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Original Article

Synthesis and characterization of PEGylated guar gum based nanoparticles: New approach in novel drug delivery system

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ABSTRACT

Objective: To investigate the synthesis of guar gum PEGylation and the nanoparticles preparation and evaluation of drug release characters were studied. **Methods:** The PEGylated particle was prepared by emulsification with cold homogenization. **Results:** The Acyclovir loaded nanoparticles were uniform and spherical with smooth surface. The entrapment efficiency (EE %) was in range of 60-95%. The entrapment was increased with increase in concentration of PEGylated guar gum. The particle size of Acyclovir nanoparticle was in range between 234.8-879.6 nm with polydespersibility range of 0.006-0.024, which reveals monodisperse nature. The PEGylated nanoparticle showed sustained drug release at 24 hours in the range of 54.28-62.58%. The drug release behaviour was reported by zero order release with diffusion and erosion mechanism. **Conclusion:** The PEGylation was done successfully with guar gum. These PEGylated guar gum was produced spherical shaped nanoparticles and it was circulating and sustained the drug release in biocompatible manner. Acyclovir PEGylated nanoparticle was best choice for prolonged the drug release in HIV treatment.

Key words: Acyclovir, Entrapment Efficiency, PeGlyation, *In vitro* Release, Guar Gum.

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INTRODUCTION

Nanoparticles are very small particles that behave as a whole unit in terms of their transport and properties. These fine particle diameter ranges is between 100–2500 nm.[1] Nanoparticle research is currently the most studied branch of science with huge application in various fields like biomedical, electronic and optical.[2] Nanoparticle research is initiated in 9th century at Mesopotaomia. The artisans used to generate a glittering effect on thesurface of pots.[3] Now it is explored by all the fields worldwide. The use of nanoparticle in biology and medicine includes creating fluorescent biological markers for diagnosis of diseases[4] and drug delivery system for all the complicated diseases including genetic diseases.[5] The nanoparticles are being ample use in drug delivery system due to properties like size, surface characteristics of nanoparticles and it can easily adapt for both active and passive targeting. These particles can be used to control and sustain the release of the drug during the transportation as well as targeted release. They can increase the drug therapeutic efficacy by altering distribution and clearance with significant reduction in side effects.

Protein, enzymes, muscle fibers, polysaccharides and gummy exudate are natural polymers used significantly in formulating the wide variety of pharmaceutical formulations. The well-known natural polymers used in pharmaceuticals and other fields are carrageenan, chitosan, acacia, agar, gelatin, guar gum, gum karaya and shellac.[6]

India is a major producer and exporter of guar gum and its derivatives. It has become the major cash crop for Indian economics, and India contributes 80% of guar gum production outof the world total production of guar gum which figures out to be 6 lakh tons.[7] Guar gum is used pharmaceutically as gelling, viscosifying, thickening agent, suspending agent, stabilizer, emulsifying agent, preservative, binder and disintegrating agent.[8]

Guar gum is a natural biodegradable polymer which can be suitable for nanoparticle formulation. It can be easily biodegraded in biological system and there by produce fast drug release.

PEGylation is the process of covalent linkage of PEG chains to another molecule, usually drug or polymer. PEGylation of drug or polymers can change physiochemical characters of either the drug or the polymer.[9] In specially it reduces immunogenicity, antigenicity and increases hydrodynamic size of the agents. This prolongs its circulatory time by reducing renal clearance.[10] PEGylation can provide significant sustain release of drug from matrix and maintains constant steady state drug level in systemic circulation.[11]

At present, research progresses in PEGylation of synthetic polymers are narrow range. Mainly some polymers like PLA,[12] PLGA,[13,14] and PMMA[15] are PEGylated. Some active drugs are PEGylated with synthetic and natural polymers which includePaclitaxel with chitosan,[16] Diphtheria toxoid with chitosan,[17] pyrazyl-carbonyl-phe-leu-boronate with PLGA,[18] SiRNA delivery with chitosan,[19] Doxorubicin,[20] gold particles coated with PEG[21,22] in delivery of mesoporous silicananoparticles.[23] These synthetic polymers are not economicfor productionof nanoparticles. In this research, we have focused on guar gum natural polymer and assessed the drug release characters of PEGylated nanoparticles containing acyclovir as model drug.

MATERIALS AND METHODS

Materials

Acyclovir and guar gum were purchased from KLH chemicals, Malaysia. Polyethylene glycol 400 and dimethyl amino pyridine (DMAP) was purchased from Suka chemicals, SDN BHD, Malaysia and all other chemicals used were of analytical grade.

Table 1: Evaluation of PEGylated guar gum loaded acyclovir nanoparticles

S.NO	Parameter	F1	F2	F3	F4	F5
1	Particle size (nm)	798.5 ± 2.6#	705.1 ± 8.2#	654 ± 2.6*	234.8 ± 2.1*	897.6 ± 18.4
2	E E (%)	72.46 ± 5.1#	75.06 ± 8.7	80.73 ± 6.4*	86.60 ± 2.2*	70.14 ± 8.3
3	PDI	0.0436 ± 0.08	0.0528 ± 0.08	0.0906 ± 0.02	0.0242 ± 0.04	0.062 ± 0.04

PDI: Poly dispersity index, EE: Entrapment Efficiency.

(The data were analyzed by two-way analysis of variance and represented as mean ± SD (N=3). Student's t2 test using graph pad prism 2.1 software was performed to determine differences between mean values at P≤ 0.001).

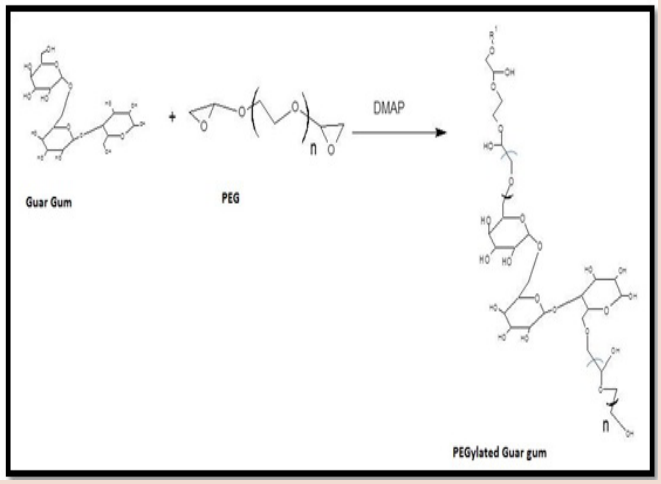

Figure 1: PEGylation reaction of Guar gum.

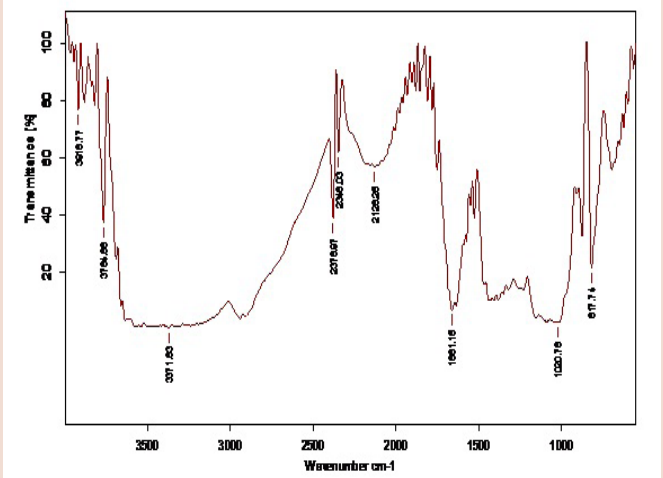

Figure 2: FTIR spectrum of pure guar gum.

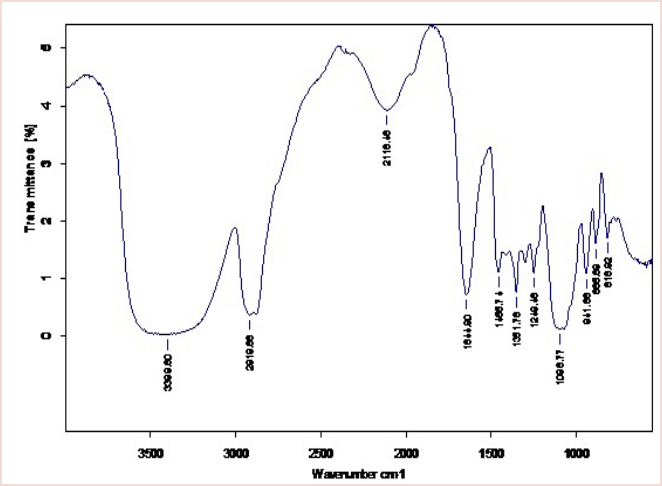

Figure 3: FTIR spectrum of PEGylated guar gum.

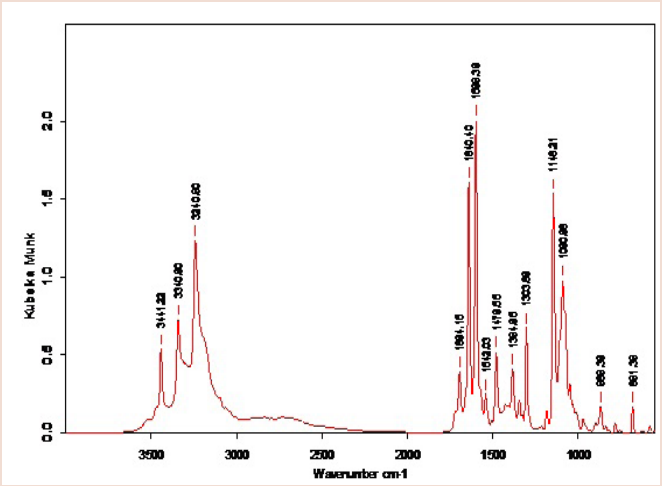

Figure 4: FTIR Spectrum of acyclovir with PEGylated guar gum.

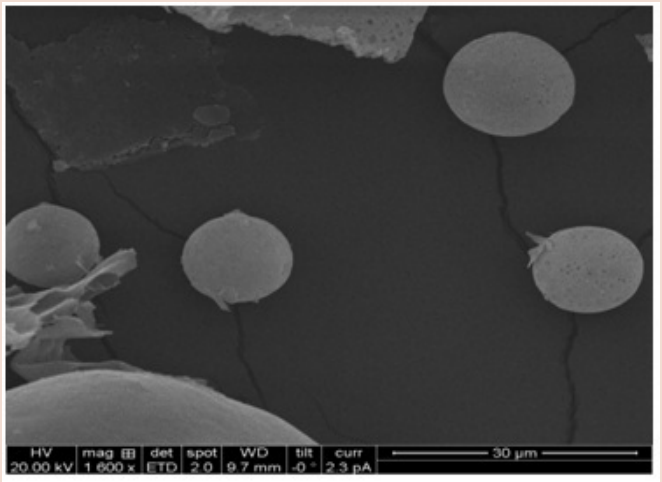

Figure 5: SEM image of PEGylated guar gum loaded acyclovir nanoparticles.

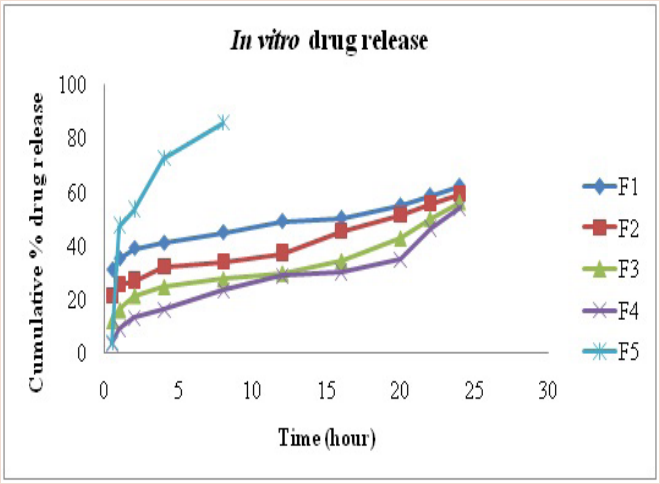

Figure 6: *In vitro* drug release of Acyclovir PEGylated Nanoparticle.

PEGylation of guar gum

The simple and commonly adopted batch process involves the mixing of reagents together in a suitable proportion, followed by the separation and purification of the desired product using a suitable technique based on its physicochemical properties. About 3.6 g of guar gum was dissolved in 30 mL double distilled water and 1.5 mL of PEG-400 was added to the guar gum mixture along with 4mL of dimethyl amino pyridine (DMAP). The mixture was stirred for 6 h in magnetic stirrer at controlled temperature (27^{0}C). The precipitate was filtered by Whatmann filter paper. The mixture was cooled to 4^{0}C until it precipitates. The separated precipitates were dried at room temperature to get free flowing powder. PEGylated guar gum was stored in desiccatorfor further studies (Figure 1).

Evaluation of PEGylated guar gum

The PEGylation of guar gum was characterized by FTIR spectrum. Pure guar gum spectrum was compared with PEGylated guar gum spectrum. The pure guar gum was mixed with 100 mg of potassium Bromide (KBr). The samples were compressed to disc by applying pressure of 5 tons for 5 min in a hydraulic press. The prepared pellet was placed in the light path and the spectrum was recorded in the region of 4000-400 cm^{-1}. Similarly PEGylated guar gum was analyzed. Functional groups were identified by interpretation of FTIR spectrum and inter linkage was confirmed.

Preparation of PEGylated nanoparticles

Nanoparticles were prepared by using cold homogenization method. In this experiment acyclovir was used as model drug where in 100 mg of drug was dissolved in 10 mL of dichloromethane (DCM). The organic phase was poured slowly in PEGylated guar gum solution (i.e. 100 mg of PEGylated guar gum in 50 mL of water with 4 mg of span 80). The mixture was ultrasonicated for 15 min at 20,000 rpmin ice bath. Finally 5 mL of glycerol and 25 mL of glutaraldehyde was added and sonication was continued for further 15 min at 20,000 rpm. The disperse system was centrifuged at 1110 X g, to separate the nanoparticles. The separated nanoparticles were washed with phosphate buffer (pH6.4) and freeze dried at -20°C. The freeze dried nanoparticles were stored in desiccator for evaluation studies.

Surface morphology

The shape and surface morphology of the PEGylated nanoparticles were examined using SEM. Appropriate samples of polymeric nanoparticles were mounted on metal stubs using double adhesive tapes. Samples were gold coated and observed for morphology, at acceleration voltage of 15kv.

Particle size analysis

Particle size of nanoparticles wasdetermined using optical binocular microscope. For particle size analysis PEGylated nanoparticles were first suspended indouble distilled water and by placing one drop on clean slide the image was observed under high power magnification using Dewinter microscopic software. Theparticle size significant ($p< 0.001$) was calculated by using graphpad prism 5.1 software.

Quantification of drug incorporation efficiency

To calculate entrapment efficiency nanoparticles were dissolved in 50 mL of dichloromethane. The solution was centrifuged at 1110 X gm and separated fluid was filtered by membrane filter, and finally the quantity of drug in the solution was measured by UV spectrophotometer at 252 nm. Quantity of drug in nanoparticles.

$$\text{Drug entrapment (\%)} = \frac{\text{Quantity of drug in nanoparticles}}{\text{Mass of drug used in formulation}} \text{x } 100$$

In vitro release study

Invitro release study of Acyclovir PEGylated nanoparticle was carried out byusing modified Franz diffusion apparatus at 37 ± 2°C. About 50 mg drug equivalent nanoparticles where suspended in donor compartment containing phosphate buffer (pH 7.4). Drug release was assessed by intermittently sampling the receptor medium (5 mL) and fresh phosphate buffer saline pH 7.4 was replaced. The samples were filtered with membrane filter (0.22 µ) and the amount of drug released was quantified by a U.V. Spectrophotometer at 252 nm.

Statistical Analysis

Particle size and entrapment efficiency data were compared using student t's test and invitro data was analyzed by ANOVA and the turkey test. Differences were considered significant when $p < 0.001$.

RESULTS AND DISCUSSION

PEGylation of guar gum

Guar gum was PEGylated due to presence of more -OH- groups of mannose and galactose in its composition. PEGylation was initiated by addition of catalyst (dimethyl amino pyridine) in guar gum with PEG solution. The PEGylation reaction was fastened by heating the mixture at 45°C for 2 h with continues stirring. The PEGylation was started after hydrolysis of guar gum into mannose and galactose. The stirring was continued until precipitates were obtained. The precipitate was cooled at 4°C for completion of the reaction. The PEGylation was significantly confirmed by FTIR spectrum interpretation with pure polymer spectrum.

FTIR spectrum of guar gum showed O-H stretching at 3764 cm^{-1}, ring stretching at 2346cm^{-1} and C-O stretching (C-O-C) at 1661cm^{-1} and primary alcohol stretching at 1020 cm^{-1} (Figure 2). On PEGylation of guar gum, the peaks were observed at 3419 cm^{-1} for O-H stretching, ring stretching at 2919 cm^{-1} and C-O stretching (C-O-C) at 1644 cm^{-1}. The primary alcohol peak at 1020 cm^{-1} was disappeared and new peak was observed at 1096 cm^{-1} due to formation of ether linkage (C-O-C) (Figure 3), because of conversion of primary alcohol to ether in presence of base. The epoxide form of poly ethylene glycol was more reactive group which can attract primary alcohol to give ether linkage. In guar gum the primary alcohol groups were more reactive than secondary alcohol. Thus polymerization occurs only at primary alcohol groups. The Figure

4 showed compatibility studies of acyclovir with PEGylated guar gum. The FTIR spectrum was confirmed that significant compatibility of drug with polymer.

Preparation of nanoparticles

The Acyclovir nanoparticles were prepared by emulsification with cold homogenization method. Aqueous phase constitutes of PEGylated guar gum with surfactant (span 80) and non- aqueous phase contains Acyclovir with dimethyl amino sulphoxide. Emulsification was done by high speed Remi homogenizer for 6 h in cold ice bath. The obtained colloidal nano emulsion was centrifuged at 1100 X gm to separatenanoparticles. The colloidal emulsion was Freeze dried at -20°C to get free flowing powder.

Morphological studies

PEGylated nanoparticles loaded acyclovir has showed smooth and spherical shaped particles. The spherical shape was produced due to high homogenization and the particles were distributed uniformly with spherical shape. Surface of nanoparticles were smooth in nature, due to polymeric saturation in both phases and diffusion rate of solvent was minimum followed by complete evaporation, which led to smooth surface (Figure 5).

Effect of polymeric concentration on entrapment efficiency

The PEGylated nanoparticles were prepared by different concentration of polymer. The entrapment efficiency of Acyclovir nanoparticle increases with increase in concentration of PEGylated guar gum. The entrapment efficiently is in the range between 72.42-86.6%. The increased entrapment was due to the number of ether linkage between acyclovir and PEGylated guar gum. The entrapment efficiently (%) was directly proportional to polymeric concentration (Table 1). Normally low entrapment efficiency was observed at low affinity between drug at different solvents (organic and aqueous).

Particle size and poly dispersity index

The acyclovir nanoparticle (F1-F5) formulations were observed spherical shape with smooth surface. The particle sizes of all the formulations (F1-F5) were between 234.8–897.6 nm (Table 1). The particle size of nanoparticles was mainly affected by homogenization speed and saturated polymeric concentration. During homogenization particlesshowed aggregation of span 80 helps to reducethe particle size by reducing interfacial tension with stabilized spherical shapedparticles. The organic solvent used in these formulations rapidly partitioned into the continuous aqueous medium and the polymer precipitated around the drug. The simultaneous evaporation of the entrapped solvent led to the formation of spherical shaped acyclovir nanoparticles.

The polydispersity index of acyclovir nanoparticles ranges in between 0.02–0.09. The PDI showed narrow size distribution in all the formulations. Thus acyclovir nanoparticle formulated had good cellular uptake by having narrow size range.

In vitro release studies

The *In vitro* release study of acyclovir nanoparticles at different polymeric concentration was evaluated by modified Franz diffusion cell. The amount of drug release from all formulation (F1-F4) was shown in Figure 6. The PEGylated nanoparticles showed initial burst release (30 min) of 3.76-31.09% depending on concentration of polymer. The 1:4 ratio formulations showed less drug release due to high polymeric matrix with slow polymeric matrix erosion. The low drug release was observed due to more compact PEGylated wall around the drug and significantly possesses sustained drug release over prolonged period.

At the end of release studies (24 h) limited percentage of drug release in range of 54.28–62.58% was noticed. The F4 formulations showed significantly less drug release over other formulations, which clearly indicated that 1:4 showed satisfied sustained action for antiviral treatment over prolonged periods.

In order to determine drug release pattern of PEGylated nanoparticles, release data wassubstituted in zero, first, higuchi and korsemeyer peppas models. The release constant was calculated from slopes of appropriate plots as well as the regression co-efficient (R^2). The acyclovir PEGylated nanoparticles were fitted by zero order release [R^2=0.9273–0.9794] followed by higuchi equation [R^2=0.9056–0.9676]. Hence the drug release kinetics demonstrates that release was independent of concentration. For explaining the mechanism of drug release from nanoparticles, korsemeyer peppas equation showed good linearity and the release exponent n=0.16-0.57. This appears to be the coupling of diffusion and erosion mechanism i.e. anomalous diffusion.

CONCLUSION

Guargum was PEGylated by using polyethylene glycol 400 with dimethyl amino pyridine. The PEGylation of guar gum was confirmed by FTIR spectrum. The OH group of guar gum was bonded with PEG by ether linkage. The PEGylated guar gum was used for preparing acyclovir nanoparticles. The nanoparticles were prepared by cold homogenization technique with glutaraldehyde as cross linker. The prepared nanoparticles were spherical shape and particle size range of 234.8-879.6 nm with monodisperse nature. The nanoparticles loaded acyclovir showed good entrapment efficiency. The PEGylated nanoparticles loaded acyclovir showed sustained drug release for more than 24 h. the acyclovir loaded nanoparticles were significantly suitable for treating viral infection, due to nontoxic circulation of carriers in biological system.

ACKNOWLEDGEMENT

Nil.

CONFLICT OF INTEREST

The authors are declared that they have no conflict of interest.

ABBREVIATION USED

PEG: Poly Ethylene Glycol; FTIR- Fourier Transform Infra Red; **PDI**:Poly Dispersity Index; **EE**:Entrapemt Efficiency; **DCM:** Dichloromethane.

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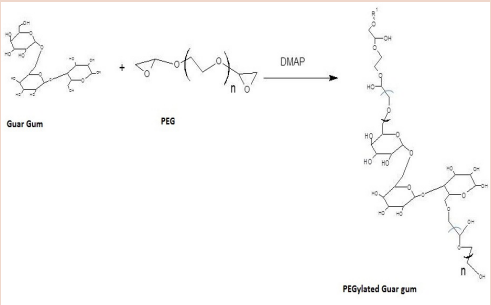
PICTORIAL ABSTRACT



SUMMARY

- For the development of natural biodegradable polymers on drug delivery systems. We converted naturally available guar gum to highly biodegradable carrier.

ABOUT AUTHOR

Dr. Venugopal Vijayan: Has been completed M.Pharm and Ph.D and Presently working as a senior lecturer in Asian Institute of medicine Science and Technology University (AIMST), Malaysia. Research interest is Nanocarriers development.