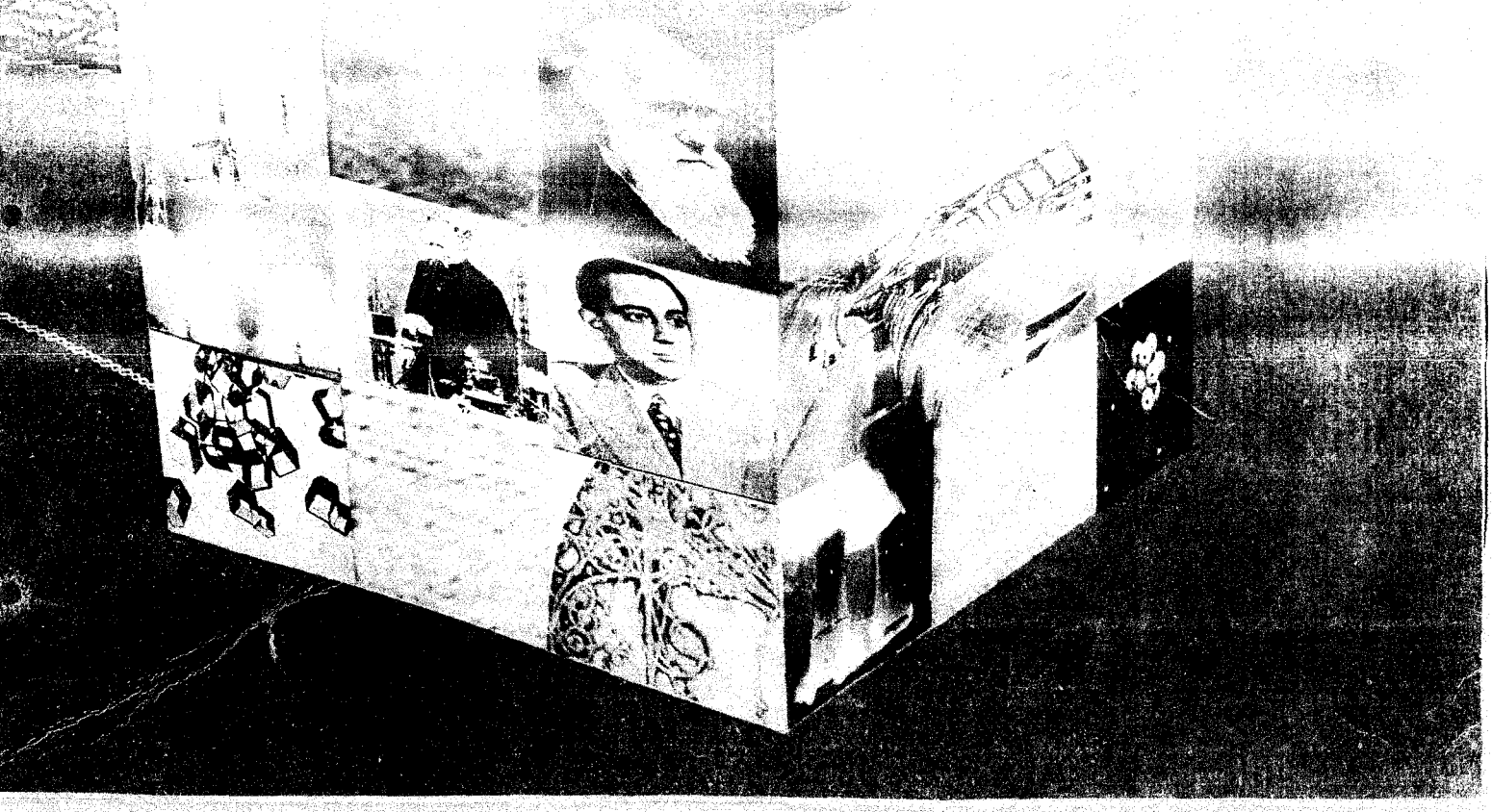

মাইক্রোপার্টিকুলেট জৈব আঠালো HIV প্রতিরোধক ভ্যাজিন্যাল জেলঃ মহিলাদের দীর্ঘস্থায়ী AIDS চিকিৎসায় একটি অভিনব প্রয়োগ

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বিশ্বব্যাপী (৩৩.২ মিলিয়ান) AIDS রোগের জীবাণুবহনকারী মানুষের মধ্যে (১৫.৪ মিলিয়ন) প্রায় অর্ধেকই মহিলা (UN AIDS পরিসংখ্যান ২০০৭) সেদিকে দৃষ্টি রেখে আমাদের মাইক্রোপার্টিকুলেটেড জৈব আঠালো জেলটি প্রস্তুত করা হয়েছে। যেটি যোনি দ্বারের মধ্যে দিয়ে প্রবেশ করালে একটি সুরক্ষিত আস্তরণ তৈরি করবে যা HIV রোগ-জীবাণু প্রতিরোধে সক্ষম। সর্বাধিক গুরুত্বপূর্ণ বিষয়টি হল যৌন ক্রিয়ার সময় মাইক্রোপার্টিকেল থেকে নির্গত Drug টি জৈব আঠালো জেলের মাধ্যমে রোগ-জীবাণু [illegible] প্রতিরোধ করে এবং মাইক্রোপার্টিকেলগুলির পৃষ্ঠতল অতি ক্ষুদ্র হওয়ায় রোগ চিকিৎসার ক্ষমতাও বৃদ্ধি [illegible] Zidovudine মাইক্রোপার্টিকেলগুলি ইথাইল সেলুলোজ পলিমার দিয়ে তৈরি করার পর আউে [illegible] Drug টি কতক্ষণ মধ্যে মোট পরিমাণ Drug টি নির্গত হয়েছে তা নির্ণয় করা হয়। তাছাড়া Drug ও পলিমারের [illegible] মধ্যে পারিপার্শ্বিক ক্রিয়া বিক্রিয়া FTIR, ডিফারেন্সিয়াল স্ক্যানিং ক্যালোরিমিটার এবং মাইক্রোপার্টিকেলগুলির পৃষ্ঠতল পরীক্ষা করা হয় স্ক্যানিং ইলেকট্রন অনুবীক্ষণ যন্ত্রের সাহায্যে। সর্বোৎকৃষ্ট জেলটির মধ্যে Drug-এর পরিমাণ প্রায় ৭৪.৭১%, জেলটি Drug ধারণ ক্ষমতা ৯১.৮৩%। সদ্য কাটা ছাগলের যোনি কলার মধ্যে দিয়ে ১২ ঘন্টায় *in-vitro* প্রবাহিতকরণে দেখা যায় Drug টি নিঃসরণের ক্ষমতা ১৫.১৫%, জেলটি জৈব আঠা ধারণ ক্ষমতা ১৬.৭ গ্রাম এবং জেলটি পেশীর মধ্যে ২২৫ গ্রাম / মিনিট হারে ছড়িয়ে পড়ে, জেলটির টিস্যু হতে বাহির হইবার ক্ষমতা ১৬৫ মিলিগ্রাম। প্রাথমিক পরীক্ষার পর আমাদের গবেষক দল এই সিদ্ধান্তে উপনিত হয়েছে এই জেলটি ভবিষ্যতে মহিলাদের কাছে একটি যুগান্তকারী সৃষ্টি হিসাবে গণ্য হবে।

MICROPARTICULATE BIOADHESIVE VAGINAL GEL: THE NEXT GENERATION TREATMENT FOR HIV INFECTED WOMEN

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Women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV world wide (UN global AIDS epidemic review 2007).Drug delivery across vagina is a challenging task. Keeping this view, a new vaginal anti-HIV microparticulated gel was engineered to coat vaginal tissue with a stable HIV protective layer. Most importantly, controlled release of anti-HIV drugs form these microparticles in gel inactivate the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. Zidovudine loaded microparticle were prepared by the solvent evaporation method using ethyl cellulose as a polymer in different drug-polymer ratio and characterized for the percentage drug content, entrapment efficiency, FTIR study, SEM for surface morphology and in-vitro release studies of AZT (2max 265 nm) using UV spectroscopy. The optimized microparticles were incorporated in bioadhesive gel and best gel was found with the percent drug content (97.67%), entrapment efficiency (97.83%), *in-vitro* diffusion studies(AZT release, only 5.13% in 12 hour) using immediately excised goat vaginal tissue with a stable gel layer with good bioadhesive strength (16.7g), good spreadability (225g/min) and minimum extrudibility(165mg).

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OSTD 03 - Prolong Release Anti-Retroviral Microparticulate Gel For Treatment Of HIV Infection In Women

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UN global AIDS epidemic review 2007 revealed, women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV. These statistics emphasize the responsibility and challenge to understand personal risks and responsibilities of our young women and girls to make healthy sexuality with a course for future action. Drug delivery across vagina, is a challenging task. Keeping this view, a new vaginal anti-HIV microparticulated gel was engineered to coat vaginal tissue with a stable HIV protective layer, retention of this gel layer before intercourse and to release entrapped anti-HIV drug in a controlled fashion in presence of the main infecting agent: semen. Most importantly, controlled release of anti-HIV drugs form these micro particles in gel inactivate the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. Zidovudine (AZT, gift sample, Aurobindo Pharma Ltd, A.P. India) loaded micro particle were prepared by the solvent evaporation method using ethyl cellulose as a controlling polymer in different drug-polymer ratio and characterized for the percent drug content, entrapment efficiency, micromeritic analysis, FTIR study, SEM for surface morphology and in vitro dissolution studies of AZT (max 265 nm) using UV spectroscopy. The micro particles were found to be nearly spherical and free flowing. The optimized micro particle was incorporated in bioadhesive gel using different bioadhesive polymer (2% carbopol 934 was found to be the best). A vaginal anti-HIV microparticulated gel was thus prepared and evaluated. Best gel was found with the percent drug content (74.71%), entrapment efficiency (97.83%), in-vitro diffusion studies (AZT release,only15.15%, up to 12 hour) using immediately excised goat vaginal tissue with a stable gel layer with good bioadhesion strength (16.7g), good spreadability (225g/min) and minimum extrudibility (165mg). Further research in this area is expected to yield technology with significant outcome for the generation-next worldwide.

Key words: HIV, Micro particle, Microparticulated vaginal gel

PP 22 - Vaginal *Zidovudine Microcapsules* For Treatment Of Heterosexually Transmitted Hiv Infection

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The women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV, reported in 2007 UN AIDS abstract. In most regions of the world, HIV is affecting women and girls in increasing numbers. To date, most vaginal drug delivery systems (VDDS) have been formulated as conventional / traditional semi-solid formulations. However, a clear rationale exists for providing long-term, controlled release of vaginal microbicides in order to provide continuous protection against heterosexually transmitted HIV infection and to improve user compliance. Most importantly, controlled release of anti-HIV drugs form this microdevice inactivates the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. The vagina is perhaps a less explored but efficient route for drug administration due to presence of dense network blood vessels and avoids liver first-pass. Zidovudine(AZT, gift sample, Aurobindo Pharma Ltd research center A.P.India) loaded microcapsules were prepared by the solvent evaporation method using ethyl cellulose as a polymer in different drug polymer(D:P) ratio and characterized for the percent drug content, entrapment efficiency, micromeritic analysis, FTIR study, SEM for surface morphology and in vitro dissolution studies. UV spectroscopy was used for the routine analysis of AZT (λmax 265 nm). The microcapsules were found to be nearly spherical and free flowing. The entrapment efficiency of the best microcapsules (D:P=1:7) was 67-98.4%. The release of drug from the microcapsules was found to be 64.84 - 97.73% over 10 hour period. FTIR study showed the stable character of Zidovudine in the microcapsules. SEM revealed that the microcapsules were porous in nature. The drug release from the microcapsules was found to be diffusion controlled-Higuchi square root kinetics. This research work suggested that ethyl cellulose microcapsule could be VDDS carrier for sustained and prolonged release for Zidovudine.

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GRYIP/REPS/OP04
HERBAL CONTRACEPTIVES

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The extraordinary growth of the world population stands as one of the significant events of the modern era to think over. The current world population is around 6.46 billion and that of India in particular is around 1.1 billion.

Due to existing and overwhelming growth rate of world population, oral contraceptives have become need of the time. But steroids have various side effects. This forced us to study the existing options of plants having anti-fertility activity. Plant plethora is rich source of plants having anti-fertility activity. Contraception is literally the prevention of conception, but generally is taken to mean the prevention of pregnancy (2). The development of new fertility regulating drug of medicinal plants is an attractive proposition, because from times immemorial humans have relied on plants and their products as sources of drugs and therapeutic agents, although in recent times, synthetic drugs are used extensively in modern medicine. However many modern medicines are developed through the clues obtained from phytochemicals. In this study, we have also covered the plants having anti-fertility activity by different mechanism in both male and female.

GRYIP/REPS/OP05
PREPARATION AND CHARACTERIZATION OF ZIDOVUDINE VAGINAL MICROCAPSULES

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The human vagina represents a potential, accessible space that offers a valuable route for drug delivery through the use of specifically designed carrier systems for both local and systemic applications. Hydro gels, vaginal tablets, pessaries/suppositories, particulate systems, and intravaginal rings are the wide ranges of drug delivery platforms suitable for intravaginal administration.

In recent years, the vaginal route has drawn the attentions of the pharmaceutical scientist and researchers, as a potential route for systemic delivery of peptides and other therapeutically important small and macromolecules, due to several uniqueness such as hepatic

first-pass avoidance, dense network of blood vessel for quick systemic entry, easy self-insertion and self-removal at time of acute toxicity and other discomfort. The rate and extent of drug absorption after intravaginal administration may vary depending on formulation factors, vaginal physiology, age of the patient and menstrual cycle.

Zidovudine (AZT)-ethyl cellulose microcapsules were successfully prepared by solvent evaporation method. The prepared vaginal microcapsules were characterized for the percent drug load, entrapment efficiency, FTIR, Scanning electron microscopy (SEM) and *in-vitro* dissolution studies. The AZT loaded vaginal microcapsules were nearly spherical and free flowing having an entrapment efficiency of 60-98 %. Infrared spectroscopy analysis confirmed the absence of any drug-polymer chemical interaction. The *in vitro* release profile could be altered significantly by changing various processing and formulation parameters to give a controlled release of drug from the microcapsules. The vaginal microcapsules were found to sustain the drug up to 10 hour thus reducing the dose frequency perhaps. The *in- vitro* release profiles from microcapsule, when treated for Zero order, First order and Higuchi kinetic models, the obtained vaginal microcapsules were found to obey Higuchi kinetic model, indicating diffusion-controlled release of drug from the microcapsules.

GRYIP/REPS/OP06
HPLC FINGERPRINTING OF VIDANG (*EMBELIA RIBS*) WITH RESPECT TO QUANTIFICATION OF EMBELIN IN VIDANG

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Vidang (*Embelia ribes*) is widely described anthelmintic herbal drugs in traditional system of medicine containing Embelin as major constituent responsible for the said activity. An attempt was made to develop HPLC method for the chemical fingerprinting of Vidang. The earlier reported HPLC methodology shows only three major peaks indicating the presence of three constitute in Vidang. But as per the herbarium specimens, Vidang contain number of chemical constitute. So it was our try to resolve as many as possible constitute in Vidang. The proposed HPLC method shows seven major peaks indicating the presence of higher number of chemical constitute than reported earlier. The

half life of 2 hrs. So it makes the drug suitable for sustained release.
Good controlled release properties were exhibited by using ethyl cellulose as polymer. The constant drug blood level from controlled release system minimizes the toxic effects like gastric irritation there by improving patient compliance and therapeutic efficiency.

GRYIP/REPS/PP33
PREPARATION AND EVALUATION OF DICLOFENAC PROLONGED RELEASE GELS FOR TOPICAL APPLICATION BY USING CARBOPOL 940 AS POLYMER

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In the present study the carbopol 940 is used as a gelling agent for the *preparation of diclofenac sodium* gel, for the treatment of analgesia. The various parameters like pH, drug content, viscosity and *in vitro* drug release study were evaluated. The pH of all the preparation shows compatible with site of application and drugs content of the gels shows uniform distribution of drug in the formulation. The viscosity of the gel was changed as the concentration of Carbopol 940 changes. *In vitro* drug release study was carried out using Keschery-Chein diffusion cell using cellophane membrane as semi permeable membrane. The drug permeation across cellophane membrane from all the formulations at the end of 8 h was found 96.29% and 77.4% for 1% and 2% Carbopol 940 formulations respectively. The marketed topical preparation of diclofenac gel was found to diffuse almost 96.49% of drug across cellophane membrane at the end of 2.5hr. Various kinetic models were applied to *in vitro* release profile of all batches and release mechanism was calculated using Korsmeyer–Peppas model.

GRYIP/REPS/PP34
STUDIES OF ANTIRETROVIRAL INTRA-VAGINAL MICROPARTICLES

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The major cause (75% of HIV cases) of HIV infection is sexual transmission. Vagina was traditionally been used as topical route to deliver locally acting drugs. This route is equally effective for systemic drug delivery due to the dense

network of blood vessels in the vaginal wall for prompt systemic entry, first-pass metabolism bypass and several other uniqueness viz. self insertion and self removal. The present study was aimed with objective to prepare and characterize anti-HIV intravaginal microparticles.

The intra-vaginal microparticles were prepared by using ethyl cellulose as rate controlling polymer in the different ratios (1:1, 1:3, 1:5 w/w) using zidovudine as a model drug. Non- aqueous solvent evaporation technology was selected among the other methods tried, as it is easy and also economical methodology.

The prepared microparticles were nearly spherical (scanning electron microscopy), discrete & free flowing in the range of 100–500 μm. High drugs content & encapsulation efficiency was observed for each formulation. The encapsulation was observed to be in the range of 67–98%. The encapsulation efficiency was found to increase with increase in polymer content. The drug release from the anti-HIV intra-vaginal microparticles exhibited a controlled release of drug sustained over a period of 10 hours. The release rates were generally increased with decreased proportion of polymer. All the microparticles followed Higuchi square root kinetic model independent of formulation variable and diffusion controlled. The bioadhesion test by modified Wilhelmy plate method, showed very good bioadhesive strength. FTIR studies at a resolution of $1cm^{-1}$ were recorded in KBr pellets of pure drug; ethyl cellulose and prepared microparticles confirmed no drug-polymer chemical interaction.

These microparticles can further be incorporated in various dosage forms like gel, tablet, and suppositories. Thus microparticles may be adopted for a successful development of newer drug delivery system of other drugs for administration to vagina.

GRYIP/REPS/PP35

INVESTIGATION OF A NEW WATER MANAGEMENT SYSTEM IN REVISED SCHEDULE M

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The water management system has become a very important part in the present implementation of Schedule M in our pharmaceutical industries. The main object of this system is to get a standard quality of Purified Water IP, BP, and USP and Water for Injection IP, BP, and USP for the preparation of medicinal products in pharmaceutical industries. In

GRYIP/REPS/PP37
FORMULATION AND EVALUATION OF ZIDOVUDINE BIOADHESIVE VAGINAL FILM FOR TREATMENT OF AIDS

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The present study concerned with the development and characterization of Bioadhesive Vaginal Film (BVF). Zidovudine containing vaginal film were prepared by Solvent casting method using combination of polymer Ethyl cellulose and Hydroxyl propyl methyl cellulose in different ratios (1:1, 3:2 and 2:3 w/w) and different plasticizer 40% w/w of polymer (Polyethylene glycol, Sorbitol, Dibutyl phthalate (DBP), Triethyl citrate (TEC), and Glycerol) in order to develop a sustained release BVF of Zidovudine for prolong period of time. The films with plasticizer DBP and TEC were found to be thin, soft, transparent, and easily remove from plate and evaluated for drug content, moisture content, swelling index, folding endurance, shape (scanning electron microscopy) and *in vitro* release performance. The drug polymer interactions were investigated by FT-IR spectroscopy. The swelling index (7-14 %), moisture content (2-5 %) and folding endurance (300 - 325) of all films were found within range, with higher drug content. The bioadhesion test by modified Wilhelmy plate method, showed very good bioadhesive strength. *In vitro* drug release rate for selected BVF (VF6, containing DBP 40 % w/w of polymer, as a plasticizer) was found to sustain Zidovudine over 12 h obeying Higuchi square root kinetic. The findings of the study were able to demonstrate, the potential of BVF to deliver Zidovudine in a controlled fashion and in constant manner for prolong period. Thus in conclusion preparation protocol of BVF studied may be adopted for a successful development of newer drug delivery system of other drugs for administration through vagina.

GRYIP/REPS/PP38
FINGERPRINTING METHOD OF AN AYURVEDIC FORMULATIONS: SPECTROPHOTOMETRIC APPROACH

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Yastimadhukadi Tailam is an Ayurvedic formulation described in Ayurvedic formulary of India and used for graying of hairs particularly of scalp, loss of hairs and falling of hairs of beard and

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DEVELOPMENT OF VAGINAL MICROCAPSULATED TABLET AND PRECLINICAL BIOADHESIVE TESTING FOR PREVENTION OF TRANSMISSION AND TREATMENT OF HIV

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Women are approximately 50 percent of total living HIV infected patients worldwide, reported in UN AIDS Update, 2007. NACO and World Bank reported the majority of people in India with HIV, are residing in four states (Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu). The recent national population based survey (NFHS-3, *2007) reported, the adult HIV* prevalence is varied from 0.62 percent in Maharashtra, 0.07 percent in Uttar Pradesh, to 0.34 percent in /Tamil Nadu, 0.69 percent in Karnataka, 0.97 percent in Andhra Pradesh and 1.13 percent in Manipur, 0.13 percent in all other states together. *Vaginal drug delivery is very challenging and less explored research* area. The vagina is an efficient route for drug administration due to presence of dense blood vessels network and avoids first pass. A clear rationale exists for providing long term, controlled release (lacunae of commercially available conventional tablet) of anti retroviral in order to provide continuous protections against heterosexually transmitted HIV infection and to improve user compliance, even during sexual activity. Zidovudine (APL, A.P.)loaded microcapsules were prepared by solvent evaporation method using ethy1 cellulose in different drug: polymer ratio and optimized from the percent drug content, entrapment efficiency, miocromeritic analysis, FTIR study, SEM for surface morphology and *in vitro* dissolution studies. The optimized microcapsules were then incorporated in tablet by direct compression with other formulation additives. The microencapsulated Bioadhesive vaginal tablet (MBVT) were evaluated for average weight, hardness, friability, drug content, swelling index and goat vaginal bioadhension testing *ex-vivo* and *in-vitro* drug release study. In vitro release study reveled best MBVT released the drug slowly, about 13.52% only in 10^{th} hr and followed diffusion controlled Higuchi Kinetics (r^2=0.981), non-Fickian n=0.529 (n>0.5) transport mechanism with a good bioadhension (13.9g). Thus in conclusion, MBVT preparation protocol studied may be adopted for the successful development of controlled release anti retroviral therapies for prevention of transmission and treatment of HIV.

DRUG RELEASE FROM POLYMERIC VAGINAL FILM FOR ANTIRETROVIRAL THERAPY

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UN global summary of the AIDS pandemic 2007 revealed that women (15.4 million) account for approximately 50 percent of people infected and living with HIV (33.2 million). More than 20 million people have died of AIDS and about 14,000 are newly infected every day. HIV is affecting women and girls in increasing numbers. These statistics emphasize the responsibility and a challenge that includes understanding personal risks of our young people and to ensure that they make healthy choices about their sexuality as well as a course for future action in designing strategies for treatment and prevention of HIV/AIDS, specifically for women. Pursuing this 2nd objective, drug delivery across cellular barriers, such as vagina, is a challenging task.

Although somewhat neglected in clinical studies, pharmaceutical characterization of vaginal polymeric films is an important step in order to optimize safety, efficacy and acceptability. Zidovudine (AZT) was used as a model drug (gift from Aurobindo Pharma Ltd Research Center, A. P. India). Vaginal films of AZT were prepared by solvent casting method, using combination of PVP with different polymer [PVA (F1), SCMC(F2), EC(F3) and Acrylcoat (F4)] using glycerol as a plasticizer. Films were observed for the high drug content, folding endurance, uniformity in thickness, weight and swelling index and *in vitro* drug release studies. UV spectroscopy method was used (λmax = 265 nm) for the routine analysis of AZT. Presence of drug particle was distinctly observed in [illegible] Microscopy of drug containing film [illegible] interaction between drug and polymer. In vaginal pH 4.7, F3 and F4 released 46.88% and 39.53 percent drug in 9 hr, when compared to F1 (67.235%) and F2 (54.43%).

Thus in conclusion, Ethyl cellulose and acrylcoat with PVP was found to release AZT slowly as compared to PVA and SCMC with PVP. Vaginal film is a good tool to provide sustained antiretroviral therapy.

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CONTROLLED RELEASE OF ANTI-HIV BIOMIMETIC MICRODEVICE IN GEL FOR INTRA-VAGINAL LUBRICATION AND AIDS THERAPY

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UN global summary of the AIDS epidemic 2007 revealed, women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV. In most regions of the world, HIV is affecting women and girls in increasing numbers. As researchers, these statistics emphasize the responsibility and a challenge that include 1. understanding personal risks and responsibilities of our young people to make healthy choices about their sexuality as well as 2. a course for future action. Pursuing this 2nd objective, drug delivery across cellular barriers, such as vagina, is a challenging task. To date, most vaginal drug delivery systems (VDDS) have been formulated as conventional / traditional semi-solid formulations. However, a clear rationale exists for providing long-term, controlled release of anti-retroviral in order to provide continuous protection against heterosexually transmitted HIV infection and to improve user compliance, even during sexual activity. Ideally, a vaginal anti-HIV microdevice in gel should provide intra-vaginal biomimetic lubrication during intercourse, uniform hydrogel-coating of vaginal tissue and retention of this gel layer before intercourse. Most importantly, controlled release of anti-HIV drugs form this microdevice in gel inactivates the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. Indeed, the simple formulation modification of a gel can lead to enhanced performance of the system containing the same amount of active substances.

In this study, a newer polymeric microdevice (microparticles) in bioadhesive vaginal gel of Zidovudine(AZT) (biological half life of 1.1 hour, 500 mg/day to be given in 2-4 divided conventional oral doses), is developed with improved efficacy, safety and controlled release profiles of the anti-HIV drug, AZT. Initially ethyl cellulose microdevices were optimized and characterized varying different drug:polymer (D:P) ratio. The best ethyl cellulose microcapsules (D:P=1:2) were found for the percent drug content (92.16%), encapsulation efficiency (99.0%), excellent flow properties, FT-IR spectroscopy, scanning electron microscopy (SEM) and *in-vitro* drug dissolution (only 65.07% AZT release, up to 12 hour) studies. The prepared microcapsules were then incorporated in bioadhesive gel prepared using different bioadhesive polymer (2% carbopol 974 was found to be the best). A vaginal anti-HIV microdevice in gel was thus prepared and best gel was found for the percent drug content(74.71%), entrapment efficiency(97.83%), *in-vitro* diffusion studies(AZT release, only15.15%, up to 12 hour) using immediately excised goat vagina and coated model goat vaginal tissue with a stable gel layer with good bioadhesion strength(16.7g), good spreadability (225g/min) and minimum extrudibility (165mg). The drug release kinetics of the developed microdeviced gel were studied. This unique VDDS, with both bioadhesive and sustained release properties, is expected to provide an efficacious anti-HIV therapeutics for lengthier duration. Impeccably, a vaginal anti-HIV microdevice in gel system will be engineered to coat vaginal tissue with a stable gel layer and to release entrapped anti-HIV drug in a controlled fashion in presence of the infecting agent: semen. CONCLUSION: We believe, the further research in this area will fill the gap that other conventional VDDS was not being able to provide and yield technology with significant outcome for the Generation-Next worldwide.

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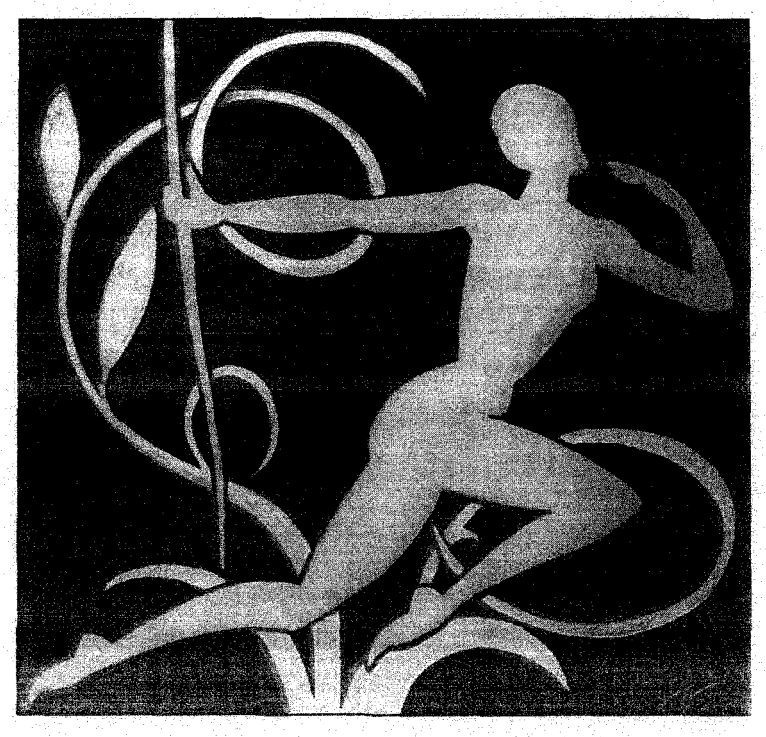
JOURNAL OF WOMEN'S HEALTH
Volume 19, Number 3, 2010

DOI: 10.1089/jwh.2010.Ab01



Abstracts from

Women's Health 2010: The 18th Annual Congress

March 26–28, 2010
Washington, DC

In collaboration with

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cancer screening and to assess maternal predictors of adolescent vaccine utilization.

Methods: We conducted a cross-sectional mailed survey of women attending breast and cervical cancer screening at two diverse institutions; an urban inner-city population (Chicago site) and a white suburban population (Michigan site). We queried the adolescent daughter's HPV vaccination status (adolescent defined as 9–17 years old) and maternal general health beliefs; HPV-specific beliefs; knowledge, perceived benefits and barriers to HPV vaccination; perceived social/peer group attitudes about HPV vaccines. HPV vaccine completion (receipt of all three doses) is the primary outcome. Correlates of the primary outcome were assessed using multivariate logistic regression.

Results: Our overall response rate was 28% at Chicago; 38% at Michigan. The percent of respondents who are mothers of adolescent daughters at Chicago attending mammography and Pap smear were 20% and 22%. At Michigan, the percent who were mothers attending mammography and Pap smear were 39% and 23%. 14.5% of Chicago adolescents completed vaccination compared to 23.7% at Michigan. In 11–12 year olds, for whom the CDC recommends universal vaccination, 7.4% Chicago and 16.7% Michigan adolescents completed vaccination. Significant independent negative correlates of adolescent HPV vaccine completion were maternal black race (AOR 0.16, $p = 0.002$), adolescents ages 12 and under (AOR 0.13, $p = 0.002$), low score on the vaccine safety scale (AOR 0.29, $p = 0.001$, alpha = 0.82), belief that her daughter was too young independent of actual age (AOR 0.37, $p = 0.001$). Maternal history of STI, abnormal Pap smear or cervical cancer trended to increase vaccine completion (AOR 2.58, $p = 0.08$).

Conclusion: HPV vaccine completion in adolescent daughters of mothers who participated in their own cancer preventive behavior remains suboptimal with significant racial disparity in vaccine use.

17. Prolonged Release Anti-HIV Microparticulate Gel: In vitro and in vivo Drug Release Study for HIV Infection in Women

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Background: Women are approximately 50% of total living HIV infected patients worldwide, reported in UN AIDS Update, 2007. Drug delivery across vagina, is a challenging task. Keeping this view, a new vaginal anti-HIV microparticulated gel was engineered to coat vaginal tissue with a stable HIV protective layer.

Methods: Zidovudine (AZT, gift sample, Aurobindo Pharma Ltd, A.P. India) loaded microparticle were prepared by the O/O solvent evaporation method using ethyl cellulose as a rate controlling polymer in different drug-polymer ratio and characterized for the percent drug content, entrapment efficiency, micromeritic analysis, FTIR study, SEM for surface morphology and in vitro dissolution studies of AZT (λmax 267 nm) using UV spectroscopy. The optimized microparticle was incorporated in bioadhesive gel using different bioadhesive polymer (2% carbopol 940P was found to be the best). A vaginal anti-HIV microparticulated gel was thus prepared.

Results: Optimized gel was evaluated for percent drug content $1.20 \pm 0.08\%$ (mg/1gm), in-vitro diffusion studies of vaginal gel AZT release, only $61.13 \pm 7.68\%$, up to 36 hour using immediately excised goat vaginal tissue with a stable gel layer with bioadhesion strength (1.69 ± 0.02 gm./cm^2), spreadability 13.74 ± 0.10 (gm.cm/sec.) and extrudibility (17.58 ± 0.08 gm./cm^2). In-vitro drug diffusion kinetic Study shows Fickian diffusion case I transport mechanism. t1/2 (1 hr), AUC in 24 hr (192.87), when compared to oral standard AZT suspension, were determined form the pharmacokinetics study of microparticulated gel, in HPLC (RPC18 column flow rate 1 ml/min) quantification with UV absorbance detection at λmax 267 nm. All values were significant $p < 0.05$ at 5% level from ANOVA study.

Conclusion: Vaginal microparticulated gel was found to be a good tool to provide sustained antiretroviral therapy.

18. The Risk for Diabetes Mellitus among Women with Gestational Diabetes: A Population-Based Study in Israel

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Background: The present study aimed to determine the incidence of postpartum diabetes mellitus (DM) in the years following a diagnosis of gestational diabetes (GDM) and to determine whether severity of GDM is associated with developing diabetes.

Methods: A retrospective cohort study was performed among 185,340 pregnant women who had glucose challenge test (OGCT) or 3-h oral glucose tolerance test (OGTT) in a large HMO in Israel. Subsequent diagnosis of diabetes was ascertained by using an automated DM registry.

Results: A total of 11,259 subjects were diagnosed as suffering from GDM comprising 6.07% of the cohort. During a total follow-up period of 946,978 Person-Years there were 1065 (1.74 per 100 Person-Years) and 1118 (0.12 per 100 Person-Years) diagnoses of postpartum DM among GDM and non-GDM women, respectively. After 10 years of follow-up, 16% of the GDM population developed diabetes mellitus, compared with 1% among the non-GDM population. GDM was associated with an 8-fold higher risk of postpartum DM after adjusting for important confounder such as socioeconomic status and BMI. Among women with GDM history, diabetes risk increased with number of abnormal OGCT values and among women with Type A2 GDM.

Conclusion: GDM, and particularly severe GDM, are important predictors of future development of DM.

Abstract Preview - Step 3/4

- print version -

Abstract category: A23 Preclinical HIV drug development

Title: **A strategy to reduce new HIV infection, prevention and treatment: Prolong release Anti-HIV polymeric vaginal film - it's *in vivo* and *in vitro* drug release study**

Authors: B.B. BHOWMIK, A. Chatterjee, R. Thakuria

Institute(s): HIMALAYAN PHARMACY INSTITUTE, DEPARTMENT OF PHARMACEUTICS, MAJHITAR, India

Text: **Background:** Women are approximately 50% of total HIV infected people worldwide. In this critical moment for universal access to HIV prevention and treatment, prolong release anti-HIV polymeric vaginal film (APVF) may become one strategy of choice, specifically for women to provide personal choice and self-control to prevent HIV transmission with antiretroviral prophylaxis. APVFs are suitable for a wide variety of drugs and release drugs in a predetermined manner with better vaginal dispersion, bioadhesion and aesthetic appeal. Teenagers will prefer the APVF because of privacy, more self-control to STDs protection, quick self-insertion and self-removal with own finger before intercourse. Drug delivery across vagina is a challenging task. Thus, a newer APVF was engineered to coat vagina with a stable HIV protective layer. **Methods:** Zidovudine (gift, APL Research Centre, India) was used as a model drug. APVFs of zidovudine, prepared by solvent-casting method using combination of different polymers [EC:HPMC(F1), Acrylcoat S100:HPMC(F2) and EC:HPMC(F3)] in1:1 ratio, were evaluated. *UV spectroscopy was used (λ_{max} 267 nm) for the routine analysis of Zidovudine.* **Results:** Drug particles were distinctly observed in Scanning Electron Microscopy of APVF. F1 was selected for detailed study due to high drug content (87.52±0.91%), folding endurance (304±15), swelling index (14.25±0.76) and good mechanical properties. *In-vitro* drug diffusion kinetic explained swelling controlled Case I Fickian diffusion mechanism. *In-vitro* permeation of APVF released only 47.57± 7.68 % Zidovudine, even after 36 hour using immediately excised goat vaginal tissue with a stable swelled layer with bioadhesion strength (22.0±1.2g). From *in vivo* pharmacokinetics of APVF, F1 demonstrated t_{max}(6hr), $t_{1/2}$ (0.38 hr), AUC in 24 hr (467.528µg.hr/ml), when compared to standard oral conventional Zidovudine tablet, in HPLC (RPC18 column flow rate 1ml/min) with UV detector at λ_{max} 267 nm. **Conclusions:** *Prolong release APVF was found to be a good strategy to reduce new HIV infection, prevention and for sustained antiretroviral therapy.*

Keywords: 1. polymeric vaginal film
2. In vivo
3. In vitro

Country of research: India

Related to women and girls? Yes

Related to children? No

Ethical research declaration: Yes

Applied for scholarship? Yes

Acceptance Status: Poster exhibition

Presentation Date: Monday, 19 July 2010

Location: Poster Exhibition Area

Conference: XVIII International AIDS Conference · Reference No: A-240-0030-06050 · **Status: Submitted**

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Pharmaceutical Development and Technology, 2010, 1–8, Early Online

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RESEARCH ARTICLE

Microparticulated anti-HIV vaginal gel: *In vitro-in vivo* drug release and vaginal irritation study

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Abstract

The aim of this study was to develop and evaluate a Zidovudine (AZT)-loaded microparticulated bioadhesive vaginal gel (MBVG) in order to obtain a controlled releasing, safe gel delivery system. AZT microparticles (ZMPs) were evaluated for encapsulation efficiency, drug loading, surface morphology and in vitro drug release profiles and drug release mechanism and optimized. The optimized ZMPs were then encompassed in bioadhesive gel using different bioadhesive polymers and evaluated for the drug encapsulation efficiency, drug loading, *in vitro* and *in vivo* drug release profiles, drug release mechanism and vaginal irritancy study. From the dissolution data of ZMP4 and MBVG4 showed a zero-order diffusion pattern and Fickian diffusion case I transport mechanism in 24 and 36 h, respectively. On the basis of a pharmacokinetic study of MBVG4 (containing ZMP: Carbopol 1:4), it was found to have better bioavailability, larger AUC and T_{max} in comparison to an oral pure suspension of AZT.

Keywords: *Zidovudine; microparticle; gel; irritation*

Introduction

The vagina is an efficient route for drug administration due to the presence of a dense blood vessels network and because it avoids first-pass metabolism.[1,2] Conventional vaginal formulations are associated with the disadvantage of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. Zidovudine (AZT), with a short elimination half-life of about 1 h, high dose 250 mg in every 4 h while 300 mg twice a day, in some cases, low systemic bioavailability (64%) due to rapid hepatic fast-pass metabolism,[3] was chosen as a model drug of choice. The antiretroviral drug, AZT, a nucleoside reverse transcriptase inhibitor, is taken up by the host cells where it is converted into its triphosphate form. Subsequently, by competitive inhibition, it inhibits the reverse transcriptase, therefore, viral replication stops. Also it is incorporated into the viral DNA chain which is growing (during replication) and terminates the lengthening of the viral DNA chain, thereby stopping viral replication.

The UN global AIDS epidemic review 2007 revealed that women (15.4 million) are approximately 50% of the people (33.2 million) infected and living with HIV.[4] The present study was designed to develop a newer microparticulated bioadhesive vaginal gel (MBVG) for prolonged release of AZT for continuous protection against heterosexually transmitted HIV infection and to improve user compliance and patient convenience even during sexual activity.[5] Ideally, anti-HIV AZT microparticles (ZMPs) in vaginal gel should disintegrate in vaginal medium; provide uniform microparticulated drug-hydrogel coating of vaginal tissue, resulting in intra-vaginal bio mimetic lubrication during intercourse, and retention of this gel layer before and after intercourse.[6] Most importantly, controlled release of anti-HIV drugs from these ZMPs in gel inactivate the viral load potentially introduced during sexual activity, due to the increase in effective micro surface area of the therapeutics. MBVG was engineered to coat vaginal tissue with a stable HIV protective layer, retention of this gel layer before intercourse, and to

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(Received 30 November 2009; revised 12 March 2010; accepted 16 March 2010)

ISSN 1083-7450 print/ISSN 1097-9867 online
DOI: 10.3109/10837450.2010.485318

http://www.informahealthcare.com/phd

release an entrapped anti-HIV drug in a controlled fashion in the presence of the main infecting agent: semen. Potentially, they can be further developed to apply vaginally to prevent both male-to-female and female-to-male sexual disease transmission.[7,8] This study was aimed to formulate and evaluate microparticulated controlled release vaginal gel of AZT using ethyl cellulose as the release decelerating polymer with high entrapment efficiency and extended release to provide continuous protection by increasing the viscosity of the cervical mucus against heterosexually transmitted HIV infection. ZMPs in general effectively control the release of drugs and produce efficacious therapeutics due to the increase in effective surface area to micron size.

In view of the above, the present study was designed to develop a newer MBVG for prolonged release of AZT to treat HIV infections with increased patient convenience.

Experimental

Materials

Zidovudine, a gift sample from Aurobindo Pharma Ltd, A.P, India and ethyl cellulose (EC) (ethoxy content - 47.5% by weight and viscosity (η) of 22 cps in a 5% concentration by a weight in toluene: ethanol 80:20 at 25°C), Carbopol 940P and HPMC (K4M, 4000 cps 2% aqueous solution) were purchased from S. D. Fine Chemicals, Mumbai, India. All other used chemicals and reagents were of analytical grade and used as received.

Preparation of ZMP

ZMP were prepared by solvent evaporation method using an acetone and light liquid paraffin. Span 60 was used as the droplet stabilizer. Different amounts of drug:ethyl cellulose (1:1, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7) were dissolved in 5 mL acetone by using a magnetic stirrer. The drug was also added into the polymer solution followed by stirring with magnetic stirrer for 15 min. The resulting dispersion was then poured into 250 mL beaker containing the mixture of 50 mL light liquid paraffin (as continuous phase) and different ratio of Span 60 for different formulations, while stirring. A mechanical stirrer with a three blade paddle was used. Stirring (at 1000 rpm) and heating (at 40°C) was continued for 2 h, until acetone evaporated completely. After evaporation of acetone, the ZMP formed were filtered using Whatman type one filter paper.[9,10] The residue was washed 4–5 times by 10 mL of ether. ZMPs (ZMP1–ZMP7) were dried at room temperature for 24 h. All batches were prepared in triplicate.

Surface morphology of ZMP

ZMP were also analyzed by Scanning Electron Microscopy (Jeol, JSM-25 SII, Tokyo, Japan) to visualize the surface quality of ZMP. ZMP were placed on double-sided tape attached onto graphite surface.[11] The samples were coated with gold/palladium using an Ion Sputter (Jeol, JFC-1100). Coating was provided at 20 mA for 4 min. Observation was performed at 15 kV.

Drug loading and encapsulation efficiency

Drug content means the actual amount of drug present in the formulation. About 50 mg of accurately weighed drug-loaded ZMP were dissolved in 2 mL of acetone and after that volume was made up to 50 mL in volumetric flask with simulated vaginal fluid[26] (SVF, acetate buffer pH 4.7). The resulting mixture was agitated on a mechanical shaker and then kept aside for 24 h.[11,12] The solution was then filtered and the absorbance was measured at 267 nm using UV-Visible spectrophotometer (UV - 1700, Shimadzu, Japan) and drug content was determined by using:

$$D_c = \frac{C_C \times D_F \times V}{C_F}$$

where D_C = drug content, C_C = concentration, D_F = dilution factor, V = volume taken, and C_F = conversion factor.

The percent entrapment efficiency of the ZMP

The percent encapsulation efficiency is calculated using following equation:[13]

$$\%E_f = \frac{A_{dc}}{T_{dc}} \times 100$$

where $\%E_f$ = entrapment efficiency, A_{dc} = actual drug content, and T_{dc} = theoretical drug content.

In vitro drug release studies of ZMP

In vitro drug release study of drug from the ZMP was studied by using USP type I dissolution test apparatus. The receptor compartment (a cylindrical vessel) was contained 900 mL SVF that was within the vaginal pH and maintained at a temperature of 37 ± 1°C.

The ZMPs, equivalent to 100 mg of AZT, were placed in a basket covered with muslin cloth. The *in-vitro* drug release studies were carried out for 24 h and the

dissolution medium was stirred at 50 rpm. At predetermined time intervals 5 mL aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium maintaining sink condition, the samples were analyzed for drug quantification at 267 nm using UV-Visible Spectrophotometer, Shimadzu, model no. 1700. The concentrations of AZT in samples were calculated.[11,14]

Preparation of microparticulated vaginal gel

Carbopol gels were prepared by cold mechanical method.[15,16] The required quantity (Table 2) of polymer (Carbopol 940 and HPMC) was weighed and it was sprinkled slowly on surface of purified water for 2 h, after which it was continuously stirred by mechanical stirrer, until the polymer soaked in the water. With continuous stirring, triethanolamine was added to neutralize the gel. It also maintained the pH of the gel. Now the appropriate quantity of DMSO (Dimethyl sulfoxide) was added to the gel, which behaves as the penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Finally, the best microparticulated formulation was added to the gel with continuous stirring until the ZMPs were dispersed in the gel completely. Eight formulations of microparticulated intra-vaginal gels were prepared by using Carbopol 940 and HPMC in different ratio. The prepared gels were packed in wide mouth glass jars covered with screw capped plastic lids after covering the mouth with an aluminum foil and were kept in dark and cool place. The formulations were preserved for further study.

Analysis of drug release mechanism from vaginal gel

In vitro drug diffusion studies were carried out by using modified Keshery-Chien (KC) cell with a semi-permeable barrier. Cellophane membrane was soaked in SVF. 1 g of MBVG was placed on the surface of the processed cellophane membrane and was fixed to one end of the cylindrical donor compartment by cyanoacrylate adhesives, such that the lower end just touched the surface of SVF medium. Also 0.5 mL of SVF was placed and maintained at the same level throughout the study in the donor compartment. Temperature was maintained at 37 ± 2°C with constant stirring at 50 rpm. A quantity of 5 mL sample was withdrawn from the receptor compartment at a definite time interval and replaced with 5 mL of SVF to maintain sink condition. The drug was estimated by using UV-Visible spectrophotometer at 267 nm (λ_{max}).

In order to investigate the mechanism of AZT release from ZMP and MBVG of different bioadhesive polymers and ZMP ratios, the release data were analyzed with the following mathematical models: Zero-order kinetic (Equation 1), first order kinetic (Equation 2), and Higuchi kinetic (Equation 3).

$$Q_t = K_0 t \quad (1)$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad (2)$$

$$Q_t = K_h t^{1/2} \quad (3)$$

The following plots were made: Q_t versus t (zero-order kinetic model), ln (Q_0 - Q_t) vs. t (first-order kinetic model) and Q_t vs. $t^{1/2}$ (Higuchi model);[14,17] where, Q_t is the percentage of drug released at time t, Q_0 is the percentage of drug present in the ZMP, K_0, K_1 and K_h are the constants of the equations. Furthermore, to confirm the mechanism of drug release, the first 60% of drug release was fitted in Korsmeyer-Peppas model (equation 4):

$$\frac{M_t}{M_\alpha} = K_p t^n \quad (4)$$

where, M_t/M_α is the fraction of the drug release at time t, K_p is the rate constant, n value is used to characterize different release mechanisms, and is calculated from the slope of log of fraction of drug released (M_t/M_α) vs. time (t). The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model); and log cumulative % of drug released vs. log time (Korsmeyer-Peppas model).[18]

In vivo study of MBVG4 in rabbit model using HPLC

Seven adult, female, New Zealand white species rabbits weighing 1.5–1.7 kg were used for the *in vivo* study. The animals were divided into two groups containing four animals each and one animal was used as control.[19] The animals were kept fasted overnight. Water was given *ad libitum* during fasting and throughout the experiment. The rabbits were not anesthetized during or prior to the experiment. The formulation was applied with the help of vaginal applicator and standard oral dose (1 mg/mL) with the help of oral cannula. The procedures employed in this study were approved by Institutional Ethical Committee (no: HPI/ 07/ 60/ IAEC/ 0013). Blood samples (2 mL) were collected from the marginal ear vein at an interval of 1, 2, 4, 6 and 24 h during the study. The same method was followed H for each group (both standard and test). The blood samples withdrawn as above were transferred to a series of

graduated centrifuge tube containing 1 mL of 10% w/v EDTA solution and 1 mL of 15% w/v trichloro acetic acid (TCA).[20] The samples were centrifuged immediately at 3000 rpm for 15 min in cooling centrifuge machine to collect plasma.[21] The plasma was separated and transferred into other set of sample tubes and stored in −20°C until assayed.

The plasma samples were analyzed for AZT by firstly passing the samples through silica gel column (for solid phase extraction) and analyzed by HPLC (LC-20 AT, Shimadzu, Japan) using mobile phase methanol:water (60:40)[22] at flow rate 1.2 mL/min. Twenty micro liters (20 µl) of injection volume was eluted in RP C(18) column (4.6 × 150 mm) at room temperature and was monitored at wavelength (λ_{max}) 267 nm using diode array UV detector.[23] The concentration was calculated from standard curve. A standard curve was first prepared using known concentrations of standard AZT against the HPLC peak area and was used throughout for analysis. Standard curve equation was $Y = 16.22x + 3.016$, $r^2 = 0.999$ (Y = HPLC peak area, x = concentration in µg/ml, r^2 = correlation coefficient).

Pharmacokinetic analysis

The primary objectives of the study were to determine AZT plasma concentration, area under the plasma AZT concentration-time curve up to 24 h $\{[AUC]_0^{24}\}$ after vaginal and oral administrations of AZT, peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}). The area under the AZT concentration curve was calculated according to the trapezoidal method. The $[AUC]_0^{24}$ was divided into trapezium segments according to the time intervals of blood sampling. The $[AUC]_0^{24}$ was calculated by summation of the trapezium segments. The highest observed concentration during the study period; C_{max}, and time, at which C_{max} observed, T_{max}, were obtained directly from the plasma concentration-time profiles. The area under the plasma concentration time curve ($AUC_{0\text{-}24h}$, and $AUC_{0\text{-}\alpha}$, µg.h/mL) was calculated based on the trapezoidal rule. The volume of distribution (V_d), total body clearance (Cl_T), elimination rate constant (K_E) and half-life ($t_{1/2}$) were also calculated.[24]

Vaginal irritation study

The vaginal irritation study was carried out by treating intra-vaginally a group of three white female Wister albino rats with 0.2 g of MBVG4, another group of three for standard irritant (benzalkonium Chloride, BZK 1% v/v) and one rat, as control (without any formulation).

Formulation was applied every day up to 10 days. All animals were killed on the 11th day. The reproductive tract was examined grossly. The vaginal tissues were rapidly removed and parts of the upper (cervico-vagina), middle and lower (uro-vagina) regions of each vagina were fixed in 10% neutral-buffered formalin for microscopic evaluation.[25] Fixed vaginal tissues were trimmed, embedded in paraffin, sectioned at a thickness of 4–6 µm and stained with hematoxylin and eosin and examined under X200 and X400 magnification using an Olympus microscope CX21 Leica light microscope (Milton Keynes, Buckinghamshire, UK) interfaced with an image analysis system (Media Cybernetics, Silver Spring, MD, USA) in conjunction with a 3-CCD camera (DAGE-MTI Inc, Michigan City, IN, USA) for observation and analysis.

Statistical analysis

For statistical analysis using MYSTAT software for univariables comparison we used the Analysis of Variance is presented as mean ± SEM and $P = 0.05$ was considered significant. Statistical data analyses were performed by statistical analysis using MYSTAT software the ANOVA one way at 5 % level of significance $P < 0.05$.

Results and discussion

The production yield was of about 65.16–83.15% for all formulations. Encapsulation efficiency was found to be 15.37–93.92% which was significantly different ($P < 0.05$) (Table 1). Actual drug contents of formulations were between 9.132 and 36.97%. Drug content and encapsulation efficiency relatively high drug content and encapsulation efficiency were observed for each formulation presented in column 4 and 5 of Table 1. The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose and sparingly soluble

Table 1. Percentage yield, drug entrapment efficiency, and drug content of ZMPs (Zidovudine microparticles).

Formulation Code	Drug/polymer ratio	Percent yield Mean ± SEM	Drug entrapment efficiency Mean ± SEM	Drug content/100 mg Mean ± SEM)
ZMP 1	1:1	76.33 ± 1.45	15.37 ± 0.45	10.07 ± 0.23
ZMP2	1:2	70.99 ± 8.13	50.62 ± 9.77	23.96 ± 4.81
ZMP3	1:3	65.16 ± 5.S9	93.92 ± 4.59	36.97 ± 5.35
ZMP4	1:4	72.93 ± 0.73	89.75 ± 3.52	24.58 ± 0.87
ZMP5	1:5	33.15 ± 6.46	83.60 ± 4.63	16.99 ± 1.42
ZMP6	1:6	74.33 ± 6.93	67.32 ± 8.86	14.44 ± 1.24
ZMP7	1:7	68.31 ± 5.54	49.85 ± 5.28	9.132 ± 0.62

Table 2. Formulation design for the preparation of MBVGs (microparticulated bioadhesive vaginal gels).

Formulation	ZMP 4 (mg)	Carbopol P940 (mg)	HPMC (mg)	Triethanolamine (ml)	Dimethyl Sulfoxide (ml)	Methyl Paraben (mg)	Distilled Water (gm)
MBVG1	407.00	100	—	0.2	2.0	15.00	Qs
MBVG2	407.00	200	—	0.2	2.0	15.00	Qs
MBYG3	407.00	300	—	0.2	2.0	15.00	Qs
MBYG4	407.00	400	—	0.2	2.0	15.00	Qs
MBYG5	407.00	100	100.00	0.2	2.0	15.00	Qs
MBYG6	407.00	100	200.00	0.2	2.0	15.00	Qs
MBYG7	407.00	100	300.00	0.2	2.0	15.00	Qs
MBYG8	407.00	100	400.00	0.2	2.0	15.00	Qs

AZT. The encapsulation efficiency was found to increase with the increase in polymer content up to ZMP4 but after that, the concentration of polymer did not affect encapsulation efficiency, perhaps due to the complete encapsulation of drug. As usual, it was not affected even if the concentration of polymer increased.

The ZMP of AZT prepared by the solvent evaporation methods was found to be discrete, nearly spherical, free flowing. The SEM photographs indicated that ZMP were spherical and completely covered the coat polymer (Figure 1).

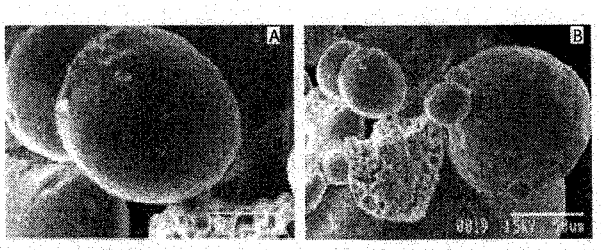

Figure 1. SEM study of ZMPs using magnification ×1500 Scanning electron microscopy of ZMP4 using magnification 600.

Figure 2 compares the in vitro drug release profiles of all the eight formulations of controlled-release MBVG (Figure 2B) with ZMP (Figure 2A). It can be seen from Figure 2 that the drug release throughout the study period was more or less steady. In formulation MBVG4, where Carbopol P940 was used, the t_{50} (time taken for 50% of drug to be released) was found to be 32 h, when compared to ZMP4 (Figure 2C) as formulation ZMP4 showed the t_{50} at the 10th h although it was significantly different (P= 0.001) from all the ZMPs formulations. The data clearly suggested that the concentration of the polymer used in ZMPs was inadequate to control the release of AZT. In the next stage, an attempt was made to control the release by using MBVG in to the vagina. It was of immense interest that the release profile of formulation MBVG4 showed an auxiliary controlled release throughout the study period as was expected. The release rate was steady from the initial hour, and only 61.13% of drug was released up to 36 h although it was significantly different (P = 0.004) among all the MBVG formulations. All the formulations were analyzed for the drug release kinetics. The calculated regression coefficients showed a higher r^2 value with zero-order kinetics (0.914 and 0.973 for ZMP4 and MBVG4, respectively) and Higuchi model (0.914 and 0.896 for ZMP4 and MBVG4, respectively).

However, the regression values were found to be low with first-order kinetics models. Thus, both zero-order and Higuchi models could be applicable, although zero-order kinetic models seem to be better (higher r^2 value for the whole release process). Although, drug release from all the MBVG formulations (MBVG1-MBVG8) was observed up to 36 h, the drug release mechanism of all MBVG formulations were found to be predominately influenced by the different bioadhesive polymer added. The mechanism of drug release from hydrophilic polymeric matrices involves solvent penetration, hydration, swelling of the polymer, diffusion of the dissolved drug in the matrix and erosion of the gel layer.

From Figure 2, the n values for all the formulations ranged from 0.227–0.529. Based on the diffusion control studies, it was observed that the MBVG4 (n = 0.433) underwent case I Fickian diffusion control, during the dissolution study. In the case of Fickian release mechanism, the rate of drug release is much less than that of polymer relaxation (swelling/erosion). So the drug release is chiefly dependent on the diffusion through the matrix. Drug release was also significantly different ($P < 0.05$) in single factor. It means null hypotheses were nullified and an alternative hypothesis is accepted, i.e. the variation in formulations in polymeric type and content (MBVG1-MBVG8) had a significant effect on drug release profile.

In vivo studies MBVG4

The mean plasma concentrations of AZT after oral administration of pure drug solution (1 mg/mL) or vaginal MBVG4 formulation containing ZMP4 (407 mg) with the ratio (1:4) are shown in Figure 3. A number of pharmacokinetic parameters were studied, namely the C_{max}, T_{max} and AUC 24 (Table 3). The C_{max} and T_{max} differed significantly between the two groups. Treatment with orally administered AZT resulted in the earliest serum C_{max} of AZT (Figure 3, Table3). Orally administration also

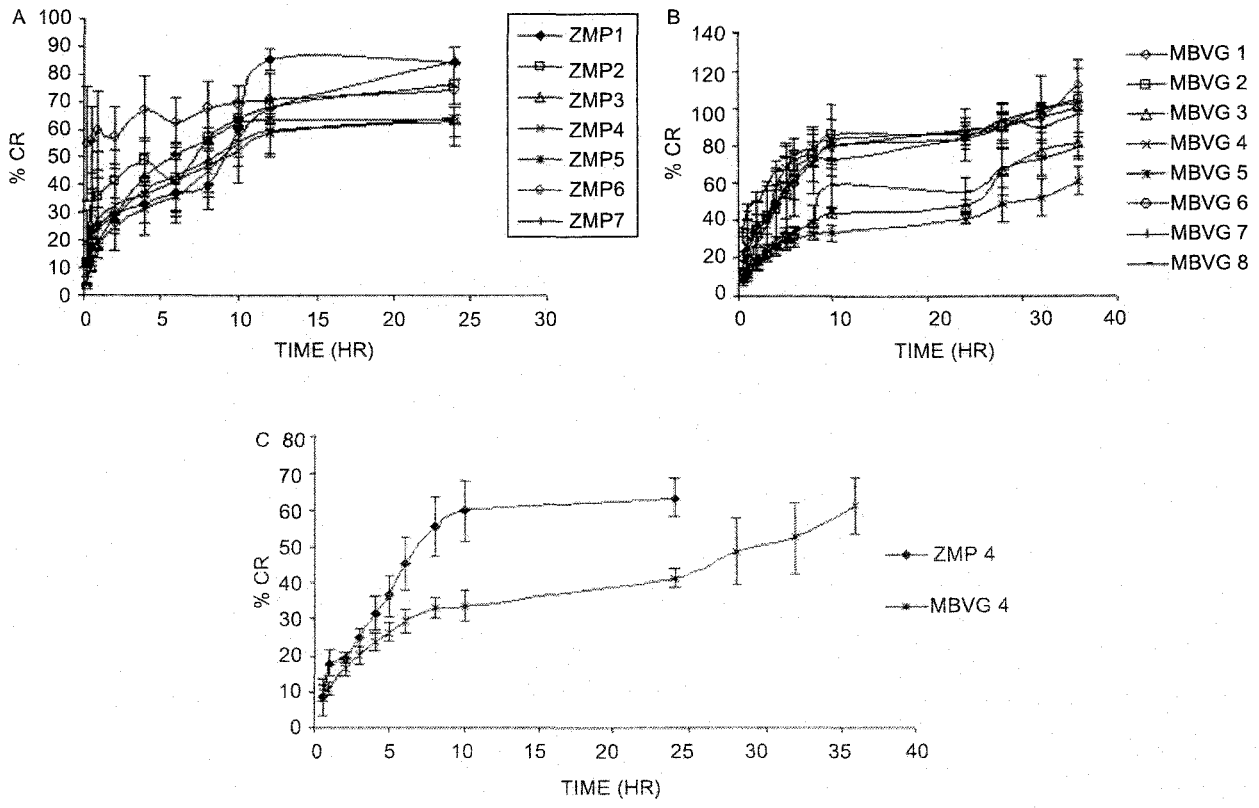

Figure 2. Comparison in vitro drug release of ZMP4 with MBVGs. Data represents the means ± SEM (A, B, C).

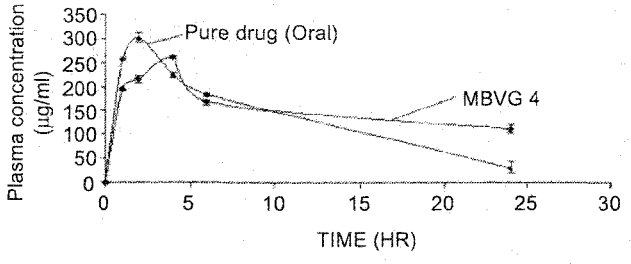

Figure 3. In vivo comparison study of MBVG4 with pure drug (oral). Data represent the means ± SEM.

resulted in the highest C_{max} 18.49 µg ml^{-1}, which was significantly higher than the peak levels following vaginal administration of AZT. There are only a few studies of the pharmacokinetic profiles of AZT depending on the administration route.

After a single oral dose, AZT is rapidly and almost completely absorbed from the gastrointestinal tract. However, the drug undergoes extensive and rapid first-pass metabolism. Following 1 mg oral AZT administration, the plasma level increases rapidly and peaks at about 2 h. However, the plasma level declines rapidly by 6 h after intake and remains low thereafter. In contrast to the oral route, vaginal administration results in plasma concentrations that increase gradually, reaching a maximum level after 4 h and slowly declining, with detectable levels of AZT remaining up to 24 h after administration. The peak plasma concentration (C_{max}) 15.38 µg ml^{-1}

Table 3. Observations of pharmacokinetic study of MBVG4 and oral suspension.

S.No	Parameters	Pharmacokinetics of Oral Suspension	Pharmacokinetics of MBV G4
1.	$\int_0^{24} AUC$ (µg.h/ml)	182.02	192.87
2.	$\int_0^{24} AUMC$ (µg.h²/ml)	848.23	1617.3
3.	$\int_0^{\infty} AUC$ (µg.h/ml)	182.02	200.79
4.	$\int_0^{\infty} AUMC$ (µg.h²/ml)	2072.21	3951.07
5.	MRT at 24 (hrs.)	4.66	9.55
6.	MRT at ∞ (hrs.)	11.38	19.67
7.	C_{max} (µg/ml)	18.49	15.38
8.	T_{max} (hrs.)	2.00	4.00
9.	$t_{1/2}$ (hrs.)	1.00	1.00
10.	K_E (hr^{-1})	0.693	0.693
11.	Vd (L/Kg)	0.068	0.081
12.	Cl_T (L/Kg/hr.)	0.047	0.056
13.	F (%)	100 (Consider here)	105.96

achieved is lower than following oral administration but bioavailability, measured as the area under the curve (AUC) of plasma AZT, is not significantly greater after vaginal administration. The greater bioavailability of vaginal AZT may help to explain why vaginal administration is more effective in inducing uterine contractions. Pharmacokinetic studies show a rapid rise to peak plasma level and a sustained elevation in AZT, resulting in a bioavailability that is higher than for the oral route. This results in the development of uterine contractility similar to that seen with vaginal treatment. However, although vaginal absorption has been shown to be slower and the C_{max} achieved by the vaginal route is lower than that of the other routes of administration, the plasma level of AZT is sustained for as long as, or possibly for longer than, with oral AZT. The effect of AZT may linger for 24 h after a single dose administered vaginally. So far, the pharmacokinetic profiles of conventional and MBVG4, AZT resulting from different administration routes have not been studied beyond 24 h. Since, the effects of vaginal and oral AZT are similar and more pronounced than for oral AZT, it seems that it is the sustained plasma levels, rather than the high peak plasma concentration, that are crucial to efficacy. The volume of distribution (V_d) 0.081 L/kg, total body clearance (Cl_T) 0.056 L/kg/h, elimination rate constant (K_E) 0.693 h^{-1} and half-life ($t_{1/2}$) one hour was found to compare with the oral pure drug. The overall release form MBVG4 formulation and pure drug solution were not significantly different ($P < 0.05$) statistically, indicating that the drug amount between the formulations did not vary.

Vaginal irritation study

Histological evaluation of three different regions of the vaginal tissues of seven rats (three for formulations, three for standard irritant and one control) given daily intra vaginal application of MBVG4 for 10 consecutive days showed lack of significant vaginal irritation (Table 4 and Figure 4A–C). Rats treated with MBVG4 revealed very mild epithelial ulceration with an absence of edema, leukocyte influx, and vascular congestion (characteristic of inflammation as quantified by histological scoring according to the method of Eckstein et al.[25]), indicating the MBVG4 formulation as safe for vaginal application.

Table 4. Histological scoring for vaginal irritation study using rat vagina.

		Histopathological parameters			
S.No	Batch	Epithelial ulceration	Neutrophil infiltration	Leukocyte infiltration	Vascular congestion
1	Normal (control)	0	−−	−−	−−
2	Standard	+++	+++	++	−−
3	Test	−−+−	+−−	−−−−	−−

+ = positive, −− = negative (n=7)

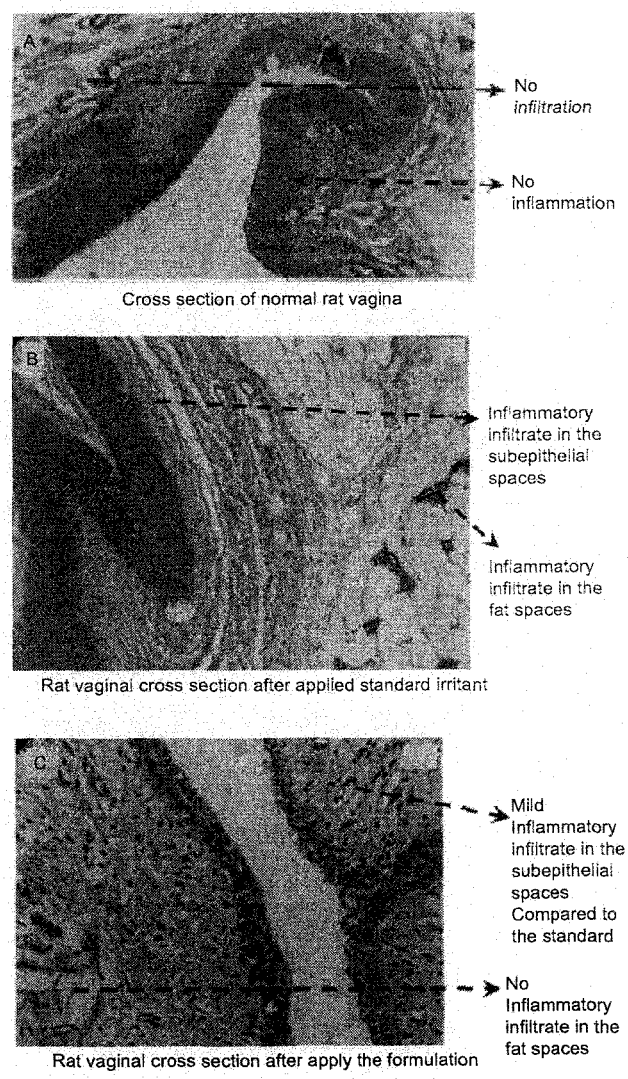

Figure 4. Rat vaginal cross sections during vaginal irritancy study. (A) For normal vagina; (B) rat vagina treatment with BZK; (C) rat vagina treatment with MBVG4.

Conclusion

Drug delivery through vaginal gel is a promising area for continued research with the aim of achieving controlled release with enhanced bioavailability over longer periods of time. In conclusion, ZMP4 containing the drug:polymer ratio 1:4 was selected and evaluated in order to achieve one objective of this study. The method of preparation of ZMPs of AZT (by solvent evaporation method) was found to be simple and reproducible. To achieve the second objective, optimized ZMP4 were blended into gel by mechanical mixing with various bioadhesive polymers. In vitro drug release study of ZMP4

showed t_{50} in 10 h drug released and MBVG4 showed t_{50} in 36 h drug released following case I Fickian ($n \leq 0.5$) transport mechanism and *in vivo* drug release it was found T_{max} 4 h and C_{max} 15 h comparison with oral pure drug solution. Very mild epithelial ulceration was observed during the 10-day irritancy study. Based on these results, it can be concluded that microparticulated vaginal gel of AZT could improve upon physicochemical and biological properties. Further research in this area will surely be expected to yield significant outcome with improved vaginal drug delivery system for treatments of AIDS and its prevention that women may control.

Acknowledgements

The authors thankfully acknowledge the APL Research Center, A.P. India., for the gift sample of AZT. The authors are grateful to the SAIF, (DST) AIIMS, New Delhi, India, and Burdwan University for help in performing the SEM studies. The authors also thankfully acknowledge AICTE, India, for supported research project, F.No:8023/BOR/RID/RPS 48/2008-09.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Asian Journal of Pharmaceutical and Clinical Research ISSN 0974-2441

OVER VIEW ON PARTICULATED BIOADHESIVE VAGINAL DELIVERY SYSTEM AND EVALUATION

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Bioadhesion in vagina is a topic of current interest in the design of particulated bioadhesive gel as drug delivery systems and different evaluations. Particulate carrier systems comprising of nanoparticles, liposomes, niosomes, microparticulates etc. are incorporated with bioadhesive gel, exhibit a prolong residence time at the site of application or absorption and hence facilitate an intimate contact with the vaginal epithelial membrane to improve the therapeutic performance of the drug. In recent years such vaginal gels are used in different diseases. The principles underlying the formulation development and characterization of particulated bioadhesive gel and the current research trends in this arena are explored in this review.

Keywords : particulate systems, bioadhesive gel, vaginal drug delivery system (VDDS), evaluations.

INTRODUCTION

Bio adhesive gel carrier technology offers a good research work for drug delivery by coupling the drug to carrier particles such as microcapsules, liposomes, nanoparticles, which modulate the release and absorption characteristics of the drug (FIG. 1).

Vaginal preparations, although generally perceived as safer, have historically been undesirable to women due to their multiple days of dosing; messy, dripping creams and requisite night-time dosing. Recognizing that the therapeutic delivery of the active agent plays a critical role in the overall success of therapy and attempting to circumvent the weaknesses of traditional vaginal drug delivery while maintaining and even improving safety profiles, the idea of new form of vaginal drug delivery was developed. Microcapsules play an important role in particulate drug delivery systems (DDS) due to their small size and efficient carrier characteristics. These types of novel DDS have limited success due to their short residence time at the site of absorption area; however this limitation can be easily maneuvered by the use of bioadhesive polymers in a localized area. A microcapsule is an upcoming area of microparticulate DDS that contains a distinct core and a discrete envelope. In current revolutionary state, microparticulate systems were used in different areas of treatment. (FIG. 2).

In general the solid, liquid, or gas core is entrapped in the envelope, made up of continuous, porous or nonporous, polymeric phase. The drug can be dispersed inside the microcapsule as solid particulates with regular or irregular shapes[1]. The advantages of these DDS are intimate contact of the dosage forms with absorbing membranes and dispense the drug slowly. It can be achieved by pairing the drug with bioadhesive polymeric gel which might be referred as bioadhesive microencapsulated gel perhaps. Microencapsulated vaginal gel is one such less explored area, as per literature survey. This unique delivery system, with both bioadhesive and sustained release properties, introduces the convenience of a single dose of medication

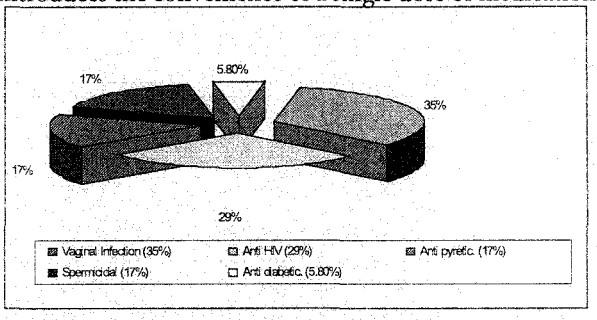

FIGURE 1: Different areas of interest using microparticulate delivery system. From 500 Scientific paper Survey ,05/05/2005 to 12/02/2007. www.pubmed.gov, www.elsevier.com etc.

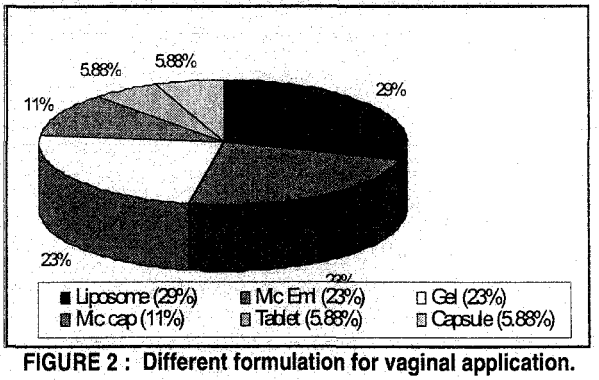

FIGURE 2 : Different formulation for vaginal application.

that can be applied at any time, with efficacy rates equivalent to lengthier durations of treatment (FIG.3)[2-4]. Bio adhesive Vaginal microcapsule of Zidovudine prepared by solvent evaporation method , using ethyl cellulose as a rate controlling polymer and hydroxy propyl methyl

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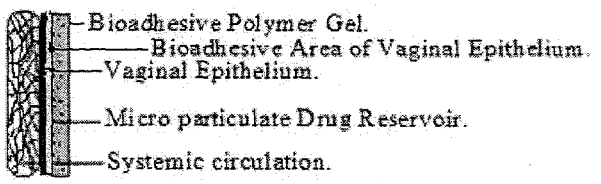

FIGURE 3 : Schematic representation of microencapsulated vaginal gel delivery system.

cellulose as a bio adhesive polymer for prevention and treatment of AIDS[5].

BIOADHESION AND BIOADHESIVE DRUG DELIVERY SYSTEMS (BDDS)

The term bioadhension refers to either adhesion between two or more biological materials (including cells, cellular secretion, mucus, extra-cellular matrix, and artificial substrate such as metal, ceramics and so on) or adhesion between polymer samples, either synthetic or natural, and soft tissue. Possible means of administration for BDDSs include the ocular, respiratory, GI, rectal, urethral and vaginal routes.[6-9]

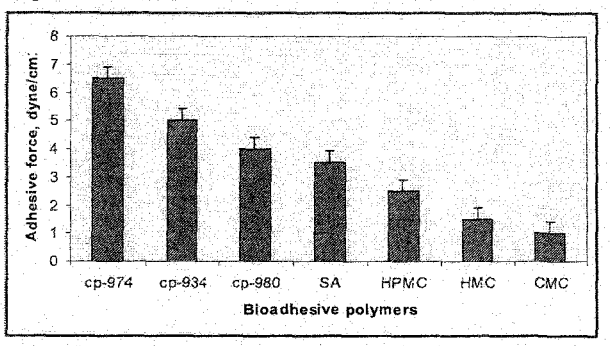

FIGURE 4 : Bioadhesive measurement of various polymers.

ADVANTAGES OF BIOADHESIVE SYSTEM

Bioadhesive drug delivery systems have four distinct advantages when compared to conventional dosage forms. First one is the enhanced bioavailability and effectiveness of drug due to targeted delivery to a specific localized region such as vagina; second one is the maximized absorption rate due to intimate contact with the absorbing membrane and decreased diffusion barriers. Third, drug protection is improved by polymer encapsulation and direct contact with absorbing cell layers. Fourth, longer residence time resulting in extended periods for absorption.[8,9].

BIOADHESIVE POLYMERS

From current scientific literatures, two classes of polymers appeared to be of interest for bioadhesion: hydrophilic polymer and hydrogel. Recent research has suggested that in the large class of hydrophilic polymers, those containing carboxyl groups exhibit the best bioadhesive properties . Other promising bioadhesive polymers have included sodium alginate (SA), methylcellulose (MC), hydroxy propyl methyl cellulose (HPMC), carboxy methyl cellulose (CMC), hydroxy methyl cellulose (HMC) and cationic hyhrogel such as chitosan. In general hydrogel have most often been used for bioadhesive drug delivery because of polymer-mucin chain entanglement, an essential component in bioadhesive bond formation. Other factor includes surface energy, surface texture, electrical charge and hydrophilic functional groups may be equally important. It was also found from literatures, non-hydrogel polymers which are having hydrophilic functional groups can also produce intense bioadhesive interactions.[10,11]

CLASSIFICATION OF BIOADHESIVE POLYMERS

Polymers can be classified as following category such as hydrogel polymer, natural polymer, natural modified polymer, Synthetic polymer. Hydrogel are water swellable material, usually a cross link polymer with limited swelling capacity. Some time their aqueous dispersion is very mildly acidic. Upon neutralization with an alkali, the resins expand extensively like a gel- like structure and their viscosity increases drastically, thus forming aqueous mucilage. As a result, the dispersion become thickened e.g Carbopol-974P, Carbopol-934, Carbopol 974P NF resin, Carbopol 980 NF etc. Various biocompatible natural polymer which are useful in bioadhesive vaginal formulation, includes pectin, guar gum etc. Another way to achieve desirable polymer properties is the modification of preformed in natural polymer. This modification may take place on the reactive sites of the polymer chain through alkylation, hydrolysis, sulfonation, esterification etc. Examples of natural polymer and their modifications are useful in bioadhesive formulation, e.g. chitosan and chitin. Various Synthetic polymer which are use full in bioadhesive vaginal preparation system are hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose, hydroxy methyl cellulose, sodium alginate and sodium polystyrene sulfonate (PSS), polyacrylic acid (PAA) or polycarbophil.

CLASSIFICATION OF POLYMERIC EMBRANES OF MICROCAPSULES

Polymeric films and membranes can be classified in various ways. One such classification is based on porosity, with the following categories.[18]

A. Macroporous membranes, which have large pores (0.1-1ìm).

B. Microporous membranes, in which the pores are

appreciably smaller (100-500Å)

C. Nonporous (gel, solution-diffusion) membranes. In the last category the "pores" are of the order of molecular dimension.

EVALUATION OF BIOADHESIVE GEL

Methods to evaluate Bioadhesive interaction

1. **Techniques Bioadhesion Test Procedure using Portable Universal Tester.**

After preparation of gel with 1.2 % polymer, measure the viscosity with a Brookfield Viscometer, which has been equipped with a suitable spindle rotating at 20 rpm. Place the sample on a brass mount piece, and screw the mount onto the platform. Level the sample with a spatula and care should be taken to avoid the presence of air bubbles. The Portable Universal Tester is then used to measure the peak adhesion force and the work of adhesion. (FIG.5).[19-21]

Here are adhesion models for biological systems listed in order of increasing complexity : Model 1 is used to predict bioadhesion by Polyacrylic acid (PAA) .Model 8 is typical of a real tablet dosage form with bioadhesive activity, while Model 3 is fairly typical of syrup, ocular or oral suspension dosage model *In vivo*. Isotropic = the same in all directions.

2. **The Wilhemy plate technique**

Traditionally this method has been used for dynamic contact angle measurement and involves a microbalance or tensiometer. A glass slide is coated with the polymer of interest and then dipped in to a beaker of synthetic or natural mucus. The surface tension, contact angle, adhesive force can be automatically measured using available in software. The shear test measures the force required to separate two polymer-coated glass slides joined by a thin film of natural or synthetic mucus. The result of this technique often correlates well with *In vivo* test result.[22]

3. **Du Nouy tensiometer :**

The bioadhesive properties of the hydrophobic, basic polyelectrolyte hydrogel disks containing crosslinked N,N-dimethyl amino ethyl methacrylate-co-methyl methacrylate 30/70mol% were evaluated in vitro using gastric (pH 1.2), sublingual (pH 6.5), vaginal (pH 4.0) and intestinal (pH 7.5) pig's mucosa. Adhesive strength was measure by using a modified Du Nouy tensiometer by measuring the force of detachment between a gel disk and the respective mucosa[22]

4. **Flow channel method:**

Mikos & Peppas, was developed a flow channel method that utilized a thin channel made of glass and filled with 2% w/w aqueous solution of bovine maxillary mucine, thermostated at 37°C. A particle of a bioadhesive polymer was placed on the mucine gel and its static and dynamic behavior was monitored at frequent interval by using a camera[23]

5. **Falling liquid method:**

This method is useful for identifying the adhesion strength of different polymer. Teng & Ho was first developed this methods. Small intestine segments from rats placed at an inclination of a tygon tube flute. The adhesion of particles to this surface was monitored by passing the particle suspension over the surface. By comparing the fraction of particles adherent to the tissue, the adhesion strength of different polymers can be determined[18]

6. **Viscometric method:**

In these methods, viscosities of 15% w/v percine gastric mucin dispersion in 0 .1N HCl (pH 1.0) or 0.1 N acetate buffer (pH 5.5) were measured by Brookfield viscometer in absence or presence of selected polymer. Viscosity component and the forces of bioadhesion were calculated [24, 25]ATR FT-IR spectroscopy method Saiano et al 2005 was work on the bioadhesive

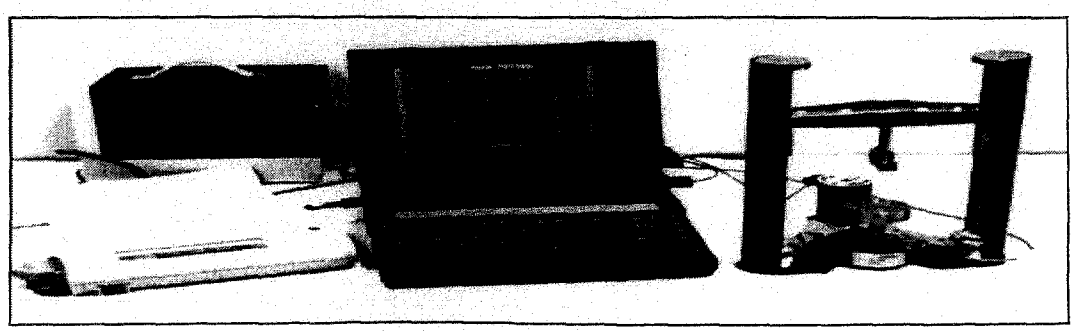

FIGURE 5 : The Portable Universal Tester.

properties of a hydrogel have been investigated by using ATR FT-IR spectroscopy. In particular, the co-polymer PHG obtained by partial derivatization of PHEA with GMA was chemically crosslinked by UV irradiation at 313 nm. Crosslinked PHG was treated with water to obtain a swelled sample, named PHG-UV gel, that was brought into contact with a phosphate buffer/ citric acid solution at pH 7.0 in the absence or in the presence of mucin at various concentrations (0.01, 0.1 and 1 wt.-%) [20]

7. **PBMC-based in vitro assay:**

It was measure anti-HIV activity of marketed vaginal gel or cream products and excipients by a PBMC-based in vitro method has been developed for the evaluation of anti-HIV activity of gel or cream marketed products and formulated drug delivery systems. Using this methodology, a number of marketed vaginal products showed 83% to 100% inactivation of a variety of X4 and R5 HIV of different clades. This knowledge of baseline anti-HIV activity of vaginal products (cream/ gel) and excipients is useful for the final formulation and development of anti-HIV microbicide [26]

8. **Vaginal tolerance tests :**

Amaral was established the vaginal tolerance tests were performed with a new potential microbicidal and spermicidal product, an acid-buffering vaginal gel (Acidform) without or with nonoxynol-9 (N-9). The potential advantages over other vaginal products include keeping a low pH, decrease of the irritating effect of N-9 on the cervix or vaginal mucosa associated with greater retention of the product after application, and decreasing "messiness" as compared to other vaginal products. Vulvar irritation was seen in seven of these 10 volunteers. [27]

9. **By Freeze-fracture electron microscopy (FFEM):**

By using Freeze-fracture electron microscopy (FFEM) Boulmedarat was found morphological changes of liposome when dispersed within the hydrogel. A hydrophilic model molecule, inulin, was encapsulated within positively charged and PEG-ylated liposomes and its release was measured in the presence of Me (beta) CD after vesicle dispersion within the bioadhesive Carbopol 974P gel Liposome-Me(beta)CD interactions were investigated by turbidity monitoring during continuous addition of Me(beta)CD to liposomes [28]

Techniques

Three models of real biological application systems are presented in (FIG. 6). , arranged in order of decreasing complexity. Model C represents the typical test performed using a mechanical tack tester and metal plates, and does not incorporate mucous into the system. In Model B, a separate "phase" of intermixed Carbopol and mucus between the mucous layer and the swollen Carbopol phase is formed. Studies performed on this type of system have shown that there is a marked increase in mucous viscosity with carbopol polymers. describes this increase in the mucous viscosity and micro viscosity as well. The bioadhesive tablet (Model A) represents the most complex case of the three [29, 30]

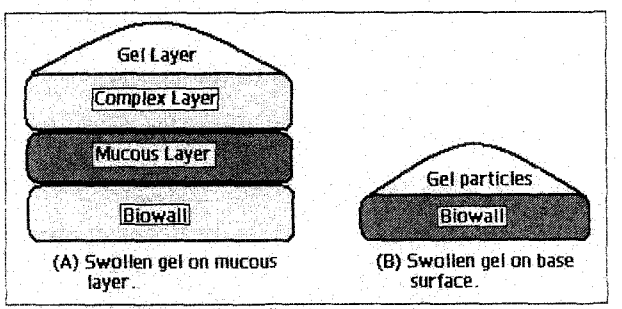

FIGURE 6 : Models for In vivo Bioadhesion.

D'Cruz were describing in vivo evaluation of a gel formulation. Rabbits in groups of four were exposed intra vaginally to a gel with 0.5- 2% drugs or no active drug for 14 consecutive days. The rabbits were euthanized on day 15 and their vaginal tissues were evaluated for histologic evidence of mucosal toxicity and immuno histochemical evidence of cellular inflammation or hyperplasia and plasma samples collected at various timepoints were assayed for drug and its major metabolites, drug can be analysis by high performance liquid chromatography (HPLC) by using of suitable mobile phase.[31]

MRI evaluation : distribution of a vaginal gel describe by Barnhart KT et al 2004 for prevent transmission of HIV. factors affecting coverage have not yet been studied. Ten women were selected for this study by with self-inserted with serial MRI scanning both before and after simulated intercourse. Gel spread was dependent upon time and volume. Simulated intercourse greatly enhances gel spread [32]

TABLE - 1 Models for Evaluation

S. No.	Model	Comment
1.	1	Viscoelastic adhesive / smooth, inflexible, impermeable substrate(s).
2.	2	Viscoelastic adhesive / rough or flexible or permeable or anisotropic substrate or adhesive specific chemical interactions with substrate.
3.	3	Viscoelastic adhesive / rough or flexible or permeable or anisotropic substrate or adhesive specific chemical interactions with substrate; with mass transfer between phases (adhesi substrate).
4.	4	Anisotropic adhesive; rough or flexible or permeable or anisotropic substrate or adhesive has sp chemical interactions with substrate.
5.	5	Anisotropic adhesive which changes with time / rough or flexible or permeable or anisot substrate or adhesive has specific chemical interactions with substrate.
6.	6	Anisotropic adhesive which changes with time / rough or flexible or permeable or anisot substrate or adhesive has specific chemical interactions with substrate; with mass transfer bet phases.
7.	7	Anisotropic adhesive which changes with time / rough and/or flexible and/or permeable a anisotropic substrate, and/or adhesive have specific chemical interactions with substrate; with transfer between phases.
8.	8	Anisotropic adhesive which changes with time / rough and flexible and permeable or anisot substrate and adhesive has specific chemical interactions with substrate; with mass transfer bet phases; substrate also changes with time.

CONCLUSION

Particulated Bioadhesive Vaginal Gel is very safe and efficient drug delivery systems. This DDS can be use for not only local therapy it may very useful in different diseases like anti-hypertensive, anticancer, anti HIV, anti malarial etc. this delivery systems not only for female it can be use full for male also.

ACKNOWLEDGEMENTS

We are giving thanks to Noveon Asia Pacific Limited, Hong Kong for supply so much information about bioadhesive gel. We also give thanks to Dr. H.P.Cheirti, Honorable Director of Himalayan Pharmacy Institute for his so much cooperation with us.

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ISSN: 0974-6943

Arkendu Chatterjee et al. / Journal of Pharmacy Research 2009, 2(4),698-700

Review Article

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An Overview of Intra-Vaginal Drug Delivery System

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Received on:11-12-2008; Accepted on :04-02-2009

ABSTRACT

Vagina is one of the best routes for drugs administration like contraceptive steroids, metronidazole, anti-retroviral. An intra-vaginal controlled-release drug delivery system is an effective means for achieving a continuous delivery of therapeutic agents, not only the systemically active drugs, such as contraceptive steroids, but also the locally active drugs, such as metronidazole and other drugs like zidovudine. This continuous penetration of drugs through the vaginal mucosa can prevent the possibility of hepato-gastrointestinal first-pass metabolism gastric irritation of drugs and fluctuation of dosaging interval. The advantage of intra-vaginal controlled drug administration over conventional/traditional oral administration is the drug absorbed systemically, because due to the presence of dense network of blood vessels in vaginal wall. This intra-vaginal drug delivery reduces the incidence of hepatic first-pass metabolism, and improves the patient compliance.

Key words: vagina,drug delivery, application

INTRODUCTION

Vaginal drug delivery systems are traditionally used to deliver contraceptive and drugs to treat vaginal infection. However, vaginal drug delivery is not limited to these drugs as the vagina has promise as a site to topically deliver drugs which will be absorbed systemically because of the dense network of blood vessels in the vaginal wall.[1] A formulation given by this route as pessaries, vaginal tablets, inserts, cream, powders, douches, gel.[2]Orally inactive progesterone was active when administered intra-vaginally. This route of administration was made practical in 1970 with the development of a medicated, recent vaginal ring, fabricated form a biocompatible silicone elastomer to contain medroxyprogestone acetate (MPA) for intra-vaginal contraception. Still tablets, creams and suppositories are the usual formulations in over-the-counter (OTC) vaginal medications while vaginal rings are the most common long-term drug delivery systems currently used.[3]The concept of controlled-release drug delivery has also been successfully applied to the intra-vaginal administration of a systemic prostaglandin derivative for abortion indication. Intra-vaginal controlled-release drug delivery system is an effective means of continues delivery of therapeutically active agents such as contraceptive steroids and prostaglandins.[4]

ANATOMY AND PHYSIOLOGY OF THE VAGINA:

The vagina is a fibro-muscular tube lined with stratified epithelium, connecting the external and internal organs of reproduction. It runs obliquely upwards and backwards at an angle of about 45° between the bladder in front and rectum and anus behind. In the adult, the anterior wall is about 7.5 cm (3 inches) long and the posterior wall is about 9.0 cm long. The difference is due to the protrusion of the cervix through the anterior wall.

The vagina has an outer covering of areolar tissue a middle layer of smooth muscle and an inner lining of stratified squamous epithelium that forms ridges or rugae. It has no secretory glands, but the surface is kept moist by cervical secretions. Between puberty and the menopause Lactobacillus acidophilus microbes are normally present and they secrete lactic acid, maintained the pH between 4.9 and 3.5. The acidity inhibits the growth of most microbes that may enter the vagina from the perineum.[5]

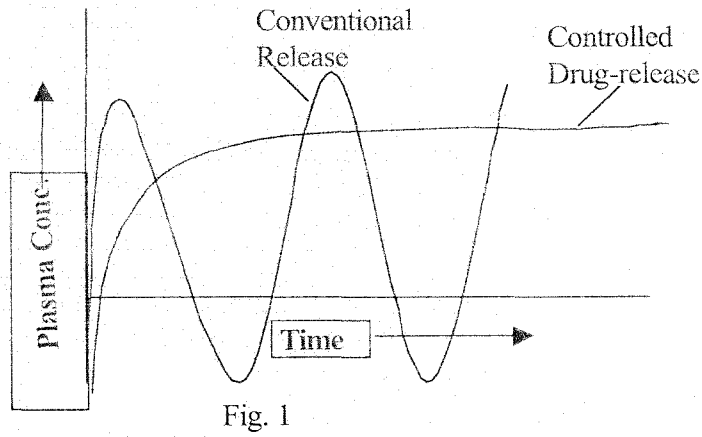

Fig. 1

ABSORPTION OF DRUGS FROM VAGINA:

Absorption of a wide range of drugs from the vagina has been studied. A detailed review describes studies on the vaginal absorption of steroids, prostaglandins, antimicrobials, antiviral, proteins and nonxynol-9.[3]

As with other mucosal drug delivery routes, drug transport across the vaginal membrane may occur by a number of different mechanisms:

a)Diffusion through the cell due to a concentration gradient (trans-

ISSN: 0974-6943

cellular route)
b)Vesicular or receptor-mediated transport mechanism, or
c)Diffusion between cells through the tight junctions (intercellular route).[6]

In some cases, drugs given by the intra-vaginal route have a higher bioavailability compared to the oral route. This is because the drug enters immediately into the systemic circulation without passing the metabolizing liver. The vaginal wall is very well suited for the absorption of drugs for systemic use, since it contains a vast network of blood vessels.[7]

FACTORS INFLUENCING VAGINAL DRUG ABSORPTION:

A good understanding of the various factors that can influence drug absorption from the vaginal cavity is very important in designing both the formulation and the device used for intra-vaginal administration. There are several factors ranging from physiological conditions, physic-chemical properties of the drug, factors related to the administration device. [4,5,8]

1) Physiological Factors:

Factors related to the vaginal physiology include pH of vagina (3.5 to 4.9), effect of the estrus cycle on the permeability of the vaginal mucosa, thickness of vaginal epithelium, vaginal fluid volume, chemical composition of fluid, pH, viscosity and surface tension and the pressure exerted on the dosage form by the rectal wall, play a vital role in vaginal drug absorption and sexual arousal, mucociliary clearance (MCC), vaginal obstruction, etc. which affect either the mucus or ciliary heating and vaginal blood flow.

2) Physicochemical Factors:

Factors related to the dosage forms are physicochemical characteristics of the active ingredients; pH and mucosal irritancy; osmolarity; viscosity (solution, gels) and density (powder, tablet) to the formulation; concentration and volume administration; and type of dosage forms; particle size of the molecule of drug, hydrophilicity or lipophilicity of drug molecule, molecular weight of drug molecule, chemical nature, ionization surface charge, etc.

3) Factors Related to the Administration Device:

It includes the viscosity of the semi-solid dosage forms (creams, gels, etc), size of the solid dosage form (vaginal tablet, etc) adhesive property of the dosage form.

IMPROVEMENT OF VAGINAL ABSORPTION[3]:

The low vaginal absorption can be the result of poor membrane permeability due to molecular size, lack of lipophilicity (e.g. steroidal drugs) fluid volume, estrus cycle and pH of vagina. To overcome this problem, most reported studies have investigated penetration enhancers to facilitate the transport of these molecules and improve the bioavailability. In general, enhancers improve the absorption of these molecules by one or several combined mechanisms:
1)By increasing intracellular transport or use of penetrating agents e.g. PEG.
2)By increasing the contact time between the dosage form and the vaginal membrane by using mucoadhesive polymers e.g. Carbopol 934, 940, 973 and formulation of gel and by increasing viscosity of formulation.
3)By increasing vaginal blood flow, thereby raising the concentration gradient across the vaginal mucosa.
4)By the use of bio-adhesive preparations have been developed as a new type of controlled release form for the treatment of both topical and systemic diseases. The greatest advantage of such dosage forms is the possibility of maintained them in the vagina for extended periods of time including day hours and night, thereby enabling lower dosing frequencies. Among the polymers poly-acrylic acid (PAA) and HPMC (Hydroxypropyl methyl cellulose) are the ideal excipients in bio-adhesive vaginal preparations due to their high bio-adhesive strength.[9]
5)By the use of chelating agents as a penetration enhancers in vaginal formulations. Vaginal administration of the protein leuprolide is much more effective when enhancers, such as carboxylic acids with chelating ability are co-administered.[3]
6)By the use of pro-drugs enhances drug permeability through modification of the hydrophilicity or lipophilicity of the drug. The method includes modification of chemical structure of the drug molecule, thus making it selective, site specific and a safe vaginal drug delivery system.
7)By the use of gel formulation is an extreme case of viscosity enhancement through the use of viscosity enhancers. So the dosing frequency can be decreased to once or twice a day. Example of vaginal gel is metronidazole gel. It is most popular vaginal gel. Mostly hydrogels are used for the intra-vaginal gel drug delivery system.[9]

CLASSIFICATION OF INTRA-VAGINAL DRUG DELIVERY SYSTEM[1,3,8]:

a)Vaginal rings.
b)Vaginal gel and creams.
c)Pessaries, tablets and suppositories.
d)Bio-adhesive micro-particulate drug delivery devices or systems and
e)Others like foams.

IDEALITY OF INTRA-VAGINAL DRUG DELIVERY SYSTEM:

1)Component should melt at vaginal temperature i.e. at 36 °C.
2)Intra-vaginal drug delivery device should be non-toxic and non-irritating.
3)It should not have any meta-stable form.
4)The preparation should have high water number.
5)The preparation should have wetting and emulsifying properties.
6)The preparation should be non-sensitized on vaginal pH (i.e. 3.5-4.9)[5].
7)It should be stable on storage.
8)The preparation should have small interval between melting and solidification point.
9)The preparation should have proper viscosity, so avoid the leakage of preparation from vagina (in case of semisolid dosage form).
10)The preparation should have proper bio-adhesive/mucoadhesive properties, so increase the contact time between the membrane and preparation[9].

ISSN: 0974-6943

ADVANTAGES OF INTRA-VAGINAL DRUG DELIVERY SYSTEM:

This route is the most preferred and targeted goal of new drugs and dosage forms, vaginal administration can be used as an alternative route in certain cases of therapeutic importance:

1)In cases of nausea and vomiting, the act of taking medication orally may induce emesis so that drug is vomiting before it is absorbed.
2)Irritation to the stomach and small intestine associated with certain drugs can be avoided.
3)Hepatic first pass elimination of high clearance drugs may be avoided partially.
4)Contact with digestive fluid is avoided, thereby preventing enzymatic degradation of some drugs.
5)The vaginal bioavailability of smaller drug molecules is good.
6)The bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.
7)Self medication is possible.

LIMITATIONS OF INTRA-VAGINAL DRUG DELIVERY SYSTEM:

1) Some of the drugs are sensitive at the vaginal pH.
2)Local irritation of some drugs.
3)Influence of sexual intercourses.
4)Gender specificity.
5)Personal hygiene.
6)Sometimes leakage of drugs from vagina and wetting of under garments.

APPLICATIONS OF INTRA-VAGINAL DRUG DELIVERY SYSTEM:

1)This route of drug administration is useful for vaginal immunization.
2)Multi-cycle administration of vaginal contraceptive rings.
3)Effective route for the treatment of HIV infection.
4)Effective route for the treatment of local fungal infection.
5)Effective for the delivery of hormones.

CONCLUSION:

The present study gives an overview of anatomy, morphology and physiology of vagina as necessary for an understanding of the function of mucoadhesive system in vaginal delivery of drugs. Absorption of drugs, factors influencing vaginal drug absorption and improvement of vaginal absorption using absorption enhancers is discussed. Vaginal pharmacokinetics and permeation of drug is also discussed. It has been shown that different types of dosage forms are delivery by using this route. Ideality of intra-vaginal drug delivery system is also discussed for particular disease or infection. It has been shown that intra-vaginal drug delivery route has more advantages over disadvantages. This type of drug delivery has more applications now-a-days. In conclusion, the vaginal drug delivery provides an effective route of drug administration with reduced side effects. Better absorption of drug due to the presence of dense blood vessels network, avoidance of first-pass metabolism, a relative permeable, self medication contribute to make the vaginal cavity an attractive for drug delivery. The intra-vaginal controlled-release drug delivery system improves the patient compliance and reduces the dose also.

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Source of support: Nil, Conflict of interest: None Declared

Research Article
ISSN: 0974-6943

Arkendu Chatterjee et al. / Journal of Pharmacy Research 2009, 2(6),1124-1128
Available online through
www.phresearchjournal.info

Drug release efficacy of zidovudine loaded microencapsulated bioadhesive vaginal tablet

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Received on: 28-03-2009; Accepted on: 25-04-2009

ABSTRACT

The objective of the present study was to formulation development, characterize and *in vitro* drug release efficacy of microencapsulated bio adhesive vaginal tablet (MBVT) for treatment of AIDS. The microcapsules were prepared by the solvent evaporation method, were characterized for the percent drug content, entrapment efficiency, micromeritic analysis, FTIR, surface morphology and *in vitro* dissolution studies. The microcapsules were compressed into in tablet by direct compression method, after blending with the bioadhesive polymers and other formulation additives. The MBVTs were evaluated for average weight, hardness, friability, drug content, bioadhesion test, swelling index study of tablet formulation and in-vitro drug release study. The microcapsules were found to be discrete with entrapment efficiency of 67.12-99.1%. FTIR study showed the no chemical interaction between drug and polymer. SEM revealed that the microcapsules were porous in nature. *In vitro* drug release study of microencapsulated tablets revealed, MBVT2 and MBVT4 released the drug only about 13.52% and 19.35% following diffusion controlled Higuchi (r^2=0.98 and 0.88) model respectively even in 10th hr, with a good bioadhesion property, confirmed by *ex-vivo* studies. Thus in conclusion, preparation protocol of MBVT studied may be adopted for a successful development of other anti-retroviral drugs for administration into vagina.

Keywords: Microcapsules, bio adhesive, vaginal tablet.

INTRODUCTION

The women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV reported in 2007 UN AIDS summary. In most regions of the world, HIV is affecting women and girls in increasing numbers. Vaginal drug delivery is very challenging and less explored research area. The vagina is an efficient route for drug administration due to presence of dense blood vessels network and avoids first-pass. Conventional vaginal formulations are associated with disadvantage of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. To circumvent these problems, bioadhesive drug delivery systems VDDS are being propagated. Various peptide protein drugs have also been attempted to administer via bioadhesive microcapsule.[1] A clear rationale exists for providing long-term, controlled release (lacunae of commercially available conventional tablet) of anti-retroviral in order to provide continuous protection against heterosexually transmitted HIV infection and to improve user compliance, even during sexual activity.

MATERIALS AND METHODS

Materials

Zidovudine (AZT), a gift sample from Aurobindo Pharma Ltd, A.P, India and ethyl cellulose (ethoxy content- 47.5% by weight and viscosity (η) of 22 cps in a 5% concentration by a weight in toluene: ethanol 80:20 at 25°C), Carbopol 934, Carbopol 940, methyl cellulose (MC) and hydroxypropylmethylcellulose (HPMC) were obtained from S.D. Fine Chem., Mumbai (India). All other chemicals and reagents used were of analytical grade and used as received.

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Preparation of microcapsules

AZT microcapsules were prepared by the solvent evaporation method.[2] Accurately weighed quantities of polymer were dissolved in acetone (20ml). The drug and polymer (1:1) ratio were dissolved or dispersed in acetone and added to light liquid paraffin with continuous stirring (1000 rpm). Stirring is continuing for 2 hrs. Microcapsules were recovered by treating with petroleum ether, then filtered, dried and kept in desiccators for further evaluations.

Percentage Yield Estimation

The yield was calculated as the weight of the microcapsules recovered from each batch divided by total weight of drug and polymer used to prepare that batch by 100.[3]

Morphological and Size Distribution Characterization

Microcapsules were observed and photographed with scanning electron microscopy (SEM) (LEO, 435 VP, U.K.) and optical microscopy (OLYMPUS BX-50, Japan).[3]

Micromeritic Studies Flow of microcapsules was investigated by determining angle of repose, bulk density, Carr's index and Hausner ratio. The angle of repose was determined by fixed funnel method. The microcapsules were tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 100 taps in a cylinder and the change in volume was measured. Carr's index and Hausner ratio were calculated by the formula: Carr's index (%) = $[(D_f - D_0) \times 100] / D_f$ and Hausner ratio = D_f / D_0, Where, D_f is tapped density; D_0 is poured density. All the experimental units were studied in triplicate (n=3).[4-6]

Drug content and Drug Encapsulation efficiency (DEE) accurately weighed amount of microcapsules 50 mg, were suspended in 50ml of methanol to dissolve the polymer coat. The drug was extracted with 50 ml of methanol in separating funnel and analyzed by using UV-Visible

spectrophotometer (UV-1700, Shimadzu, Japan) 267nm after suitable dilution . The drug entrapment efficiency (DEE) was calculated by the following equation[3]

DEE = (Pc / Tc) X 100 where Pc is practical drug content; Tc is the theoretical drug content. The entire test was performed in triplicate.

***In vitro* drug release studies of microcapsule**

In vitro drug release study was carried out in USP type II dissolution test apparatus using SVF (simulated vaginal fluid) as dissolution medium (900 ml acetate buffer I.P. pH 4.7, at 37±1 °C , Peddle speed was adjusted to 50 rpm). An aliquot sample (5 ml) was withdrawn at intervals of 1 h with replacement by fresh medium and analyzed for Zidovudine content by UV-Visible spectrophotometer at 267 nm. The entire release tests were performed in triplicate.[7,16]

Release Kinetic studies of microcapsule

In order to study the exact mechanism of drug release from the Vaginal Microcapsules, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsmeyer - Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test. [8-10]

Fourier Transformed Infrared spectroscopy (FT-IR) IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and 2mg of pure drug, empty microcapsules and drug loaded microcapsules were selected and measured in the range the spectra were scanned in the wave number range of 4000-600 cm^{-1}.[11]

Preparation of microencapsulated bioadhesive vaginal tablet

Selected batches of Zidovudine microcapsule were incorporated in tablet by direct compression method using various bioadhesive polymers, such as Carbopol 934, Carbopol 940, methyl cellulose (MC), hydroxypropylmethylcellulose (HPMC) along with other formulation additives. The prepared tablets were kept in a decicator for further study.[12-14]

EVALUATION OF MICENCAPSULATED BIOADHESIVE VAGINAL TABLET

Weight variation of tablet

Twenty tablets were randomly selected form each batch and weighed individually. The average weight was calculated. Then the deviation of individual weight from the average weight and the standard deviation were calculated .[4, 14]

Disintegration of tablet

The modified disintegration apparatus(B.P) was designed in a vessel of suitable diameter containing water at 36° to 37° C. The level of the liquid was adjusted by the gradual addition of water at 36° to 37° C until the perforations in the metal disc are just covered by a uniform layer of water. One vaginal tablet on the upper perforated disc was place and the apparatus was covered with a glass to maintain appropriate conditions of humidity. The operation was repeated with two more vaginal tablets.[14,15]

Hardness of tablet

The hardness of the tablet was calculated with the help of a Monsanto hardness tester. Five tablets from each batch of formulations were tested. Then average hardness and standard deviation were calculated.[4]

Friability of tablet

The friability test was done using Roche's Friabilator. Twenty tablets were selected and weighed individually. Then the friability test was carried out at 25 rpm for 4 minutes. These tablets were then again weighed and percentage loss in weight was calculated.[4]

Drug content of the tablet

Tablets of each formulation were ground in a mortar to a powder form. An accurately weighed amount of the powder was transferred to a 100-mL volumetric flask. The powder was dissolved in methanol overnight. After filtration, the solution was assayed spectrophotometrically for Zidovudine at 267 nm against methanol as blank. The content was calculated using a preconstructed calibration curve for the drug.[14]

***In vitro* Dissolution study of tablet**

In vitro drug release study from bioadhesive vaginal tablet was carried out in USP type II dissolution test apparatus using SVF as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout study. Peddle speed was adjusted to 50 rpm. At an interval of 1 hr, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for Zidovudine content by UV-Visible spectrophotometer at 267nm. The entire release tests were performed in triplicate.[14]

Release Kinetic studies of tablet

In order to study the exact mechanism of drug release from the Vaginal Microcapsules, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsmeyer - Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.[12-14]

Bioadhesive strength of tablet

The bio adhesion measurement was performed by using a modified balance method intact with mucosal membrane of goat vagina *in vitro* model. The two pan of physical balance were removed. Right side pan was replaced with a 100 ml beaker and on left side, glass slide was hanged, on which vaginal membrane was attached. For balancing the assembly a weight was hanged on left side. A glass block was kept inside the glass container .Above this glass block a glass slide was placed on which vaginal membrane also attached. The height of this set up was adjusted to leaving a space of about 0.2 cm between two vaginal membrane faces. The set up was balanced by hanging a weight of 20 gm on left side. One tablet was placed between two vaginal membrane faces, little pressure was applied to form bioadhesion bond, then slowly drop of water was added on right side beaker, till the tablet was separated from one side vaginal membrane. Volume of water added was converted to mass .This gave the bioadhesive strength of tablet in gm. An initial investigation examined the reproducibility of the system using 5 same formulations .Then study was carried out for different formulations.[17]

Swelling index of tablet

The weight of microencapsulated tablets was determined (W_1). Each tablet was placed separately in a 25-ml beaker containing 5 ml buffer acetate pH 4.7. The beakers were stored at 25°C and 37°C ± 0.1°C. Tablets were removed at different time intervals (0.25, 0.5, 1, 2, and 4 hours), wiped with filter paper, and reweighed (W_2). The swelling index was calculated as follows.

Swelling index = $(W_2 - W_1)/W_1$ each experiment was performed in triplicate using 400-mg tablets.[14]

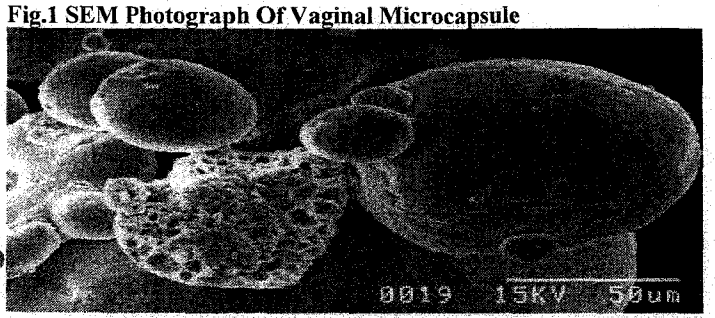
RESULTS AND DISCUSION

Percentage yield of microcapsules

Relatively high 67.2± 1.04 to 98.5±1.73% yield were observed for each formulation presented in column 3 of Table 1. Morphological and Size

Distribution Characterization: The SEM as given in Fig. 1 revealed that all microcapsules thus obtained were opaque, discrete and nearly spherical particles. The particle size of the microcapsules was found to be increased with increase in proportion of coat material as expected.

Fig.1 SEM Photograph Of Vaginal Microcapsule



Flow properties: The flow properties of the microcapsules were shown in column 6,7,8 and 9 of Table 1. As usual, the flow properties increase with polymer ratio. Most of the formulations are having excellent to good flow properties as represented in Table 1.

Drug content and Encapsulation efficiency: Relatively high drug content and encapsulation efficiency were observed for each formulation presented in column 4 and 5 of Table 1. Although there is no significant difference among the encapsulation efficiency of different formulations, except MC7. The DEE was found to be within the range of 67.12-99.1%.The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose, its content and Zidovudine.

FTIR studies: FTIR studies the characteristics of -C-O stretching at around 1095 cm^{-1} and-C=O stretching at around 1658 cm^{-1} was clearly distinguished in all the formulation. Additionally peak at 2087 cm^{-1} due to azide group and 3398 cm^{-1} due to O-H stretching were also observed unchanged in all formulation, suggesting no drug polymer chemical interaction.

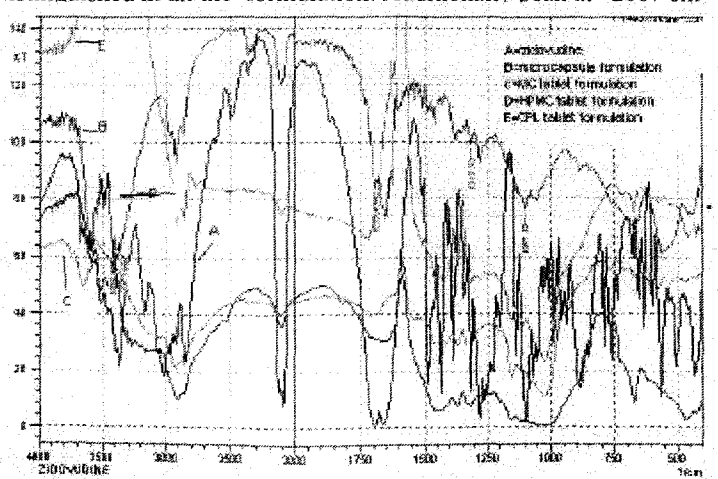


Fig no 2 :- FTIR of microcapsule formulation

In vitro drug release of prepared microcapsules: The *in vitro* drug releases of acquired microcapsules were shown in Fig no 3. In all the cases, the release rate was sustained with increased proportion of polymer. The microcapsule formulation MC_7 was found to release the drug of about 97.73% only, even after 10hrs, thus concluded to have sustained drug release for longer period of time when compared to other microcapsules formulations.

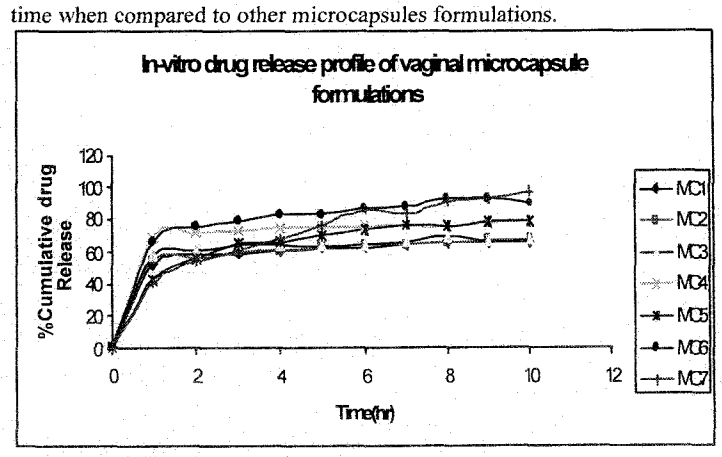


Fig no 3:- *In-vitro* drug release of prepared microcapsules

Table 1: composition, % yield, drug content, and DEE of vaginal microcapsule and flow properties of vaginal microcapsules

Formulation code	Drug/ Polymer ratio	Yield%	Drug content (mg) /50mg	Encapsulation efficiency (%)	Carr's index (X ± S.D.)	Hausner's ratio	Angle of Repose (°)	Comment (U.S.P)
MC1	1:1	98.5±1.73	18.20± 0.18	67.12±0.38	10.12	1.08	24.7°	Excellent
MC2	1:2	86.7±0.85	18.63± 0.05	96.9±0.92	13.38	1.09	23.2°	Excellent
MC3	1:3	86.1± 0.90	12.52± 0.07	79.28±0.54	11.97	1.01	20.1°	Excellent
MC4	1:4	97.6±0.90	10.36± 0.11	99.1± 1.48	09.34	1.26	20.3°	Excellent
MC5	1:5	67.2± 1.04	11.76± 0.18	96.9± 1.01	15.32	1.04	26.4°	Good
MC6	1:6	75.5±1.15	10.37± 0.19	97.4± 1.05	09.19	1.05	27.1°	Good
MC7	1:7	87.6±0.89	15.07± 0.52	80.14± 0.60	08.17	1.01	21.7°	Excellent

Drug release kinetics of microcapsules: The release rate was inversely proportional to polymer amount. The *in vitro* drug release of all the formulations (MC1-MC7) was found constant for each formulation and influenced by the polymer added. The *in vitro* drug release profile was 64.84 - 97.73 %. To categorize the kinetics of drug release from microcapsule, release data was verified with different kinetic models. Indicated that drug releases from all microcapsule formulations obeyed diffusion controlled Higuchi kinetics and release rate was depended on concentration of polymer with process variables. The *in vitro* drug releases of kinetic study of various microcapsule formulation were shown in Table no 2.

Table no:- 2 kinetic study of various microparticulate formulation

Formulation code	Zero order release model	First order release model	Higuchi square root model	Korsmeyer and peppas model	
		regression co-efficient(r^2)		n	
MF1	0.707	0.788	0.955	0.924	0.096
MF2	0.691	0.773	0.946	0.959	0.097
MF3	0.585	0.608	0.885	0.325	0.067
MF4	0.593	0.638	0.915	0.769	0.036
MF5	0.900	0.971	0.998	0.967	0.270
MF6	0.757	0.901	0.968	0.978	0.136
MF7	0.945	0.946	0.994	0.988	0.370

Table 3 Formulation Design Microencapsulated Bioadhesive Vaginal tablet

Formulation	MCS: Polymer	MC (mg)	HPMC (mg)	CPL (mg)	Starch (mg)	Mg.stearate (mg)
MBVT 1	1:1	100	-	-	140	15
MBVT 2	1:1.5	150	-	-	90	15
MBVT 3	1:2	200	-	-	40	15
MBVT 4	1:2.5	240	-	-	-	15
MBVT 5	1:1	-	100	-	140	15
MBVT 6	1:1.5	-	150	-	90	15
MBVT 7	1:2	-	200	-	40	15
MBVT 8	1:2.5	-	240	-	-	15
MBVT 9	1:1	-	-	100	140	15
MBVT 10	1:1.5	-	-	150	90	15
MBVT 11	1:2	-	-	200	40	15
MBVT 12	1:2.5	-	-	240	-	15

MCS-Microcapsule,MC-methyl cellulose ,

HPMC- hydroxypropylmethylcellulose,CPL- Carbopol.

Weight variation test :The total weight of MBVT was 400mg. Weight variation of the formulated tablets (20 in number) was tested in according official monograph in India. Average weight of different formulation was found 399.75±0.177 to 400.15±0.01 mg. Hardness: the formulated tablets (10 in number) of each batch were evaluated using the Monsanto hardness tester. The hardness of different formulation was found 9.25±0.132 to 6±0.2 kg/cm. Friability: was determined according to the prescribe monograph of USA 0.001±0.000252 to 0.0025±0.000557 % friability of different MBVT. Disintegration: Of MBVT was determined according to prescribed monograph of B.P. Disintegration time of different MBVT was found to be 12 - 2.6 hr. Drug content: drug content of microencapsulated tablet formulations. The drug contents of the prepared microencapsulated tablet were found to be in the range of 58.62± 0.021 to 90.40± 0.011 % indicating the applications of the present method for the preparation of novel MBVT.

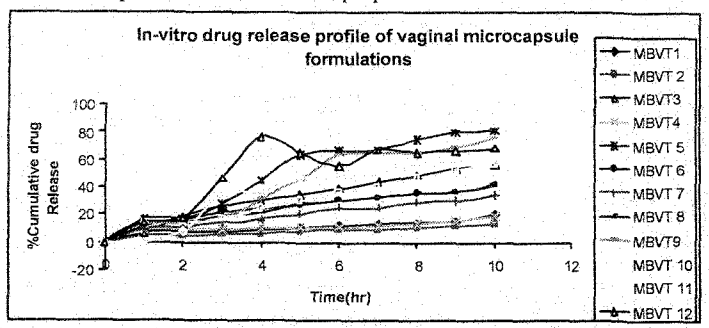


Fig.4 *in vitro* dissolution profile of MBVT1- MBVT12 formulations

In vitro dissolution and release kinetics of MBVT: Although, constant drug release from all the MBVT formulations (MBVT1-MBVT12) was observed after 10 h, the drug release mechanism of all MBVT was found to be predominately diffusion controlled and influenced by the different bioadhesive polymer added. To categorize the complex kinetics of drug release from microencapsulated vaginal tablet containing microcapsules, release data was verified with different kinetic models.Table. 4 indicated that drug release from all formulations obeyed diffusion controlled Higuchi kinetic equation. Except MBVT 3, 5, 9, 10.When treated with Korsmeyer and Peppas equation ($m_t / m_\infty = kt^n$), showed that all formulations released the drug by diffusion following non-Fickian (n>0.5) transport mechanism except the formulation MBVT1 and MBVT4 which follow Fickian (n<0.5) transport mechanism.

Table 4:-*in vitro* drug diffusion kinetic study of microencapsulated vaginal tablet

Formulation	Zero order release	First order release	Higuchi square root equation	Korsmeyerand Peppas equation	
		r^2		r^2	n
MBVT 1	0.935	0.936	0.956	0.899	0.479
MBVT 2	0.965	0.966	0.981	0.928	0.529
MBVT 3	0.992	0.994	0.990	0.953	0.907
MBVT 4	0.814	0.824	0.875	0.717	0.298
MBVT 5	0.972	0.976	0.954	0.923	0.817
MBVT 6	0.975	0.978	0.991	0.991	0.566
MBVT 7	0.980	0.983	0.990	0.965	0.593
MBVT 8	0.981	0.985	0.992	0.974	0.559
MBVT 9	0.955	0.955	0.945	0.908	0.932
MBVT 10	0.936	0.961	0.921	0.879	1.269
MBVT 11	0.983	0.989	0.984	0.900	0.695
MBVT12	0.864	0.760	0.799	0.772	0.733

Swelling index study of MBVT formulation: Swelling index plays an important role in the drug release pattern. The swelling index lied in the range of 0.08 to 5.77 as given in the table no 5. The highest swelling achieved by the microencapsulated bioadhesive vaginal tablet formulation 12 (MBVT12).This data reflect that swelling index was dependent on polymer concentration.

Table no 5 swelling index study

Formulation	Swelling index			
Time (hr)	0.5(hr)	1(hr)	2(hr)	4(hr)
MBVT 1	0.08	0.232	0.530	0.620
MBVT 2	0.182	0.270	0.600	0.710
MBVT 3	0.252	0.355	0.640	0.722
MBVT 4	0.327	0.465	0.755	2.252
MBVT 5	*	*	*	*
MBVT 6	*	*	*	*
MBVT 7	0.102	0.215	*	*
MBVT 8	0.245	0.350	*	*
MBVT 9	*	*	*	*
MBVT 10	*	*	*	*
MBVT 11	1.222	1.512	2.975	4.257
MBVT 12	1.315	1.702	3.300	5.770

Vaginal bioadhesion measurements: vaginal bioadhesive properties of the prepared MBVT (MBVT1-MBVT12) using goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order MBVT11>MBVT10>MBVT9>MBVT12>MBVT4> MBVT3>MBVT2 >MBVT 8> MBVT 1> MBVT7> MBVT6> MBVT5> MBVT12 was concluded that Carbopol 1:2 (MBVT11) Vaginal bioadhesion measurements. The bioadhesive property of CP is reported to be due to carboxyl groups present on its acrylic acid backbone, which possess an ability to interact with sialic acid molecules present in the mucus layer.

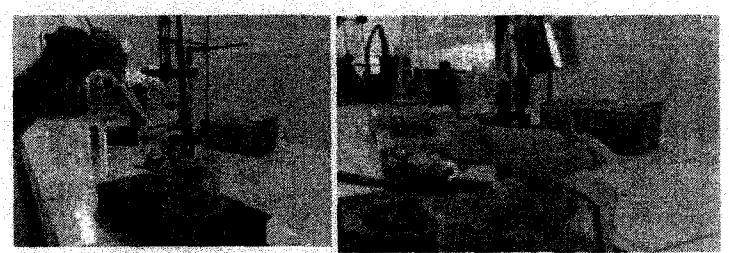


Fig 5.Vaginal bioadhesion measurements

Acknowledgement

The authors greatly acknowledged the APL RESERCH CENTERE, A.P. India., for the gift sample of Zidovudine. The authors gratefully acknowledge the AICTE RPS(C) grants as a part funding to this work. The authors are grateful to the SAIF, (DST) AIIMS, New Delhi and University of Burdwan, West Bengal, India for help in performing the SEM studies.

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Source of support: Nil, Conflict of interest: None Declared

International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 1, July-Sep. 2009

Research article

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF METRONIDAZOLE MICROENCAPSULATED BIOADHESIVE VAGINAL GEL

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Received- 14 March 09, Revised and Accepted- 05 April 09

ABSTRACT

The present study concerned with the development and characterization of Microencapsulated Bioadhesive Vaginal Gel (MBVG). Metronidazole encapsulated microcapsules were prepared by thermal change method using ethyl cellulose as rate controlling polymer in different ratios. The microcapsules were found to be discrete, spherical with free flowing properties and evaluated for particle size analysis, shape (scanning electron microscopy), flow properties, wall thickness, drug encapsulation efficiency, and *in vitro* release performance. The selected microcapsule formulation (MC_3, containing drug: polymer ratio 1:4) was incorporated in gels with a variety of bioadhesive polymers. The MBVGs were evaluated for pH, spreadability, extrudability, viscosity, vaginal irritation test, *in vitro* drug release, drug release kinetics, bioadhesion test, accelerated stability of selected gel formulation. *In vitro* drug release rate for selected MBVG (F5 gel, containing 1 % w/w of drug loaded microcapsules and 0.6 % w/w of carbopol 974) was found to sustain metronidazole over 36 h obeying zero order kinetic with a good bioadhesion quality. The results were compared statistically and found with satisfactory correlation. Thus in conclusion preparation protocol of MBVG studied may be adopted for a successful development of newer drug delivery system of other drugs for administration to vagina.

Keywords: Microencapsulated bioadhesive vaginal gel (MBVG); Microcapsules; Vaginal bio adhesion, Vaginal irritation.

INTRODUCTION

The vaginal route has been traditionally used for the conventional delivery of several locally acting drugs like antimicrobial agents[1]. However conventional vaginal delivery systems such as creams, foams, pessaries and jellies reside at the targeted site for relatively shorter retentivity because of the self cleaning action of the vaginal tract which limits effective drug levels for a shorter period and fluctuation in

drug dose level leads to increased dose frequency of the drug. This ultimately results into patient inconvenience and toxic conditions[2]. The use of prolong-release bioadhesive vaginal gel was thought to offer numerous benefits including prolong residence time of the dosage form at the site of absorption due to bioadhesion to the vaginal mucosa, prolong drug release, improved bioavailability and decreased side effect of drug and ultimately improved patient compliance. Metronidazole was used as a model drug in this study due to its bacteriostatic and bactericidal activity against gram negative bacteria and also effective against various vaginal infections[3]. Another important rationale of using metronidazole, is its unique, low molecular weight offering the greater permeation benefit through vaginal epithelial membrane. Ethyl cellulose was assumed to offer the control release behavior of drug due to its hydrophobic coating over metronidazole[4]. Bioadhesive polymer carbopol presumed to provide better vaginal bioadhesion.[5] Keeping in view of the above uniqueness, the present study was designed to develop a newer Microencapsulated Bioadhesive Vaginal Gel (MBVG) for prolong release of metronidazole to treat vaginal infections with increased patient convenience.

Experimental

Materials and methods

Metronidazole was received as a gift sample from Aristo Pharmaceutical Ltd., Kolkata (India). Ethyl cellulose (BDH, ethoxy content- 47.5% by weight, viscosity (ŋ), and 22 cps) was purchased from S.D. Fine Chem., Mumbai (India). All grades of Carbopol were received as gift sample from Corel Pharma Chem., Ahmedabad (India). All other chemicals and reagents used were of analytical grade and used as received.

Preparation of vaginal microcapsules Metronidazole

Vaginal microparticles were prepared by the solvent evaporation method. Accurately weighed quantity of polymer was dissolved in acetone, Metronidazole was dispersed slowly in polymer solution and this solution was added to heavy liquid paraffin with stirring (800 rpm). Microparticles were recovered by treating with petroleum ether. Then filtered, dried in a desicator. Microcapsules of various drug polymer ratios prepared accordingly for further evaluations. Metronidazole vaginal microcapsules were prepared by thermal change method. Accurately weighed quantity of ethyl cellulose and cyclohexane (50 ml) was heated in water bath. The temperature was gradually raised to 70°C over 20 min under

constant stirring (500 rpm). Metronidazole was dispersed slowly with maintaining temperature at 80°C for 30 min and it was cooled slowly under continuous stirring and temperature was dropped to 5°C in order to hardening of ethyl cellulose coated microcapsules. Then filtered, dried in a desiccator. Microcapsules of various drug polymer ratios prepared accordingly for further evaluations[6, 7].

Morphological and topographical characterization

Microcapsules were observed and photographed with scanning electron microscopy (LEO, 435 VP, U.K.) and optical microscopy (OLYMPUS BX-50, Japan). Their diameters were determined with a pre-calibrated graduated eyepiece of the optical microscope. One hundred measurements were averaged for each microcapsule formulation prepared[8].

Wall thickness of microcapsules

Wall thicknesses of the microcapsules were determined by the method as suggested by Luu et al.[9], using equation, $h = r\,(1\text{-}P)\,d_1 / 3[Pd_2 + (1\text{-}P)\,d_1]$, Where, h is wall thickness; r is mean radius of microcapsules from optical microscopic observations; d_1 is density of the core material; d_2 is density of coat material; P is proportion of medicament in the microcapsules. All the test sample was examined for three times (n=3).

Flow properties

Flowability of microcapsules was investigated by determining angle of repose, bulk density, Carr's index and Hausner ratio[10]. The angle of repose was determined by fixed funnel method. The microcapsules were tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 1000 taps in a cylinder and the change in volume were measured. Carr's index and Hausner ratio were calculated by the formula: Carr's index (%) = $(D_f - D_0) \times 100 / D_f$ and Hausner ratio = D_f / D_0,

Where, D_f is tapped density; D_0 is poured density. All the experimental units were studied in triplicate (n=3).

Drug content and drug encapsulation efficiency (DEE)

Accurately weighed microcapsules equivalent to 50 mg, were suspended in 10ml of diethyl ether to dissolve the polymer coat. The drug was extracted with 50 ml of simulated vaginal fluid (SVF, phosphate buffer I.P., pH 4.9) in separating funnel and analyzed by using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) after suitable dilution at 320 nm. Drug encapsulation efficiency was calculated using the formula[11].

DEE (%) = (Practical drug content/Theoretical drug content) × 100, each sample was analyzed in triplicate (n=3).

***In vitro* drug release studies of microcapsule formulations**

In vitro drug release study was carried out in USP XXI paddle type dissolution test apparatus using SVF as dissolution medium (900 ml phosphate buffer I.P. pH 4.9, at 37±1 °C was adjusted to 100 rpm). An aliquot sample (5 ml) was withdrawn at an interval of 1 h with replacement of fresh medium and analyzed for metronidazole content by UV-Visible spectrophotometer at 320 nm[8]. The same method was adopted for each batch of microcapsules.

Release kinetic studies of microcapsule formulations

In order to study the exact mechanism of drug release from the Vaginal Microcapsules, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer - Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test[12, 13, 14].

***In vitro* polymer degradation**

In vitro degradation study of placebo microcapsules was carried out in the same *in vitro* SVF medium. Accurately weighed 100 mg of microcapsules in 150 ml of the SVF was shaken at 72 rpm and 37.0°C. At pre-set intervals, the vials were centrifuged at 5000 rpm for 20 min. After removing the upper clear solution; the microcapsules were dried under vacuum for 48 h. Then mass loss of the dried microcapsules was determined by digital microbalance[15].

Fourier transformed infrared spectroscopy (FT-IR)

IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu, Japan). The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000-600 cm^{-1}.[8]

Scanning electron microscopy (SEM)

The SEM analysis was carried out using a scanning electron microscope (LEO, 435 VP, U.K.). Prior to examination, samples were mounted on an aluminium stub using a double sided adhesive tape and making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The scanning electron microscope was operated at an acceleration voltage of 05 kV and resolution of 4000[8].

Preparation of microencapsulated vaginal bioadhesive gels

Selected batches of metronidazole microcapsule were incorporated in gels by mechanical stirring method using various grades of bioadhesive polymer[5], such as carbopol 934, 940, 974 and 980 with other formulation additives. For all

batches, the microcapsules were mixed with prepared bioadhesive gels[16]. The prepared gels were packed in wide mouth plastic jars covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in cool place for further study.

Estimation of metronidazole in vaginal gels

Accurately weighed gel (0.5 g) was suspended in 25 ml of SVF. It was filtered after constant stirring and analyzed by using same UV-Visible spectrophotometer after suitable dilution at 320 nm[17].

Drug content uniformity

Initially the formulations were tested for homogeneity by visual inspection. To further ensure the homogeneity of drug content in the formulation of the gel, six tubes were sampled from the different locations in the mixer and assayed for the drug content as stated above. Studies were performed in triplicate for all the formulations[18].

Determination of pH

The pHs of the microencapsulated carbopol gels were determined by digital pH meter (Model MK–VI, Kolkata, India). One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated three times[19].

Determination of spreadability

Spreadability of the formulations was determined by an apparatus suggested by *Mutimer et al.*[20] Each formulation was replicated for three times.

Extrudability study

In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the microencapsulated gel was extrudes until the pressure was dissipated[20, 21].

Viscosity measurement

A Brookfield digital viscometer (Brookfield Engineering Laboratories, Model DV-II, Mumbai) with a suitable sample adaptor was used to measure the viscosities in cps of the micro-encapsulated gel prepared[19].

Vaginal irritation test

The study protocol (Regd. No. HPI / 07 / 60 / IAEC / 0013) was approved by the Institutional Animal Ethics Committee. Microencapsulated gels (0.5 g) were applied in to the vagina of the New Zeeland white rabbits. After 72 hours, the microencapsulated gel was removed and the following characteristics such as sensitization (allergic reaction),

photosensitization, edema and excess redness were observed in test animals and in control by visual inspection[23].

***In vitro* drug diffusion studies of microencapsulated vaginal gels**

In vitro drug release study was carried out in KC-Diffusion cell using SVF as diffusion medium. The processed cellophane membrane was used, simulating the vaginal i*n vivo* condition like vaginal epithelial barrier. The drug content in withdrawn sample was estimated by UV-Visible spectro-photometer at 320 nm[19]. The same method was adopted for each batch of microencapsulated gels.

Release Kinetic studies of micro-encapsulated vaginal gels

In order to study the exact mechanism of drug release from the microencapsulated gels, drug release data was analyzed according to zero order, first order, Higuchi square root and Korsemeyer-Peppas equations. The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test[12, 13, 14].

Vaginal bioadhesion measurements

The bio adhesion measurement was performed by using a modified balance method intact with mucosal membrane of goat vagina *in vitro*[25, 24].

Accelerated stability studies of microencapsulated vaginal gel

Stability studies were performed according to ICH guidelines[21]. The formulations were stored in hot air oven at 37 ± 2°, 45 ± 2° and 60 ± 2° for a period of 12 weeks. The samples were analyzed for drug content every two weeks by UV-Visible spectrophotometer at 320 nm. Stability study was also carried out by measuring the change in pH of gel at regular interval of time.

Statistical Analysis Statistical data analyses were performed using the ANOVA one way at 5 % level of significance $p < 0.05$[22].

Results and discussion

The obtained microcapsules were found to be none aggregated. The generalized microparticulation protocol depends on, choice of ingredient, successful preparation of microcapsules and optimization at every preparative steps. The formulation code and composition of vaginal microcapsules were presented in column 1 and 2 of Table 1.

Morphological and topographical characterization

That all microcapsules thus obtained, were opaque, discrete and spherical particles with smooth surfaces further confirmed by SEM study. The results of all particle size (mean diameter) were

given in column 4 of Table. 2. Particle size distribution of selected microcapsules and the mean particle size for all formulations. The mean diameter of the microcapsules was found to be increased with increase in proportion of coat material as expected.

Wall thickness

The wall thickness of the microcapsules was shown in column 3 of Table. 1. The wall thickness was found to be highest 3.888 ± 0.25 μm for MC_3 in comparison to others. The wall thickness of the microcapsules mainly built up with increase in polymer content.

Flow properties

The flow properties of the microcapsules were shown in column 4, 5 and 6 of Table 1. As usual, the flow properties increase with polymer ratio. Most of the formulations are having excellent (MC_1, MC_2 and MC_3) to good (MC_4 and MC_5) flow properties as represented in column 7 of Table 1.

Drug content and Encapsulation efficiency

Relatively high drug content and encapsulation efficiency were observed for each formulation presented in column 2 and 3 of Table 2. Although there is no significant difference among five different formulation in encapsulation efficiency, the DEE was found to be within the range of 70-80% and highest for MC_5 (lowest polymer content in comparison to others) perhaps. The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose and metronidazole. The encapsulation efficiency was found to be increased with decrease in polymer content.

Table 1 : Composition, flow properties and wall thick ness of vaginal microcapsules.

Formulation code	Drug/ polymer ratio	Wall thickness (μm) (X ± S.D.)	Carr's index	Hausner's ratio	Angle of repose (°) (X ± S.D.)	Comment (U.S.P)
MC1	1:1	2.512 ± 0.21	08.600	1.093	24.8 ± 0.11	Excellent
MC2	1:2	3.567 ± 0.18	10.790	1.107	21.6 ± 0.09	Excellent
MC3	1:4	3.888 ± 0.25	07.525	1.081	20.0 ± 0.12	Excellent
MC4	2:1	1.848 ± 0.14	12.880	1.148	26.4 ± 0.14	Good
MC5	4:1	0.843 ± 0.27	13.630	1.158	27.9 ± 0.25	Good

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.156.

Table 2 : Physical properties and drug release data of Vaginal Microcapsules.

Formulation code	Drug content (mg) (X ± S.D.)	Encapsulation efficiency (%) (X ± S.D.)	Mean diameter (μm) (X ± S.D.)
MC1	19.03 ± 0.92	75.988 ± 0.91	45.950 ± 0.92
MC2	12.04 ± 0.56	69.601 ± 0.65	53.633 ± 1.05
MC3	07.53 ± 0.48	75.250 ± 0.84	68.830 ± 0.98
MC4	23.67 ± 0.83	69.048 ± 0.71	39.933 ± 1.11
MC5	33.98 ± 0.75	80.713 ± 0.95	24.016 ± 1.07
ANOVA			
F	19.716		
df	19		
p	1.27		

Each value represents as mean ± standard deviation. n=3. Standard error mean < 0.641.

***In vitro* drug release of prepared microcapsules**

The *in vitro* drug releases of acquired microcapsules were shown in column 2 of Table. 3 and Fig 1. In all the cases, the release rate was increased with decreased proportion of polymer. The microcapsule formulation MC_3 was found to release the drug only about 59.367 % even after 12hrs, thus concluded to have sustained release of drug for longer period of time when compared to other microcapsules formulations.

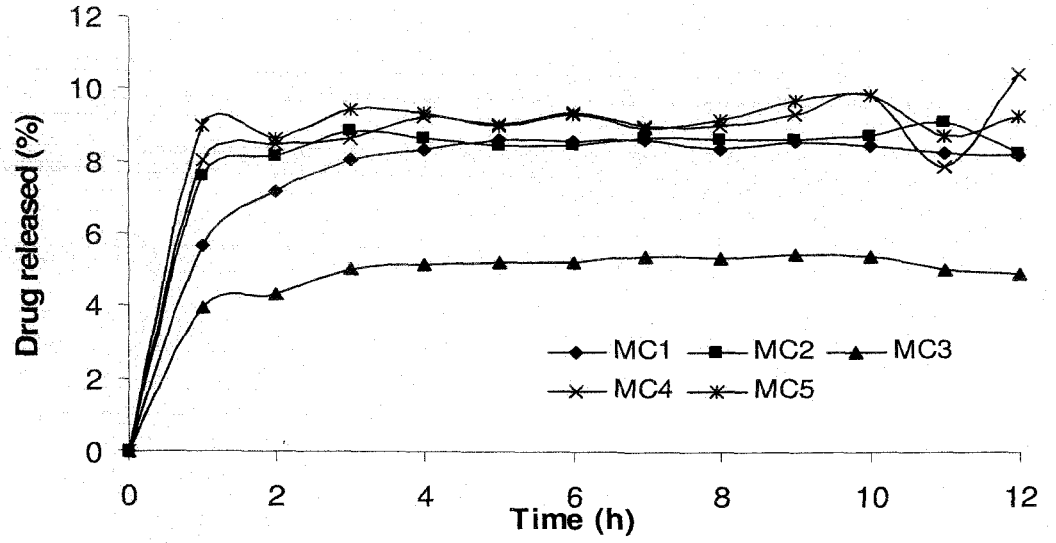

Fig. 1 : It Shows Dissolution profile of various vaginal microcapsule formulations. Each point represents as mean ± S.D., n=3.

Drug release kinetics

The release rate was inversely proportional to wall thickness. The *in vitro* drug release of all the formulations (MC1-MC5) was found constant for each formulation and influenced by the polymer added. The *in vitro* drug release profile was presented in Table. 3, Fig. 1. To categorize the kinetics of drug release from microcapsule, release data was verified with different kinetic models. The column 3,4,5,6 of Table. 3 indicated that drug release from all formulations obeyed Higuchi kinetic equation, have diffusion controlled release rate which is depend on concentration of release regarding polymer with process variable. Column 7 of Table 3 showed that all the formulations released the drug by swelling followed by diffusion as per super Case II (n > 1) transport mechanism the release mechanism was not significantly influenced by formulation variables and was predominately swelling controlled, the drug is dispersed within a glassy polymer. Initially the polymer begin to swell in contact of water, .as the penetrant enters the glassy polymer , the glass transition temperature (T_g ,120-124°C,)[29] of the polymer is lowered and become rubbery show diffusion allowing relaxation of macromolecular chains and drug diffuse out from the swollen rubbery area of polymer wall[30]. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (20.252) at 5 % level of significance ($p < 0.05$).

Table 3 : Drug release and *in vitro* release kinetics data of Vaginal Microcapsule.

Formulation	Cumulative % Drug release (X ± S.D.) (12 h study)	Zero order equation	First order equation	Higuchi Square root eq.	Korsmeyer and Peppas equation	
		Regression co-efficient (r)			(n)	
MC 1	96.456 ±1.16	0.755	0.767	0.988	0.999	1.145
MC 2	101.731 ± 0.98	0.572	0.575	0.919	0.999	1.043
MC 3	59.367 ± 0.83	0.744	0.769	0.982	0.999	1.103
MC 4	104.551 ± 1.13	0.554	0.558	0.875	0.999	1.053
MC 5	100.752 ± 0.75	0.509	0.522	0.870	0.999	1.018
ANOVA						
F	57,95					
df	3					
p	6.12					

n- Diffusion exponent related to mechanism of drug release, according to Korsmeyer and Peppas equation, $\mathbf{m_t / m_\alpha = kt^n}$. Each value represents as mean ± standard deviation, n=3.Standard error mean < 0.756.

Infrared spectroscopy (IR)

The interaction between the drug and the carrier often leads to identifiable changes in the FT-IR profile of solid systems. FT-IR spectra at 45 scans and a resolution of 1 cm^{-1} were recorded in KBr pellets for pure drug (Fig. 2A), polymer (Ethyl cellulose) (Fig. 2B) and the selected (MC_3) microcapsule formulation (Fig. 2C) of 1:4 drug / polymer ratios, as represented in fig. 2. In FT-IR studies, the characteristic C-N stretching at around 1159 cm^{-1} was clearly distinguishable in the selected formulation (MC_3). Additionally characteristics peak of drug C=N stretching vibration at around 1487 cm^{-1}, N=O symmetrical and asymmetrical stretching at around 1369 cm-1 and 1535. cm^{-1} respectively, and characteristics peak of polymer C-O-C asymmetrical and symetrical stretching at around 1269 cm-1 and 1072 cm-1, cyclic alkanes C-H bending at around 1458 cm-1 and cyclic alkanes C-H stretching at around 3099 cm-1 were also observed unchanged in the formulation suggesting no drug polymer chemical interaction. The drug was therefore considered to have been encapsulated in unbound form in microcapsule formulation.

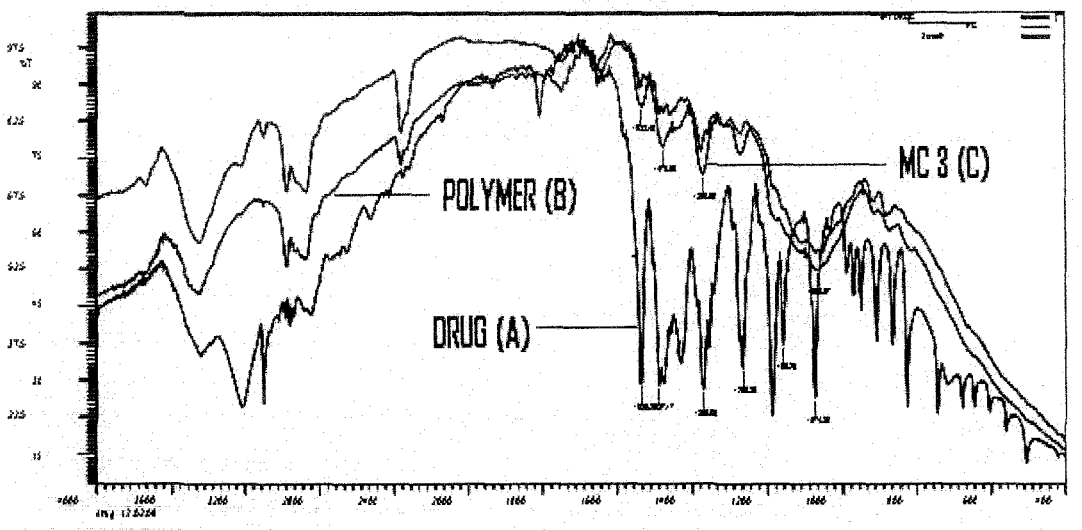

Fig. 2 : It Shows Entire FT-IR spectra and analysis region (In inset) of pure drug (A), ethyl cellulose (B), vaginal microcapsule formulation (C).

Scanning electron microscopy (SEM)

The morphology of the ethyl cellulose metronidazole systems prepared by thermal change method was investigated by SEM analysis (Fig. 3). Microcapsules appear as small spherical particle with smooth surfaces of homogenous morphology and no aggregation was seen.

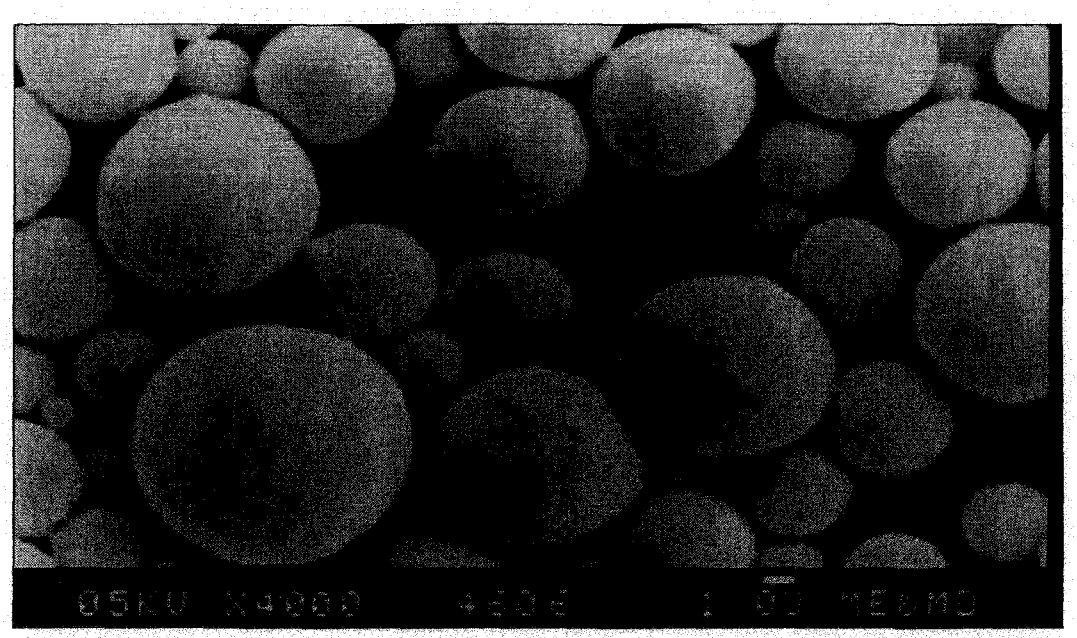

Fig. 3 : It shows scanning electron microscopy photograph of vaginal microcapsule at 05KV × 4000.

Preparation of microencapsulated gel

Selected batches of prepared vaginal microcapsules were then incorporated in gels prepared by mechanical stirring with various grades of bioadhesive[5] polymer, such as carbopol 934 (ŋ 37200 cps), 940(ŋ 49000 cps), 974 (ŋ 35850 cps) and 980(ŋ 47200 cps) and other formulation additives. The experimental design of the formulated gels was expressed in Table. 4.

Table 4. Experimental design of Microencapsulated Bioadhesive Vaginal Gels.

Formul-ation	Microencapsulated Bioadhesive Vaginal Gels compositions Amount taken in percentage (w/w)					
	Micro-capsules	Carbopol	Triethanol-amine	Alcohol	Propylene glycol	Distilled Water
F1	1	0.6	0.5	20	10	q.s.
F2	1	0.8	0.6	20	10	q.s.
F3	1	0.6	0.5	20	10	q.s.
F4	1	0.8	0.6	20	10	q.s.
F5	1	0.6	0.5	20	10	q.s.
F6	1	0.8	0.6	20	10	q.s.
F7	1	0.6	0.5	20	10	q.s.
F8	1	0.8	0.6	20	10	q.s.

F1, F2: Carbopol 934, F3, F4: Carbopol 940, F5, F6: Carbopol 974 and F7, F8: Carbopol 980. q.s. quantity sufficient.

Drug content and uniformity

The column 2 and 3 of Table. 5 showed the drug content and homogeneity of microencapsulated gel formulations. The drug contents of the prepared microencapsulated gels were found to be in the range of 53.433 - 94.188 % indicating the applications of the present method for the preparation of novel semi-solid MBVG system with high drug content uniformity.

pH measurement

The pH of gels as showed in column 4 of Table. 5 were found to be within the range of 6.8 to 7.8 which is within the limit of semisolid specifications. The almost neutral pH reflected, the gel will be non irritant to vagina. This was further confirmed by vaginal irritation study in rabbit.

Spreadability and extrudability

The spreadability plays an important role in patient compliance and helps in uniform application of gel to the skin. A good gel takes less time to spread and will have high spreadability. The spreadabilty of formulated gels was decreased as the concentration of polymer increased. The extrusion of gel from tube is important during application and for the patient compliance. Extrudability of gel formulations with low polymer content was found satisfactory Fig. 8. Dissolution profile of various Microencapsulated Bioadhesive Vaginal Gels.

Each point represents as mean ± S.D., n=3.

while the high polymer content, good extrudability was observed. From the data of spreadability and extrudability as given in column 2 and 3 of Table. 6, among all the formulations, formulation F5 having good spreadability and extrudability and selected.

Table 5. Physical properties of microencapsulated bioadhesive vaginal gels.

Formulation	Drug content (%) (X ± S.D.)	Drug content uniformity	pH (X ± S.D.)
F1	78.72 ± 0.030	**	7.5 ± 0.011
F2	81.93 ± 0.042	***	7.4 ± 0.024
F3	53.33 ± 0.055	**	7.3 ± 0.016
F4	79.74 ± 0.021	***	7.1 ± 0.025
F5	94.52 ± 0.043	***	6.8 ± 0.027
F6	78.67 ± 0.051	**	7.1 ± 0.033
F7	72.98 ± 0.029	**	7.2 ± 0.025
F8	76.40 ± 0.054	*	7.3 ± 0.015

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.317. * (good), ** (very good), ***(excellent).

Viscosity

Viscosity is an important parameter for characterizing the gels as it affects the spreadability, extrudability and release of drug. The column 4 of Table. 6 showed the data of viscosity. The viscosity of gels was increased with the increase in carbopol content which may be due to the increase in formation of three dimensional cross linking structure of gel, as expected.

Vaginal irritation study

The result of vaginal irritation study was shown in column 5 of Table. 6. All formulations were found to be non irritant to vagina of New Zeeland white rabbits.

Table 6 : Rheological properties and vaginal irritation data of microencapsulated bioadhesive vaginal Gels

Formulation	Spreadability (g.cm/sec) (X ± S.D.)	Extrudability	Viscosity (cps) (X $\times 10^4$)	Irritation
F1	046.87 ± 0.098	*	2.015	-
F2	028.85 ± 0.181	**	4.175	-
F3	075.02 ± 0.134	**	1.742	-
F4	057.69 ± 0.174	**	2.397	-
F5	150.01 ± 0.324	***	1.802	-
F6	166.67 ± 0.112	**	1.645	-
F7	187.51 ± 0.315	***	1.555	-
F8	125.12 ± 0.114	**	2.702	-

Each value represents as mean ± standard deviation, n=3. Standard error mean < 0.187.
* (good), ** (very good), *** (excellent) and - (no irritation).

***In vitro* drug diffusion studies and release kinetics**

The release mechanism was not significantly influenced by formulation variables and was predominately diffusion controlled. The release rate was inversely proportional to wall thickness. The *in vitro* drug release of all the formulations (F1-F8) was found constant for each formulation and influenced by the polymer added. The *in vitro* drug release profile was presented in column 3 of Table. 7, Fig. 4 and Fig.10 indicated release from microcapsule retarded by incorporating in gel network. To categorize the kinetics of drug release from microencapsulated gel, release data was verified with different kinetic models. The column 4, 5, 6, 7 of Table. 7 indicated that drug release from all formulations obeyed Higuchi kinetic equation except formulation F1, F4 and F5 which obeyed Korsemeyer and Peppas kinetics. The column 8 of Table 7 showed that all the formulations

released the drug by diffusion following Fickian (n<0.5) transport mechanism except the formulation F2, F3 and F6 which follow non-Fickian (n>0.5) transport mechanism. Statistical verification with one way ANOVA method attested the fact that the drug release data were found significant for F (20.252) at 5 % level of significance ($p < 0.05$).

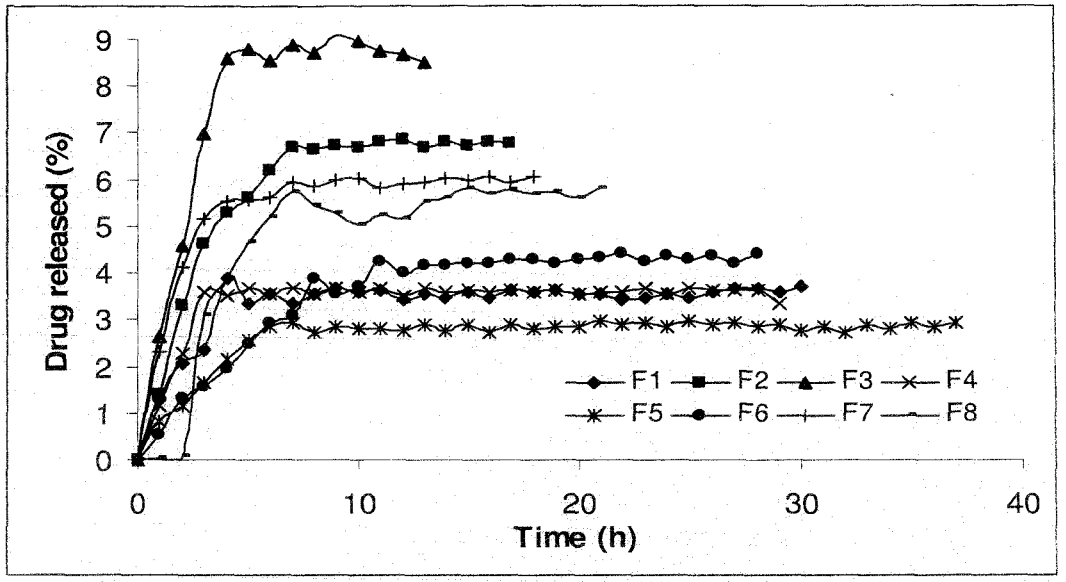

Fig. 4 : It shown dissolution profile of various microencapsulated bioadhesive vaginal gels. Each point represents as mean ± S.D., n=3.

Table 7 : Vaginal bioadhesive strength, drug release and *in vitro* release kinetics data of microencapsulated bioadhesive vaginal gels.

Formulation	Vaginal Bioadhesive Strength (Kg) (X ± S.D.)	Cumulative % Drug release (X ± S.D.) (12 h study)	Zero order equation	First order equation	Higuchi Square root eq.	Korsmeyer and Peppas equation	
			Regression co-efficient				
				(r)		(n)	
F1	0.069 ± 0.011	37.985 ± 1.12	0.560	0.716	0.858	0.883	0.403
F2	0.100 ± 0.010	66.823 ± 1.31	0.784	0.750	0.930	0.911	0.608
F3	0.080 ± 0.015	93.168 ± 0.98	0.751	0.702	0.889	0.848	0.624
F4	0.120 ± 0.013	39.460 ± 1.25	0.486	0.656	0.824	0.846	0.401
F5	0.210 ± 0.014	28.097 ± 0.88	0.581	0.797	0.925	0.942	0.456
F6	0.190 ± 0.016	33.271 ± 1.09	0.819	0.876	0.979	0.971	0.682
F7	0.170 ± 0.020	63.815 ± 1.14	0.676	0.658	0.871	0.818	0.444
F8	0.140 ± 0.009	48.869 ± 1.05	0.760	0.649	0.892	0.791	1.376
ANOVA							
F	78.023						
df	39						
p	6.12						

n- Diffusion exponent related to mechanism of drug release, according to Korsmeyer and Peppas equation, $\mathbf{m_t / m_\alpha = kt^n}$. Each value represents as mean ± standard deviation, n=3.Standard error mean < 0.756.

Vaginal bioadhesion measurements

Figure. 9 and column 2 of Table. 7, indicates the vaginal bioadhesive properties of the prepared gels (F1-F8) in goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order F5>F6>F7>F8>F4>F2>F3>F1. It was concluded that carbopol 974 (F5) showed the highest bioadhesive property.

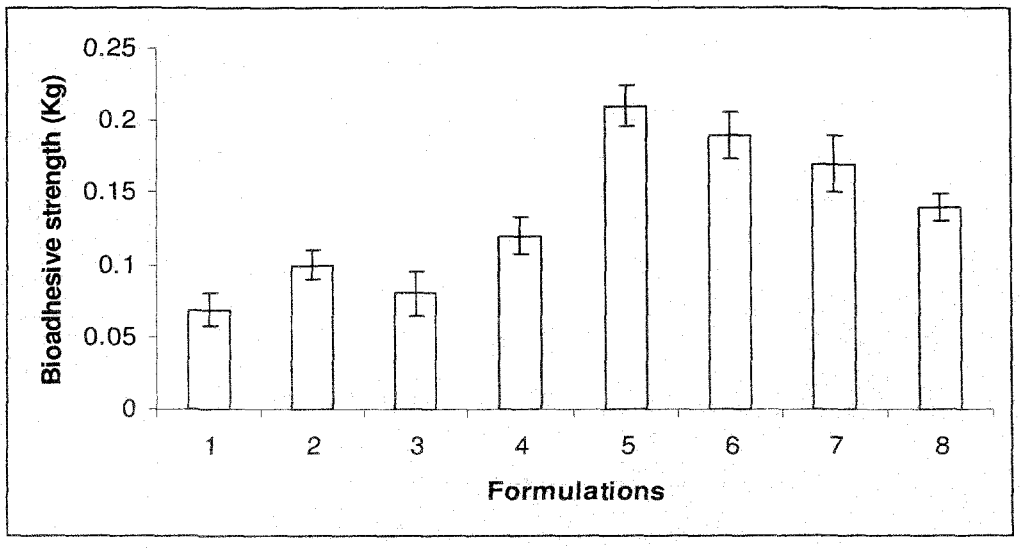

Fig. 5 : It shows vaginal bioadhesion measurement of various microencapsulated bioadhesive vaginal gels. Each point represents as mean ± S.D., (n=3).

Accelerated stability studies of microencapsulated gel

The accelerated stability studies were performed according to ICH guidelines for 12 weeks and the results were found to be stable in varying temperature as shown in Table. 8, which further verified with one way ANOVA method, found to be significant for F (3.395) at 5 % level of significance ($p < 0.05$).

Table 8 : Accelerated stability study of selected microencapsulated bioadhesive vaginal gels

Storage Temp. (°C)	Potency of formulation (%) Period of studies in week						
	1st day	2nd	4th	6th	8th	10th	12th
37 ± 2	99.56	99.31	99.12	99.05	98.87	98.51	98.39
45 ± 2	99.56	99.17	98.94	98.76	98.61	98.29	98.15
60 ± 2	99.56	99.08	98.84	98.54	98.33	98.13	97.98
pH	6.8	6.9	6.7	6.6	6.7	6.8	6.9

CONCLUSION

In conclusion, MC_3 containing drug: polymer ratio 1:4 was found to be the best microcapsule formulation, regarding all the properties evaluated in order to achieve one objective of this study. Formulation MC_3 was selected on basis of its slower release rate, higher entrapment efficiency, excellent flow property and higher wall thickness for its use in next objective.

Another objective was to further incorporation of selected microcapsules in gel by using different carbopol polymers for prolonging the bioadhesion and release of representative drug. The evaluation reports of microencapsulated gel explained F5 gel (containing 1 % w/w of drug loaded microcapsules and 0.6 % w/w of carbopol 974) was found to be the best, releasing about 100 % of metronidazole over a period of 36 hours in SVF successfully. The novel formulation design facilitated the optimization and successful development of MBVG formulations for enhanced vaginal drug delivery by optimum vaginal bioadhesion and longer retention. Our data concluded that MBVG protocol may be an effective strategy for the development of easy, reproducible and cost effective method to prove its potential for safe and effective vaginal delivery therapy. This technique can be further tested for the development of different vaginal carrier therapeutics.

ACKNOWLEDGEMENTS

The authors wish to thank Aristo Pharmaceutical Ltd., Kolkata, for gift sample of Metronidazole. Words insufficient to express our thanks to Corel Pharma, Ahmedabad, for gift sample of polymers. The authors are also indebted to Himalayan Pharmacy Institute, Sikkim, for their precious peripheral support in carrying out this work.

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Research Article
ISSN: 0974-6943

Chatterjee A et al. / Journal of Pharmacy Research 2010, 3(1),183-192

Available online through
www.jpronline.info

Formulation, *In Vitro Evaluation* and Stability of Prolong Release Anti-HIV Bioadhesive Microencapsulated Vaginal Gel

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Received on: 20-09-2009; Revised on: 16-10-2009; Accepted on:19-12-2009

ABSTRACT

Gel as a dosage forms are successfully used as drug delivery systems which considering their ability to prolong the drug release. HIV microbicides are topical, self-administered products aimed to preventing or reducing HIV infection in women and may represent the most promising strategy for combating the HIV/AIDS epidemic at the present time. Drug delivery across vagina, is a challenging task. Keeping this view, a new vaginal anti-HIV microencapsulated vaginal gel (AZMBVG) was engineered to coat vaginal tissue with a stable HIV protective layer. Ideally, drug will be release from this gel layer to improve controlled fashion due to presence of microcapsules and bioadhesive polymers. The whole work was divided between two phases. In first phase, Zidovudine loaded microcapsules (AZMC) were prepared by O/O single emulsion solvent evaporation method. The encapsulation efficiency was observed into the range of 15.37 ± 0.45 to 93.92 ± 4.59 %. The FTIR study showed that no chemical interaction between drug and polymer. SEM study depicted that drug loaded ethyl cellulose AZMC was in micro size. AZMCs wear shown drug release into the range of 63.41± 5.36 to 85.46± 7.14% up to 24 followed by Fickian case I release transport mechanism. To achieve second objective, optimized AZMC4 was incorporated into the gel with various bioadhesive polymers. From *In vitro* drug release profile data, it was evidenced that the formulation AZMBVG4 showing only 63.41± 5.36% drug release up to 28 hrs in constant manner in comparison to other formulations followed by Fickian case I release transport mechanism. It was also shows 64% Swelling index in 6hr study with good extrudability 17.58 ± 0.08 gm /cm^2 and spreadability 13.74 ± 0.10 gm.cm/sec, Bioadhesive strength 1.69 ± 0.02 gm./cm^2 and viscosity 29400cps. Shelf life of AZMBVG4 was found 6.579 and 3.990 years in 25±2°C and 50±2°C temperatures. So AZMBVG was successfully developed and standardized for improved vaginal therapeutics for HIV infected women's.

Keywords: AIDS, Zidovudine, Bioadhesive vaginal gel, Stability

INTRODUCTION

The women (15.4 million) are approximately 50% of people (33.2 million) infected and living with HIV, as reported in 2007 UN AIDS summary[1]. All most regions of the world, HIV is affecting women and girls are increasing numbers. Vaginal drug delivery is a very challenging and less explored research area, that women may control themselves. The vagina is an efficient route for drug administration due to presence of dense blood vessels network and avoids first-pass[2,3]. Conventional vaginal formulations such as tablets, pessaries, suppositories, gels, creams and foams, are associated with disadvantage of low retention to the vaginal epithelium, leakage and messiness thereby causing inconvenience to the user. To circumvent these problems, bioadhesive drug delivery systems are being propagated. Various peptide protein drugs have also been attempted to administer via vaginal drug delivery systems (VDDS)[4].

A clear rationale exists for providing long-term, controlled release (lacunae of commercially available conventional dosage forms) of anti-retroviral for local therapy as well as systemic administration in order to provide continuous protection against heterosexually transmitted HIV infection and to improve user compliance, even during sexual activity[5]. Ideally, anti-HIV AZMC in vaginal gel should disintegrate in the vaginal medium; provide uniform microencapsulated drug-hydrogel coating of vaginal tissue, resulting in intra vaginal biomimetic lubrication during intercourse, and retention of this gel layer before and after intercourse[6]. Most importantly, controlled release of anti-HIV drugs form this microencapsulated bioadhesive vaginal gel (AZMBVG) inactivates the viral load potentially introduced during sexual activity, due to increase in effective micro surface area of the therapeutics. Potentially, they can be further developed to apply vaginally to prevent both male-to-female and female-to-male sexual disease transmission[7,8]. An antiretroviral, zidovudine, a nucleoside reverse transcriptase inhibitor, with short elimination half life of about 1hour, high dose (250mg in every 4 hours while 300mg twice a day, in some cases), low systemic bioavailability (64%) due to rapid hepatic fast-pass metabolism, was chosen as a model drug of choice. Bioavailability is 60-80% at nearly complete absorption, followed by first pass metabolism. Peak plasma concentration occurs at 30 minute. Most of the drug is metabolized to the inactive glucouronide in the liver[9]. Keeping in view of the above uniqueness in mind, the present

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study was designed to develop a newer AZMBVG for prolong release of zidovudine to treat HIV infections with increased patient convenience.

MATERIALS AND METHODS

Materials:

Zidovudine was obtained from Aurobindo Pharma Ltd., A.P, India as a gift sample. Ethyl cellulose (EC) (ethoxy content- 47.5% by weight and viscosity (η) of 22 cps in a 5% concentration by a weight in toluene: ethanol 80:20 at 25°C), Carbopol P940 and Hydroxypropylmethylcellulose (HPMC) (K4M,4000 cps 2% aqueous solution) were purchased from S.D. Fine Chem., Mumbai (India). All other chemicals and reagents used, were of analytical grade and used as received.

Preparation of Zidovudine microcapsules:

Zidovudine AZMCs were prepared by O/O single emulsion solvent evaporation method. Accurately weighed, different quantities of EC were dissolved in 20 ml acetone (HPLC Grade) by using a magnetic stirrer. The drug was mixed with the polymer solution followed by stirring with magnetic stirrer for 15 minutes. The resulting dispersion was then poured into 250 ml beaker containing the mixture of 50 ml light liquid paraffin (as continuous phase), while stirring. A mechanical stirrer with a three bladed paddle was used for stirring (at 1000 rpm) with heating (at 40°C) and was continued for 2 hours, until complete evaporation of acetone. After evaporation of acetone, the AZMCs formed were filtered using filter paper[9,11,12]. The residue was washed for 4-5 times by 10 ml of ether. AZMCs were dried at room temperature for 24 hours and kept in desiccators for further evaluations. All batches were prepared in triplicate as shown in table no.1.

Percentage Yield Estimation:

The yield [10] was calculated as

% yield= (Weight of the AZMCs recovered from each batch / Total weight of drug and polymer used to prepare that batch) x100

Morphological Characterization:

Microcapsules were analyzed in Scanning Electron Microscopy (LEO, 435 VP, U.K.) to reveal the surface morphology of the AZMC[10,7]. The AZMCs were placed on double-sided tape attached onto graphite surface. The samples were coated with gold using an ion sputter. Coating was provided at 20 mA for 4 min. Observation was performed at 15 kV and ~ X600 and X 1500 magnification.

Fourier Transformed Infrared spectroscopy (FT-IR):

IR spectroscopy was performed on Fourier transformed infrared spectrophotometer (840, Shimadzu,Japan).The pellets of drug and potassium bromide were prepared by[13,14]compressing the powders at 20 psi for 10 min on KBr-press and the spectra were scanned in the wave number range of 4000-600 cm^{-1}

Micromeritic Studies:

Flow properties of AZMCs were investigated by determining angle of repose, bulk density, Carr's index and Hausner ratio. The angle of repose was determined by fixed funnel method [15,16]. The AZMC were placed in a measuring cylinder and tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 100 taps and the change in volume was measured. Carr's index and Hausner ratio were calculated by the formula

Carr's index (%)=$(D_f - D_0) / D_f$ x100 and Hausner ratio = D_f / D_0, Where, D_f is tapped density; D_0 is poured density. All the experimental units were studied in triplicate (n=3).

Drug content and Drug Encapsulation Efficiency (DEE):

About 50 mg of accurately weighed AZMCs were suspended in 2 ml of methanol to dissolve the polymer matrix and then its volume was made up to 50 ml in volumetric flask with acetate buffer pH 4.7, I.P. (Simulated vaginal fluid, SVF)[17]. The resulting mixture was agitated on a mechanical shaker and then kept aside for 1 hour. The solution was then filtered and the absorbance was measured at 267 nm after suitable dilution using UV-Visible spectrophotometer (UV1700, Shimadzu, Japan) and drug content was determined by using the following formula.

DrugContent = (Concentration x Dilution Factor x Volume taken) / Conversion Factor

The drug entrapment efficiency (DEE) was calculated by the equation [10,17], DEE = (Pc/ Tc)x100 , where Pc is practical drug content; Tc is the theoretical drug content. The entire test was performed in triplicate.

In vitro Dissolution study of AZMC:

In vitro drug release study of AZMCs were carried out in USP Type II dissolution test apparatus (TDT-08L USP, Electrolab, Kolkata, India) using SVF as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout study. Paddle speed was adjusted to 50 rpm. At an interval of 1 hr, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for Zidovudine content by UV-Visible spectrophotometer at 267nm. The entire release tests were performed in triplicate[18].

Drug release kinetic studies of AZMCs and AZMBVGs:

In order to investigate the mechanism of zidovudine release from different AZMC and AZMBVG, the release data was analyzed with the following mathematical model, zero order kinetic equation ($Q_t=K_0t$), first order kinetic equation ($\ln Q_t = \ln Q_0 - K_1t$) and Higuchi kinetic equation ($Q_t=K_Ht^{1/2}$), where Qt is the percent of drug release at time t, Q_0 is the initial amount of drug present in the AZMCs and AZMBVGs. K_0, K_1 and K_H are the constants of the equations. Further to confirm the mechanism of drug release, the first 60% of drug release was fitted in Korsmeyer Peppa's model, $M_t/M_a= K_Pt^n$ where M_t/M_a is the fraction of drug release at time t and K_P is the power law constant and n is the release exponent. The n value is used to characterize different release mechanisms and was calculated from the slope

of the plot of log of M_t/M_a vs. log of time (t). The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test [18,19].

Stability studies of AZMC4:

Stability studies of AZMC4 was done according to the International Conference on Harmonization (ICH) harmonized [21] guidelines on stability testing of new drug substance and product.Stability studies were conducted to find out stable product under storage. The AZMCs were stored in amber colored glass bottles at elevated temperature i.e. 4 ± 1°C (FT), 25 ± 1 °C (RT) and 50 ± 1 °C (HT) for a per iod of two month and observed for change in drug content.

PREPARATION OF AZMBVG:

Carbopol 940P gels were prepared by cold mechanical method[22,23] described by *Schmolka et al.* Required quantity of polymer (Carbopol 940P and HPMC) was weighed and it was sprinkled slowly on surface of purified water for 2 hrs. After which it was continuously stirred by mechanical stirrer, till the polymer soaked in the water. With continuous stirring, triethanolamine was added for the maintained the pH of the gel. Appropriate quantity of DMSO (Dimethyl sulfoxide) was added for penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Finally the optimized microencapsulated formulation was added to the gel with continuous stirring till the microparticles get dispersed in gel completely. Eight formulations of microencapsulated intra-vaginal gel were prepared by using Carbopol 940P and Hydroxyethyl cellulose in different ratio (table 8). The prepared gel were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place.[24,25] The formulations were preserved for further study.

EVALUATION OF AZMBVG:

Percent yield of AZMBVG:

The Percent yield was calculated [4] as the weight of the AZMBVGs recovered from each batch divided by total weight of drug containing microparticles and other all ingredients used to prepare AZMBVGs multiplied by 100. The percentage yield of each formulation was replicated three times. The yield of AZMBVGs was calculated using the following formula:

$Y = \{ P_m (Z_G) \} / T_m [P+M+I_g] \times 100$ Where as Y=Yield, P_m=Practical mass, Z_G =Microencapsulated vaginal Gel, T_m =Theoretical mass , P =Polymer, M =Microparticles, I_g= Ingredients

Drug content evaluation:

Drug content was determined by [26] dissolving accurately weighed quantity of gels in SVF. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 267 nm. Drug content was determined using slope of standard curve; previously given. The drug content was determined by using following equation:

$D_C = (C_C \times D_F \times V) / C_F$ Where as D_C =Drug Content, C_C = Concentration, D_F= Dilution Factor, V =Volume taken, C_F=Conversion Factor

Bioadhesive strength of AZMBVGs using isolated goat vagina:

Isolated goat vaginal tissue (*Capra hircus*, local breed, obtained immediately after sacrifice of animals at a slaughterhouse) was cleaned, separated from the supporting muscular and connective tissues taking care to maintain integrity of mucosa, and kept at 0°C till further use. Before experiments, goat vaginal tissue was thawed in normal saline. The bioadhesion measurement was performed by using a modified balance method intact with freshly excised goat vaginal mucosal membrane as an *in vitro* model[27]. The two pans of physical balance were removed. Right side pan was replaced with a 100 ml beaker and on left side, a glass slide was hanged. For balancing the assembly a weight of 20g was hanged on left side. Another glass slide was placed below the hanged slide. Portions of vaginal membranes were attached with both slides. The height of this set up was so adjusted, leaving a space of about 0.2 cm between two vaginal membrane faces. One gm of gel was placed between two vaginal membrane faces. Little pressure was applied to form bio adhesion bond, and then slowly drop of water was added on right side beaker, till the gel was separated from one face of vaginal membranes attached. Volume of water added was converted to mass .This gave the bioadhesive strength of gel in gm. An initial investigation examined the reproducibility of the system using five same formulations. Then the study was carried out for all formulations [28].

pH and color evaluation of microencapsulated vaginal gel:

2.5 gm of gel was accurately weighed and dissolved in 25 ml of distilled water and stored at 4°C for two hours. The pH of dispersions was determined using digital pH meter (Digital pH meter MK VI, Systronics, Naroda, Ahemdabad). The measurement of pH of each formulation was in triplicate and the average values are presented. The color of gel is also visualize by naked eyes.[26]

Swelling index:

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared microencapsulated vaginal gel, 1 gm of gel was taken on porous aluminum foil, and then placed separately in a 50 ml beaker containing 10 ml SVF. The beakers were removed at different time intervals, put it on dry place for some time and then reweighed. Swelling index was calculated as follows[37]
Swelling Index (S_W) % = $[(W_t - W_o) / W_o] \times 100$. Where, (S_W) % = Equilibrium percent swelling, W_t = Weight of swollen gel after time t, W_o = Original weight of gel at zero time.

Spreadability of AZMBVGs:

Spreadability was determined[29] by apparatus suggested by *Mutimer et al* which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of gels. A ground glass

slide was fixed on this block. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability [29,30].

Spreadability was then calculated using the following formula:

S= (Mg x Ls)/ Tm. Where as, S = is the spreadability, Mg = is the weight in the pan (tied to the upper slide), Ls = is the length moved by the glass slide and Tm = represents the time taken to separate the slide completely from each other.

Extrudability of AZMBVGs:

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination[32]of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by *wood et al.* In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented.[36] The extrudability was than calculated by using the following formula[31]:

$E_p = G_m / A$, Where as E_p =Extrudability, G_m = Applied weight to extrude gel from tube (in gm) / A =Area (in cm^2)

***In vitro* drug diffusion study of AZMBVGs:**

In vitro drug diffusion studies were carried out by using biochambered donor receiver compartment model (K.C.diffusion cell).Cellophane membrane was stored in SVF the cellophane membrane act like a barrier between the gel and SVF (sink phase).1gm of gel was place on the surface of processed cellophane membrane and membrane was fixed to one end of the cylindrical donor compartment by adhesive tape such that the lower end of tube containing film just touched the surface of SVF medium. SVF volume 0.5 ml of SVF was placed and maintained at same level throughout the study in donor compartment. Temperature was maintained at 37 ± 2°C with constant stirring at 50 ± 10 rpm. A quantity of 5 ml sample was withdrawn from the receptor compartment at definite time interval and replaced with 5 ml of SVF to maintain sink condition. The drug was estimated by using schimadzu UV-Visible spectrophotometer at 267 nm (λmax).[32,33]

Stability studies of gel:

Stability studies of gel was done according to the International Conference on Harmonization (ICH) harmonized guidelines on stability testing of new drug substance and product.[30,34] The formulated gel were filled in the sterile lacquered collapsible aluminum tubes and stored at different temperature condition viz. 25 ± 2°C (refrigerator temperature) and 40 ± 2°C (condition of accelerated stability testing)[34,35,36] for a period of three months and studied for changes in color , pH, extrudability and variation of drug content.

Viscosity measurement

A Brookfield digital viscometer (Brookfield Engineering Laboratories, Model DV-E, Mumbai) with a suitable sample adaptor and Spindle (S64) was used to measure the viscosities in cps of the microencapsulated gel. [38]

Statistical analyses

Statistical data analyses were performed by using Mystat statistical software. One way ANOVA was performed and was considered significant ($p < 0.05$) at 5 % level.

RESULT AND DISCUSSION

Formulation design of microcapsules:

The microcapsules were prepared by O/O single emulsion solvent evaporation method using ethyl cellulose as polymer in different ratio with other ingredients and solvents as given in Table1. All the prepared microencapsulated formulations contains different drug: polymer ratio and coded as AZMC1 (1:1), AZMC2 (1:2), AZMC3 (1:3), AZMC4 (1:4), AZMC5 (1:5),AZMC6 (1:6) and AZMC7 (1:7).

Percent yield, drug content, percent drug entrapment efficiency and average particles diameter of AZMCs:

The percent yields of microparticles were calculated and found to be into the range of 65.16 ± 5.89 to 83.16 ± column 2 in Table2. Drug content for all microencapsulated formulations was observed good.The observations given in the column 3 in Table 2. The drug content of all microcapsules was obtained between the ranges of 9.13 ± 0.62 to 36.97 ± 5.35 and the highest for AZMC 3 and lowest for AZMC 7. All the microcapsules formulation with high percent entrapment efficacy are observed (three times for each batch) for the loading efficiency. The observed data given column 4 in Table 2, the percent drug entrapment efficiency of all microcapsules were observed between the 15.37 ± 0.45 to 93.92 ± 4.59 and the highest for AZMC 3 and lowest for AZMC 1. The particle size analysis of microparticles was studies by using optical microscopy and SEM with magnification power 600X and 1500X. The average diameter of all different microencapsulated formulations has shown column 5 in Table2 Polymer concentrations have a positive effect on mean particle size.

Micromeritic Studies:

The flow properties of the AZMCs were shown in column 2-5of table 3.Form the Carr's Index, most of the AZMCs were having excellent to good flow properties as represented in column 5 of table 3.Form the Hausner's ratio, all AZMCs were found to have good flow properties as represented in column 4 and column 5 of table 3. From the angle of repose data as in column 5 of table 3 all AZMCs possessed the free

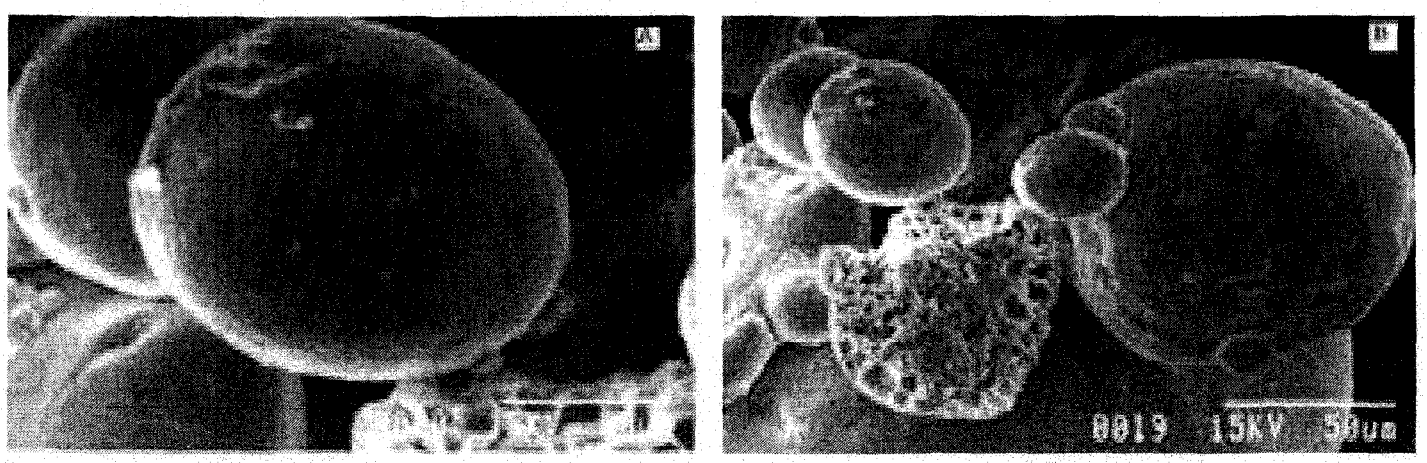

Figure 1: SEM study of AZMCs using magnification X 600 Scanning electron microscopy of AZMC4 using magnification 1500

Table 1 Formulation design of AZMCs

Formulation Code	Drug/polymer ratio	Drug (in mg)	Polymer (in mg)	Span 60 (in mg)	Light liquid paraffin (ml)
AZMC 1	1:1	100	100	10	50
AZMC 2	1:2	100	200	10	50
AZMC 3	1:3	100	300	10	50
AZMC 4	1:4	100	400	10	50
AZMC 5	1:5	100	500	10	50
AZMC 6	1:6	100	600	10	50
AZMC 7	1:7	100	700	10	50

flowing properties. As a general guide, values of Hausner ratio 1.25 indicate good flow (=20.0% Carr's index), while greater than 1.25 indicates poor flow (=33.0 % Carr's index). Between 1.25 and 1.5, added glidant normally improves flow. While for angle of repose = 30° usually indicating the free flowing material and angle =40° suggested the poorly flowing material. Powder with angle of repose >50° have unsatisfactory flow properties, where as minimum angle close to 25° corresponds to very good flow property. AZMC4 has the good angle of repose, Carr's index and Hausner ratio values and having the good flow properties.

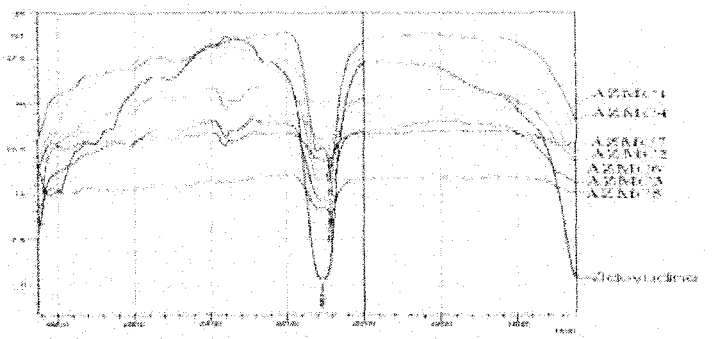

Figure 2: Combined FTIR graph of Zidovudine and all formulations

Table 2: Percent yield of AZMCs formulation:

Formulation code	Percent yield (mean ± sem)	Drug content (mg/100mg ofmicrocapsules)	Percent drug entrapment efficiency (Mean ± SEM)	Average particles diameter (µm) (Mean ± SEM)
AZMC 1	76.33 ± 1.45	10.07 ± 0.23	15.37 ± 0.45	84.50 ± 2.00
AZMC 2	70.99 ± 8.18	23.96 ± 4.81	50.62 ± 9.77	87.80 ± 5.71
AZMC 3	65.16 ± 5.89	36.97 ± 5.35	93.92 ± 4.59	94.64 ± 3.21
AZMC 4	72.93 ± 0.73	24.58 ± 0.87	89.75 ± 3.52	84.33 ± 4.35
AZMC 5	83.15 ± 6.46	16.99 ± 1.42	83.60 ± 4.63	92.34 ± 4.18
AZMC 6	74.33 ± 6.98	14.44 ± 1.24	67.32 ± 8.86	84.49 ± 2.03
AZMC 7	68.31 ± 5.54	9.132± 0.62	49.85 ± 5.28	70.12 ± 9.17

All values are expressed in mean ± standard deviation (n=3)

Table 3: Observation table for bulk density, tapped density, Carr's index, Hausner ratio and angle of repose

Formula Code	Bulk Density	Tapped Density	Carr's Index	Hausner Ratio	Angle of Repose
AZMC 1	0.31 ± 0.001	0.37 ± 0.001	17.27 ± 0.496	1.21 ± 0.007	28.38 ± 0.118
AZMC 2	0.31 ± 0.002	0.37 ± 0.001	16.99 ± 0.258	1.21 ± 0.004	28.673 ± 0.526
AZMC 3	0.31 ± 0.002	0.37 ± 0.000	17.30 ± 0.468	1.21 ± 0.007	28.676 ± 0.366
AZMC 4	0.31 ± 0.002	0.36 ± 0.002	14.24 ± 0.080	1.17 ± 0.001	28.15 ± 0.000
AZMC 5	0.31 ± 0.002	0.36 ± 0.002	16.28 ± 0.936	1.19 ± 0.001	29.084 ± 0.479
AZMC 6	0.31 ± 0.001	0.36 ± 0.004	16.27 ± 0.990	1.19 ± 0.014	29.372 ± 0.63
AZMC 7	0.31 ± 0.001	0.37 ± 0.000	17.57 ± 0.270	1.21 ± 0.004	29.89 ± 0.172

All values are expressed in mean ± standard deviation (n=3)

FTIR studies:

In FT-IR studies, -C-O stretching at around for Carbohydrates groups 1066.67 cm^{-1}, O-H deformation (for 1°alcohol) at around 1095.60 cm^{-1}, C-N stretching at around for 3°amine 1278.85 cm^{-1},C=O stretching at around for six member ketones 1697.41 $cm^{-1}$and $-N_3$ (for azide group) stretching at around 2085.12 cm^{-1} was clearly distinguished in all the drug AZMCs formulations. All the IR peaks present in different formulations were matched with the drug peaks, were observed suggesting no drug-polymer chemical interaction figure 2 and table 4.

Table 4: Peaks table for Zidovudine and AZMCs formulations

Formulation Code	C-O str (for Carbo hydrates) cm-1	O-H def (for1° alcohol) cm-1	C-N str (for 3°amine) (cm-1)	C=O str (for six membered ketones) (cm-1)	-N3 (for azide group)(cm-1)
Zidovudine	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC1	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC2	1066.67	1097.53	1278.85	1697.41	2087.05
AZMC3	1066.67	1097.53	1278.85	1697.41	2088.98
AZMC4	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC5	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC6	1066.67	1097.53	1278.85	1697.41	2087.05
AZMC7	1066.67	1097.53	1278.85	1697.41	2087.05

Table 5: Drug release and kinetic study of AZMCs

Formulations	%CR (24 hrs)	Zero order release		First order release		Higuchi square root equation		Korsmeyer and Peppas equation	
	%CR	K_0	r^2	K_1	r^2	K_H	r^2	r^2	n
AZMC1	84.42±5.77	3.13	0.81	1.93	0.78	0.18	0.91	0.82	0.349
AZMC2	76.81±7.37	2.79	0.74	1.83	0.87	0.14	0.89	0.85	0.213
AZMC3	64.18±1.54	2.79	0.65	1.88	0.71	0.24	0.91	0.98	0.564
AZMC4	63.41±5.36	3.96	0.97	1.91	0.86	0.18	0.90	0.97	0.432
AZMC5	64.24±9.82	1.89	0.84	1.88	0.84	0.12	0.92	0.96	0.264
AZMC6	74.85±7.05	0.84	0.77	1.62	0.82	0.03	0.90	0.89	0.071
AZMC7	85.46±7.14	3.27	0.88	1.91	0.94	0.16	0.94	0.99	0.394

All values are expressed in mean ± standard deviation (n=3)

***In vitro* dissolution and drug release kinetics of AZMCs:**

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in Higuchi, first order and followed by zero-order equations as given figure 3 and table 5 between the ranges of 74.85±7.05 to 85.46±7.14 up to 24 hrs. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the Higuchi square root equation rather than first-order and zero-order models. The drug release was proportional to square root of time, indicating that the drug release from ethyl cellulose AZMCs was diffusion controlled. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of AZMCs of different drug to polymer ratio was ranged of 0.071 to 0.564, indicating that the mechanism of the drug release was diffusion controlled. The release also showed higher correlation with the Korsmeyer- Peppas model, as shown in Table 5. In conclusion, the attempt to prepare controlled release AZMC4 of zidovudine with high entrapment efficiency was successful, even though the entrapment efficiency was still lower compared to the same process reported for other hydrophilic drugs. Further studies are required to find out the exact cause for the difference and to improve the entrapment efficiency. The overall effect of polymer on release (AZMC1-AZMC7) was also significantly different ($P<0.05$, single factor ANOVA). It means null hypothesis is nullified and alternative hypothesis is accepted i.e. the variation in formulations in polymeric type and content (AZMC1-AZMC7) have significant effect on release profile.

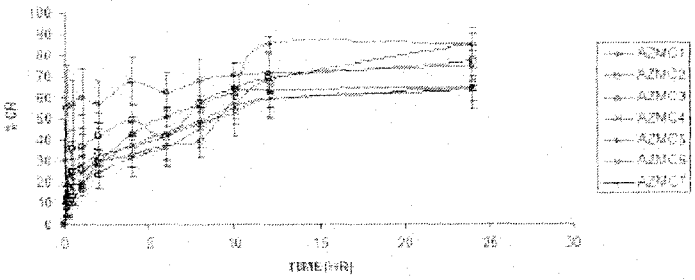

Figure 3: *In-vitro* drug release profile of AZMCs formulations

Accelerated stability studies of AZMC4:

Table6 shows the concentration, potency and log percent concentration of AZMCs for 60 days stability study, and table7 shows the parameters determined for the stability of AZMC4. Shelf life in year in different temperatures like 4± 1°C, 25± 1°C and 50± 1°C result were found 1.468 years and 2.207 years respectively. Degradation half life also calculated in year of AZMC4 in different temperatures like 4± 1°C, 25± 1°C and 50± 1°C results were found 9.608 years, and 14.438 years for respective temperatures.

Table 6: Stability studies (concentration, potency and log % concentration) of AZMC4

Temperatures Time(in Days)	4± 1°C Conc. (in mg /100mg)	Poten-cy (%)	Log % Conc.	25± 1°C Conc. (in mg/ 100mg)	Poten-cy (%)	Log % Conc.	50± 1°C Conc. (in mg/ 100mg)	Poten-cy (%)	Log % Conc.
0	33.99	100.00	2.000	33.99	100.00	2.000	33.99	100.00	2.000
7	33.86	99.63	1.998	33.99	100.00	2.000	33.86	99.63	1.998
14	33.86	99.63	1.998	33.99	100.00	2.000	33.86	99.63	1.998
21	33.86	99.63	1.998	33.99	100.00	2.000	33.99	100.00	2.000
30	33.99	100.00	2.000	33.99	100.00	2.000	33.99	100.00	2.000
38	33.73	99.23	1.997	33.86	99.63	1.998	33.86	99.63	1.998
45	33.86	99.63	1.998	33.86	99.63	1.998	33.86	99.63	1.998
52	33.59	98.84	1.995	33.86	99.63	1.998	33.86	99.63	1.998
60	33.59	98.84	1.995	33.73	99.23	1.997	33.73	99.23	1.997

Table 7: Observation table of parameters determined for stability studies of AZMC4

Parameters	Temp. 4± 1°C	Temp. 25± 1°C	Temp. 50± 1°C
Zero – order (r^2)	0.554	0.776	0.210
First – order (r^2)	0.594	0.783	0.382
First order rate constant (k1) in day-1	1.976 × 10-4	1.315 × 10-4	1.315 × 10-4
Degradation Half life (in year)	9.608	14.438	14.438
Shelf life (in year)	1.468	2.207	2.207

Table 8: Formulation design for the preparation of microencapsulated vaginal gel:

Ingredients	AZMBVG 1	AZMBVG 2	AZMBVG 3	AZMBVG 4	AZMBVG 5	AZMBVG 6	AZMBVG 7	AZMBVG 8
Microparticles(AZMC4) (mg)	407.00	407.00	407.00	407.00	407.00	407.00	407.00	407.00
Carbopol P940 (mg)	100.00	200.00	300.00	400.00	100.00	100.00	100.00	100.00
Hydroxyethyl cellulose (mg)	———	———	———	———	100.00	200.00	300.00	400.00
Triethanolamine (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dimethyl Sulfoxide(ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Methyl Paraben (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled Water (gm)	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100

Table 9: Percent yield,drug content,spreadability,extradability and bioadhesive strength of AZMBVGs

Formulation code	% Yield (Mean ± SEM)	Drug Content (mg/1gm of gel) (Mean ± SEM)	Spreadability (gm.cm/sec.) (Mean ± SEM)	Extrudability (gm./cm²) (Mean ± SEM)	Bioadhesive strength (gm./cm2) (Mean ± SEM)
AZMBVG 1	99.63 ± 0.32	1.16 ± 0.08	15.00 ± 0.22	16.17 ± 0.08	1.20 ± 0.02
AZMBVG 2	99.33 ± 0.33	1.21 ± 0.12	14.38 ± 0.12	16.67 ± 0.08	1.31 ± 0.02
AZMBVG 3	99.40 ± 0.31	1.18 ± 0.03	14.16 ± 0.10	17.42 ± 0.08	1.61 ± 0.03
AZMBVG 4	99.40 ± 0.31	1.20 ± 0.08	13.74 ± 0.10	17.58 ± 0.08	1.69 ± 0.02
AZMBVG 5	99.40 ± 0.31	1.11 ± 0.05	13.54 ± 0.10	17.33 ± 0.08	1.41 ± 0.02
AZMBVG 6	99.10 ± 0.06	1.15 ± 0.05	13.54 ± 0.21	17.66 ± 0.08	1.52 ± 0.02
AZMBVG 7	99.13 ± 0.05	1.07 ± 0.01	13.16 ± 0.12	17.92 ± 0.08	1.59 ± 0.02
AZMBVG 8	99.40 ± 0.15	1.02 ± 0.09	12.86 ± 0.09	18.08 ± 0.08	1.67 ± 0.03

All values are expressed in mean ± standard deviation (n=3)

Percent yield, drug content, spreadability, extrudability and bioadhesive strength of AZMBVG:

The percent yields of AZMBVGs were calculated and found to be into the range of 99.10 ± 0.06 to 99.63 ± 0.32 given in column 2 of table 9. The drug content of AZMBVGs were found to be in the range of 1.07 ± 0.01 to 1.21 ± 0.12 given in column 3 table 9. Observations for spreadability study are in column 4 table 9. Spreadability of all microencapsulated vaginal gels was in between range of 12.86 ± 0.09 to 15.00 ± 0.22. As the concentration of polymer increases the spreadability of AZMBVGs decreases the spreadability. Extrudability study shown was column 5 table 9. Extrudability of all AZMBVGs was in between range of 16.17 ± 0.08 to 18.08 ± 0.08. As the concentration of polymer increases the extrudability of AZMBVGs also increases, because as the concentration of polymer increases weight required to extrude gel from tube also increases. Vaginal bioadhesive strengths of all AZMBVGs, using goat vagina, were found in the following order AZMBVG 4> AZMBVG8 > AZMBVG3 > AZMBVG7 > AZMBVG 6> AZMBVG5 > AZMBVG2 > AZMBVG1 accordingly. Thus it was concluded that AZMBVG 4 showed the highest bioadhesive strength as in column 6 of table 9. The bioadhesive property of Carbopal was reported due to carboxyl groups present on its acrylic acid backbone, which possess an ability to interact with sialic acid molecules present in the vaginal mucus layer.

***In vitro* drug diffusion and drug release kinetics study of AZMBVGs:**

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in Higuchi, first order and followed by zero-order equations as given Table10 and Figure 4 and drug release ranges of 48.58 ± 8.91 to 92.88 ± 4.76 up to 28 hrs. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the Higuchi square root equation rather than first-order and zero-order models. The drug release was proportional to square root of time, indicating that the drug release from ethyl cellulose AZMCs and AZMBVGs were diffusion controlled. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n values of AZMBVGs were ranged of 0.227 to 0.529, indicating that the mechanism of the drug release was diffusion controlled. The release also showed higher correlation with the Korsmeyer- Peppas model, as shown in Table 10. In conclusion, the attempt to prepare controlled release AZMBVG4 with AZMC4 of Zidovudine lorded with high entrapment efficiency was successful. Further studies are required to find out the exact cause for the difference and to improve the entrapment efficiency. The overall effect of polymer on release (AZMBVG1-AZMBVG8) was also significantly different ($P<0.05$, single factor ANOVA). It means null hypothesis is nullified and alter-

native hypothesis is accepted i.e. the variation in formulations in polymeric type and content (AZMBVG1-AZMBVG8) have significant effect on drug release profile.

Table 10: Drug release and kinetic study of AZMBVG

Formulations	Cumulative % Drug release (28 h) study	Zero order release		First order release		Higuchi square root equation		Korsmeyer and Peppas equation	
	%CR	K_0	r^2	K_1	r^2	K_H	r^2	r^2	n
AZMBVG1	90.72 ± 1.84	2.08	0.81	1.89	0.36	0.13	0.82	0.91	0.440
AZMBVG2	90.57 ±12.79	2.01	0.75	1.89	0.77	0.12	0.90	0.94	0.422
AZMBVG3	65.89 ± 1.59	1.74	0.91	1.93	0.91	0.15	0.89	0.97	0.483
AZMBVG4	48.58 ± 8.91	1.19	0.91	1.92	0.90	0.13	0.91	0.99	0.433
AZMBVG5	92.82 ± 3.79	2.03	0.66	1.89	0.88	0.15	0.93	0.85	0.529
AZMBVG6	90.40 ± 7.68	1.86	0.80	1.85	0.91	0.11	0.91	0.95	0.369
AZMBVG7	92.88 ± 4.76	1.31	0.77	1.70	0.91	0.07	0.90	0.95	0.227
AZMBVG8	67.81 ± 3.78	1.62	0.88	1.92	0.91	0.14	0.90	0.96	0.446

All values are expressed in mean ± standard deviation (n=3)

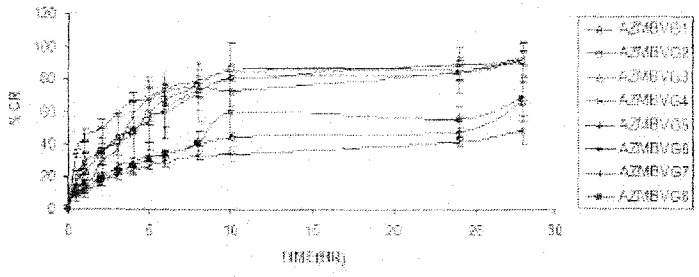

Figure4: *In-vitro* drug release profile of AZMBVGs formulations

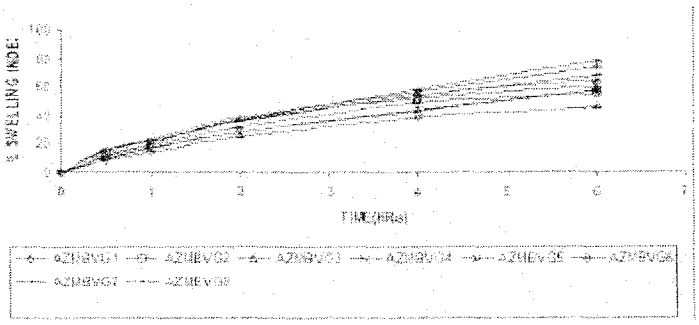

Figure 5: Swelling index profile of AZMBVGs formulations

Table11: pH and color evaluation study of AZMBVG1- AZMBVG 8:

Formulation code	pH	Color of Vaginal Gel
AZMBVG1	6.9±0.05	Transparent
AZMBVG2	7.2±0.09	Transparent
AZMBVG 3	7.3±0.08	Transparent
AZMBVG 4	7.0±0.03	Transparent
AZMBVG 5	7.2±0.13	Light Brownish-White
AZMBVG 6	7.2±0.09	Light Brownish-White
AZMBVG 7	7.3±0.11	Light Brownish-White
AZMBVG 8	7.5±0.05	Light Brownish-White

All values are expressed in mean ± standard deviation (n=3)

Swelling index:

Table12: Percent swelling index of AZMBVG 1- AZMBVG8

Formulation code	Swelling Index (%) (X± SD)				
Time(hrs)	0.5	1	2	4	6
AZMBVG1	08.40 ± 0.15	14.80 ± 0.12	25.30 ± 0.40	38.67 ± 0.29	46.07 ±0.93
AZMBVG 2	10.70 ± 0.29	19.33 ± 0.26	32.03 ± 0.67	49.07 ± 0.49	55.43 ±0.45
AZMBVG 3	12.17 ± 0.37	20.60 ± 0.46	37.23 ± 0.67	52.90 ± 0.78	60.40 ±1.28
AZMBVG 4	16.34 ± 0.67	21.70 ± 0.59	36.17 ± 0.61	54.23± 1.28	64.70±0.95
AZMBVG 5	09.67 ± 0.29	17.40 ± 0.61	28.43 ± 0.79	42.80 ± 0.55	57.57 ±1.25
AZMBVG 6	09.17 ± 0.23	17.10 ± 0.12	29.10 ± 0.12	44.43 ± 1.69	57.73 ±0.75
AZMBVG 7	13.33 ± 0.47	23.33 ± 0.72	39.03 ± 0.09	56.60 ± 0.61	73.37 ±0.38
AZMBVG 8	16.77 ± 0.09	22.93 ± 0.46	36.90 ± 0.55	58.10 ± 0.45	78.03 ±0.83

All values are expressed in mean ± SEM (n = 3).

In order to understand the influence of the polymer system on drug release and swelling study on gel matrices containing the polymers (HPMC and Carbopol P940) was evaluated. It is clear that the gel matrices underwent swelling at the same time as it was placed in the dissolution media. The pH of the media influenced swelling. On the other hand, the percentage of matrix swelling as a function of pH ranged from 78.03% at pH 7.5 to 46.07% at pH 6.9 (Table11 and Table 12). This result demonstrates that gel matrix swelling depends on the pH of the media. As the pH of the media increases, swelling of the matrix increases. The Table 12 and Figure 5 showed swelling indexes of showed swelling indexes of different formulations. Swelling index increased in the following order of formulations AZMBVG1 <AZMBVG2<AZMBVG5<AZMBVG6<AZMBVG3 <AZMBVG4 <AZMBVG7<AZMBVG8. Formulation AZMBVG8 (1:4) was contain highest proportion of HPMC and lowest proportion of carbopol showed highest swelling index. Other hand formulation AZMBVG1 was contain lowest proportion of carbopol, showed lowest swelling index respectively. It was indicated that as the proportion of HPMC and carbopol increased, swelling index increased.

Effect of swelling index on Zidovudine (AZT) release

In all the cases, the release rate was increased with increased proportion of hydrophilic polymer carbopol and HPMC due to more swelling. Initially, the diffusion coefficient of drug in the dehydrated polymer will be less and increases significantly as the polymer imbibes more and more water, and forms a gel, as the time progresses. The hydration rate of the polymer and thereby the gel formation significantly depended on polymer proportion. The overall effect of polymer was observed as follows. Formulation AZMBVG4(Carbopol) with swelling index (64.70±0.95) at pH7.0, showed lowest % cumulative drug release (48.58±8.91% up to 28thhr) Thus concluded that carbopol contend EC microcapsule has an effect on drug release and it was as a batter rate controlling polymer which gave better drug release. AZMBVG8 was (Carbopal:HPMC=1:4) highest swelling index (78.03 ±0.83) at pH7.5 showed 67.81 ± 13.78% drug release up to 28th hrs. while formulation AZMBVG 5 (Carbopal : HPMC=1:1) with swelling

Table 13: Stability studies (drug concentration, potency and log % concentration) of AZMBVG 4

Temperatures	25± 2°C			50± 2°C		
Time (in Days)	Conc. (in mg/gm gel)	Potency (%)	Log % Conc.	Concentration (in mg/gm gel)	Potency (%)	Log % Conc.
0	1.20	100.00	2.000	1.20	100.00	2.000
7	1.20	100.00	2.000	1.19	99.17	1.996
14	1.20	100.00	2.000	1.19	99.17	1.996
21	1.19	99.17	1.996	1.18	98.33	1.992
30	1.20	100.00	2.000	1.19	99.17	1.996
38	1.20	100.00	2.000	1.17	97.50	1.989
45	1.19	99.17	1.996	1.18	98.33	1.992
52	1.19	99.17	1.996	1.17	97.50	1.989
60	1.18	98.33	1.992	1.16	96.67	1.985
68	1.19	99.17	1.996	1.16	96.67	1.985
75	1.17	97.50	1.989	1.17	97.50	1.989
82	1.18	98.33	1.992	1.15	95.83	1.982
90	1.17	97.50	1.989	1.15	95.85	1.982

Table14: Parameters determined for stability studies of AZMBVG 4

Parameters	Temp. 25± 2°C	Temp. 50± 2°C
Zero – order (R2)	0.738	0.828
First – order (R2)	0.749	0.856
First – order rate constant (k1)(in day-1)	2.886 / 10-4	4.758 / 10-4
Degradation Half life (in year)	1.006	0.610
Shelf life (in year)	6.579	3.990

Viscosity:

Table 15: showed the data of viscosity of AZMBVG4

Sample	Spindle	RPM	cP	% (T)
AZMBVG4	64	20	29400	99.2

index (57.57 ±1.25) at pH7.2, was found to release the drug only about 92.82 ± 3.79% up to 28th hrs. This finding can be attributed to the higher water retentions property of carbopol and HPMC, thus concluded that in present of EC microcapsule drug release was significantly influence of both the polymers. Carbopol contained EC microcapsules was a better rate controlling polymer to sustain the release of drug for longer period of time when compared to formulations containing HPMC and carbopol with EC microcapsules.

Accelerated stability studies of AZMBVG4:

Table 13 shows the concentration, potency and log percent concentration of AZMBVG4 for 90 days stability study, and table 14 shows the parameters determined for the stability of AZMBVG4. Shelf life in year in different temperatures like 25±2°C and 50±2°C result were found 6.579 and 3.990 years respectively. Degradation Half life was also calculated in year of AZMBVG4 in different temperatures like 25± 1°C and 50± 1°C results were found 1.006 years, and 0.610 years for respective temperatures like 25± 1°C and 50± 1°C results were found 1.006 years, and 0.610 years for respective temperatures.

Viscosity is an important parameter for characterizing the gels as it affects the spreadability, extrudability and release of drug. The Table15 showed the data of viscosity. ZMBVG4 showed 29400cps indicated that the viscosity of gels was increased with the increase in carbopol content which may be due to the increase in formation of three dimensional cross linking structure of gel, as expected.

ACKNOWLEDGEMENT

Our Research Team thankfully acknowledged the APL Research Center (A Division of Aurobindo Pharma Ltd) A.P. India., for the gift sample of Zidovudine, SAIF,(DST) AIIMS, New Delhi and University of Burdwan,University of Calcutta and Jadavpur University, West Bengal, India, for help in performing the SEM, viscosity and DSC studies.

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Source of support: Nil, Conflict of interest: None Declared

ISSN: 0975- 8232

IJPSR (2010), Vol. 1, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received 28 January, 2010; received in revised form 20 February, 2010; accepted on 26 February, 2010

PROLONG RELEASE BIOADHESIVE VAGINAL FILM OF ANTI-HIV DRUG (ZIDOVUDINE): FORMULATION AND *IN- VITRO* EVALUATION

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Keywords:

AIDS ,

Zidovudine ,

Bioadhesive ,

Film

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ABSTRACT

The present study concerned with the development and characterization of bioadhesive vaginal film (VF). Zidovudine containing VF were prepared by solvent casting method using different ratios of Acrycoat S 100 (AC) or Ethyl cellulose (EC) to Hydroxy propyl methyl cellulose (HPMC) and di butyl phthalate(DBP) as a plasticizer. The optimized films were found to be transparent, flexible and soft and evaluated for mechanical properties by modified instrument, drug content, folding endurance, *in vitro* drug release with release kinetic and % moisture content bioadhesive strength by modified pan balance method. The films were found higher drug content and flexible. The VF10 (containing AC: HPMC 4:1) was selected. *In vitro* drug was found of Zidovudine over 11 hr obeying zero order followed by Higuchi kinetics and Case II non-Fickian (anomalous) diffusion control, indicating the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation with a sufficient bio adhesion quality with good mechanical properties. The results were compared statistically and found with satisfactory correlation. Thus in conclusion preparation protocol of VFs studied may be adopted for a successful development of newer drug delivery system for treatment and prevention for AIDS.

INTRODUCTION: UN global summary of the AIDS pandemic 2007 revealed, women (15.4 million) account for approximately 50% of people (33.2 million) infected and living with HIV. More than 20million people have died of AIDS and about 14,000 are newly infected every day[1]. HIV is affecting women and girls increasing in numbers. As researchers, these statistics emphasize the responsibility and a challenge that includes understanding personal risks of our young people, to make healthy choices about their sexuality as well as a course for future action in designing safe, effective, acceptable and affordable vaginal microbicide to reduce the risk of STD transmission, particularly HIV, specifically for women[2].

Access to a safe and effective microbicide would benefit both women and men. Although somewhat neglected in clinical studies, pharmaceutical characterization of vaginal polymeric films, is an important step in order to optimize safety, efficacy and acceptability [3]. Vagina is explored as an effective site for local and systemic drug delivery due certain unique features such as presence of dense network of blood vessels, lacking of GI and liver first pass effect.[4,5,6] The primary objective of this study was to develop VF of Zidovudine. The VF were targeted to control the release of Zidovudine in a predetermined manner for a prolong time through vagina. We had prepared VF of Zidovudine by solvent casting method with reduced initial burst, increased prolonged cumulative release *in vitro* to achieve controlled release over a period of 11 hr and improved vaginal bio adhesive strengths in goat vagina *ex- vivo*. The film also possessed aesthetic appeal such as good appearance, softness, flexibility and free from of any sharp edge to avoid mechanical injuries during insertion for ease of administration and user convenience after administration.

MATERIALS AND METHODS:

Materials: Zidovudine was obtained as a gift sample from Aurobindo Pharma Ltd, A.P, India and EC (ethoxy content- 47.5% by weight and viscosity [η] of 22 cps in a 5% concentration by a weight in toluene: ethanol 80:20 at 25°C), HPMC (K4M,4000 cps 2% aqueous solution) and AC were obtained from S.D. Fine Chem., Mumbai, India. All other chemicals and reagents used were of analytical grade and used as received.

Methods: VF of Zidovudine was prepared by solvent casting method containing different ratios of AC or EC and HPMC in di-butyl phthalate or glycerol or sorbitol or PEG 400 as a plasticizer. 40 % w/w of polymeric solution was allowed to stir for 1 h. After that, drug and plasticizer were added with constant stirring and this solution was allowed to stir until we got clear solution. The solution was allowed to stand overnight to remove all the air bubbles. The solution was then casted onto a petri dish and dried in the oven at 60°C until complete drying. The film was carefully removed from the petri dish, checked for any imperfections and cut according to the size required for testing. The films thus prepared, were wrapped in a aluminum foil and kept in a desiccators for further study. Each formulation was replicated three times[7,8].

Morphological characterization: Films were analyzed in Scanning Electron

Microscopy (LEO, 435 VP, UK) to reveal the surface morphology of the films. The films were placed on double-sided tape attached onto graphite surface. The samples were coated with gold using an ion sputter. Coating was provided at 20 mA for 4 min. Observation was performed at 15 kV and ~ X550 magnification.[9]

Measurement of mechanical properties: Mechanical properties of the films were evaluated using a modified instrument based on the similar working principle as reported by *Kok Khiang Peh et al.* Film strip in dimension of 50x10 mm and free from air bubbles or physical imperfections, were held between two clamps positioned at a distance of 3 cm. A cardboard was attached on the surface of the clamp via a double-sided tape to prevent the film from being cut by the grooves of the clamp. One clamp remains fixed and another one is movable.

During measurement, the strips were pulled by the movable clamp at a rate of 2.0 mm/s to a distance of 5 mm before returning to the starting point. The force and elongation were measured when the films broke. Results from film samples, which did not broke at between the clamps, were not included in calculations. Measurements were triplicate for each film. The following equations[10]were used to calculate the mechanical properties of the films:

The reproducibility of the system was examined in the initial investigations using three same formulations of VFs. Then the study was carried out for different formulations.

Folding endurance: The folding endurance is expressed as the number of folds (no. of times the film is folded at the same place) either to break the film or to develop visible cracks. This test is important to check the ability of sample to withstand folding during handling and transport. The measurements of folding endurance of each formulation was replicated three times.[8]

$$\text{Tensile strength}(kg/mm^2) = \frac{\text{Force at break}(kg)}{\text{Initial cross-sectional area of the sample}(mm^2)} \quad --(1)$$

$$\text{Elastic modulus}(kg/mm^2) = \frac{\text{Force at corresponding strain}(kg)}{\text{Cross-sectional area of the sample}(mm^2)} X \frac{1}{\text{Corresponding strain}} \quad --(2)$$

$$\text{Elongation break}(\%mm^{-1}) = \frac{\text{Increase in length}(mm)}{\text{Original length}(mm)} X \frac{100}{\text{Cross sectional area}(mm^2)} \quad --(3)$$

$$\text{Strain} = \frac{\text{Tensile strength}}{\text{Elastic modulus of the sample}} \quad --(4)$$

Estimation of drug content: Zidovudine content in film was estimated by UV-Visible spectrophotometric method in simulated vaginal fluid (SVF, phosphate buffer I.P., pH 4.7)[18].The accurately weighed film strip in dimension of 50x10 mm, were dissolved first in solvent (2ml methanol) so that polymer get dissolved to release drug into the solution. Then volume was made up to 25 ml with SVF and kept for 1 hr under stirring. Similarly, a blank was carried out using drug free film. The solution was filtered and absorbance was measured at 267nm (λmax) using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) [8, 11].

Estimation of moisture Content: The prepared films were cut into 50 ×10 mm strips. The films were weighed individually and kept in a desiccator containing Calcium Chloride as desiccant at 37° c for

24hr. The films were reweighed individually until a constant weight was obtained. Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film [12, 13].

Determination of swelling index: Each film sample was weighed and placed in SVF for 25 min. The swelling index of film was calculated using following formula.[10]

Swelling index $(Sw) = \frac{W_t - W_0}{W_0} \times 100$;

Where Wt = weight of film at time t, Wo = initial weight of film

***In vitro* drug release and release kinetics of VFs:** *In vitro* drug diffusion studies were carried out by using K.C. cell with a semi permeable barrier. Cellophane membrane was soaked in SVF. Film of specified diameter was placed on the surface of processed cellophane membrane and was fixed to one end of the cylindrical donor compartment by cyanoacrylate adhesives, such that the lower end just touched the surface of SVF medium. Also 0.5 ml of SVF was placed and maintained at same level throughout the study in donor compartment. Temperature was maintained at 37 ± 2°C with constant stirring at 50 ± 10 rpm. A quantity of 5 ml sample was withdrawn from the receptor compartment at definite time interval and replaced with 5 ml of SVF to maintain sink condition. The drug was estimated by using UV-Visible spectrophotometer at 267 nm (λmax) [11, 14]. In order to investigate the mechanism of Zidovudine release from different VFs, the release data was analyzed with the following mathematical model, zero order kinetic equation ($Q_t = K_0 t$), first order kinetic equation ($\ln Q_t = \ln Q_0 - K_1 t$) and Higuchi kinetic equation ($Q_t = K_H t^{1/2}$), where Qt is the percent of drug released form VF at time t, Q_0 is the initial amount of drug present in VF. K_0, K_1 and K_H are the constants of the equations. Further to confirm the mechanism of drug release, drug release was fitted in Korsmeyer Peppa's model, $\frac{M_t}{M_\infty} = K_P t^n$, where $\frac{M_t}{M_\infty}$ is the fraction of drug release at time t and K_P is the power law constant and n is the release exponent.

The power law is valid only for the first 60% of the release profile. The n value is used to characterize different release mechanisms and was calculated from the slope of the plot of log of $\frac{M_t}{M_\infty}$ vs. log of time (t). The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test. [15,16]

Bioadhesion strength in goat vaginal mucosa: Isolated goat vaginal tissue (*Capra hircus*, local breed, obtained immediately after sacrifice of animals at a slaughter house) was cleaned, separated from the supporting muscular and connective tissues taking care to maintain integrity of mucosa, and kept at 0°C till further use. Before experiments, goat vaginal tissue was thawed in SVF medium. The bio adhesion measurement was performed by using a modified balance method intact with goat vaginal tissue. The two pans of physical balance were removed. Right side pan was replaced with a 100 ml beaker and on left side, a glass slide was hanged. For balancing the assembly a weight of 20g was hanged on left side. Another glass slide was placed below the hanged slide. Portions of vaginal membranes were attached with

both slides. The height of this set up was so adjusted, leaving a space of about 0.2 cm between two vaginal membrane faces. One film was placed between two vaginal membrane faces. Little pressure was applied to form bio adhesion bond, and then slowly drop of water was added on right side beaker, till the VF was separated from one face of vaginal membranes attached. Volume of water added was converted to mass. This gave the bioadhesive strength of film in gm. An initial investigation examined the reproducibility of the system using five same formulations. Then the study was carried out for all formulations [17].

Statistical analyses: Statistical data analyses were performed by using Mystat statistical software. One way ANOVA was performed and was considered significant ($p < 0.05$) at 5 % level.

RESULTS AND DISCUSSION: Antiretroviral drug, Zidovudine, a nucleoside reverse transcriptase inhibitor, is taken up by the host cells where it is converted into in its tri-phosphate form. Subsequently, by competitive inhibition, it inhibits the reverse transcriptase, therefore, viral replication stops. Also it is incorporated into the viral DNA chain which is growing (during replication) and terminates the lengthening of the viral DNA chain, thereby stops viral replication. Zidovudine was chosen as a model drug of choice due to its short half life of about 1hour, high oral dose, low systemic bioavailability (only 64%) due to rapid hepatic first-pass metabolism. Thin and soft transparent Bioadhesive vaginal film released drug in a predetermined manner. Film has advantages among bioadhesive vaginal formulations with better dispersion throughout vagina, better aesthetic appeal, more comfortable, less interference during intercourse, dose removal possibility in emergency situations, suitability for a wide variety of drugs and excellent drug content uniformity. The formulation code and composition of VF were presented in (Table 1).

Physical characteristics of films: Physical characteristics of different VF were shown in column 4 and 5 of (Table 1) to optimize plasticizers. Films containing PEG 400 and sorbitol as plasticizers could not be removed from glass plate after drying. Film containing Glycerol as a plasticizer, was appeared transparent and easily removed from plate but was brittle. Films containing DBP as a plasticizer appeared transparent, easily removed from plate and were soft. So for such composition of Film, DBP was selected as plasticizer of choice.

Table 1: Composition and plasticizer of Bio-adhesive vaginal films

Formulation	Polymer	Drug: Polymer
VF 05	EC: HPMC (4: 1)	1: 5
VF 08	EC: HPMC (1: 4)	1: 5
VF 10	AC: HPMC (4: 1)	1: 5
VF 13	AC: HPMC (1: 4)	1: 5

Morphological Characterization: SEM photographs of blank and drug loaded films were shown in (Figures 1a and 1b) accordingly. Films appeared to be homogenous and continuous. Drug was distributed on the surface, over the drug loaded film.

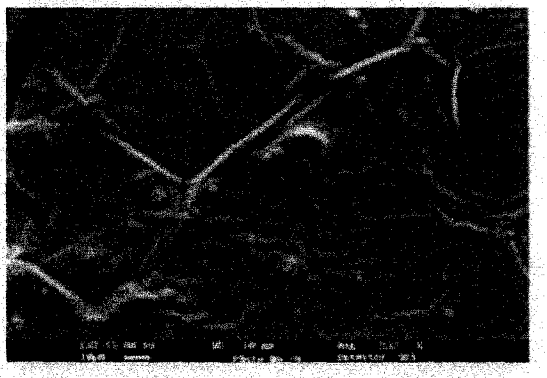

Fig.1a: SEM photograph of Blank

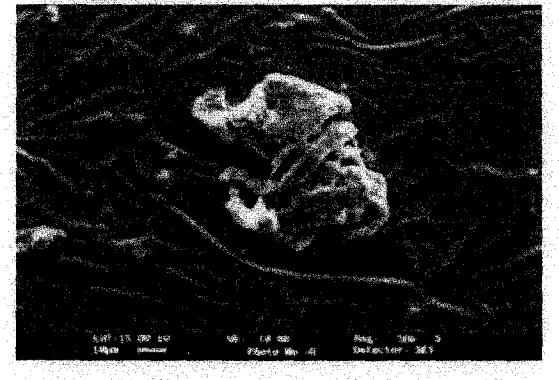

Fig. 1b: Drug loaded vaginal film

Measurement of mechanical properties: The tensile testing is an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength (TS), elastic modulus (EM) and elongation at break (E/B). A soft and weak film is distinguished by a low TS, EM and E/B; a hard and brittle film is defined by a moderate TS, high EM and low E/B; a soft and tough polymer is characterized by a moderate TS, low EM and high E/B; whereas a hard and tough polymer is characterized by a high TS, EM and E/B. Another parameter, Strain has been used as an indicator of the overall mechanical quality of the film. A high strain value indicates that the film is strong and elastic. Hence, it is suggested that a suitable vaginal film should have a relatively high TS, E/B and Strain but a low EM. (Table 2) showed mechanical properties of different formulations. For EC film, decrease HPMC content TS and EM decreases, E/B increases but no significant difference in case of strain. For AC film, decrease TS, EM and strain, E/B increases.

These results indicated that HPMC generally increase the strength while decreased the softness, elasticity and flexibility of both EC as well as AC films. The greater elasticity exhibited by films containing lower HPMC content. From Table 2, VF 8 (EC:HPMC=1:4) with moderate TS and EM with low B/Band low strain indicated that film were soft & weak nature, while formulation VF10(AC: HPMC=4:1) with low TS, low EM, high E/B with high strain was found indicated film were soft, strong and elastic; while Formulation VF 05 (EC: HPMC= 4: 1) with low TS ,low EM and high E/B with low strain indicated soft & weak film ,whereas formulation VF13(AC:HPMC=1:4) high TS and high EM, with low E/Band low strain indicated that film was hard , brittle elastic nature. These results indicated that AC generally reduced the strength while increased the softness, elasticity and flexibility of both EC as well as AC films. The greater elasticity exhibited by films containing higher AC content could be related to its conformation and configuration, which is highly cross linked. In comparison, the mean TS values of

both EC and AC films were closely comparable for similar compositions. Increase in AC content rendered the HPMC films more elastic than EC films.

Table 2: Mechanical properties of different vaginal formulations

Formulation	Tensile Strength	Elastic Modulus	Elongation Break	Strain
VF 05	1.23 ± 0.17	3.41 ± 0.23	12.57 ± 0.20	0.36 ± 0.09
VF 08	2.45 ± 0.65	5.83 ± 0.32	10.99 ± 0.09	0.42 ± 0.09
VF 10	1.24 ± 0.18	2.88 ± 0.21	12.54 ± 0.08	0.43 ± 0.05
VF 13	2.71 ± 0.32	6.15 ± 0.42	11.67 ± 0.14	0.44 ± 0.12

Each value represents as mean ± standard deviation (n= 3)

Estimation of drug content: The drug content of all the prepared VFs was found to be satisfactory and each formulation demonstrated high drug contents, as summarized in column 2 of (Table 3). The drug contents of the prepared VFs were found to be in the range of 77.87(VF 05) - 97.65% (VF 13). The formulation VF 13 showed highest drug contents among all the formulations. Further, as shown in (Table 3), the drug content analysis of the prepared films showed that the process used to prepare the films in this investigation is capable of giving optimum drug content and minimum batch variability.

Folding endurance: Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme conditions of folding. The column 4 of (Table 3) showed folding endurance of different formulations. The folding endurance the prepared VFs were found to be 296-324 numbers of times for all formulation indicating that all formulations were flexible and soft. This also gives an indication of brittleness; less folding endurance indicates more brittleness.

Table 3: % Drug content, moisture content, folding endurance, bioadhesive strength and swelling index of different formulations

Formulation	% w/w Drug Content	% Moisture Content*	Folding Endurance *(no. of times)	*Bioadhesive Strength	*Swelling Index (Upto 25 mins.)
VF 05	77.87 ± 0.74	2.94 ± 0.65	308 ± 21	5.1 ± 3.2	19.72 ± 0.83
VF 08	87.75 ± 0.75	4.13 ± 0.95	324 ± 15	17.5 ± 2.9	56. 56 ± 77
VF 10	87. 44 ± 0.45	1.23 ± 1.11	321 ± 23	4.4 ± 1.8	17.08 ± 67
VF 13	97.65 ± 1.32	3.43 ± 0.84	296 ± 76	14.6 ± 2.6	51.32 ± 1.56

*Each value represents as mean ± standard deviation (n= 3)

% Moisture content: The column 3 of (Table 3) showed % moisture content of different formulation. The moisture content in the formulations was found to increase with the increasing concentration of drug and hydrophilic polymer HPMC. Formulation containing EC and HPMC showed higher % moisture content than formulation containing AC and HPMC. Formulation VF 8 showed highest (4.13 %) moisture content and Formulation VF 10 showed lowest (1.231%) moisture content indicating that as ratio of HPMC increases % moisture content increases and vice-versa

Table 4: Drug release profile and kinetics of different formulations

Formulation	% Cumulative Drug Release (11 hr study)	Zero Order Eq.		First order Eq.		Higuchi Square Root Eq.		Korsmeyer and Peppas Eq.	
		r^2	K_1 (hr^{-1})	r^2	K_{2-1} ($\%/h^{1/2}$)	r^2	K_{2-1} ($\%/h^{1/2}$)	r^2	n
VF 05	21.30 ± 0.96	0.991	2.055	0.99	0.010	0.926	7.300	0.988	0.979
VF 08	59.30 ± 0.84	0.873	5.185	0.91	0.033	0.952	19.89	0.924	0.766
VF 10	13.54 ± 1.12	0.981	1.229	0.97	0.005	0.889	4.298	0.939	0.795
VF 13	44.86 ± 1.11	0.823	3.015	0.99	0.014	0.980	11.96	0.952	0.304

Each value represents as mean ± SD. n=3

***In- vitro* drug release of prepared Film:** The *in- vitro* drug releases of acquired films were shown in column 2 of (Table 4) and (Fig. 2).

Effect of swelling index on Zidovudine release: In all the cases, the release rate was increased with increased proportion of hydrophilic polymer (HPMC) due to more swelling. Initially, the diffusion coefficient of drug in the dehydrated polymer will be less and increases significantly as the polymer imbibes more and more water, and forms a gel, as the time progresses. The hydration rate of the polymer and thereby the gel formation significantly depended on polymer proportion[14]. The overall effect of polymer was observed as follows. Formulation VF 8 (EC: HPMC= 1:4) with highest swelling index (56.56 ± 0.77), showed highest % cumulative drug release (59.30 ± 0.84% up to 11th hr) while formulation VF10 (AC: HPMC= 4: 1) with lowest swelling index (17.08 ± 0.67), was found to release the drug only about 13.54± 1.12% upto 11 hrs. Formulation VF 05 (EC: HPMC= 4:1) with swelling index of 19.72 ± 0.77, showed 21.30 ± 0.96% % cumulative drug release up to 11th hr whereas formulation VF13 (AC:HPMC=1:4) with swelling index of 51.32 ± 1.56, was found to release the drug only about 44.86± 1.11% upto11hrs. This finding can be attributed to the higher water repelling property of AC, thus concluded that AC was a better rate controlling polymer to sustain the release of drug for longer period of time when compared to formulations containing EC as a rate controlling polymer.

Different kinetic models (zero- order, first-order and Higuchi's) were applied to interpret the release profile from VFs. The best fit with higher correlation (r^2> 0.9) was found with the Higuchi's equation. The rate constants were calculated from the slope of the respective plots. The best fit with the highest correlation coefficient was shown in Higuchi followed by first order and zero-order equations as given in Table 4. The drug release was proportional to square root of time, indicating that the drug release from

VFs was diffusion controlled. However, two factors diminish the applicability of Higuchi's equation to matrix systems as this model fails to allow the influence of swelling of the matrix (upon hydration) and gradual erosion of the matrix. Therefore, the dissolution data were also fitted according to the well-known power law equation (Korsmeyer Peppas' equation).The drug release mechanism of all VFs was found to be predominately influenced by the different bioadhesive polymer added. The mechanism of drug release from hydrophilic-hydrophobic polymeric films involves solvent penetration, hydration and swelling of the polymers, diffusion of the dissolved drug in the matrix and erosion of the gel layer. Form (Table 4 and Figure 2), the *n* values for all the formulations ranged from 0.304 to 0.979 indicating different release patterns viz. Case I Fickian release (n = 0.5), Case II non-Fickian (anomalous) release (0.5≤ n ≤ 0.89), super case II type of release (≥0.89). It was observed that the VF 13 (n= 0.304) films underwent Case I Fickian diffusion control, during the diffusion study. In case of Case I Fickian release mechanism, the rate of drug release is much lesser than that of polymer relaxation (swelling/erosion). So the drug release was chiefly dependent on the diffusion through the films.

Also it was observed that the formulations VF 8(n= 0.766) and VF10 (n= 0.785), underwent Case II non-Fickian (anomalous) diffusion control, indicating the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation. Further VF 05 (n= 0.979) endured super case II release, denoting polymer relaxation had a significant role in the drug release mechanism. Super Case II release generally refers to the polymer relaxation. The overall effect of polymer on release (VF 04- VF 13) was also significantly different ($P< 0.05$, single factor ANOVA). It means null hypothesis is nullified and alternative hypothesis is accepted i.e. the variation in formulations in polymeric type and content (VF4-VF13) have significant effect on release profile.

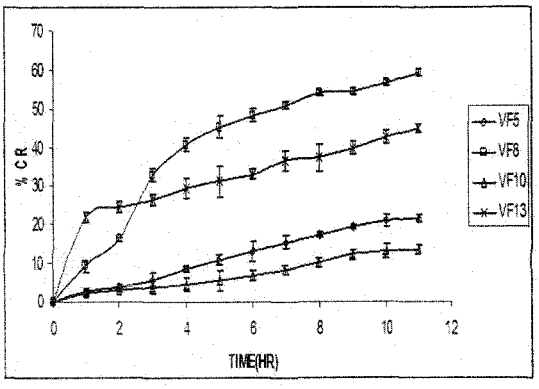

Fig 2: Drug release profile of different formulations. Each value represents as mean ± standard deviation. n= 3

Selection of Film-Forming Polymer: Combination of hydrophobic (EC or AC S 100) and hydrophilic (HPMC or PVP) polymer was experimented with different plasticizer (DBP, glycerol, sorbitol, PEG 400) for film formation. Solvent casting techniques was employed for the preparation of VF. From the results of the present study it appears that the release of Zidovudine was significantly influenced by the characteristics of the polymer used AC and HPMC (4:1) shown greater rate retarding property in comparison with EC and HPMC (4:1) .Thus we chosen AC and HPMC (1:4) as a film forming polymers.

Vaginal bio adhesion measurements: (Figure 3 and column 2 of Table 4), indicates the vaginal bioadhesive properties of the prepared VF (VF 04- F 13) in goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order VF 08 >VF 13 > VF 05 > VF10. It was

concluded that Bioadhesive strength proportional to the proportion of HPMC in formulation VF 08 (EC: HPMC 1: 4) showed the highest bioadhesive property.

ACKNOWLEDGEMENT: The authors thankfully acknowledged the APL Research Center, A.P. India., for the gift sample of Zidovudine. The authors are grateful to the SAIF, (DST) AIIMS, New Delhi, India, helping in performing the SEM studies. Research Project supported By AICTE, India, and F.No:8023/BOR /RID /RPS 48 / 2008-09.

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Dear Mr. Chatterjee,
The Editorial Board of Journal of Young Pharmacists is pleased to inform you that your manuscript entitled Formulation, In vitro and In vivo Pharmacokinetics of Anti-HIV Vaginal Bioadhesive Gel, with manuscript number JYP_10_10, is acceptable for publication in the Journal.
We will be sending you the page proofs through the manuscript management site before publication of the manuscript. At that time, you may place the order for the extra reprints.
If you have not sent the copyright form signed by all the contributors and the images, if any, till now, you are requested to do so at the earliest. Copyright form can be emailed to copyright@medknow.com Copyright form can be emailed to copyright@medknow.com
Please note that the journal reserves the rights to make changes in the language, grammar, presentation, etc. to suit the journal's requirements.
We thank you for submitting your valuable research work to Journal of Young Pharmacists.
With warm personal regards,
Yours sincerely,
The Editorial Team
Journal of Young Pharmacists

-----END OF MESSAGE ---

HIMALAYAN PHARMACY INSTITUTE

(Approved by A. I. C. T. E. & P.C.I)
Majhitar, E. Sikkim – 737136
Ph. No. 03592-246462

Self-financing Institution

Ref. HPI/IAEC/F-60- 16/2006 Date: 11.05.2007

To,

Dr. B.B. Bhowmik
Lecturer
Himalayan Pharmacy Institute
Majhitar, East Sikkim- 737 136

Sub: Sanction of research proposals and IAEC No for your proposals regarding

Sir,

I am forwarding herewith the minutes of Institutional Animal ethics committee, which met on 07.05.2007 in the Himalayan Pharmacy Institute Majhitar East Sikkim – 737136

1. **Study of formulation and evaluation of submicron vaginal gel .**

Principal Investigator: **Dr. B.B. Bhowmik & Mr. Arkendu Chatterjee.**

The research proposal was sanctioned under the registration no of the institute 1028/C/07/CPSEA date: 24.01.2007 and IAEC approval No. is ***HPI/07/60/IAEC/0002 dt 07.05.2007***

Kindly intimate the date of Commencement of the project and also your revised animal stock. Kindly furnish your updated stock of animals every quarter to the veterinary surgeon, which is mandatory. Failure to do so may not enable you to procure animals for further experimentation.

Member Secretary
IAEC

CHAIRMAN
IAEC

Dr. K. Gauthaman, M. Pharm, P.
Principal
Himalayan Pharmacy Institute
Majhitar-737136, East Sikkim

IAEC – Minutes

Minutes of the IAEC meeting held on 07/05/2007 at 11.00 am in the Office of the Principal, Himalayan Pharmacy Institute, Majhitar, East Sikkim – 737136.

Members Present:

Dr. P.K. Mitra, Dr. B.P. Saha, Dr. K. Gauthaman, Dr. A. Jha, Mr. P.K. Kar, Dr. D.S. Tewari, Dr. S.K. Dash & Mrs. M. Cintury.

The Principal Dr. K. Gauthaman, welcomed the gathering and requested Mr. P.K. Kar Senior Lecturer, Department of Pharmacology and in-charge for the animal experiments, to give a brief review of the previous meetings. Following this,IAEC members are introduced by the Secretary Mr. P.K. Kar to Dr. P.K. Mitra, CPCSEA Nominee.

Following this, the proposals submitted by the Principal Investigators were discussed and adhoc numbers were issued for obtaining the required animals for research from authorized institutions.

It was unanimously decided that the Director, Himalayan Pharmacy Institute, Majhitar, Sikkim will be a member of IAEC. Proposal regarding this will be forwarded to CPCSEA for approval.

The proposals submitted, discussed and cleared as follows:-

1. Non-Lipid lowering properties of HNG-CoA Reductase Inhibitors.
   Principal Investigator & Co – investigator:Dr. K. Gauthaman, S. Vijay Kumar
   Species – Wistar Rats and Swiss Albino Rabbits
   Requested – 360/ 3 years and 156/ 3 years
   Sanctioned – 120/year and 52/year
   IAEC No. HPI/07/60/IAEC/0001

2. Study of formulation and evaluation of submicron vaginal gel.
   Principal Investigator & Co – investigator: Dr. B.B. Bhowmick, Arkendu Chatterjee
   Species – Albino Rat and Newziland White Rabbit
   Requested – 12/ 1 year and 12/ 1 years
   Sanctioned – 12/year and 12/year
   IAEC No. HPI/07/60/IAEC/0002

3. Formulation, evaluation & optimization of matrix type transdermal drug delivery system.
   Principal Investigator & Co – investigator:Mr. Biplab Kumar Dey
   Species – Albino Wistar Rats
   Requested – 6/1month
   Sanctioned – 6/month
   IAEC No. HPI/07/60/IAEC/0003

10. Cardioprotective Properties of Pyrazolone Derivatives in Myocardial Ischemic Reperfusion Injury.
Principal Investigator & Co - investigator:Dr. K. Gauthaman, G. Mariappan
Species - New Zealand Rabbit and Wistar Rats
Requested - 156 Rabbits/ 360 Rats
Sanctioned - 52/year and 120/year
IAEC No. HPI/07/60/IAEC/0010

11. Development and Evaluation of Propranolol Hydrochloride transdermal patch
Principal Investigator & Co - investigator: Dr. L.K. Nath, Mr. B.K. Dey,& Mr. P.K. Kar.
Species - New Zealand Rabbits
Requested - 30
Sanctioned - 30/year
IAEC No. HPI/07/60/IAEC/0011

12. Formulation. Evaluation & optimization of matrix type transdermal drug delivery system
Principal Investigator & Co - investigator:Mr. Biplab Kr. Dey & Mr. Sudip Das
Species - Albino Wistar Rats
Requested -06
Sanctioned - 06/year
IAEC No. HPI/07/60/IAEC/0012

13. Studies on Formulation Development and Evaluations of Anti HIV Bioadhesive Microencapsulated Vaginal Gel.
Principal Investigator & Co - investigator: Dr. B.B. Bhowmick, Arkendu Chatterjee
Species - New Zealand Rabbits and Swiss Albino Mice
Requested - 48 Mice & 48 rabbits
Sanctioned - 48/year & 48 year
IAEC No. HPI/07/60/IAEC/0013

14.Studies on Pharmacological Screening of the Bioactive Molecules from some selected plants.
Principal Investigator & Co - investigator: Dr. L.K. Nath & Mrs. J.P. Mohanty
Species - Albino Wistar Rats
Requested - 30/year
Sanctioned -30/year
IAEC No. HPI/07/60/IAEC/0014

15. Studies on Formulation Development and Evaluation of Herbal Male Contraceptive.
Principal Investigator & Co - investigator: Mr. P.K. Kar, Mr. Arkendu Chatterjee & Dipankar Dey
Species - Albino Rats
Requested - 12/year
Sanctioned - 12/year.
IAEC No. HPI/07/60/IAEC/0015

16. Cardioprotective Effect of *Hibiscus rosasinensis* flowers: A Pharmacological and Molecular Biological study.
Principal Investigator & Co - investigator: Dr. K. Gauthaman.
Species - Rats and Rabbits
Requested - 360/ 3 years and 156/ 3 years
Sanctioned - 120/year & 52/year
IAEC No. HPI/07/60/IAEC/0016

The meeting was closed by Mr. P.K. Kar, Secretary, IAEC and in-charge for the animal experiments thanking to all the members for sharing their valuable suggestions and the successful conduct of the meet.

1. Dr. P.K. Mitra 7/5/07
(CPCSEA Nominee)

5. Mr. P.K. Kar,
Member (Secretary, IAEC)

2. Dr. K. Gauthaman
(Principal & Chairman, IAEC)

6. Dr. D.S. Tewari.
(Veterinary Surgeon)

3. Dr. B.P. Saha
(Member) - Scientist

7. Dr. S.K. Dash,
(Member) - Scientist

4. Dr. A. Jha
(Member)
Scientist outside).

8. Mrs. M. Cintury,
(Social Member)

A..L INDIA COUNCIL FOR TECHNICAL EDUCATION
4th Floor BCC Place, Bhishma Pitamah Marg, Lodhi Road, Pragati Vihar
New Delhi– 110 003 Telefax: 011-24369632

F.No.:8023/BOR/RID/RPS-48/2008-09 Dated: January 6, 2008

The Drawing and Dis' rsing Officer
All India Council for ..hnical Education
7th Floor, Chanderlok B..ding, Janpath
Connauht Place, N.. Delhi – 110 001

Subject: Releas.. of Grants under Research Promotion Scheme (RPS) Scheme for the financial year 2008-0.. ..er plan.

Sir,

This is to convey the sanction of the Council for payment of Rs.19.00 Lakhs only (Rupees Nineteen Lakhs only) during 2008-09 under the Research Promotion Scheme (RPS) as Grant-in-aid to Himalayan Pharmacy Inst., R..gpo, Sikkim for meeting the expenditure for implementing the Scheme as per details given below:

1.	Name of the Beneficiary Institution (University / College / Institution)	:	HIMALAYAN PHARMACY INST MAJHITAR, EAST RANGPO- 737136, SIKKIM
2.	Principal Investigator Name & Deptt.	:	DR. BENOY BRATA BHOWMIK PHARMACEUTICS
3.	Co-Principal Investigator Name	:	MR. ARKENDU CHATTERJEE
4.	Grant-in-aid Sanctioned Non-Rec..ring Recurring	: : :	Rs.19.00 Lakhs only Rs.19.00 Lakhs only Nil
5.	Amount to be released	:	Rs.19.00 Lakhs only
6.	Approved Duration	:	2 Years (Two Years)
7.	Title of the Project	:	STUDIES ON EFFICACY OF NANOPARTICULAR VAGINAL ANTI-HIV DRUG DELIVERY SYSTEM

1. The sanctioned grant-in-aid is debitable to the major Head RPS/Plan grant and is valid for payment during the financial year 2008-09.

2. The grant-in-aid of the grant shall be drawn by the Drawing and Disbursing Officer (DDO), All India Council for Technical Education, New Delhi on the Grants-in-aid bill and shall be disbursed to and credited to the Principal / Director / Registrar, HIMALAYAN PHARMACY INST, MAJHITAR, EAST, RANGPO- 737136, SIKKIM through demand draft/Cheque.

3. The date of release of the grant by AICTE shall be taken as the date of commencement of the project. The Registrar/Principal/Director shall intimate about the receipt of the grant to AICTE. Any Expenditure uncured prior the issuance of the approval letter is not allowed to be adjusted in the grant and if the University/Institution do not take the project work within 6 months of the receipt of the grant, approval shall ipso facto lapse.

Contd.2

4. Each project sanctioned by AICTE is assigned a specific file no which is on pre-page. Al correspondence addressed to AICTE regarding the project must quote this number along with yea of sanction of the project; otherwise the correspondence may not be entertained.

5. The accounts of the grantee will be open for test check by the Council or Comptroller and Audite General of India or by any Officer designated by them.

6. The Institute/University shall not charge any overheads on this project and will provide all th administrative support for completion of the project.

7. The grantee shall utilize grants on only approved items of expenditure (list enclosed). However, i case of the grantee wishes to recast the project, approval of Council must be obtained for th revised item of expenditure and they will maintain proper accounts of the expenditure as per th norms/ procedures of AICTE / Government of India.

8. The assets acquired wholly or substantially out of All India Council for Technical Education's gran shall not be disposed or encumbered or utilize the purposes other than those for which the Gran was given without proper sanction of the All India Council for Technical Education.

9. The grantee shall maintain an audited record of assets acquired wholly or substantially out of th grant-in-aid and a register of assets shall be maintained by the Institute in the prescribed form i.e GFR-19.

10. Interest on the sanctioned grant-in-aid will be treated as part of the grant and shall be used fo project purposes only and the same shall be mentioned in the audited statement of accounts.

11. The Annual Progress Report in the prescribed format along with Statement of Expenditure an Audited Utilization Certificate shall be submitted to AICTE not later than one month after completio of each financial year.

12. Project Completion Report (PCR) in the prescribed format along with the Audited Statement Expenditure indicating expenditure incurred in the total duration of the project in the prescribe format, utilization in the format and GFR-19 shall be submitted to the Council.

13. The Utilization Certificate (U.C.) supported by Audited Statement of Expenditure to the effect tha the grant has been utilized for the purpose for which it has been sanctioned shall be furnished to th All India Council for Technical Education as early as possible after completion of the project. should contain the head-wise break up of expenditure made from the grant-in-aid provided by th Council.

14. The grantee shall follows the terms and conditions of Research Promotion Scheme (RPS) as lai down by the Council from time to time. The detailed terms and conditions for implementing RP can be obtained from booklet as well as website www.aicte.ernet.in.

15. The Grantee shall fully implement to the official language policy of Union Government and comp with the official language Act, 1963 and official language (use of official purposes of the Unic Rules, 1976 etc.)

Contd....

16. The funds to the extent are available under the scheme.

17. The sanction issues in exercise of the powers delegated to the Council. It is also certified that grant-in-aid is being released in conformity with the rules and the Principle of the scheme.

Yours sincerely,

(Swadesh K. Gupta)
Advisor (RID)

Note: The prescribed formats & Terms & Conditions are available in the application brochure.

Copy forwarded for information and necessary action to:

1 **The Principal/Registrar/Director**
HIMALAYAN PHARMACY INST
MAJHITAR, EAST
RANGPO-737136 SIKKIM

2. **DR. BENOY BRATA BHOWMIK**
Principal Investigator
DEPT OF PHARMACEUTICS
HIMALAYAN PHARMACY INST
MAJHITAR, EAST
RANGPO-737136 SIKKIM

3. MR. ARKENDU CHATTERJEE
CO-PRINCIPAL INVESTIGATOR
HIMALAYAN PHARMACY INST
MAJHITAR, EAST
RANGPO-737136 SIKKIM

4. Office of Director General of Audit,
General Revenues,
AGCR Building
I.P. Estate. New Delhi – 110 002

5. Guard File.

ALL INDIA COUNCIL FOR TECHNICAL EDUCATION
4th Floor, NBCC Place, Bhishma Pitamah Marg, Lodhi Road, Pragati Vihar
New Delhi – 110 003

File no.	:	8023/BOR/RID/RPS-48/2008-09
Name of the Principal Investigator & Deptt	:	DR. BENOY BRATA BHOWMIK DEPT OF PHARMACEUTICS
Name of the Co-Principal Investigator:		MR. ARKENDU CHATTERJEE
Name of the Beneficiary Institution (University / College / Institution)	:	HIMALAYAN PHARMACY INST MAJHITAR, EAST RANGPO-737136 SIKKIM
Title of the Project	:	STUDIES ON EFFICACY OF NANOPARTICULAR VAGINAL ANTI-HIV DRUG DELIVERY SYSTEM

List of approved Items under Non-recurring Head

Sl. No.	Approved Items
1	Zetasizer Nano Zs 90

(Sulata Dandapat)
Asst. Director

Government of India
Ministry of science & Technology
Department of Science & Technology

(CASTECH/GY-B)
Telegram:SCIENCETECH
Phone:26567373,Fax:2211(
26502216(
Fax: 26567(
Technology Bhawan,
New Mehrauli Road,
New Delhi-110016
Date:28/1/2010

D/c No:SR/ITS/03634 /2009-2010

To
Dr. A. Chatterjee
D/O PHARMACEUTICS
Himalayan Pharmacy Institute
Rangpo -737136(Sikkim)

Sub: Financial Assistance to Dr. A. Chatterjee for attending in Int. Annual conf on women health to be held from 26/03/2010 to 28/03/2010 in U S A

Sir/Madam,

We are happy to inform you that your application seeking financial grant to attend the above mentioned international scientific event has been recommended for support by our Department. We will provide you economy class air-fare by the shortest route, airport tax, visa fees and registration fees. We hope this support will provide you an opportunity to interact with leading international experts in the area. The support, however, is subject to the following conditions:

1. You should not have received financial support during last three years under this scheme
2. The air tickets are to be booked in economic class by the shortest route in a National Carrier (i.e., Air India).For Travel to stations not connected by Air India, the officials may travel by Air India to the hub/point closet to their eventual destination, beyond which they may utilize the services of another airline which should also preferably be an alliance partner of Air India.
3. E-ticket is acceptable provided the amount of the fare is clearly reflected on the ticket.
4. You will submit your report and other documents in the enclosed proforma within 30 days of your return to India.
5. The claim-sheet along with all documents must be tagged/stapled properly before sending it to the Department. Otherwise, it is not possible for us to process the claim with loose documents.
6. All other expenses such as per diem, taxi fare, bus fare etc.will not be reimbursed by the Department.
7. You have to make your own arrangements for foreign exchange required for the purpose.
8. You will not be treated as a delegate sponsored by the Government of India.

Based on this offer letter, your institute may consider advancing necessary funds to enable you to attend the above event.

We request you to intimate to us within two weeks, if you are not availing this offer.

With kind regards,

Yours faithfully,

(A.B. ROY CHOWDHURY)
CONSULTANT

Encl : Claim Sheet

पी.एबक्स./PABX : 26588980, 26588707, 26589336, 26589745,
26589873, 26589414
: 011-26588662, 011-26589791, 011-26589258

तार / GRAM : विज्ञानी / SCIENTIFIC
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भारतीय आयुर्विज्ञान अनुसंधान परिषद
INDIAN COUNCIL OF MEDICAL RESEARCH

स्वास्थ्य अनुसंधान विभाग (स्वास्थ्य एवं परिवार कल्याण मंत्रालय)
वी. रामलिंगस्वामी भवन, अन्सारी नगर, नई दिल्ली - 110 029

DEPARTMENT OF HEALTH RESEARCH (MINISTRY OF HEALTH & FAMILY WELFARE)
V. RAMALINGASWAMI BHAWAN, ANSARI NAGAR, NEW DELHI - 110 029

SANDHYA DIWAKAR
Scientist- E

No.3/2/TG-4/MPD-2010
Dated: 29.1.2010

Dr.Arkendu Chatterjee,
Lecturer,
Dept. of Pharmaceutics,
Himalayan Institute, Majhitar,
Rangpo, East Sikkim, India-737136

Subject: prolong release anti-HIV microparticulate gel: In vitro and In vivo drug release study for AIDS infection in women abstract submitted to The 18th Annual congress on Women's health 2010 ,Washington DC, USA, March 26-28, 2010

Dear Sir,

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of Rs. 49,995/- (forty nine thousand nine hundred and ninety five only) to you towards air fair to attained international conference/workshop/training.

If, you are willing to avail the assistance, you may convey your acceptance within 15 days of issue of this communication, failing which it will be assumed that you are not interested to avail the grant. In the event of your not being able to utilize this amount for various reasons even after confirming your acceptance, please inform us immediately for necessary action at our end.
We have following comments to make:

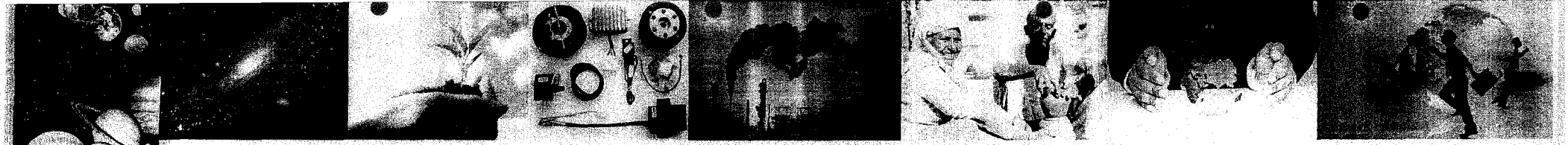
The actual amount will be reimbursed after your return from the conference and receiving the required travel documents. Please find enclosed herewith accounts proforma in which you will have to submit your claim along with a copy of:
-Award letter
-Participation certificate and copy of presented paper in the proceedings/abstract book
-Participation report and air ticket (from Air India as per Govt orders)
-Award letter from other agencies.
-Any other relevant documents

You are requested to produce the original bills/vouchers. The claim should be forwarded to us through competent authority and should reach this office within one month after the completion of the scientific conference/workshop/training.

The financial assistance is governed by the terms and conditions as mentioned in enclosed form.

Yours faithfully,
S Diwakar
Sandhya Diwakar
For Director General ICMR

Copy to: Prof. Amitava Ghosh, M. Pharma, Ph.D, Director, Dept. of Pharmaceutics,
Himalayan Institute, Majhitar, Rangpo, East Sikkim, India-737136 (Sikkim)



ষোড়শ পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেস

আয়োজক

পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি সংসদ ও দপ্তর, পশ্চিমবঙ্গ সরকার

এবং

বর্ধমান বিশ্ববিদ্যালয়, বর্ধমান

সহযোগিতায়

পশ্চিমবঙ্গ বিজ্ঞান মঞ্চ

শ্রী/শ্রীমতী এ. চট্টোপাধ্যায় .....কে

২৮ ফেব্রুয়ারি - ১ মার্চ, ২০০৯ -এ বর্ধমান বিশ্ববিদ্যালয়ে অনুষ্ঠিত ষোড়শ পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেসে ভূতত্ত্ববিজ্ঞান বিদ্যা (মৌখিক) বিভাগে শ্রেষ্ঠ গবেষণাপত্রের স্বীকৃতি স্বরূপ এই অভিজ্ঞানপত্র প্রদান করা হল।

সুব্রত পাল

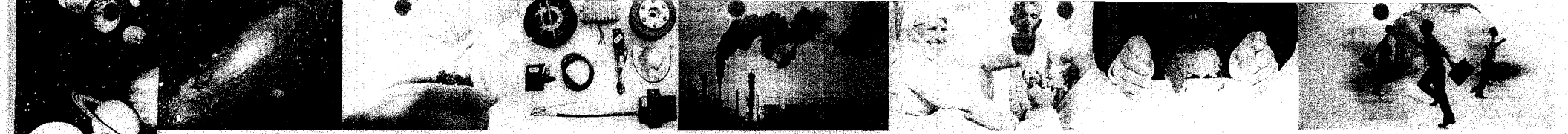
ব্রত পাল

উপাচার্য, বর্ধমান বিশ্ববিদ্যালয়

হেমন্ত কুমার মজুমদার

ডঃ হেমন্ত কুমার মজুমদার

কার্যনির্বাহী সভাপতি, পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি সংসদ,
পশ্চিমবঙ্গ সরকার এবং কার্যনির্বাহী সভাপতি,



ষোড়শ পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেস

আয়োজক

পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি সংসদ ও দপ্তর, পশ্চিমবঙ্গ সরকার

এবং

বর্ধমান বিশ্ববিদ্যালয়, বর্ধমান

সহযোগিতায়

পশ্চিমবঙ্গ বিজ্ঞান মঞ্চ

শ্রী/শ্রীমতী .....A. Chatterjee..... কে

২৮ ফেব্রুয়ারি - ১ মার্চ, ২০০৯ -এ অনুষ্ঠিত ষোড়শ পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেসে

অংশগ্রহণ ও .....Microparticulate.....Women.....শীর্ষক গবেষণাপত্র পাঠ/ পোস্টার

অধিবেশনে অংশগ্রহণের স্বীকৃতি স্বরূপ এই অভিজ্ঞানপত্র প্রদান করা হল।

সুব্রত পাল

সুব্রত পাল

উপাচার্য, বর্ধমান বিশ্ববিদ্যালয়

হেমন্ত কুমার মজুমদার

ডঃ হেমন্ত কুমার মজুমদার

কার্যনির্বাহী সভাপতি, পশ্চিমবঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি সংসদ,

পশ্চিমবঙ্গ সরকার এবং কার্যনির্বাহী সভাপতি,

ষোড়শ পশ্চিম বঙ্গ রাজ্য বিজ্ঞান ও প্রযুক্তি কংগ্রেস

First National Hands-On-Workshop

Organized by

Department of Ocular Pharmacology & Pharmacy

Dr. Rajendra Prasad Centre for Ophthalmic Sciences

All India Institute of Medical Sciences (AIIMS), New Delhi

on

"Bioanalytical Techniques in PK Studies – From Method Development to Data Interpretation"

8-9th February, 2008

Certificate

This is to Certify that

Dr. / Mr. / Ms. Arkendu Chatterjee

Attended the Hands-On-Workshop as a Participant

Prof. K K Sharma	**Prof. S Ghose**	**Dr. T Velpandian**
President, IPS-Delhi Branch	**Chief,** Dr. R P Centre, AIIMS	**Gen. Secy.,** IPS-Delhi Branch

LOUIS PASTEUR MEMORIAL SYMPOSIUM - 2008

NATIONAL SYMPOSIUM ON INFECTIOUS DISEASES

(Sponsored by Indian Council of Medical Research, New Delhi)

Department of Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore - 641 048. Tamilnadu.

Certificate

This is to certify that ~~Dr.~~ / ~~Ms.~~ / Mr. LALIT KUMAR

has participated as Invited speaker / delegate / organizer in the National Symposium on Infectious Diseases

Organized by

Department of Microbiology, Dr. N.G.P. Arts and Science College

in association with

Indian Council of Medical Research (ICMR), New Delhi

On August 8th & 9th 2008.

He / She has also presented a ~~Paper~~ / Poster / ~~Model~~ / ~~Movie~~ on Vaginal Zidovudine microcapsules for treatment of heterosexually transmitted HIV *and awarded as* BEST POSTER

Mr. N. Prabhu	**DR. S.S. Sudha**	**DR. P.R. Muthuswamy**	**Dr. Thavamani D. Palaniswami**
Organizing Secretary	Convener	Principal	Secretary

CERTIFICATE

NATIONAL SEMINAR ON

RESEARCH AND EDUCATION IN PHARMACEUTICAL SCIENCES

13th APRIL 2008

GRY INSTITUTE OF PHARMACY, VIDYA VIHAR, BORAWAN, KHARGONE (M.P.)

www.grypharmainst.org; principalgry@gmail.com

GRY INSTITUTE OF PHARMACY विद्या ददाति विनयम् KHARGONE

This certificate is awarded to Dr./Mr./Ms. ...Dharmendra Solanki.....*For*

participation as delegate and for oral/~~poster~~ presentation entitled..."Preparation & Chara

-terization of Zidovudine vaginal microcapsules" and was adjudged 'BE

Mr. Amit Roy
Convenor

Shri Subhash Yadav
Chairman, JNCET, Borawan

COLLEGE OF PHARMACEUTICAL SCIENCES, PURI

BIDYANIKETAN, MARINE DRIVE ROAD
BALIGUALI, DIST.- PURI, PIN - 752002 (ORISSA)
(Approved by A.I.C.T.E., BPUT. and Govt. of Orissa)
Phone : (06752) 251559, Mob : 98610 85889 Fax : (06752) 251559
E-mail - puri_cps@yahoo.co.in

Ref. No. ..... Date 29/6/09

TO WHOM IT MAY CONCERN

Sri Arkendu Chatterjee, Lecturer, Himalayan Pharmacy Institute, Majhitar, Sikkim, India as an invited Lecturer presented his seminar entitled **"Microencapsulated anti-HIV bioadhesive Vaginal Gel"** in our Institution, College of Pharmaceutical Sciences , Marine Drive Road, Baliguali, Puri on 29.06.09.

29/6/09

Principal
C.P.S. Puri

PRINCIPAL
COLLEGE OF PHARMACEUTICAL SCIENCES
PURI

BioConferences International, Inc.

140 Huguenot St New Rochelle, NY 10801 Tel. 914-740-2100 Fax. 914-740-2105

March 26-28, 2010

March 28, 2010

To Whom It May Concern:

This is to certify that Arkendu Chatterjee attended *Women's Health 2010: The 18th Annual Congress* in Washington, DC for the entire length of the conference on March 26th, March 27th and March 28th, 2010.

Sincerely,

Sara McCarthy
Conference Coordinator
Women's Health 2010
BioConferences International, Inc.
140 Huguenot Street, 3rd Floor
New Rochelle, NY 10801-5215
Tel: 914-740-2180
Fax: 914-740-2105
smccarthy@bioconferences.com
www.bioconferences.com

ery household has a bank account and all beneficiaries of various government-sponsored schemes get their

ing in the village on September 11, 2009. They were also explained the grievance redressal mecha-

sitized on the salient features of genuine Indian currency notes. The pamphlets and the booklets were also distrib-

rency notes, comic booklet 'Money Kumar' and RBI's Platinum Jubilee caps, said the release.

HIV/AIDS awareness camp held

SE Report

GANGTOK, September 20: The Red Ribbon Club of Himalayan Pharmacy Institute, Majhitar, Rangpo organized its first programme on HIV/AIDS Prevention and Awareness Camp on September 18 sponsored by Sikkim State Aids control Society (SSACS).

A press release informs that the students were the major participants to bring awareness among them and to help eradicate this disease from the society. The guest speakers were Dr. DSK Kerongi, Chung Chung La Bhutia, Consultant, Youth Affairs and Namrata Sharma from SSACS. The programme was presided over by the College Principal Dr. Amitava Ghosh and seminars on AIDS were presented by senior faculty members of the institutions, said the release.

It was also informed that a blood donation camp will be organised by the Red Ribbon Club in the third week of November in the College campus in collaboration with SSACS.

Simple wa

Washington, September 20: As swine flu continues to infect school kids, parental anxieties have stirred up with each one trying to be cautious enough to avoid the spread of HINI virus that has caused severe illness and deaths worldwide.

Dr Galit Holzmann-Pazgal assistant professor of pediatrics at The University of Texas Medical School at Houston suggest parents can ease their anxieties by arming themselves with facts about H1N1 and using some commonsense tips.

***Wash your hands after you touch...and Twitter**

Washing your hands is the single-most important step to prevent the spread of H1N1. The virus is spread by droplets from coughs and sneezes as well as touching hands and objects contaminated with these droplets such as each other"s phones, computer keyboards, iPods and

Not enough evidence yet to

ISSN: 0975-8232

International Journal of Pharmaceutical Sciences and Research

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To,

Mr. Arkendu Chatterjee

Department of Pharmaceutics,

Himalayan Pharmacy Institute,

Majhitar, Rangpo,

East-Sikkim (SIKKIM), India

This is to certify that [redacted] has published a research article entitled [redacted] in Vol. 1, Issue 3, March, 2010 of online International Journal of Pharmaceutical Sciences and Research.

This certificate is issued only after considering the originality and authenticity of the manuscript submitted by the author to the International Journal of Pharmaceutical Sciences and Research. This certificate is provided for informative purpose only on behalf of his article publication in IJPSR.

Acknowledgement no: IJPSR/RA-142/01-10

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Web site: www.ijpsr.com

Regards,

EDITOR
IJPSR