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Formulation and evaluation of *Albendazole microcapsules* for colon delivery using chitosan

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ABSTRACT

Objective: To formulate and evaluate Albendazole microcapsules using chitosan, a natural polymer for colon-specific delivery for better treatment of helminthiasis, filariasis, colorectal cancer, avoiding the side effects. **Methods:** The Albendazole microcapsules were prepared by the use of different concentrations of sodium alginate, chitosan and hydroxypropyl methylcellulose (HPMC). The polysaccharides chitosan reacted with sodium alginate in the presence of calcium chloride to form microcapsules with a polyelectrolyte complex membrane by electrostatic interactions between the two oppositely charged polymers. The microcapsules were then studied for entrapment efficiency, drug-polymer compatibility and surface morphology. *In vitro* drug release study in presence and absence of cecal content were also studied. Further, kinetic modellings were employed to find out release mechanisms. **Results:** Albendazole loaded microspheres showd high entrapment efficiency (72.8%) and the microcapsules were free flowing, non aggregated and spherical, between 600 and 1 000 μm in diameter. The surface of microcapsules were found to be porous and wavy. The FT-IR spectrum showed that there is no interaction between the polymer and the drug. The in vitro drug release study found to be affected by change in chitosan, sodium alginate and HPMC concentration. The microcapsules with 2.5% sodium alginate and 0.4% chitosan shown minimum release in gastrointestinal simulated condition but shows maximum drug release at the end of 24th hour in presence of cecal content. The rate of drug release follows Korsmeyer-peppas model that was the drug release is by diffusion and erosion. **Conclusions:** The study reveals that Albendazole loaded chitosan-alginate based microsphere can be used effectively for the colon targeting.

1. Introduction

Oral route is considered to be most convenient for administration of drug to patients. Oral administration of conventional dosage forms normally dissolves in the stomach fluid or intestinal fluid and absorption from these regions of the gastrointestinal tract depends upon the physicochemical properties of the drug. Dosage forms that deliver drug into the colon rather than upper gastrointestinal tract offers number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (Ulcerative colitis, Crohn´s disease, carcinomas, and infections such as helminthiasis and amoebiasis) whereby high local concentration can be achieved while minimizing side effects that occur because of release of drug in the upper gastrointestinal tract or unnecessary systemic absorption[1]. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. This region of the colon is recognized as having a somewhat less hostile environment with less diversity and intensity of activity than the stomach and small intestine. Additionally, the colon has a long retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Apart from retarding or targeting dosage forms, a reliable colonic drug delivery could also be an important starting position for the colonic absorption of per orally applied, undigested, unchanged and fully active peptide drugs. As the large intestine is relatively free of peptidases such special delivery system will have a fair chance to get their drug sufficiently absorbed after per oral application. The different approaches for targeting orally administered drugs to the colon include coating with pH-dependent polymers, design of timed-release dosage forms and utilization of carriers that are degraded exclusively by colonic bacteria[2]. Micro-encapsulation is now the most frequently employed method of producing controlled release dosage forms. Microcapsules developed for use in medicine consisting of solid or liquid core material containing one or more drugs enclosed in coating. For example in ionic cross-linking technique, dropping or spraying a sodium alginate solution into a calcium chloride or barium chloride solution produces microcapsules[3]. The divalent calcium or barium

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ions crosslink the alginate formed gelled droplets. The polysaccharide chitosan reacted with sodium alginate in the presence of calcium chloride to form microcapsules with a polyelectrolyte complex membrane by electrostatic interactions between the two oppositely charged polymers[4].

Chitosan is a natural polymer obtained by deacetylation of chitin[5]. Chitin is the second most abundant polysaccharides in nature after cellulose and biologically safe. The main commercial sources of chitin are the shell wastes of shrimp, crab, lobster, krill, and squid. This polymer exhibits several favorable properties, such as biodegradability and biocompatibility. It also has mucoadhesive properties due to its positive charges at neutral pH that enable an ionic interaction with the negative charges of sialic acid residues of the mucus.Sodium alginate (NaAlg),a water soluble salt of alginic acid, is a natural polysaccharide extracted from marine brown algae[6], Albendazole is a broad-spectrum anthelminthic agent used for the treatment of Neurocysticercosis , hydatid disease, giardia infection and filariasis[7,8]. Albendazole is poorly absorbed from the gastrointestinal tract due to its low aqueous solubility. This study selected Albendazole as a drug for formulating colonic drug delivery system to obtain better pharmacological effect because most of the worms reside in large intestinal part and to avoid side effects associated with Albendazole therapy. In addition it was reported that Albendazole can also be used to treat regional peritoneal carcinomatosis arising from colorectal origin[9]. Thus the above observation prompted us to formulate and evaluate natural polymer based microcapsules of Albendazole, which targeted to colon for improving the pharmacological effect and avoiding the side effects. Different batches of Albendazole microcapsules were prepared using different concentration of polymers.The microcapsules were then studied for Entrapment efficiency, drug-polymer compatility, surface morphology, *in-vitro* drug release studies with and without cecal content and kinetic studies.

2. Materials and methods

2.1. Preparation of chitosan-alginate microcapsules

The codes and contents of formulations prepared are shown in Table 1. Chitosan dispersions of 0.1%, 0.2% and 0.4% w/v were prepared by dispersing chitosan 0.1 g, 0.2 g, 0.4 g, respectively in 2% v/v acetic acid(100 mL)with continuous stirring using a magnetic stirrer followed by dissolving calcium chloride(1.5 g). The pH of the solution was adjusted to 5.5 with 10% w/v sodium hydroxide solution and this solution was used in the encapsulating procedure. Alginate solutions of 1.5%, 2.0% and 2.5% w/v were then prepared by dissolving sodium alginate 1.5 g, 2 g and 2.5 g in distilled water (100 mL). Albendazole(0.4 g) was dispersed in to 20 mL of this solution using a magnetic stirrer. Chitosan-alginate microcapsules (Mc) were prepared by following the reported method with slight modification. The sodium alginate solution containing Albendazole was loaded into a syringe fitted with 23 G needle.100 mL of chitosan-calcium chloride solution was taken in a beaker. Alginate-Albendazole solution was added drop wise at constant rate of 30 mL/hr to chitosan-calcium chloride solution with constant stirring (100 rpm). Reaction time of 1 hr was used. After forming microcapsules it was filtered and washed three times with distilled waster. The microspheres were then hardened with acetone and dried by keeping it in a vaccum desiccator.

2.2. Preparation of chitosan and sodium alginate+ hydroxypropyl methylcellulose (HPMC) microcapsules

Chitosan dispersions of 0.1%, 0.2% and 0.4% w/v were prepared by dispersing chitosan 0.1 g, 0.2 g, 0.4 g, respectively in 2% v/v acetic acid (100 mL)with continuous stirring using a magnetic stirrer followed by dissolving calcium chloride(1.5 g). Different concentration of HPMC(0.0125 g/mL, 0.006 25g/mL) and Albendazole(0.4 g) was dispersed in to 20 mL of this solution using a magnetic stirrer. Chitosan-alginate+HPMC microcapsules (Mc) were prepared by following the reported method with slight modification. The others were familiar with steps above mentioned.

2.3. Morphological evaluation of Mc

Shape and surface characteristics of Mc were studied using Scanning Electron Microscope (SEM Philips 200 FEI). Sizes of Mc were evaluated using optical microscope. Since Mc were irregular after drying the longest diameter was measured. Fifty Mc per formulation were evaluated. Average diameter was then calculated.

2.4. Compatibility study using fourier transform infrared (FT-IR)

An infrared (IR) study was carried out to see whether there is any incompatibility between the selected polymers sodium alginate, chitosan and the drug Albendazole. The IR study was also helpful to know whether there is complete physical adsorption (entrapment) of the drug on to the polymer matrix without any mutual interaction.

2.5. Drug loading

25 mg of microcapsule were crushed in to powder and treated with 47.5 mL 0.1N hydrochloric acid+2.5mL methanol. The resulting mixture was stirred at 250 rpm. The temperature was maintained at (37.0±0.2) ℃. At the end of 2 hrs, it was filtered, filtrate was analyzed spectrophotometrically at 309 nm (Shimadzu UV 1700, Japan).

Table 1
Ingredients used for each formulations.

S.NO	Ingredients used	Formulations						
		A	B	C	D	E	F	G
1	Albendazole(g)	0.4	0.4	0.4	0.4	0.4	0.40	0.40
2	Sodium alginate(%)	1.5	2.0	2.5	2.5	2.5	2.50	2.50
3	Chitosan(%)	0.4	0.4	0.1	0.2	0.4	0.40	0.40
5	HPMC(g)	--	--	--	--	--	0.25	0.12
6	Calcium chloride(g)	1.5	1.5	1.5	1.5	1.5	1.50	1.50

2.6. *In vitro* drug release study under simulated gastrointestinal conditions

In vitro drug release characteristics of Mc were evaluated following reported method with little modification. 50 mg of the prepared microcapsules were placed in a 100 mL beaker and contacted with 50 mL elution medium with maintained temperature (37.0±0.2) ℃ and stirring(50 rpm) with small Teflon coated magnetic bead, by placing the beaker on energy regulated temperature controlled hot plate magnetic stirrer. The dissolution was conducted in dissolution medium of pH1.2 for the first two hours followed by dissolution medium of pH 6.8 up to 24 hrs. The dissolution medium of pH 1.2 was prepared by first making 100 mL solution containing 2.5 mL methanolic HCl and 97.5 mL 0.1N HCl, 5 mL of this solution make upto 50 mL with 0.1N HCl, The dissolution medium of pH 6.8 was prepared by taking 49 mL of the above medium and 51 mL of 0.1 N NaOH. Samples were withdrawn every half an hour for 2 hrs in the case of dissolution medium of pH 1.2. After two hours the medium was replaced with dissolution medium of pH 6.8 and samples were withdrawn at one hour interval upto 6 hrs and then samples were withdrawn at 8th, 12th and 24th hour. 5 mL of the samples were withdrawn and replaced with 5 mL of the dissolution medium. The samples were analyzed spectrophotometrically at 309 nm with suitable dilution.

2.7. *In vitro release study under gastrointestinal simulated conditions in presence of cecal content*

To access the susceptibility of chitosan and guar gum being acted upon by the colonic bacteria drug release studies were carried out in the presence of rat's cecal contents, because of the similarity with the human intestinal microflora. The cecal content was collected from rat. As the cecum is naturally anaerobic, all those operations were carried out under anaerobic conditions. The drug release studies were carried out in a closed sterile beaker. The anaerobic condition is maintained by bubbling CO_2 into the dissolution medium. The other steps were the same as the process above mentioned.

2.8. *Mathematical modeling of Albendazole microcapsules*

In order to investigate the mode of release from the microcapsules, the release data were analyzed with the following mathematical models.

$Q_t = K_0\ t$ (Zero Order Kinetics)

$\text{Log}\ (Q_t / Q_0) = -K_1\ t / 2.303$ (First order Kinetics)

$Q_t = K_{KP}\ t^n$ (Korsmeyer and Peppas equation)

$Q_t = K_H\ t^{1/2}$ (Higuchi's equation)

Where, Q_t is the percent of drug released at time "t", K_0, K_1, K_{HC}, K_{KP} and K_H are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

3. Results

3.1. *Entrapment efficiency of Albendazole microcapsules*

Among the different batches of microcapsules, minimum entrapment efficiency was found to be 38.45% (Batch B) and maximum 72.84% (Batch C). Entrapment efficiency of Batch A was 49.89%, 54.23% in Batch D, 48.08% in Bacth E, 33.29% in Batch F, and 44.89% in Batch G. Batch C containing 2.5% sodium alginate and 0.1% chitosan was found to posses better entrapment efficiency (72.84%). From the results it was found that the entrapment efficiency increased with increase in concentration of sodium alginate and decreasing concentration of chitosan. The entrapment efficiency was to be decreased in Chitosan formulations containing HPMC (Batch F, Batch G) ie 33.29% for Batch F and 44.89% for Batch G. For Batches A, D and E The entrapment efficiency was 49.89%, 54.23% and 48.08% respectively.

3.2. *Compatibility study using FT-IR*

After interpretation through the spectra it was confirmed that there were no major shifting of functional peaks between the spectra of drug, polymer and the drug loaded microcapsules. The characteristic peak due to pure Albendazole at 3 330.3/cm, 2 956.8/cm, 1 710.6/cm,1 524.0/cm, 1 325.3/cm, 731.9/cm was founded in Albendazole microcapsules spectrum peak. It can be concluded from IR spectroscopic studies that the drug Albendazole was entrapped in to the polymer matrix and there was no chemical interaction, because there is no major shifting of the functional peaks between the drug, polymer and drug loaded microcapsules and it was ensured that the polymers chitosan and sodium alginate are compatible in entrapping the drug Albendazole.

3.3. *Particle size analysis of microcapsules*

The particle size analysis of formulations was carried out by optical microscopy. The results shown in Figure 1. From the results it was found that the maximum particle size range was found to be 800-850 μm in Albendazole microcapsules with sodium alginate and chitosan for Batch E. So it can be concluded that the particle size range was maximum for microcapsules with high concentration of sodium alginate and chitosan. HPMC had no effect on the particle size.

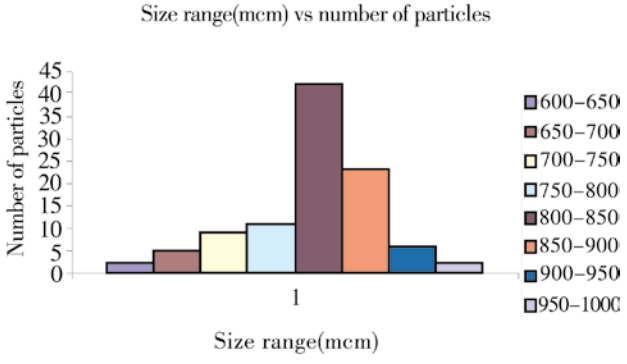

Figure 1. Particle size analysis.

3.4. *Morphological evaluation of microcapsules*

The surface morphology of Albendazole microcapsules was seen by Scanning Electron Microscope. The surface morphology was done for Batch E with magnifications 30×, 100×, 500× and 1 500× and shown in Figures 2 & 3. Albendazole microcapsules was found almost spherical, free flowing and non-aggregated. The surfaces of microcapsules are porous and wavy.

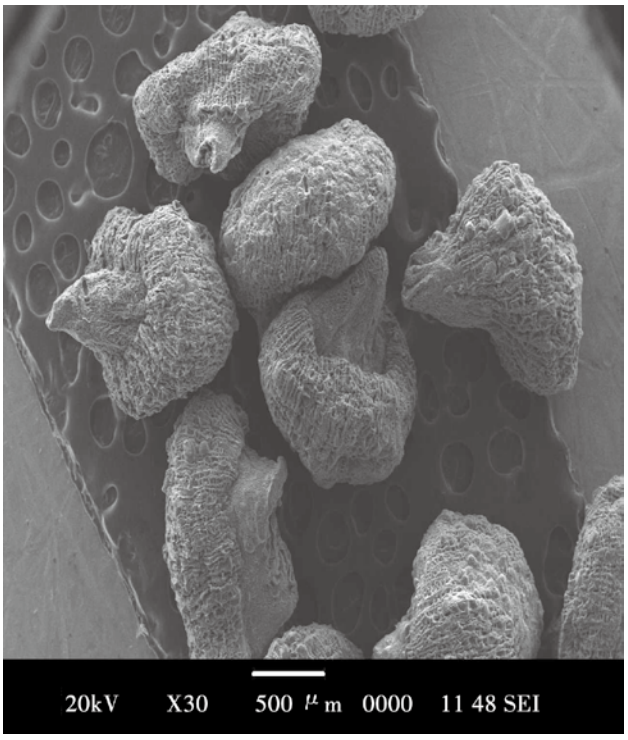

Figure 2. SEM of microcapsules.

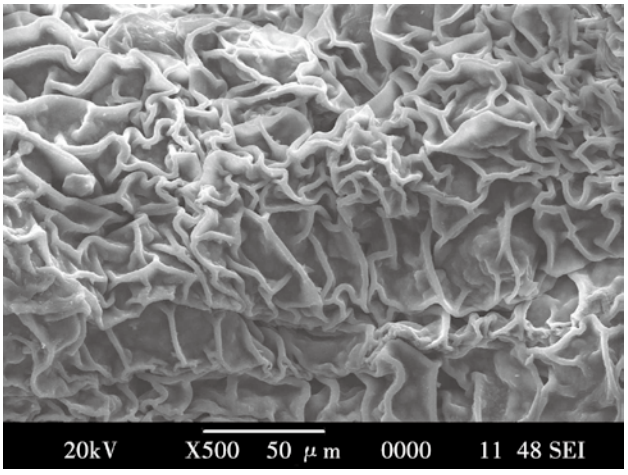

Figure 3. SEM of surface of microcapsules.

3.5. In vitro drug release study under simulated gastrointestinal conditions

It was seen from the observation data that the cumulative percentage release from all the drug loaded batches of microcapsules fell within the range of 53% to 99% in 24 hours study and it was found that the %cumulative release in microcapsules encapsulated with chitosan was maximum for Batch B (98.66%) and minimum for Batch E (53.82%).

The results of *in vitro* release study indicated that the amount of drug release decreased significantly with an increase in chitosan concentration and decrease in sodium alginate concentration and was attributed to increase in the diffusional path length, which the drug molecule had to traverse. It was found that the release of drug from all drug loaded batches was found to follow a biphasic pattern, that was an initial burst release of 34% to 65% during the second hour and the remaining amount of drug was found to be released in a slow or sustained manner for a period of 24 hours (Table 2).

Table 2
% cumulative release of all formulations.

S NO	TIME (Hrs)	% Cumulative drug release						
		A	B	C	D	E	F	G
1	0.5	6.38	9.9	7.4	6.5	6.2	6.3	5.9
2	1.0	18.51	24.2	17.4	15.6	14.5	16.1	15.3
3	1.5	33.61	42.8	28.5	27.2	24.9	30.2	30.9
4	2.0	51.62	65.2	40.7	40.3	36.9	47.8	47.5
5	3.0	53.06	71.3	44.7	42.6	39.6	56.5	54.6
6	4.0	55.07	78.4	48.0	45.1	41.4	64.5	60.2
7	5.0	57.36	83.8	51.2	47.4	43.3	71.8	65.8
8	6.0	60.81	89.2	53.4	50.1	45.8	79.7	71.3
9	8.0	63.82	91.3	56.1	51.9	48.4	84.3	75.5
10	12.0	68.42	94.7	58.9	54.1	51.0	89.4	81.7
11	24.0	74.45	98.6	61.8	56.6	53.8	94.5	91.6

3.6. In vitro release study under gastrointestinal simulated conditions in presence of cecal content

As the polymers chitosan and guar gum were mainly degraded by the colonic bacteria, simulated in vitro release in presence of rat caecal content were conducted. The in vitro drug release in presence of cecal contents showed rapid increase in percentage cumulative release from 5th hour. The study shows that the release of Albendazole in the physiological environment of colon is due to the microbial degradation of chitosan. The comparative in vitro release of Batch E with and without cecal contents (Figure 4) were carried out. A significant difference was noted in release data obtained with and without cecal content. It was found that the in vitro drug release of Albendazole from Batch E microcapsules was only 53.82% without cecal content at the end of 24 h where as with cecal content it was 99.7%.

So, incorporation of chitosan and guar gum as coating showed maximum release in anaerobic colonic condition with caecal content as well as lower release in gastrointestina; simulated condition.

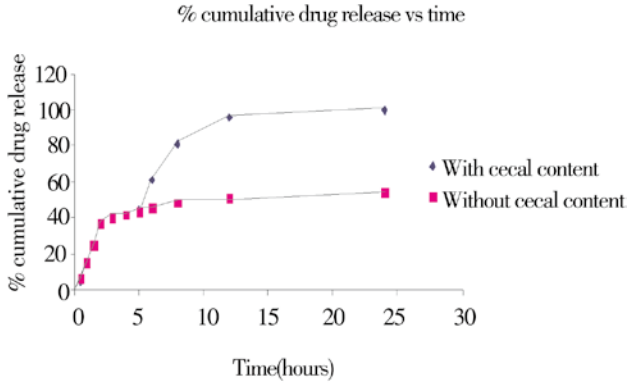

Figure 4. Comparative % cumulative drug release of Batch E with and without cecal content.

3 .7. Mathematical modeling of Albendazole microcapsules

Various kinetic data studies were done including zero order, first order, Higuchi model, Korsmeyer-peppas model and the results are shown in Figure 5. The results showed that almost all formulations followed Korsmeyer-peppas model that is the drug release by diffusion and erosion.

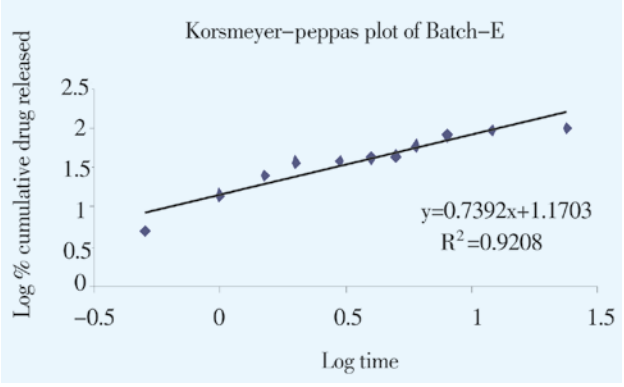

Figure 5. Korsmeyer-peppas plot of Batch E.

4. Discussion

The microcapsules are formulated using different concentration of polymers (sodium alginate, chitosan and HPMC).The entrapment efficiency of all the formulations are determined .In formulations with chitosan, maximum entrapment efficiency is found to be 72.84% (Batch C). This may be due to maximum availability of polymers for encapsulation and strong cationic chitosan and anionic sodium alginate ionic bonding.Increase in concentration of sodium alginate reveals better platform for encapsulation because of low viscosity when compared to chitosan. But at the same time adding HPMC (Batch K,Batch L) to chitosan coated sodium alginate micro capsule shows less entrapment. This may be due to decrease in ionic linkage because of nonionic HPMC. IR study is carried out to see whether there is any incompatibility between the selected polymers sodium alginate, chitosan and the drug Albendazole. It can be concluded from IR spectroscopic studies that the drug Albendazole is entrapped into the polymer matrix and there is no chemical interaction, because there is no major shifting of the functional peaks between the drug, polymer and drug loaded microcapsules and it is ensured that the polymers chitosan, and sodium alginate are compatible in entrapping the drug Albendazole. The particle size analysis of formulations is carried out. From the results it is found that the particle size range is maximum for microcapsules with high concentration of sodium alginate, chitosan. HPMC has no effect on the particle size. The surface morphology of Albendazole microcapsules is seen by Scanning Electron Microscope. From the results Albendazole microcapsules is found almost spherical, free flowing and non-aggrigated. The surface of microcapsules are porous and wavy. The invitro drug release of microcapsules in gastrointestinal simulated conditions are done. The results of in vitro release study indicate that the amount of drug release decrease significantly with an increase in chitosan concentration and decrease in sodium alginate concentration and is attributed to increase in the diffusional path length, which the drug molecule have to traverse. As the polymer chitosan mainly are degraded by the colonic bacteria. Simulated in vitro drug release were conducted in presence of rat cecal content for batch E(2% of sodium alginate and 0.4% of chitosan,) It is found that the in vitro drug release of albendazole from batch E is only 53.82% with out cecal content at the end of 24 hour whereas with cecal content, it is 99.7%. So incorporation of chitosan as coating show maximum release in anaerobic colonic condition as well as lower release in gastrointestinal simulated conditions. Various kinetic data studies are done including zero order, first order, Higuchi model,Korsmeyer-peppas model and the results showed that almost all formulations follows Korsmeyer-peppas model that is the drug release by diffusion and erosion in gastrointestinal simulated conditions.

It is concluded that Albendazole microcapsules from chitosan with free flowing, spherical and maximum entrapment efficiency are prepared and analyzed for in vitro drug release and found to be maximum in colonic simulated conditions. So, our objective of delivering Albendazole to colon for Helmintiasis and other diseases by minimizing the side effects were achieved through optimized microcapsules parameters from natural polymer chitosan.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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