Stomach-Specific Drug Delivery of 5-Fluorouracil Using Floating Alginate Beads

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Shishu,[1] Neeta Gupta,[1] and Nidhi Aggarwal[1]

[1]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India

ABSTRACT

A multiple-unit-type oral floating dosage form (FDF) of 5-fluorouracil (5-FU) was developed to prolong gastric residence time, target stomach cancer, and increase drug bioavailability. The floating bead formulations were prepared by dispersing 5-FU together with calcium carbonate into a mixture of sodium alginate and hydroxypropyl methylcellulose solution and then dripping the dispersion into an acidified solution of calcium chloride. Calcium alginate beads were formed, as alginate undergoes ionotropic gelation by calcium ions and carbon dioxide develops from the reaction of carbonate salts with acid. The evolving gas permeated through the alginate matrix, leaving gas bubbles or pores, which provided the beads buoyancy. The prepared beads were evaluated for percent drug loading, drug entrapment efficiency, image, surface topography, buoyancy, and in vitro release. The formulations were optimized for different weight ratios of gas-forming agent and sodium alginate. The beads containing higher amounts of calcium carbonate demonstrated instantaneous, complete, and excellent floating ability over a period of 24 hours. The optimized formulation was subjected to in vivo antitumor studies to check the therapeutic efficacy of the floating dosage forms containing 5-FU against benzo(a)pyrene-induced stomach tumors in albino female mice (Balb/C strain). The multiple-bead FDF was found to reduce the tumor incidence in mice by 74%, while the conventional tablet dosage form reduced this incidence by only 25%. Results indicate that FDF performed significantly better than the simple tablet dosage form.

KEYWORDS: 5-Fluorouracil, floating dosage form, calcium alginate beads, gastric residence time, buoyancy.

INTRODUCTION

Gastric emptying is a complex process that is highly variable and makes the in vivo performance of drug delivery systems uncertain. To overcome this physiological problem, several drug delivery systems with prolonged gastric retention time have been investigated.[1-3] Attempts are being made to develop a controlled drug delivery system that can provide therapeutically effective plasma drug concentration levels for longer durations, thereby reducing the dosing frequency and minimizing fluctuations in plasma drug concentration at steady state by delivering drug in a controlled and reproducible manner.[4] Lately, a wide variety of both natural and synthetic hydrophilic polyionic systems like alginates have been investigated for preparation of multiple-unit floating dosage forms (FDFs).[5]

In the present study, a multiple-unit FDF was designed, keeping in view the "all or nothing" response of single-unit systems.[6] Literature reports indicate widespread use of sodium alginate for achieving sustained release of drugs,[7,8] targeting gastric mucosa,[9,10] and increasing the bioavailability of drugs[11] because of sodium alginate's ability to form a stable and bioadhesive gel with calcium ions.[12] Hydroxypropyl methylcellulose (HPMC) has been reported to enhance the sustained-release properties of alginate by providing a denser inner matrix.[13] Also, the preparative methodology of alginate beads involves the use of aqueous solvents, avoiding exposure of ingredients to high temperatures and toxic organic solvents.[14,15] Moreover, the resulting preparation is nonimmunogenic, with bioadhesive properties that could serve as a potential advantage in stomach targeting.

One of the major antimetabolites used in a variety of solid cancers, such as stomach, colon, lung, and breast cancer, is 5-fluorouracil (5-FU).[16] It is usually given intravenously, as absorption of 5-FU from the gastrointestinal tract is erratic and unpredictable. The intravenous route of administration is associated with severe systemic side effects because of 5-FU's cytotoxic nature when it reaches unwanted sites. After oral administration, gastrointestinal absorption is rapid, and peak levels in the blood are reached between 15 and 60 minutes after ingestion, but much variability is seen between individuals because of first-pass metabolism in the liver. After intravenous administration, the drug diffuses equally in all the compartments in a volume equivalent to the body fluid volume. Peak plasma levels are reached within minutes, and plasma half-life is 10 to 20 minutes. The drug, despite low lipid solubility, enters the cerebrospinal fluid and the brain.[17] Therefore, a stomach-specific multiple-unit FDF of 5-FU has been prepared to reduce its unwanted side

Corresponding Author: Shishu, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014. Tel: ++91-172-2534112; Fax: ++91-172-2541142; E-mail: shishugoindi@yahoo.co.in

Table 1. Formulation Variables and Evaluation Parameters of Various 5-FU Floating Bead Formulations*

Formulation Code	Calcium Carbonate: Sodium Alginate (wt/wt)	Mean Surface Diameter (mm)	Drug Loading (%)	Drug Entrapment Efficiency (%)	*Flotation Property	Duration of Flotation (h)
N_1	0:1	1.41 ± 0.07	45.35	86.45	–	–
N_2	0.25:1	1.45 ± 0.09	45.00	83.41	+–	–
N_3	0.5:1	1.52 ± 0.08	43.20	79.96	++	24
N_4	0.75:1	1.69 ± 0.08	40.98	75.40	++	24
N_5	1:1	1.81 ± 0.09	39.80	71.85	++	24

*5-FU indicates 5-fluorouracil; ++ completely float; – completely sink; +– partially sink or float.

effects at other sites by localized and sustained delivery to gastric tumors. This stomach-specific oral delivery may serve as an alternative to inconvenient and painful conventional intravenous therapy.

Materials and Methods

Materials

The 5-FU was a gift from Biochem Pharmaceutical Industries (Mumbai, India), and HPMC K15M was a gift from Colorcon Asia Pvt Ltd (Mumbai). Sodium alginate was purchased from SD Fine Chem Ltd (Mumbai). All other reagents and chemicals used were of analytical grade.

Preparation and Evaluation of Floating Beads of 5-FU

Exactly 0.2 g of 5-FU was dissolved in 15 mL of distilled water. This solution was dispersed in 12.5 mL of 1.6 wt/vol alginate solution containing HPMC K15M (alginate:HPMC = 9:1 wt/wt). Then, the gas-forming agent calcium carbonate ($CaCO_3$) was added to the solution in weight ratios ranging from 0:1 to 1:1 ($CaCO_3$:alginate wt/wt). The resulting solution was dropped through a 26-G syringe needle into 50 mL of calcium chloride solution (15% wt/vol) containing 10% vol/vol acetic acid.[5] The beads were allowed to remain in the same solution for 2 hours to improve their mechanical strength. The formed beads were separated, washed initially with ethanol and subsequently with distilled water, and then freeze-dried. Table 1 lists the formulation variables for different formulations of 5-FU-loaded floating beads. Blank beads without 5-FU were also prepared using the same technique.

Evaluation of Floating Beads

The prepared beads were evaluated for percent drug loading and drug entrapment efficiency. An accurately weighed sample of beads (10 mg) was crushed in a mortar and added to 10 mL of Sorenson's phosphate buffer pH 7.4. This mixture was centrifuged at 4200 rpm for 30 minutes, filtered, and analyzed spectrophotometrically at λ_{max} 266 nm against buffer as blank. Blank beads were treated similarly. The percent drug loading was calculated by dividing the amount of drug in the sampled beads by the weight of beads.[18] The particle size and the size distribution of beads were determined in the dry state using the optical microscopy method. The mean surface diameter was calculated arithmetically. The external and cross-sectional morphology of beads was characterized by scanning electron microscope (SEM).

Dissolution Studies

In vitro dissolution studies were performed for all the formulation combinations in hexaplicate using US Pharmacopeia XXIII Dissolution Apparatus II (paddle type). An accurately weighed sample (40 mg) of floating alginate bead formulations N_1 to N_5 (containing 16-18 mg of active drug) was dropped into 900 mL of HCl buffer pH 1.2 maintained at a temperature of 37ºC ± 0.5ºC and stirred at a speed of 50 rpm. At different time intervals, a 10-mL aliquot of the sample was withdrawn and the volume was replaced with an equivalent amount of plain dissolution medium kept at 37ºC. The collected samples were filtered and analyzed at λ_{max} 266 nm using a UV-visible spectrophotometer against HCl buffer pH 1.2 taken as blank. Drug release data were corrected for the values of the drug loss during sampling. The effect of different weight ratios of $CaCO_3$ on the release rate of 5-FU from the beads was also determined. Dissolution studies with placebo beads were performed in a similar fashion.

Floating Properties

The time between the introduction of the FDF into the medium and its buoyancy to the upper one third of the dissolution vessel (buoyancy lag time) and the time for which the formulation constantly floated on the surface of the medium (duration of buoyancy) were measured simultaneously as a part of dissolution studies.[5]

Antitumor Activity

Albino female mice (Balb/C strain) aged 8 to 9 weeks old weighing 20 to 30 g were used. The animals were kept under

a standard 12/12 light/dark cycle and were given food and water ad libitum. The animals were administered 2 doses of 3 mg of benzo(a)pyrene [B(a)P] in 0.25 mL of corn oil orally with 2 weeks between doses. The vehicle alone in an equal quantity was administered to the control group.[19] The B(a)P-treated mice were divided into 3 groups (n = 10): 5-FU treatment, FDF of 5-FU treatment, and no treatment. The treatment groups were administered 20 mg/kg of the drug in a corn oil suspension or equivalent (in the case of FDF) orally for 5 consecutive days followed by 2 drug-free days. The dosage regimen was repeated till the end of the experiment.[19]

Tumor Determination

Animals were sacrificed by cervical dislocation 10 weeks after the last dose of B(a)P. The presence of stomach tumors was determined as described by Wattenberg.[20] The forestomach was separated and was cut longitudinally and fixed in 10% buffered formalin–phosphate. Stomach papillomas measuring 1.0 mm or larger were counted using a magnifying glass.[19,21] All the data were statistically analyzed by 1-way analysis of variance followed by Fischer's least square difference (LSD) test.

Results and Discussion

Drug Loading and Drug Entrapment Efficiency

The percent drug loading of various 5-FU bead formulations ranged between 39.8% and 45.35% (the active drug content varied between 4 and 4.5 mg in a 10-mg sample). The entrapment efficiency for various 5-FU floating bead formulations was found to vary between 71.85% and 86.45% (Table 1). It was observed that an increase in the ratio of $CaCO_3$:alginate from 0:1 to 1:1 resulted in a decrease in the entrapment efficiency of 5-FU in floating beads.[22] The beads without $CaCO_3$, because of the highly dense internal structure of the alginate matrix, were able to retain 5-FU more effectively. During the preparation of beads, $CaCO_3$ reacts with acetic acid to release carbon dioxide, which permeates the alginate matrix, leaving pores. These porous beads, with a less dense internal structure, result in decreased entrapment efficiency of the drug.

Particle Size Analysis

The formed beads were almost spherical. The mean particle size of 5 formulations was between 1.41 ± 0.07 and 1.81 ± 0.09 mm. It was found that the particle size distribution of each formulation was within a narrow range. Different weight ratios of $CaCO_3$ to alginate were used to determine the effect of the gas-forming process on the size of the floating beads (Table 1). It was observed that an increase in the proportion of $CaCO_3$ (0:1 to 1:1) led to an increase in the size of beads. Also, when $CaCO_3$ was added to the alginate solution in a ratio of 1:1, spherical beads could not be obtained because evolved carbon dioxide caused bursting of the beads before their walls were sufficiently hardened.

Surface Characterization

The surface and cross-sectional SEM pictures for different formulations of floating beads are shown in Figures 1 to 3. The beads without $CaCO_3$ possessed a rough surface (Figure 1A). However, incorporation of $CaCO_3$ resulted in the formation of smooth beads (Figures 2A and 3A). The addition of Ca^{2+} ions might have contributed to the homogenous alginate bead formation. In fact, $CaCO_3$ has been reported to be used as a gelling agent to aid the internal gelation of the alginate.[12] In spite of improved gelation, cracks were observed on the surface of formulation N_5,

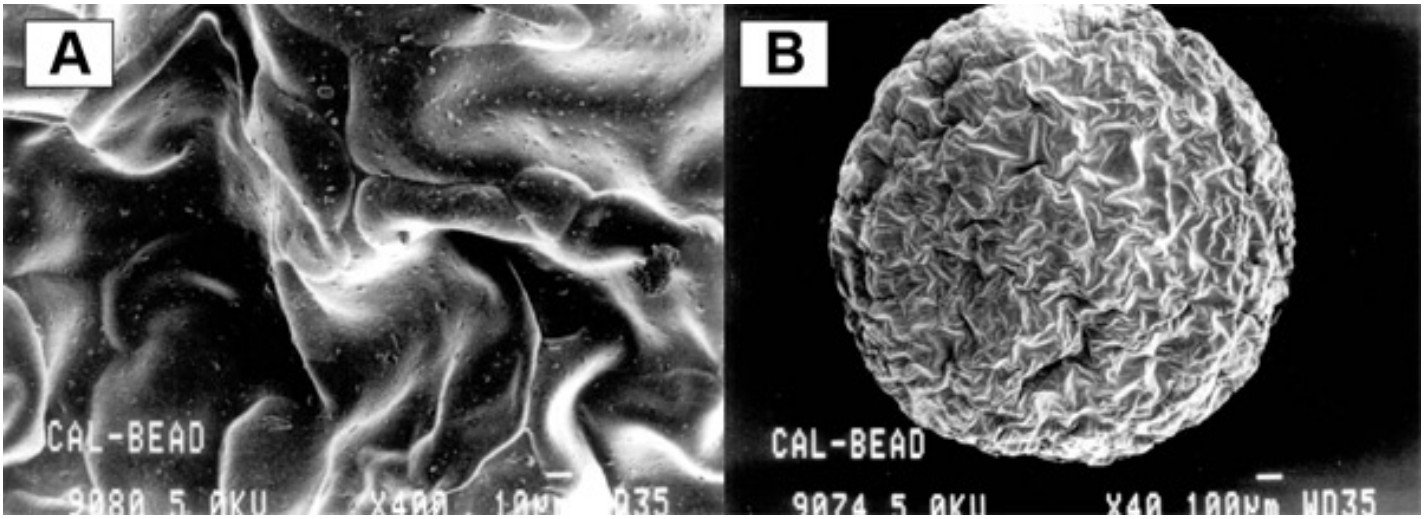

Figure 1. Scanning electron microscopy photomicrographs of formulation N_1 (without calcium carbonate): (A) surface morphology, (B) cross-section.

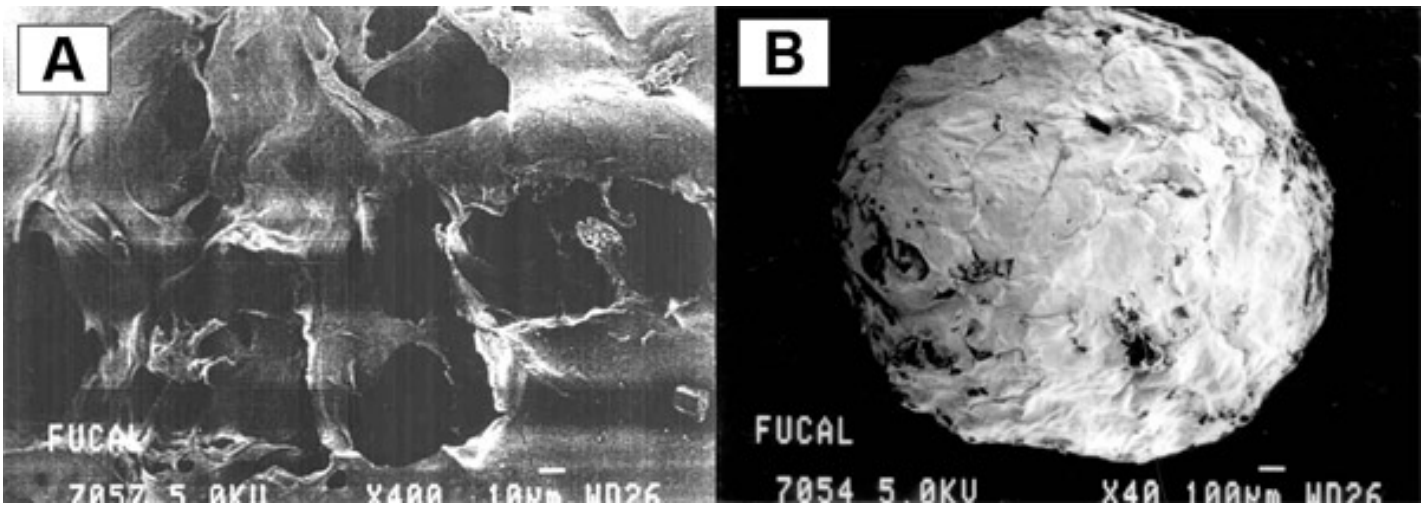

Figure 2. Scanning electron microscopy photomicrographs of formulation N_3: (A) surface morphology, (B) cross-section.

containing 1:1 $CaCO_3$ and alginate (Figure 3B). These cracks may have been formed because of the bursting effect of the larger amounts of carbon dioxide before the walls were sufficiently hardened. The cross-sectional morphologies of floating beads were also examined with SEM (Figures 1B, 2B, and 3B). Many large closed pores were present in the alginate gel matrix. The number of observed pores appeared to be directly related to the amount of incorporated $CaCO_3$.

Dissolution Studies

Drug release data were analyzed using ZOREL software[23] after correcting the values for the drug loss that occurred during sampling. Based primarily on the algorithms proposed by Peppas and Sahlin,[24] the software reports the values of the release exponent (n) indicating the kinetics of drug release, the kinetic constant (k), the magnitudinal contributions of the Fickian diffusion (k_1), and the polymer relaxation (k_2). The various dissolution parameters computed for all the formulations are listed in Table 2. The values of the release rate exponent calculated as per the algorithm proposed by Peppas and Sahlin ranged between 0.378 and 0.561. In the case of porous floating bead formulations (N_2-N_5) containing a gas-forming agent, the value of the release rate exponent was less than 0.5, confirming Fickian diffusion. Also, negligible values of k_2 clearly indicate that the drug release was predominantly through Fickian diffusion. The formulation N_1 (free from $CaCO_3$), with a highly dense external structure of alginate, exhibited non-Fickian release.

Figure 4 exhibits the in vitro release profiles obtained for various formulations. The values of $t_{50\%}$ were increased markedly, from 10 minutes for the control formulation to as high as 9.55 hours, observed for floating beads at varying levels of the polymer. This finding indicated that there was considerable release-retarding potential of the polymer for 5-FU. An initial rapid release was seen in all floating bead

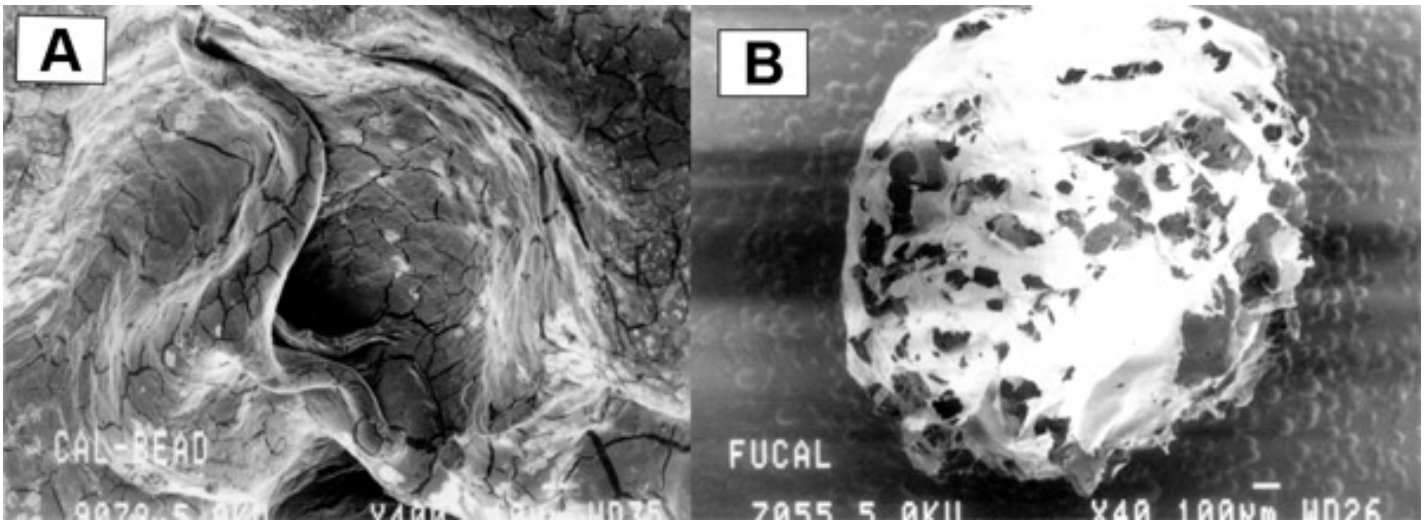

Figure 3. Scanning electron microscopy photomicrographs of formulation N_5: (A) surface morphology, (B) cross-section.

Table 2. Drug Release Parameters of Various 5-FU Floating Bead Formulations*

Formulation Code	Release Exponent (n)	Kinetic Constant (k)	Fickian Diffusion Constant (k_1)	Polymer Relaxation Constant (k_2)	$t_{50\%}$ (h)	$t_{80\%}$ (h)	Overall Rate of Drug Release (mg/h) Mean ± SD (n = 6)
Control (plain 5-FU)	—	—	—	—	0.003	0.310	31.88 ± 16.54
N_1	0.561	0.141	1.127	0.025	9.547	21.643	1.35 ± 0.04
N_2	0.501	0.174	1.176	0.015	8.213	20.994	1.56 ± 0.09
N_3	0.451	0.236	1.275	0.000	5.305	15.056	1.86 ± 0.04
N_4	0.419	0.263	1.330	0.000	4.632	14.203	1.96 ± 0.07
N_5	0.378	0.302	1.399	0.000	3.784	13.114	2.31 ± 0.06

*5-FU indicates 5-fluorouracil.

formulations, presumably because of the rapid release of 5-FU from the surface. Freeze-dried beads disintegrate more readily in the dissolution tester because of increased water uptake. In addition, dry beads are more fragile and susceptible to shear stress from rotating paddles.[5,25] The formulation N_1 (free from $CaCO_3$), because of more effective entrapment of drug in the highly dense external structure of alginate, exhibited a slow and extended release of 1.35 ± 0.038 mg/h and $t_{80\%}$ = 21.64 hours, but these beads were nonbuoyant. The gas-forming agent was added in varying amounts to make these beads float in the medium. It was observed that with increasing weight ratios of $CaCO_3$: alginate, from 0.25:1 (N_2) to 1:1 (N_5), there was an increase in buoyancy (Table 1) as well as in the rate of drug release when compared with formulation N_1 (without the gas-forming agent).

When compared with control (plain drug), a delay in percent drug release ($t_{50\%}$ and $t_{80\%}$) with increased $CaCO_3$ concentration was observed. This may be attributed to the internal ionotropic gelation effect of $CaCO_3$. The gas-forming agent is present as an insoluble dispersion in neutral pH aqueous alginate solution; however, in acidic media, the $CaCO_3$ becomes water-soluble. The ionized Ca^{2+} ions then promote internal gelation by cross-linking with the alginate carboxyl group.[26]

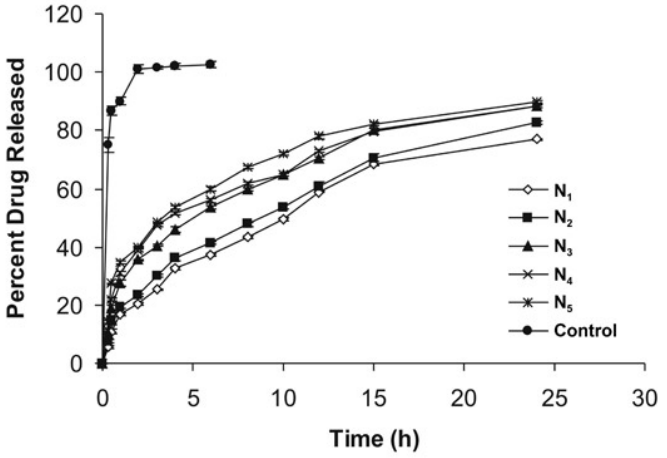

Figure 4. In vitro drug release profile of percent drug released vs time for different formulations of 5-fluorouracil floating beads.

The formulation N_3 ($t_{80\%}$ = 15.06 hours) was observed to be optimum with respect to floating ability and extended release and was used in further antitumor studies.

Floating Properties

The floating ability of the prepared beads was evaluated along with dissolution studies (Table 1). The beads without $CaCO_3$ (N_1) sank immediately in HCl buffer pH 1.2, while beads containing $CaCO_3$ (N_3-N_5) demonstrated instantaneous and excellent floating ability. Thus, floating ability was found to be directly related to the gas content of the polymer matrix, which might have imparted buoyancy to the formulations for a sufficient time period.

Antitumor Activity

The studies indicated that treatment of mice with B(a)P resulted in 100% incidence of forestomach tumors after 10 weeks with an average of 1.78 tumors per mouse compared with corn oil–treated control animals.

The treatment of mice with plain 5-FU and FDF of 5-FU (N_3) after the last dose of B(a)P (ie, during the initiation period) resulted in 25% and 74% reduction in tumor incidence (percentage of number of mice with tumors), respectively (Figure 5). The number of tumors per tumor-bearing mouse was reduced to 24% and 44%, respectively. The statistically significant ($P < .05$) reduction in the number of tumors obtained with formulation N_3 as compared with control and plain 5-FU treatments indicates site-specific delivery of 5-FU through floating dosage forms.

These results can be correlated with in vitro release studies of 5-FU from floating beads, where an extended release up to 24 hours was observed. Maintenance of a local concentration of 5-FU for a longer period of time from floating

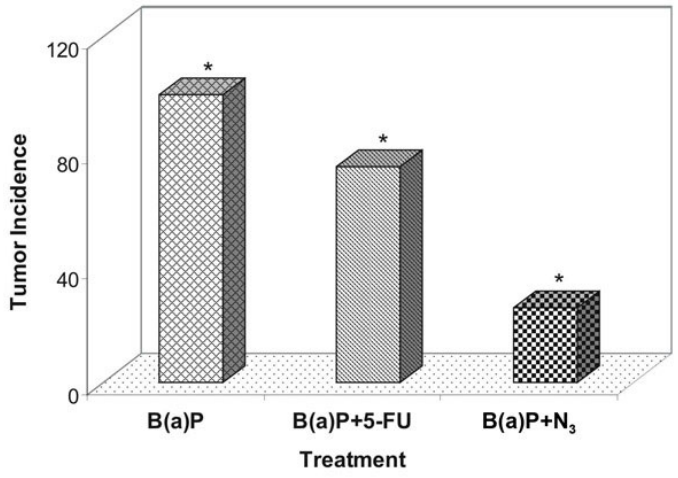

Figure 5. Comparison of tumor incidence for different treatment groups. B(a)P indicates benzo(a)pyrene; 5-FU, 5-fluorouracil. $*P < .05$.

beads in the stomach might sustain therapeutic levels to treat stomach tumors more effectively than with pure 5-FU.

CONCLUSION

In the present work, multiple-unit floating beads of 5-FU were formulated to provide sustained release of drug with a view to providing an effective and safe therapy for stomach cancer with a reduced dose and reduced duration of therapy. The formulation N_3 exhibited the optimum sustained release of 5-FU, with excellent floating properties. Also, in vivo antitumor studies confirmed that the overall rate of tumor incidence and number of tumors/mouse was less in the animal group treated with FDF of 5-FU than in the animal group treated with pure 5-FU in the B(a)P-induced tumor model of mice. Therefore, the floating-type gastroretentive dosage form of 5-FU may be better for treating gastric tumors.

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