FORMULATION AND CHARACTERIZATION OF RANITIDINE MUCOADHESIVE MICROSPHERES

Tushar Kishore Sambre [1]*, Tarak Mehta [1], Tanvi Sambre [2]

ABSTRACT

The purpose of this study was to create ranitidine mucoadhesive microspheres to block H2 receptors. To speed up the start of action, boost medication bioavailability, and get around the notoriously difficult-to-cross blood-brain barrier, a number of formulations were created utilising spruce gum polymer in drug:polymer ratios ranging from 1:1 to 1:4. Drug loading, entrapment efficiency, histopathological features, in vitro drug release, mucoadhesion in vitro, particle size, manufacturing yield, and swelling property were all measured for the generated mucoadhesive microspheres. Differential scanning calorimetry, scanning electron microscopy, and X-ray diffraction analysis were used to characterise the microspheres after they were created. The manufactured microspheres were perfectly spherical and had a high swelling capacity and a smooth exterior. When mucoadhesive microspheres formulations reach the mucosa, they come into touch with the fluid (containing cations), which causes spontaneous viscous gelation (reducing the clearance rate) in the cavity and increases the residence time, hence increasing the activity by several-folds. This research paved the way for new strategies to improve the therapeutic effectiveness of ranitidine.

Keywords: Ranitidine, Mucoadhesive, Microsphere, Drug absorption, Bioavailability.

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1. INTRODUCTION

Anti-retroviral ranitidine, licenced by the US Food and medication Administration (USFDA), is a medication in the same family as cimetidine and famotidine. It blocks the action of histamine by binding to the H2 receptors on the surface of cells. This medication's capacity to reduce stomach acid output makes it useful for the prevention and treatment of disorders related to excess gastric acid, such as ulcers. Zantac, or ranitidine, is a brand name for a medication that comes in tablet, injectable, and effervescent tablet forms.[1] It is estimated that 10%-20% of the western population suffers with GERD. As a result of its efficacy in treating GERD and other gastric-acid related diseases, ranitidine is often prescribed for patients suffering from these illnesses. Due to its short half-life of 1.2-1.9 hrs, it must be administered repeatedly to keep therapeutic medication plasma levels constant.[2]

Due to its many therapeutic benefits, pharmacotherapeutics has become an essential component of the contemporary drug delivery module. The nasal mucosa, as well as the buccal, gastrointestinal, and other mucosal areas of the human body, have a high surface area and high permeability, allowing for rapid systemic absorption of the drug and rapid initiation of drug action.[3] Nasal mucosa is rich in blood vessels, allowing for direct systemic circulation and, as a consequence, increased systemic bioavailability of medicines by bypassing hepatic metabolism.[4]

When compared to the other common methods of microsphere development, the emulsification cross-linking technique stands out as a significant procedure for the preparation of microspheres because of its high feasibility, easy scale-up, excellent drug loading ability, and good reproducibility at the laboratory scale.[5] To speed up the onset of action, boost drug bioavailability, and get around the notoriously difficult-to-cross blood-brain barrier, the current study set out to create ranitidine mucoadhesive microspheres out of spruce gum polymer in drug: polymer ratios ranging from 1:1 to 1:4. Drug loading, entrapment efficiency, drug penetration ex vivo, histopathological features, drug release in vitro, mucoadhesion in vitro, particle size, manufacturing yield, and swelling property were all measured for the generated mucoadhesive microspheres. Differential scanning calorimetry, scanning electron microscopy, and X-ray diffraction analysis were used to characterise the microspheres after they were created.

2. MATERIALS AND METHODS

2.1. Materials

Alembic® India Limited of Ahmedabad, India was contacted and asked to provide us with pharmaceutical grade ranitidine and spruce gum. Analytical grade calcium chloride, Span-80, n-octanol, and dichloromethane were all provided by Sigma Aldrich® Limited, Mumbai, India.

2.2. Drug and polymer interaction studies

Ranitidine and spruce gum were tested for compatibility with an FT-IR spectrometer (GX-FT-IR, Perkin Elmer®, USA) to determine whether or not the polymer was appropriate for the microsphere manufacturing process. Scaning was done between 4000 and 500 using KBr discs containing the drug, polymer, and physical combination samples.[6]

2.3. Preparation of mucoadhesive microsphere

Without emulsification cross-linking was used to create mucoadhesive microspheres. In double-distilled water, spruce gum was dissolved with mild heat. Weighed amounts of ranitidine were added to the spruce gum solution, which had been previously been homogenised by steady agitation at 40°C. In a 250 mL beaker, 2% w/v span-80 was added to 100 mL of n-octanol: water system (99:1) with continual agitation at 1700 rpm using a mechanical stirrer. The aforesaid solution was swiftly injected using a 5 mL syringe. Thirty minutes were spent stirring the w/o emulsion. Dispersion was then agitated for 5 mins while 4% $CaCl_2$ solution was dropwise added. After being prepared, the microspheres were filtered using Whatman filter paper no. 41 and washed two to three times in isopropyl alcohol. The microspheres were then desiccated at room temperature after being dried in a hot air oven at 40°C.[7] The formulation chart is given in **Table 1**.

Table 1. Formulation batches of spruce gum microspheres of ranitidine.

Formulation code	Drug (mg)	Spruce gum (mg)
F1	20	20
F2	20	40
F3	20	60
F4	20	80

2.4. Evaluation of mucoadhesive microspheres

The mucoadhesive microspheres of ranitidine were evaluated as per Nayak *et al*., 2010.

2.4.1. Production yield

The proportion of the original ranitidine and spruce gum polymer weight that was recovered after drying the end product (formulation) was used to

calculate the manufacturing yield of several batches of microsphere formulation.

2.4.2. Drug loading

For 24 hours, each formulation's microspheres were extracted in double-distilled water using a mechanical shaker, releasing all of the ranitidine that had been encapsulated within. No. 41 Whatman filter paper was used to filter the solution. A 1 mL sample was taken and diluted to 10 mL using 10 times as much double-distilled water. We measured the concentration of the medication in this solution using a UV spectrophotometer (Shimadzu® UV-1800, Japan) set to 313 nm.

2.4.3. Entrapment efficiency

For 24 hours, each formulation's microspheres were extracted in double-distilled water using a mechanical shaker, releasing all of the ranitidine that had been encapsulated within. No. 41 Whatman filter paper was used to filter the solution. A 1 mL sample was taken and diluted to 10 mL using 10 times as much double-distilled water. We measured the concentration of the medication in this solution using a UV spectrophotometer set to 313 nm.

2.4.4. Particle size analysis

A Motic digital microscope (DMW2-223, Motic® Instruments Inc., Canada) equipped with a 1/3" CCD camera imaging attachment and computer-controlled image analysis software was used for microscopic image analysis for the assessment of particle size. The produced microspheres were evenly spread out on a standard-sized microscope slide, and the video camera was used to scan the microscopic field. Within the scanned area, the programme evaluated the photos.

2.4.5. Degree of swelling

Allowing the formulations to swell in the phosphate buffer pH 6.6 established the ranitidine microspheres' swellability in the physiological medium. For twenty-four hours, a measured volume of microspheres was submerged in a phosphate buffer with a pH of 6.6 and then completely rinsed.

2.4.6. *In-vitro* mucoadhesive study

The falling liquid film method was used to evaluate the mucoadhesive quality of the microspheres. After being washed in isotonic saline solution, a 2 cm^2 section of goat nasal mucosa was removed from a recently slaughtered goat. A mucosal surface was put over a polyethylene plate, and 100 mg of microspheres were carefully placed on top of it. For the spruce gum (polymer) to interact with the nasal mucosa membrane, 100 µL of a simulated nasal electrolyte solution was added to the microspheres, and the mixture was incubated for 15 minutes in a desiccator at 90% relative humidity. Finally, the membrane was fastened at a 45° to the horizontal. At a rate of 1 ml/min, a phosphate buffer with a pH of 6.6 and a temperature of 37°C was pumped through the microspheres and membrane. The concentration of the medication in the perfusate was measured spectrophotometrically after 1 hour.

2.4.7. Differential scanning calorimetry (DSC)

We used a differential scanning calorimeter (Mettler Toledo®, USA) to investigate the thermal properties of the pure drug, polymer, physical mixture, and optimised microsphere formulation by heating them at a rate of 10°C/min from 30 to 300 degrees Celsius while maintaining an inert nitrogen atmosphere with a flow rate of 20 millilitres per minute.

2.4.8. X-Ray diffraction study (XRD)

Samples of pure medication, polymer, physical mixture, and optimised formulation were irradiated with Cu-K radiation (monochromatized) in the 2θ range of 3-60° on an X-ray diffractometer (Ultima-III, Rigaku®, Japan) and analysed for X-ray diffraction patterns.

2.4.9. Scanning electron microscopy (SEM)

The surface morphology of the microspheres was examined using a scanning electron microscope (Jeol®, JSM-5610 LV, Japan) at 400x and 2000x magnifications. To assess the surface morphology, gold-coated (4A° thickness) microspheres were powdered and then sifted over double-sided tape on the aluminium stub of the SEM chamber. At a working accelerating voltage of 6 kV, photomicrographs of the microspheres in development were captured.

2.4.10. *In-vitro* drug release study

The manufactured microspheres were used in an in vitro drug release investigation using a Franz diffusion cell made of glass (Electrolab®, Mumbai, India). The dialysis membrane's diffusion barrier, which has a molecular cut-off between 12,000 and 14,000, was used in this experiment. Careful distribution of the manufactured mucoadhesive microspheres into the donor compartment equilibrated the dialysis membrane. The pH 1.2 phosphate buffer solution was poured into the receptor well. The donor chamber was maintained such that it makes contact with the diffusion medium of the receptor chamber. The circulating

water bath allowed for a steady 37°C to be maintained. Periodically, samples were taken from the receptor section, and the sink state was kept constant. The UV spectrophotometer measured the samples at 313 nm.

3. RESULTS AND DISCUSSION

3.1. Drug-polymer compatibility study

FTIR spectroscopy was used to investigate a potential interaction between the pure medication and the polymer. Figure 1A shows the FTIR spectrum of pure ranitidine; Figure 1B shows that of polymer spruce gum; Figure 1C shows that of a physical mixture; and Figure 1D shows that of an optimised formulation F2 (cm^{-1}: 3626, 3526, 2362, 1697, 1265, 896, and 453). The peaks of the pure drug and the polymeric peaks in the spectra of the physical mixture and the optimised formulation were quite comparable, indicating that there was no significant drug interaction between the drug and the polymer.

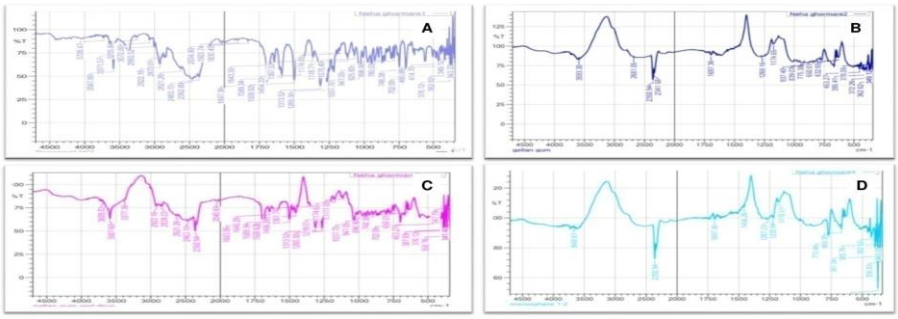

Figure 1. Drug compatibility studies: (A) pure ranitidine; (B) spruce gum; (C) physical mixture; and (D) optimized formulation N2.

3.2. Characterization of mucoadhesive microspheres

Drug loading, entrapment efficiency, ex vivo drug penetration, histopathological features, in vitro drug release, in vitro mucoadhesion, morphology, particle size, production yield, and swelling property were all measured for the four different mucoadhesive microsphere formulations (F1-F4) that were created. X-ray diffraction, scanning electron microscopy, and differential scanning calorimetry were used to analyse the produced microspheres.

3.2.1. Production yield

Ranitidine mucoadhesive microspheres had a yield between 40.26 and 55.93 percent, as shown in Table 2. It was discovered that the yield improved somewhat when the polymer concentration was raised.

3.2.2. Drug Loading

Table 2 displays the observed drug loading of the produced mucoadhesive microspheres, which varies from 49.21% to 74.60%. Drug loading was found to be greatest in the 1:1 formulation compared to the 1:4 formulation because an increase in polymer concentration alters the fabricated microsphere's morphology and, at the same time, decreases the microspheres' surface area, leading to a lower drug loading.

Table 2. Pharmaceutical properties of prepared ranitidine mucoadhesive microsphere formulations.

Formulation code	Production yield (% ± SD)	Drug loading (% ± SD)	Entrapment efficiency (% ± SD)	Particle size (µm ± SD)	% Swelling (% ± SD)	Mucoadhesion (% ± SD)
F1	40.26 ± 0.1	74.60 ± 0.01	34.5 ± 1.02	17.3 ± 0.3	82 ± 0.1	45.6 ± 0.1
F2	44.83 ± 0.3	64.62 ± 0.01	40.5 ± 0.15	14.3 ± 0.2	83 ± 0.2	57.6 ± 0.2
F3	50.45 ± 0.2	57.77 ± 0.02	42.3 ± 0.15	17.6 ± 0.1	88 ± 0.1	62.2 ± 0.1
F4	55.93 ± 0.1	49.21 ± 0.03	53.6 ± 0.21	18.3 ± 0.5	91 ± 0.1	79.6 ± 0.1

3.2.3. Entrapment efficiency

Table 2 displays the range of entrapment efficiency for mucoadhesive microsphere formulations, which is 34.5–53.6%. It was found that increasing the concentration of the polymer improved the effectiveness of the entrapment process. An increased rate of drug entrapment due to faster hardening of the larger particles could account for the increased encapsulation efficiency observed at high polymer levels in formulations. This would be accomplished by minimising the time the drug has to diffuse out of the particles.

3.2.4. Particle size

The average particle size of microspheres for intravenous delivery must be between 10 and 20

µm. The prepared microspheres had an average perimeter of 500 µm, radius of 75 µm, and area of 20,000 sq. µmm (Table 2), with a typical particle size ranging from 14.3 µm to 18.3 µm. The concentration of mucoadhesive polymer has little to no effect on particle size, since the particle size decreases with increasing stirring rates. In the case of the emulsification cross-linking approach, the stirring process plays a significant role in managing the particle size of the formulation, in contrast to the spray drying method, where the diameter of the nozzle plays a vital role in determining the particle size of the formulation.

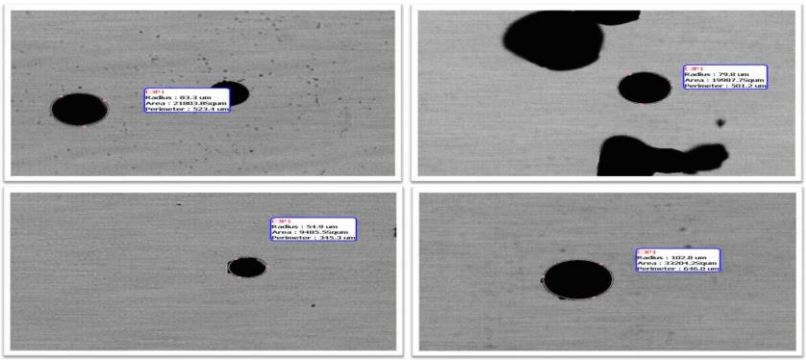

Figure 2. Microscopic image of microspheres.

3.2.5. Swelling property

Table 2 shows that the swelling property of the manufactured mucoadhesive microsphere is between 82% and 91%. In the Simulated Nasal Fluid (SNF), all four microsphere formulations (F1-F4) quickly expanded. The quantity of polymeric content was used to calculate the swelling capacity of the ready-made microspheres. Since the polymeric matrix retains fluid (water), swelling increases in tandem with an increase in polymer concentration (spruce gum).

3.2.6. Mucoadhesion potential

The in vitro mucoadhesion investigation was carried out to guarantee the formulation's long-term adherence to the mucosal membrane at the site of absorption. The entire mucoadhesive microsphere formulations tested showed strong adherence to the nasal mucosal membrane. Attachment of microspheres to the nasal mucosa, as a percentage of the total applied mass, varied from roughly 45.6% to 79.6% across all batches (Table 2). Microsphere mucoadhesion on goat nasal mucosa was evaluated by varying the polymer concentration (spruce gum) from a 1:1 ratio to a 1:4 ratio. One probable explanation is that the nasal mucosa membrane might come into touch with a high concentration of polymeric material.

3.2.7. Thermal characteristics

Figure 3 shows the differential scanning calorimetry (DSC) thermograms of the unmodified drug, polymer, physical combination, and F2 optimised formulation. The melting point of ranitidine, as seen by a strong endotherm peak in the thermogram at 225.08°C (Figure 3A), is consistent with the purity of the medication. In the thermogram (Figure 3B), the polymer spruce gum showed a wide endotherm peak at 55.64°C. Figure 3C shows a thermogram of a physical mixture of ranitidine and the polymer spruce gum, which displays the signatures of both substances (a sharp endotherm peak at 221.52°C for ranitidine and a broad endotherm peak at 90.62°C for spruce gum). The optimised mucoadhesive microsphere formulation F2, however, showed a very broad endothermic peak at 69.16°C in its thermogram (Figure 3D), indicating that the drug was molecularly dispersed (high disordered amorphous state) inside the microspheres.

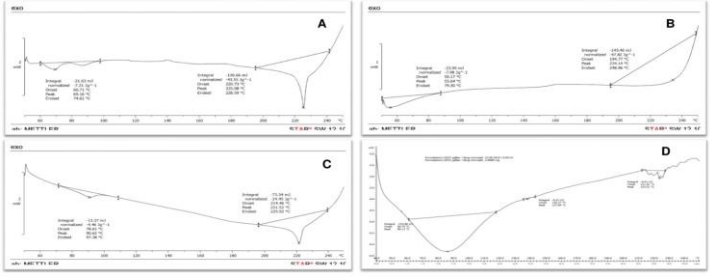

Figure 3. DSC thermograms: (A) pure ranitidine; (B) spruce gum; (C) physical mixture; and (D) optimized formulation F2.

3.2.8. Physical state examination

Figure 4 displays the results of X-ray diffraction analyses conducted on pure medication, polymer, and the F2 optimised formulation. The crystalline form of the pure medication ranitidine was shown in Figure 4A, with peaks at 6.64°, 6.87°, and 13.00° on the 2θ scale. Figure 4B's diffractogram of the polymer spruce gum, which lacks a crystalline peak, indicates that the component is amorphous. Molecular dispersion in the polymeric matrix and subsequent conversion to the highly disordered amorphous form of the drug are shown by the lack of prominent crystalline peaks in ranitidine-loaded mucoadhesive microspheres (Figure 4C).

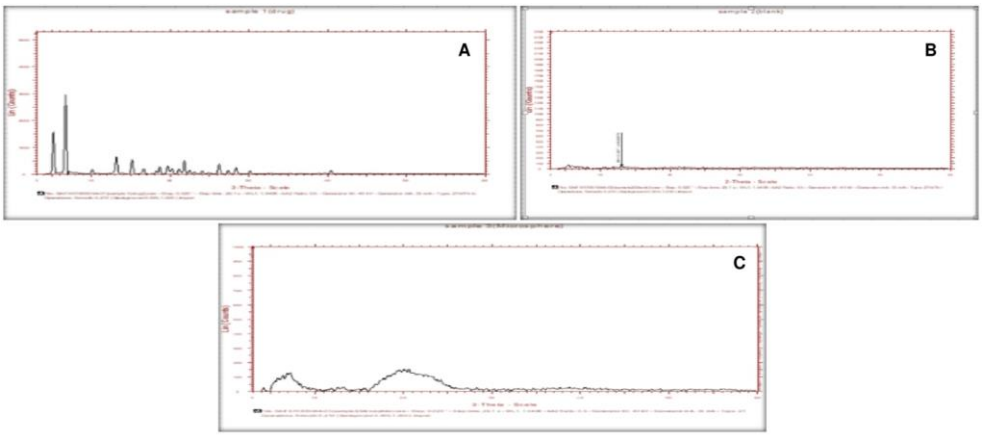

Figure 4. Powder X-Ray diffractogram: (A) pure ranitidine; (B) spruce gum; and (C) optimized formulation N2.

3.2.9. Morphological examination

Micrographs taken using a scanning electron microscope (SEM) revealed that the microspheres are consistently round and round (Figure 5A), confirming the SEM's study of their morphological and surface properties. Figure 5B shows that although the bulk of the microspheres' surfaces are smooth, a few areas are rough or irregular. Good deposition, increased retention, and sluggish removal in the nasal cavity are predicted due to the lack of pores, holes, or rupture across the formulation surface (Figure 5C). Microspheres, representing ranitidine encapsulation in a polymeric matrix (Figure 5D), show no evidence of drug particles adhering to their surface.

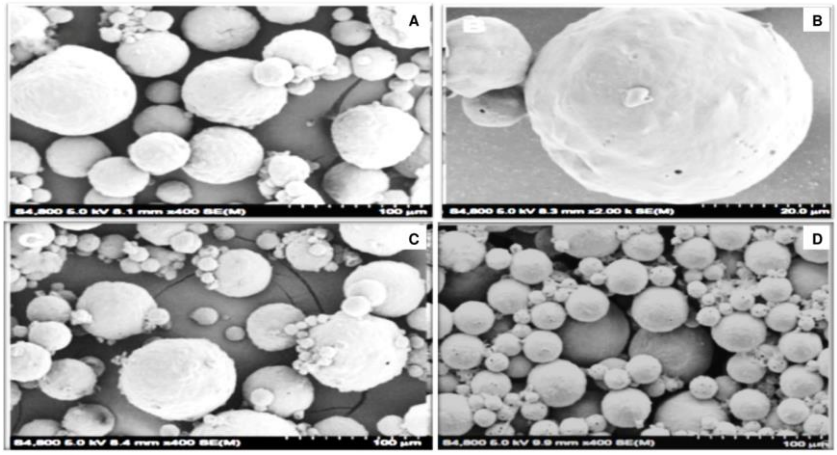

Figure 5. Scanning electron photomicrographs: (A) 400x maginification; (B) 2000x maginification; (C) 400x maginification; and (D) 400x maginification.

3.2.10. *In-vitro* release of ranitidine

Table 3 details the variations in drug release characteristics across four different batches of mucoadhesive microsphere formulation (F1-F4). In an in vitro dissolving assay lasting 5 hours, Formulation F2 had the greatest cumulative drug release of 98.26%. The best combination of polymer concentration allowed for optimised ranitidine release, which may account for the high rate of release. However, after 300 minutes of dissolution, the formulation F4 with a 1:4 ratio showed the lowest release rate. The microsphere system's controlled release properties, supplied by the polymer (spruce gum), may have restrained the free release of ranitidine into the medium, leading to the slower release rate.

Table 3. *In vitro* drug release profile of ranitidine mucoadhesive microsphere formulations.

Time (min)	F1	F2	F3	F4
15	15.52 ± 0.1	19.69 ± 0.1	9.78 ± 0.3	12.07 ± 0.2
30	31.93 ± 0.1	41.20 ± 0.1	25.67 ±0.2	25.55 ± 0.1
60	38.07 ± 0.2	47.92 ±0.3	37.71 ± 0.1	31.29 ± 0.1
90	44.43± 0.1	54.48 ± 0.1	44.16 ± 0.2	36.39 ± 0.5
120	50.39 ± 0.1	62.94 ± 0.1	49.11 ± 0.1	44.05 ± 0.1
150	53.72± 0.2	67.94 ± 0.2	53.93 ± 0.4	50.09 ± 0.2
180	57.81± 0.2	72.86 ± 0.1	55.93 ± 0.4	53.61 ± 0.1
210	63.06± 0.2	79.03 ± 0.1	59.58 ± 0.5	59.27 ± 1.1
240	66.41± 0.1	85.07± 0.2	64.79 ± 0.1	65.31 ± 0.1
270	72.62 ± 0.2	92.87 ± 0.3	71.97 ± 0.2	68.16 ± 0.2
300	78.24 ± 0.1	98.26 ± 0.1	77.21 ± 0.1	76.92 ± 0.1

CONCLUSION

The current study comprised the emulsification cross-linking technique's assistance in developing spruce gum-based mucoadhesive microspheres loaded with ranitidine for potential drug administration in the prospective treatment of GERD. The manufactured microspheres were perfectly spherical, and they inflated properly and had smooth exteriors throughout. When mucoadhesive microspheres formulations reach the nasal mucosa, they come into touch with the fluid (containing cations), which causes spontaneous viscous gelation (reducing the clearance rate) in the mucosa and increases the residence time, hence increasing the activity by several-folds.

CONFLICT OF INTEREST

No conflict of interest is declared.

FUNDING INFORMATION

No funding is received from any agency.

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