In vitro release characteristics of 5-fluorouracil from Aloe vera gel /guar gum blend as a carrier matrix

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Aloe vera gel (AVG) which is increasingly used as an excipient in sustained release pharmaceutical dosage forms due to its health benefits is sporadically studied as a drug carrier for controlled delivery. Guar-gum (GG) used for colon specific delivery is not a desirable matrix for the sustained release of 5-Fluorouracil (5-FU) in stomach and small intestine (SI) for treatment of stomach cancer as only 2% of 5-FU is released. Hence AVG is investigated as a co-carrier with GG to facilitate a sustained release of 5-FU in stomach in the present study. GG is solution blended with AVG in different proportions to achieve sustained release of 5-FU, in simulated biofluids. FT-IR, TG and DSC studies reveal only a mild physical interaction of the drug with the matrix through weak hydrogen bonding and there is no drug-matrix chemical interaction. The temperature of major weight loss degradation decrease both on the addition of drug and AVG. The in vitro release characteristics of the drug imply that addition of AVG to GG facilitate sustained release of 5-FU in stomach and SI due to enhanced matrix swellability. Blend composition and pH of the fluid control the release kinetics and the mechanism of release is found to be non-Fickian. The study demonstrates that AVG/GG blend of appropriate composition may serve as a promising matrix for the sustained release of 5-FU in stomach and SI which is difficult with pristine GG and AVG

Keywords: Aloe vera gel, Composite matrix, FT-IR, Guar gum, Sustained 5-Fluorouracil release

Synthetic and natural biocompatible/biodegradable polymers are widely used as drug carriers for controlled release. But synthetic polymer carriers may not impart any health benefits other than as matrix for controlled and targeted release of drugs. Although synthetic polymers are effective, the possibility of side effects imposes restrictions on their acceptable use and dose limits. Thus, a new drug carrier system that is safe to handle and free from side effects is very much in need, and food and medical grade polysaccharides stand high as worthy alternatives. The use of naturally occurring biocompatible hydrophilic polymers such as gums, mucilages etc., have been the focus of recent research activity in the design of dosage forms for oral controlled release administration\(^1\) and they are widely used because of their flexibility to provide a desirable drug release profile, cost effectiveness, and broad regulatory acceptance. Natural gums (guar gum) and mucilages (Aloe vera gel) are the sources for water soluble polysaccharides of medicinal values and are used in pharmaceutical formulations, as emulsifiers, suspending agents in tablets, binders, sustaining agents in modified release tablets due to its high hydration rate and safety. Guar gum, a non-ionic polysaccharide and its derivatives are used as binder and disintegrator in tablets to add cohesiveness to drug powder, form a protective layer and facilitate sustained release of drug with the desired kinetics effect. It masks the unpleasant taste and odour of drug and improves its stability and eliminates the danger to health and environment. The role of guar gum and its derivatives to control blood sugar is well known\(^3\). Guar gum is used in the treatment of diarrhea, irritable bowel syndrome and obesity, and in prevention of atherosclerosis. It helps to curb the appetite, as well as to bind and remove toxins from the body and brings down the cholesterol level. Guar gum is a well-known drug carrier for controlled drug delivery system (CDDS) via its enzymatic breakdown at colon\(^4\)\(^-\)\(^9\). In addition, CDDS would be advantageous when delay in absorption is desirable from a therapeutical point of view, such as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis\(^10\)\(^,\)\(^11\). Mucilages like AVG which is innocuous for tropical applications and oral use possesses a wide spectrum of health benefits such as wound healing, the abilities to penetrate and anesthetize tissue, preclude bacterial,
fungal and viral growth, anti-inflammatory agent, blood flow enhancer etc., and is being used in various liquid and solid medicine formulations. It is reported to be used for the treatment of cancer, tumor etc. It enhances the bioavailability of vitamin C and E by protection against degradation and by binding with polysaccharides.

Polysaccharide of natural origin enhances the intestinal absorption of co-administered drugs by transient opening of the tight junctions between adjacent epithelial cells to allow for paracellular transport across the intestinal epithelium. AVG has been reported to be a safe and effective absorption enhancer in vivo. The mucilage produced direct compressible matrix tablets had showed good swelling and sustained release of the model drugs. It is reported that addition of AVG to other natural polymers such as chitosan improved the mechanical properties of composite film. Due to the aforementioned health benefits of GG and AVG, the objective of the present investigation involves the characterization and evaluation of these polysaccharide rich plant materials as drug carriers in the blend form for controlled release of drugs with simultaneous harvesting of their inherent health benefits taking 5-FU as typical model drug. It is the drug of choice in the treatment of carcinoma of stomach, colon, rectum, breast, ovary and urinary bladder. 5-FU is toxic and hence the drug release rate has to be reduced to minimize side effect. But the high water solubility of AVG will promote a high drug release rate with 100% AVG as carrier. With 100 % GG as a drug carrier has the inherent drawback of very slow drug release rate in aqueous medium to meet the minimum dose requirement at stomach and intestine. Hence in the present investigation an attempt is made to add AVG as a co-carrier in guar gum core matrix as blend to facilitate a sustained slow release of 5-FU. The GG /AVG blend as a carrier will be evaluated by in vitro drug release kinetics.

Experimental Section

Materials

Healthily grown aloe vera leaf was collected from our own medicinal plant field. GG (Himedia), disodium hydrogen orthophosphate, monosodium dihydrogen orthophosphate (SD fine), hydrochloric acid and sodium chloride (Rankem), pepsin (Loba), potassium bromide (Merek) were used as received. SGF (pH 1.2) and SIF (pH 7.2) were prepared as per United States Pharmacopoeia. The model drug 5-FU (>99%, Himedia) were used as received. The structure of the drug is shown in Fig. 1(a).

Preparation of AVG/GG blend

AVG has partially acetylated glucomannan (acemannan also known as carrysin) responsible for mucilage like and medicinal properties. It has the backbone of β-(1→4)-D-mannosyl residues acetylated at the C-2 and C-3 positions that exhibit a mannose monomer: acetyl ratio of approximately 1:1. It contains galactose side chain attached to C-6. The repeating units of glucose and mannose exist in a ratio of 1:3 and the approximate molecular weight is 30-40 kDa. The chemical structure of polysaccharide present in AVG is shown in Fig. 1(b). GG has straight chains of α-D-mannopyranosyl (M) units linked together by β-D-(1-4)-glycosidic linkage with α-D-galactopyranose (G) linked along this chain. These side groups appear on both sides of the mannose chain at about every other mannose link in the main chain. The ratio of mannose to galactose in galactomannan of GG is approximately 2:1. The reported molecular weight of GG was in the range of 200 to 300 kilo Daltons and the chemical structure of GG is shown in Fig. 1(c).

Freshly harvested aloe vera leaves were washed with millipore water and the outer part of the leaf was removed to collect the highly hydrated gel. The gel was washed with distilled water twice to remove greenish latex and it was homogenized at 400 rpm. A known volume of homogenized AVG was taken in a weighed petridish and dried at 50°C to constant weight. From this weight and volume data a known volume of homogenized AVG corresponding to the required weight of AVG powder was taken and added to a known weight of guar-gum in a stainless steel (SS) vessel and blended by vigorous mechanical stirring for 45 min to get an homogeneous blend. Then it was allowed to air drying under ambient conditions for two days and then at 50°C under vacuum for four days to get a solid bone dried mass which was then fine powdered in a mortar and pestle, and sieved (mesh size: 150 micron). The AVG /GG blend powder is then stored under refrigeration for subsequent drug delivery applications. Typical blend ratios of AVG/GG prepared were 2/98, 5/95, 10/90, 25/75, 50/50, 75/25 and the corresponding blends were designated as AG-2, AG-5, AG-10, AG-25, AG-50 and AG-75 respectively.
UV-Visible spectrophotometer
UV spectra of pure drug and the drug released from tablet during release studies were recorded for the spectral width 200-400 nm on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer (UVWINLAB software). The released 5-FU from tablet during in vitro drug release studies was estimated by measuring the absorbance of the drug solution at 266 nm ($\lambda_{max}$).

Fourier Transform-Infrared (FT-IR) spectroscopy
FT-IR spectra of dry polymer samples, drug and drug-loaded polymer were recorded on a KBr pellet for the spectral width 400 to 4000 cm$^{-1}$ using Shimadzu FT-IR-8400S at a resolution of 2 cm$^{-1}$ by accumulating 48 scans.

Thermogravimetry (TG)
TG/DTG studies were performed on TGA Q500 V20.10 Build 36 with a sample size of 1.5-3.5 mg under nitrogen atmosphere at a heating rate of 10°C/min for the temperature range from ambient to 800°C.

Differential Scanning Calorimetry (DSC)
DSC thermograms of the dry AVG, GG, drug-loaded AVG/GG blend and the drug were recorded on a Perkin-Elmer Pyris 6DSC model in nitrogen atmosphere at a heating rate of 10°C per min for the temperature range 35 to 200°C.

X-ray diffraction (XRD)
X-Ray diffractogram of drug carrier was recorded on a Shimadzu XRD-6000 diffractometer with Cu Kα radiation operated at the voltage and current values of 40 kV and 30 mA respectively for the 20 values in the range 5-90° at a scan speed of 10°/min.

Tablet preparation
In a typical tablet (2.5 mm thickness and 13 mm diameter) formulation, 200 mg of the homogenized, dried, powdered and sieved blend of GG and AVG powder was mixed with 35 mg of 5-FU and ground in a mortar and pestle to ensure homogeneity of mixing and then compressed at a force of 5 ton using a KBr press (Techno Search M15). The prepared tablet was also weighed using a five decimal electronic balance (Mettler Toledo AB265-S). The tablets made from AG-2, AG-5, AG-10, AG-25, AG-50, AG-75, GG and AVG were designated as AGF-2, AGF-5, AGF-10, AGF-25, AGF-50, AGF-75, GGF and AVGF, respectively. GG and AVG pellets without drug were also made similarly for swelling studies.

Fig. 1 — Chemical structure of (a) 5-Fluorouracil (b) acemannan present in aloe vera gel and (c) guar gum
**Swelling studies**

Swelling studies were performed in both SGF and SIF (PBS) at 37°C on the pellets (200 mg, 2.55 mm thickness and 13 mm diameter) of AVG-GG powder blend. The pellet was kept in a stainless-steel (SS) cylindrical mesh (30 mm diameter; 50 mm height) immersed in 20 mL of SGF or SIF taken in a 25 mL beaker and allowed to swell. The weights of the swelled AVG-GG powder blend as pellet at predetermined time intervals were calculated after wiping the mesh containing the swelled polymer with a tissue paper. Then a graph was drawn between the degree of swelling (= (W_t – W_0)/W_0) and time, where W_t and W_0 are the weights of the blend after and before swelling, respectively.

**In vitro drug dissolution study**

In vitro drug dissolution studies were performed in an USP apparatus Type II (Veego Model VDA-6DR) in SGF and SIF at 37°C by embarking the compressed tablet inside the rotating (50 rpm) SS basket immersed in a thermostated biological fluid. The tablets maintained their integrity and shape during the swelling time >2 h. The amount of drug released was estimated UV spectrophotometrically by withdrawing aliquots of sample from the drug release vessel at different known time intervals and measuring their absorbance values at 266 nm. An average of three identical experiments was taken to determine the amount of drug released for a given set of experimental conditions. To maintain constant volume of the experimental solution a volume equivalent of aliquot sample as incubated fresh fluids was added to the solution after each withdrawal.

**Results and Discussion**

**FT-IR studies**

The FT-IR spectra of GG, AVG, 5-FU, AVG/GG blend were studied. Both GG and AVG have mannose and galactose moieties in their chemical structure respectively. In addition, the absorptions in the region 1350-1450 cm\(^{-1}\) were due to symmetrical deformations of CH\(_2\) and C-OH groups. The bands at 1080 and 1020 cm\(^{-1}\) were more likely due to the primary alcoholic –CH\(_2\)OH stretching mode and >CH\(_2\) twisting vibrations respectively. The band around 890 cm\(^{-1}\) can be attributed to the C–H ring vibration (deformation). The weak bands around 770 cm\(^{-1}\) were due to ring stretching and ring deformation of α-D-(1-4) and α-D-(1-6) linkages. In AVG the band around 1065 cm\(^{-1}\) and at 1033 cm\(^{-1}\) were due to C-O-C stretching in mannopyranose component and glucan units respectively. Specifically, the peaks at 1740 and 1250 cm\(^{-1}\) ascertain the presence of o-acetyl ester. Comparison of FT-IR spectra of GG and AVG with that of blend and drug loaded blend indicated a shift in the O-H band stretching, broadening of peaks around 1500 and 1100 cm\(^{-1}\) indicating possible interaction between AVG-GG.

**TG analysis**

TG thermograms of AVG, GG, AVG-GG blend and AVG-GG blend with 5-FU and their DTG traces were studied. In all the samples the weight loss around 100°C was attributed to the residual moisture. Analysis of the thermograms also implied that AVG is thermally less stable than pure GG. But addition of AVG to GG had only marginally affected the thermal stability of GG. Moreover 5-FU loaded AVG-GG blend was moderately more stable than AVG-GG blend as indicated by the onset degradation temperature perhaps through weak interaction such as H-bond. These observations corroborate the weakening of intermolecular interactions in GG by the hydrophilic AVG in AVG-GG. Moreover, the major weight loss temperature decreased in the order GG>AG-25>AGF-25. This may be attributed to the reduction in the H-bonding network in GG by the addition of AVG and 5-FU. These thermal features also implied that there is no chemical interaction between AVG-GG-5-FU.

**DSC studies**

The DSC traces of GG, GG-AVG and AVG-GG-5-FU were studied. The broad endotherms around 80-110°C in these traces were attributed to moisture loss. Comparison of DSC traces of GG and AVG also revealed that the moisture in AVG is strongly bound than that in GG as the corresponding endotherm is shifted towards right in AVG compared to GG. The transition around 160°C in AVG may be due to glass transition and this had broadened when it was mixed with GG and 5-FU. This again supported that the interaction among AVG-GG-5-FU was physical through weak forces such as H-bonding.

**XRD analysis**

XRD patterns of AVG, GG, 5-FU tablet and swelled AVG/GG revealed that these polymers were predominantly amorphous and AVG was more amorphous than GG. Addition of AVG to GG increased the amorphous character. It was anticipated
that the increased amorphous character may decrease the chain flexibility.

Swellability of AVG-GG matrix
Swelling profiles of AVG, GG and AVG-GG composite matrix both in SIF and SGF are shown in Fig. 2. In both fluids the swellability increased with the addition of AVG to GG. But in SIF the swelling rate was less than that in SGF.

Drug release studies
The 5-FU release profiles measured in SGF and SIF for the AVG-GG-5-FU tablet for typical composition of AVG-GG matrix are displayed in Figs. 3 and 4 respectively. The release rate was maximum in AVG and minimum in GG carriers both in SIF and SGF. The initial release rate was more in SGF compared to that in SIF. Addition of AVG to GG enhanced the 5-FU release rate due to the greater water solubility of AVG compared to GG. Comparison of release profiles measured for different composition of AVG-GG matrix implied that the sustained release of 5-FU can be achieved under physiological pH conditions of stomach and small intestine.

Drug release mechanism
The drug release mechanism was analyzed using drug release modeled as per the following empirical kinetic equation:

\[ \log \frac{M_t}{M_{\infty}} = k t^n \]

where \( M_t \) is the amount of drug released at time \( t \), \( M_{\infty} \) is the total amount of drug, \( k \) is the release rate constant, and \( n \) is the diffusion exponent. The release profiles displayed in Figs. 3 and 4 were fitted to this equation to determine the diffusion exponent and release rate constant.
$M_t / M_\infty = k t^n$

where $M_t$ - amount of drug released at time ‘$t$’, $M_\infty$ - amount of drug released at equilibrium, $M_t / M_\infty$ - fractional release of drug at time ‘$t$’ and ‘$n$’ is the diffusional exponent. The exponent provides an indication of the release mechanism and generally ranges from 0.5 to 1. It was the slope of the Korsemeyer-Peppas plot log $t$ vs ln $(M_t / M_\infty)$ (Fig. 5). An ‘$n$’ value of 0.5 indicates Fickian diffusion\(^{40,42}\), whereas a value of 0.45-0.89 indicates anomalous transport due to swelling and/or erosion. In the present study, for the AVG-GG matrix the $n$ values were in the range 0.4-0.63 indicating an anomalous mechanism (non-Fickian)\(^{40,42}\) for the drug release.

**Conclusion**

The study demonstrate that the addition of AVG powder to GG matrix facilitates the sustained release of 5-FU in stomach and SI that is difficult with GG which is widely used for colon specific drug delivery. The release follows a non-Fickian mechanism. Apart from sustained release, AVG and GG provide numerous health benefits envisaged under introduction.

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**References**