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INFLUENCE OF METHOD OF PREPARATION ON INCLUSION COMPLEXES OF NAPROXEN WITH DIFFERENT CYCLODEXTRINS

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SUMMARY

The aim of this study is to increase the solubility of naproxen by inclusion complex formation with α, β, γ, hydroxypropylbeta and dimethylbetacyclodextrin . The apparent stability constants were calculated from the slope and intercept of the AL-solubility diagrams. The solid inclusion complexes of naproxen with cyclodextrins in 1:1 molar ratio were prepared by the kneaded-mix, spray-drying and freeze-drying method. The formation of inclusion complexes in the solid state were confirmed by X-Ray diffractometry I.R. spectroscopy and differential scanning calorimetry. The dissolution rate of naproxen from the inclusion complexes was much more rapid than naproxen alone. The best results were obtained with β-cyclodextrin inclusion complex prepared by the spray-drying method.

INTRODUCTION

Oral administration of most drugs generally causes no problems, although the usefullness of this route can be limited by certain undesirable effects on the gastric mucosa. In an attempt

to reduce these effects attention has turned towards modifying the chemical structure of the drug molecule, to give a new compound which may or may not have the same pharmacological properties. One of the most active fields in pharmaceutical technology is concerned with the study of new excipients that will reduce these effects to acceptable levels, even eliminating them, without compromising the pharmacological properties of the active principle.

Over the past few years there has been growing interest in the cyclodextrins (1-5) because, owing to the ease with which they form inclusion complexes, it is possible: to increase the dissolution rate of drugs (6, 7), to alter membrane permeability (8, 9), to increase the bioavailability of sparingly soluble drugs (10-12), to increase drug stability (13, 14) and to reduce gastric irritancy, in particular that of the non-steroidal antiinflammatory drugs. The drug chosen for this paper falls in this last category: naproxen, a antiinflammatory, analgesic and antipyretic frequently used in the treatment of rheumatic diseases. Naproxen is only slightly soluble in water and administered orally frequently gives rise to gastric irritation. The aim of this paper is to increase the solubility of naproxen in artificial gastric medium by forming inclusion complexes with cyclodextrins and using different methods of preparation.

MATERIALS AND METHODS

Materials: Naproxen (Sigma), α, β, γ, cyclodextrins (Chinoin) hydroxypropyl and dimethylbeta cyclodextrins (Celdex). All other reagents and solvents were of analytical grade.

Differential Scanning Calorimetry: A Perkin Elmer DSC-4 was used with 5 mg samples and scanned at 10ºC min^{-1} up to 170ºC.

X-Ray diffraction studies: Diffraction patterns were obtained by scanning at 1º/min through the 2θ angle on a Siemens D-500 diffractometer using Cu-K_α radiation.

Infrared spectra: A Perkin Elmer 1330 IR spectrophotometer was used.

Dissolution assays: These were obtained via apparatus II of the USP XXI at a temperature of 37ºC and 75 r.pm. in 900 ml of 0.1 N

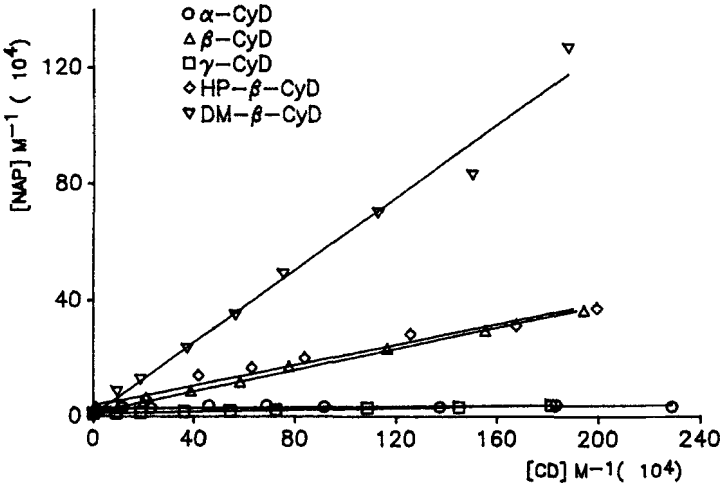

FIGURE 1

Phase-solubility diagrams of naproxen-cyclodextrin inclusion complexes at pH = 1 and 25º C.

ClH solution. Naproxen assays of collected samples were made spectrophotometrically at 272 nm ($E^{1cm}_{1\%}$ = 218) after filtration. Dissolution was characterised through two parameters: the percentage of naproxen dissolved after 5 minutes (indicative of the rate of dissolution), and the Dissolution Efficiency (15)(showing the totallity of the dissolution process). Similarities in behaviour between the complexes were revealed by taking the analysis of variance corresponding to the two characterising parameters chosen. The test for the minimum significant difference (MSD) was applied to see which inter-treatment differences were significant.

EXPERIMENTAL RESULTS AND DISCUSSION

1-. Phase-solubility studies: The naproxen solubility curves (25ºC pH=1) for the different cyclodextrins (Figure 1) show that soluble complexes were formed in each case, corresponding to the type A_1 diagrams in Higuchi and Connor's terminology (16). Furthermore, there is clear evidence that the solubility of naproxen dependes on the type of host molecule and that betacyclodextrin and its derivatives function best in this respect; quantitative

evidence being provided by the stability constants, which reflect the magnitude of the interactions between host and guest molecules. The high value for the complex formed with dimethylbetacyclodextrin (K = 26988 M^{-1}) indicates an inclusion complex of very high stability, owing to a large number of intermolecular interactions. The inclusion complexes formed with hydroxypropylbeta cyclodextrin and betacyclodextrin (K = 624 M^{-1} and 1379 M^{-1} respectively) are also stable, whereas the values obtained for alphacyclodextrin (K = 19 M^{-1}) and gammacyclodextrin (K = 141 M^{-1}) relate to the formation of complexes with very low stabilities.

It is beyond doubt that the stability constant of an inclusion complex depends on the degree to which the two molecules interact, dependent in turn on the relation between the size of the guest molecule and the size of of the cavity in the cyclodextrin. Considering the cyclodextrins used here: naproxen would have difficulty fitting into the small cavity within alphacyclodextrin (4.5 Å), while gammacyclodextrin would present a cavity that was too large (8.5 Å). Betacyclodextrin and its derivatives, however, by virtue of their cavity size (7.5 Å), would provide a space for naproxen molecules which could then interact with the functional groups rimming the cavity.

Uekama et al. (1) have put forward a scheme which emphasises those factors that are influential in the absorption of drugs administered as inclusion complexes with cyclodextrins. There is a series of consecutive stages in the absorption process (Figure 2) which are of prime importance when the drug passes into the circulatory system. It hardly needs pointing out that, for those drugs whose rate of absorption is limited by the rate of dissolution, it is advisable to increase the dissolution rate by forming inclusion complexes with the cyclodextrins. We should also be aware however, of the importance of the stability constant of the complex formed; complexes with low stability constants will, when administered, rapidly release the drug, possibly, reducing the effect that complexation has on the bioavailability of

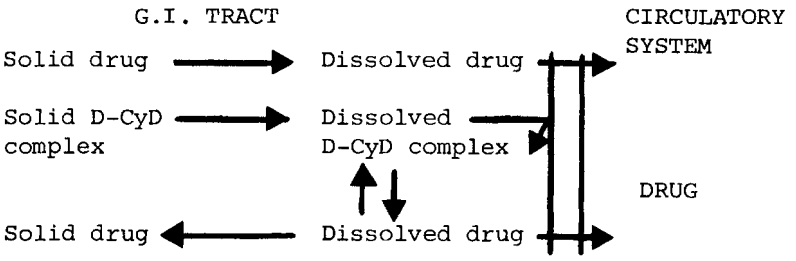

FIGURE 2

Schematic representation of dissolution-dissociation-absorption process of a drug-cyclodextrin (D-CyD) complex

the drug. If the stability constant of the complex is high, the drug release rate will be slow and the quantity of free drug around the area where it is absorbed will be low. We can reasonably conclude therefore, that the cyclodextrins can only be acting as carriers that take the drug from the aqueous nedium to the lipidic gastrointestinal membrane. In view of all this Szejtli (3) has stablished that only inclusion complexes with stability constants between 200 and 5000 M^{-1} can be used to improve the bioavailability of hydrophobic drugs.

The experimental data obtained lead us to reject dimethylbetacyclodextrin, while bearing in mind that it has been described how the interaction of this carrier with components of biological membranes altered their structure and helped in the absorption of certain drugs (1), which would relate to descriptions of gastric irritation produced by this cyclodextrin (1), and our reason for rejecting it in complex with naproxen.

With regards to hydroxypropylbetacyclodextrin this was also rejected owing to its high cost and small efect on the solubility of naproxen compared to betacyclodextrin.

Preparation of inclusion complexes with betacyclodextrin: With betacyclodextrin we went on to form inclusion complexes by means of three techniques taken from the bibliography (17): freeze-drying, spray-drying and kneaded-mixing.

Freeze-drying: A solution of 1:1 naproxen and betacyclodextrin was prepared in distilled water with a small amount of ammonia to help the active principle to dissolve. The solution was agitated 24 h and then freeze-dried.

Spray-drying: The method described above was followed up to and including the agitation stage after which the solution was spray-dried in a Mini Spray Dryer Büchi 190 under the following conditions: air temperature= 120ºC, air temperature on leaving = 40ºC, air flow rate = 600 $l.min^{-1}$.

Kneaded-mixing: Naproxen and betacyclodextrin were put together in a mortar in a stoichiometric proportion of 1:1. The same weight of 50% ethanol/water mixture was added and the contents are dround togetheruntil homogenous and all the solvent had evaporated and dried in a vacuum oven. Once dry the product was sifted twice between 125 and 250 meshes.

Naproxen and betacyclodextrin were also subjected separately to the three treatments.

Characterisation of the inclusion complexes: The thermograms for the complexes are shown for the three methods used in Figure 3 The endothermic peak at 156ºC corresponding to naproxen desappears in the inclusion complexes made by freeze-drying and spray-drying, showing that the drug had become incorporated in the cyclodextrin cavity. The complex given by kneaded-mixing shows two characteristic endothermic peaks: the first at 100ºC, is due to the evaporation of water in the cyclodextrin and the second to naproxen; so this method, in contrast to the other two, does not provide complete encapsulation, naproxen exists dispersed in the free state between inclusion complex.

Looking at the X-Ray diffractograms (Figure 4) shows that the inclusion complexes have an amorphous structure when obtained via spray-drying or freeze-drying, but maintain some cristalline structure when obtained via kneaded-mixing. Of course these results could arise from the formation of an amorphous solid dispersion and not true inclusion of naproxen in the betacyclodextrin cavity. For this reason we made recourse to the

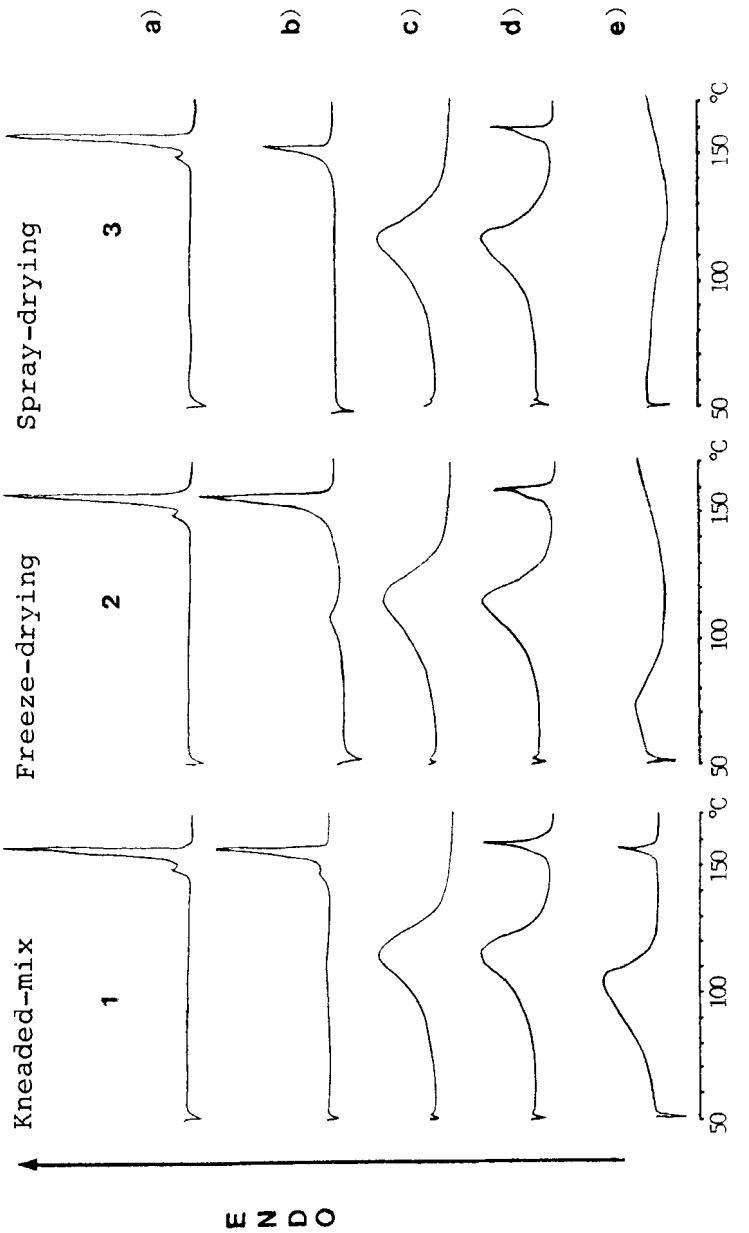

FIGURE 3

Thermograms of naproxen (a); naproxen treated (b); β-cyclodextrin Physical mixture naproxen-β-cyclodextrin (d); Inclusion complexes (e)

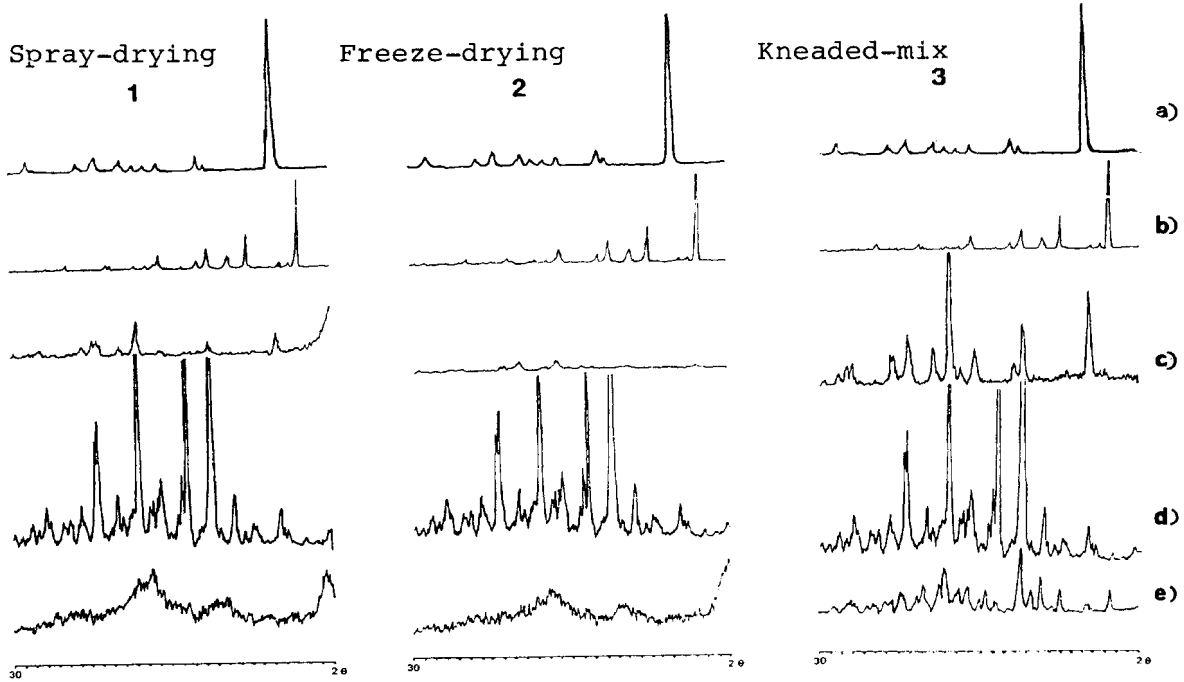

FIGURE 4

X-Ray diffraction patterns obtained for naproxen (a); β-cyclodextrin (b); naproxen treated (c); Physical mixture (d) and inclusion complexes (e)

FIGURE 5

I.R. absorption bands in the 1500-2000 cm^{-1} region: a) Naproxen b) β-cyclodextrin; c) kneaded-mix inclusion complex; d) Spray-dried inclusion complex; e) Freeze-dried inclusion complex.

corresponding infrared spectra (Figure 5): the naproxen peak at 1728 cm^{-1} is absent for the inclusion complexes obtained via spray drying and freeze-drying but it is evident for the complex obtained via kneaded-mixing.

Dissolution studies: Figures 6 and 7 show mean dissolution curves for naproxen on its own and in the form of the inclusion complexes With regards to naproxen it can be observe that different dissolution profiles are obtained according to the treatment. Table 1 lists the percentages of naproxen dissolved after 5 minutes and the dissolution efficiency values for naproxen on its own and subject to the three different treatments.

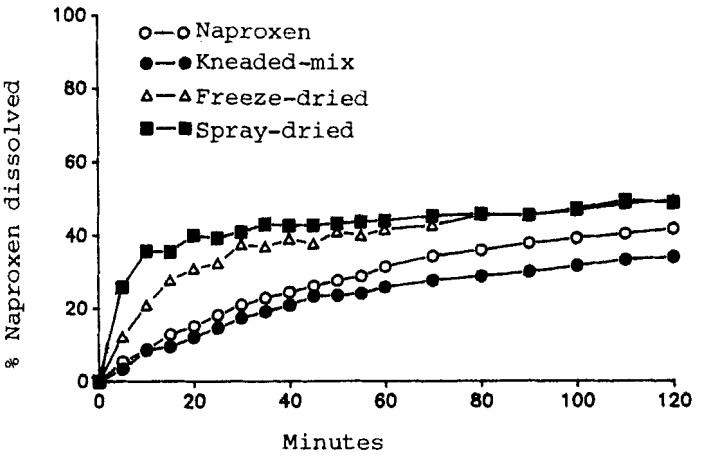

FIGURE 6

Mean dissolution curves (6 experiments) of naproxen

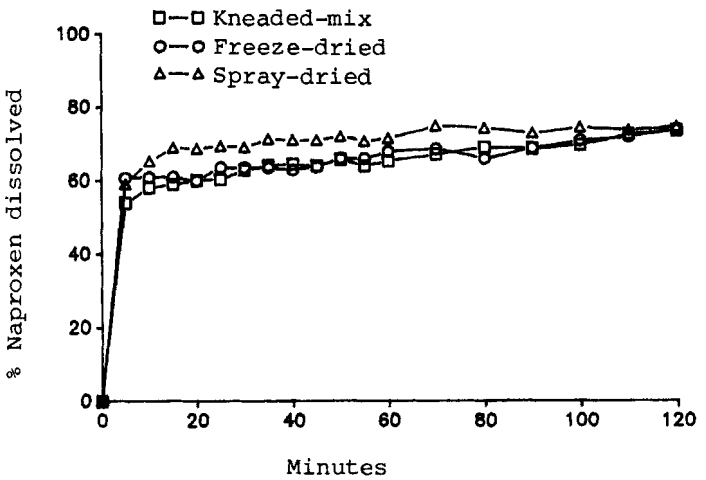

FIGURE 7

Mean dissolution curves (6 experiments) of naproxen-β-cyclodextrin inclusion complexes.

TABLE 1

Percentages of naproxen dissolved after 5 minutes and dissolution efficiencies for naproxen on its own and subject to 3 different treatments.

	% naproxen dissolved after 5 minutes	Dissolution Efficiency
Naproxen	5.43	0.2783
	5.48	0.3113
	6.45	0.2729
	5.36	0.2626
	5.21	0.2706
	5.02	0.2982
Naproxen kneaded-mixing	8.03	0.2671
	4.50	0.2392
	10.13	0.2002
	8.08	0.2107
	5.21	0.2441
	3.21	0.2409
Naproxen Freeze-Dried	8.82	0.4657
	10.25	0.4316
	9.62	0.3426
	14.76	0.3888
	16.34	0.3540
	13.52	0.3503
Naproxen Spray-Dried	23.32	0.4210
	22.79	0.4238
	27.69	0.4215
	33.98	0.4301
	24.26	0.4181
	23.34	0.4225

The ANOVA for the % of naproxen dissolved after 5 minutes gives a F value of 61.57 which is significant at $\alpha = 0.01$. For the Dissolution Efficiency values the ANOVA gives a F value of 10.04 which is also signifficant at $\alpha = 0.01$ level, so we used the test for minimum significant difference (MSD) in order to see which of the treatments gave significant differences (Table 2).

From these results it can be seen that, with regards to the percentage of naproxen dissolved after 5 minutes, naproxen and kneaded-mix naproxen present no significant difference, whereas the freeze-dried and spray-dried naproxen do show significan-

TABLE 2

Average values for parameters corresponding to the treatments applied to naproxen and values of the MSD test

Parameter	Naproxen	Kneaded-mix	Spray-dried	Freeze-dried	MSD
% Naproxen dissolved after 5 min	5.49	6.52	25.89	12.21	4.869
Dissolution Efficiency	0.2845	0.2337	0.4231	0.3855	0.108

TABLE 3

Values of dissolution parameters for naproxen in inclusion complexes made by three different methods

Inclusion Complex	% naproxen dissolved after 5 minutes			Dissolution Efficiency		
Kneaded-mix	52.45	51.60	53.01	0.6466	0.6295	0.6600
	54.87	54.98	57.42	0.6355	0.6375	0.6467
Freeze-dried	62.65	59.20	56.46	0.6364	0.6305	0.6412
	67.05	60.34	59,10	0.6253	0.6461	0.6959
Spray-dried	62.22	67.78	64.07	0.6959	0.7052	0.7295
	53.01	53.87	56.42	0.6856	0.6865	0.6896

tly higher values due to the structural changes produced by these treatments. The values for naproxen and kneaded-mix naproxen corresponding to the dissolution efficiency are statistically equal but lower than those for freeze-dried and spray-dried naproxen.

Table 3 lists the percentages of naproxen dissolved after 5 minutes and the dissolution efficiency values corresponding to the naproxen-betacyclodextrin complexes.

The ANOVA for the percentage of naproxen dissolved after 5 minutes gives a F value of 4.38 which is significant at $\alpha = 0.05$ level and for the dissolution efficiency values the ANOVA gives a F value of 10.95, significant at $\alpha = 0.01$ level.

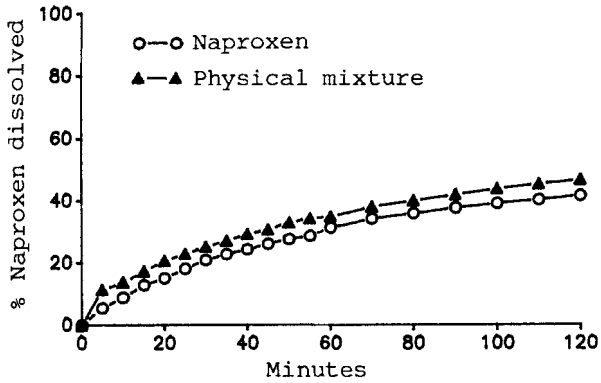

FIGURE 8

Mean dissolution curves of naproxen and physical mixture

The percentage of naproxen in solution after 5 minutes parameter, at the 0.05 significance level, shows a minimum significant difference of 5.24; therefore significant differences exist between the values due to the complexes made by kneading-mixing (53.96%) and those made by frezze-drying (60.80) and spray-drying (59.56).

With regards to the efficiency of dissolution parameter at the 0.01 significant level, the minimum significant difference is 0.0386; therefore the values corresponding to the kneaded-mix method (0.6426) and the freeze-drying method (0.6493) method are considered equal, whereas the mean value corresponding to the spray-drying method (0.6987) is significantly greater.

It has already been shown that, for the inclusion complex made by mixing, not all the naproxen is incorporated into the betacyclodextrin cavity; that the dissolution efficiencies for complexes made by kneaded-mixing and freeze-drying should be statistically similar is therefore of interest and can be explained when the increased solubility of naproxen when in a physical mixture with betacyclodextrin is considered (Figure 8).

A similar occurence has been described by Szejtly for indomethacin (18) and so, for bioavailability enhancement, the preparation of a real cyclodextrin complex is therefore not always necessary.

Acknowledgement

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