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DESIGN AND OPTIMIZATION OF CAPECITABINE NIOSOMES DERIVED FROM PRONIOSOMES

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ABSTRACT: The aim of this investigation was to design and optimize the Capecitabine niosomes derived from proniosomes using central composite design. Two independent variables *viz*., the molar ratio of drug to cholesterol (X_1), surfactant loading (X_2) and two dependent variables *viz*., the percentage drug entrapment (PDE) and mean volume diameter (MVD) were selected for the study. Proniosomes were prepared by a conventional slurry method and evaluated for the percentage drug entrapment (PDE) and mean volume diameter (MVD). The PDE dependent variables and the transformed values of independent variables were subjected to multiple regressions to establish a second order polynomial equation. Contour plots were constructed to elucidate the relationship between the independent and dependent variables further. From the computer optimization process and contour plots, predicted levels of independent variables X_1, X_2 (-0.77, -0.8 respectively), for an optimum response of PDE with constraints on MVD were determined. The polynomial equations and contour plots developed using central composite design allowed us to prepare niosomes derived from proniosomes with optimum responses.

INTRODUCTION: Most of the active pharmaceutical ingredients currently available in the market and those under development have poor and variable bioavailability. This problem can be overcome by entrapping the drug into niosomes. Niosomes are non-ionic surfactant vesicles that can entrap a solute in a manner analogous to liposomes. They are osmotically active and are stable on their own, while also increasing the stability of the entrapped drugs [1, 2]. Handling and storage of surfactants require no special conditions.

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Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together and as a result, can accommodate drug molecules with a wide range of solubilities [3]. Although, niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation, and leakage on storage.

All methods traditionally used for the preparation of niosomes are time-consuming and may involve specialized equipment. Most of these methods allow only for a predetermined lot size, so the material is often wasted if smaller quantities are required for particular dose application [4]. The proniosome approach minimizes these problems as it is a dry and free-flowing product which is more stable during sterilization and storage.

Ease of transfer, distribution, measuring, and storage make it a versatile delivery system. Proniosomes are water-soluble carrier particles coated with a surfactant, which can be measured out as needed and hydrated to form niosomes immediately before use on brief agitation in hot aqueous media [5, 6].

Capecitabine is a prodrug of 5'-deoxy-5-fluorouridine (5'-DFUR) **Fig. 1**, which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows the growth of tumor tissue [7, 8]. It is an orally administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers [9]. In the present research work, the conventional slurry method was used for the preparation of Capecitabine niosome derived from proniosome and optimized. The proniosomes are thus needed to be optimized for the desired response; many statistical experimental designs have been recognized as useful techniques to optimize the formulation and process variables [10].

Different types of experimental design include 3-level factorial design [11]. D-optimal design [12], Box Behnken design and central composite design [13]. Central composite design requires fewer runs in a 3-factor experimental design and hence was selected for the present research work. The independent variables selected for the present study are the molar ratio of drug to lipid (X_1), surfactant loading (X_2). The dependent variables included are PDE and MVD of niosomes derived from niosomes. The optimization is done using Design Expert 11 (Trial Version 11, Stat-Ease Inc., and Minneapolis, MN) to interpret the results and easy scale up.

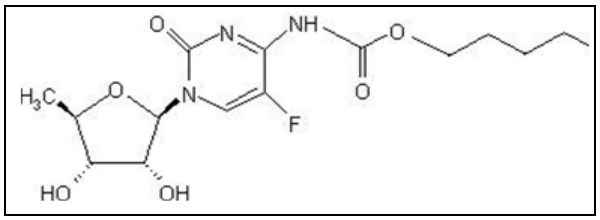

FIG. 1: STRUCTURE OF CAPECITABINE

MATERIALS AND METHODS

Materials: Capecitabine gift sample was obtained from Shilpa antibiotic Pvt. Ltd., Raichur. Maltodextrin was procured from Himedia, Hosur, cholesterol, span 40 and DCP (Dicetyl phosphate) were purchased from Loba chem Pvt. Ltd., Mumbai. All the other ingredients and reagents used were of analytical grade.

Methods:

Central Composite Experimental Design: Traditionally pharmaceutical formulations are developed by changing one variable at a time, but this approach does not give an idea about the interactions among the variables and difficult to develop an optimized formulation. Hence, a central composite experimental design with 2 factors, 3 levels, and 20 runs was selected for the optimization study. This design consists of 8 full factorial design points, 8 axial points, and 4 center points. Independent variables with their levels and the dependent variables selected are listed in **Table 1**. The second order polynomial equation generated from these experimental designs as,

$$Y_1 = b_0+b_1X_1+b^2X_2+b_3X_3+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_1^2 +b_{22}X_2^2+b_{33}X_3^2.....$$

Where Y_1 is the dependent variable while b_0 is the intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2 are the independent variables levels of which were selected from the preliminary experiments.

Preparation of Proniosomes: The proniosomes were prepared by the slurry method [14]. 250 µmol stock solution of span 40 and cholesterol was prepared in chloroform: methanol (2:1). The accurately measured volumes of span 40 and cholesterol stock solutions and Capecitabine (50mg) dissolved in chloroform: methanol (2:1) solutions were added into a 250 ml round bottom flask containing previously 2 g of maltodextrin powder used as the carrier.

Additional chloroform: methanol (2:1) solution added to form a slurry. Further, the flask was attached to a rotary flash evaporator rotated at 60 to 70 rpm. The solvent is allowed to evaporate at a temperature of 45 ± 2 ºC in a reduced pressure of 600 mm/Hg until the mass in the flask had become a dry, free-flowing product. The obtained proniosomes powder was further dried overnight in desiccators under vacuum at room temperature. The obtained dry proniosomes powders were stored in airtight amber colored vials kept in a refrigerator for further evaluation.

TABLE 1: VARIABLES AND THEIR LEVELS IN CENTRAL COMPOSITE DESIGN

Independent variables	Low	Medium	High
X_1= Molar ration of drug lipid	40%	60%	90%
X_2= Surfactant loading	10%	30%	60%
Transferred values	-1	0	1
Dependent variables		Goals	
Y_1= Percentage drug entrapment (%)		Maximize	
Y_2= Mean volume diameter (µm)		Minimize	

*1.5X corresponds to 15 mmol per g of carrier

These proniosomes were used for the preparation of niosomes and characterization of the surface characteristics by scanning electron microscopy. Proniosomes were transformed to niosomes by hydrating with phosphate buffer saline (PBS) with a pH of 7.4 at 80 °C using vortex mixer for 2 min. The niosomes were sonicated twice for a specified time using a 250W probe-type sonicator (MAGNA-PAK-250, Libra Ultrasonic, India).

Niosomes were prepared in such a manner that total surfactant concentration remained at 10 mmol in all the batches. Niosomes were characterized for morphology, PDE and vesicle size in terms of MVD.

Scanning Electron Microscopy: Proniosomes were sprinkled on to the double sided tape that was affixed on aluminum stubs. The aluminum stub was placed in the vacuum chamber of a scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands).

The samples were observed for morphological characterization using a gaseous secondary electron detector (working pressure: 0.8 torrs, acceleration voltage: 30.00 KV) XL 30, (Philips, Netherlands).

TABLE 2: CENTRAL COMPOSITE EXPERIMENTAL DESIGN WITH MEASURED RESPONSES OF CAPECITABINE PRONIOSOME

Run	X_1	X_2	Y_1	Y_2
	A: Cholesterol (%)	B: Surfactant (%)	PDE (%)	MVD(µm)
1	-1	-1	59.2 ± 0.11	7.55 ± 0.12
2	0	0	71.1 ± 0.31	6.75 ± 0.21
3	1	0	70.21 ± 0.82	6.85 ± 0.14
4	1	1	80.32 ± 0.21	3.98 ± 0.32
5	1	-1	59.21 ± 0.22	7.6 ± 0.25
6	0	0	70.21 ± 0.30	6.8 ± 0.35
7	1	1	81.02 ± 0.12	3.99 ± 0.51
8	0	1	84.12 ± 0.25	4.12 ± 0.21
9	1	0	70.95 ± 0.32	6.86 ± 0.25
10	0	0	70.85 ± 0.25	6.9 ± 0.25
11	0	-1	60.25 ± 0.52	7.6 ± 0.32
12	-1	1	80.95 ± 0.41	4.01 ± 0.13
13	0	-1	62.21 ± 042	7.65 ± 0.11
14	-1	1	80.21 ± 0.35	4.02 ± 0.22
15	-1	-1	59.31 ± 0.25	7.7 ± 0.15
16	-1	0	70.21 ± 0.24	6.96 ± 0.25
17	0	1	82.21 ± 0.15	4.02 ± 0.14
18	-1	0	71.21 ± 0.41	7.02 ± 0.15
19	1	-1	60.1 ± 0.23	7.75 ± 0.12
20	0	0	71.21 ± 0.32	7.1 ± 0.01

Optical Microscopy: The hydrated niosome dispersions prepared from proniosomes were observed using optical microscopy. After suitable dilution, the noisome dispersions on a glass slide and viewed by a microscope (Medilux-207R (II), Kyowa-Getner, India) with a magnification of 1200X.

Percentage Drug Entrapment: The entrapped Capecitabine within niosomes was determined after removing the unentrapped drug by dialysis [15]. The dialysis was carried out by taking niosome dispersion in a dialysis tube (donor compartment), which was dipped in a beaker containing 400 ml of PBS with a pH 7.4 (receptor compartment). The beaker was placed on a magnetic stirrer run for 4 h with a speed of 80-120 rpm.

Then, the solution inside the receptor compartment was studied for unentrapped capecitabine at 303 nm using a UV spectrophotometer (UV 1601, Shimadzu, Japan) [16]. The PDE in the niosomes was calculated from the ratio of the difference of the total amount of drug added and the amount of

unentrapped drug detected, to the total amount of drug added.

Measurement of Vesicle Size: The vesicle dispersion was diluted about 100 times in the same buffer used for their preparation. Vesicle size was measured on a particle size analyzer (Laser diffraction particle size analyzer, Sympatec, Germany). The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 mW using a Fourier lens [R-5] to a point at the center of the multielement detector and a small volume sample holding cell (Su cell). The sample was stirred using a stirrer before determining the vesicle size.

RESULTS AND DISCUSSION:

Morphology of Dry Proniosomes and Niosomes Derived Proniosomes: Proniosomes were prepared by the slurry method using maltodextrin as a carrier. Scanning electron microscopy (SEM) of uncoated maltodextrin powder **Fig. 2a** shows the highly porous surface, which would provide more surface area to be coated with a surfactant mixture. Proniosomes were made with different proportions of drug and surfactant coating **Fig. 2b**, **c,** and **d** are SEM images of different proniosome batches made at different surfactant loading.

The surface of the proniosomes batches F1 and F20, made at 1.5X and 3X respectively, was observed as being smooth and uniform while that of batch PA8, made at 4.5X surfactant loading was seen rough, thick and uneven. Morphology of proniosome derived niosomes was studied under the optical microscope. Niosomes prepared from proniosomes were spherical **Fig. 3**.

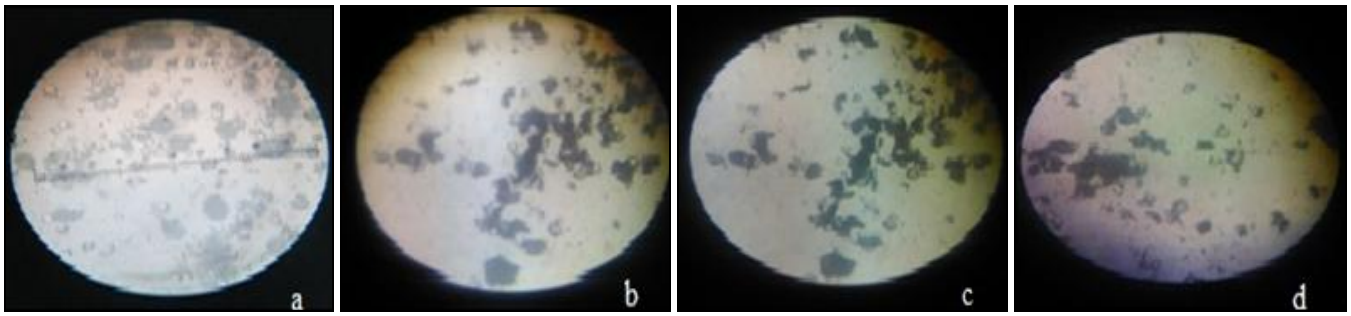

FIG. 2: SCANNING ELECTRON MICROGRAPHS OF PRONIOSOMES PREPARED: (a) WITH PURE MALTODEXTRIN, (b) AT 1.5X SURFACTANT LOADING, (c) AT 3X SURFACTANT LOADING, AND (d) AT 4.5X SURFACTANT LOADING

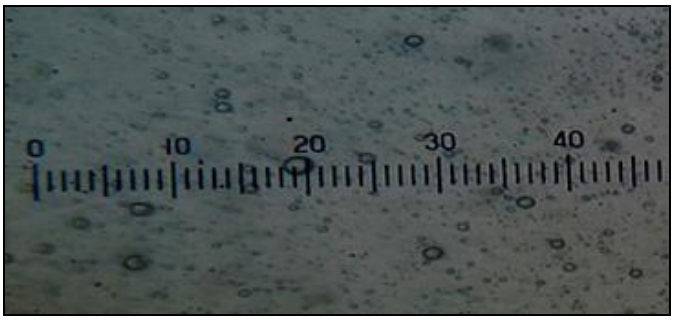

FIG. 3: OPTICAL PHOTOMICROGRAPH OF PRONIOSOME-DERIVED NIOSOMES (BATCH F8)

Optimization Study of Proniosomes: An optimization using the central composite design for 3 factors, 3 levels offers an advantage of fewer experimental runs (20 runs). The experimental runs and the observed responses for the 20 batches are given in **Table 2**. The different levels of independent variable combinations resulted in different PDE and MVD values. The PDE values observed were in the range of 59.20% in batch F1 (minimum) to 84.12% in batch F8 (maximum). This indicates selected two independent variables to have a profound effect on the PDE within proniosome-derived niosomes. The second order polynomial equation relating the response PDE and the independent variables was:

$$PDE = 71.3375 + 0.06A + 10.6367B - 0.0775AB - 1.1875A^2 + 0.1375B^2 .....$$

The values of the coefficients X_1-X_2 are related to the effect of these variables on the PDE. Coefficients of more than one terms represent interaction and show how the response changes when two factors are simultaneously changed. Coefficients of higher order terms represent quadratic relationship and are included to investigate nonlinearity. The polynomial equation can be used to conclude after considering the magnitude of each coefficient and the mathematical

sign it carries (*i.e*., positive or negative). The high value (0.98) of the correlation coefficient (R^2) in the above equation indicates a good fit. Proniosomal batches F2, F4, F7, F8, F12, F14, and F17 exhibited high PDE value, *i.e*., more than 70% **Table 2**. A negative sign of coefficient for the molar ratio of drug: lipid (X_1) and surfactant loading (X_2) represents the antagonistic effect of these variables. In this study at different levels of X_1, lipid was kept constant, and the amount of drug was increased for each level to give a different molar ratio. So at a low level of X_1 high PDE value might be due to more availability of lipophilic ambiance for the drug entrapment. The significance of the different formulation variables and their interactions was compared using analysis of variance (ANOVA) at a significance level of $P<0.05$. From the P value for PDF analysis given in **Table 3**, it can be concluded that the molar ratio of drug: lipid have significant effects on the PDE of Capecitabine proniosome-derived niosomes and no interaction term has a significant effect on the PDE.

TABLE 3: ANALYSIS OF VARIANCE FOR PDE

Source	Sum of Squares	df	Mean Square	F-value	*P*-value	
Model	1364.35	5	272.87	459.98	< 0.0001	significant
A-Cholesterol	0.0432	1	0.0432	0.0728	0.7912	
B-Surfactant	1357.66	1	1357.66	2288.62	< 0.0001	
AB	0.0481	1	0.0481	0.0810	0.7801	
A^2	6.58	1	6.58	11.09	0.0050	
B^2	0.0882	1	0.0882	0.1487	0.7056	
Residual	8.31	14	0.5932			
Lack of Fit	3.59	3	1.20	2.79	0.0904	not significant
Pure Error	4.72	11	0.4288			
Cor Total	1372.66	19				

Vesicle size (MVD) of the niosome batches was measured by low angle laser light scattering technique and was found to be in the range of 3.98µm to 7.75µm. A polynomial equation was developed for MVD, described as:

$$MVD = 6.8936 - 0.0191A - 1.819B - 0.02AB + 0.0192A^2 - 1.085B^2 .....$$

The value of the correlation coefficient (R^2) in the above equation was found to be 0.99, indicating a good fit. A positive sign of the coefficients for the molar ratio of drug: lipid and surfactant loading indicates favorable effects on MVD. Positive effects of X_1 could be attributed to hydrophobic interaction between drug and surfactant. Favourable effect of X_2 may be due to adequate hydration of the uniform and the thin film of surfactant at low surfactant loading compared to the film obtained at a high surfactant loading.

As shown in **Table 4**, among the independent variables selected the terms X_1 and X_2 were found to be significant ($P<0.05$) in predicting the MVD. It is also evident from **Table 4** that the quadratic effects of all the independent variables *i.e*. X_1^2, X_2^2 have significant effects on MVD.

TABLE 4: ANALYSIS OF VARIANCE FOR MVD

Source	Sum of Squares	df	Mean Square	F-value	*P*-value	
Model	45.35	5	9.07	1118.95	< 0.0001	significant
A-Cholesterol	0.0044	1	0.0044	0.5439	0.4730	
B-Surfactant	39.71	1	39.71	4899.63	< 0.0001	
AB	0.0032	1	0.0032	0.3948	0.5399	
A^2	0.0017	1	0.0017	0.2141	0.6506	
B^2	5.50	1	5.50	678.70	< 0.0001	
Residual	0.1135	14	0.0081			
Lack of Fit	0.0157	3	0.0052	0.5887	0.6350	not significant
Pure Error	0.0978	11	0.0089			
Cor Total	45.46	19				

As the central composite design includes two center points, we can estimate the pure error of the experiments and enable the model's to be checked for lack of fit. For the experimentally obtained data, the test for lack of fit did not yield statistical significance ($P>0.05$), and the results indicated that

the models for PDE and MVD were satisfactory **Table 3** and **4**.

Three-dimensional and Contour Plots: Presentation of the data as graphs can help to show the relationship between the independent and dependent variables. **Fig. 4** is a contour plot drawn at 0 levels showing the effect of X_1 and X_2 on MVD and PDE of proniosome-derived niosomes. The contours for all the values of MVD were found to be nonlinear. It was evident from **Fig. 4** that low value of MVD could be obtained with low level of both X_1 and X_2 and that high values of PDE (≥72%) can be obtained for different combinations of the two independent variables, X_1 in the range of less than -0.8 level and X_2 in the entire range of -1 level to 1 level.

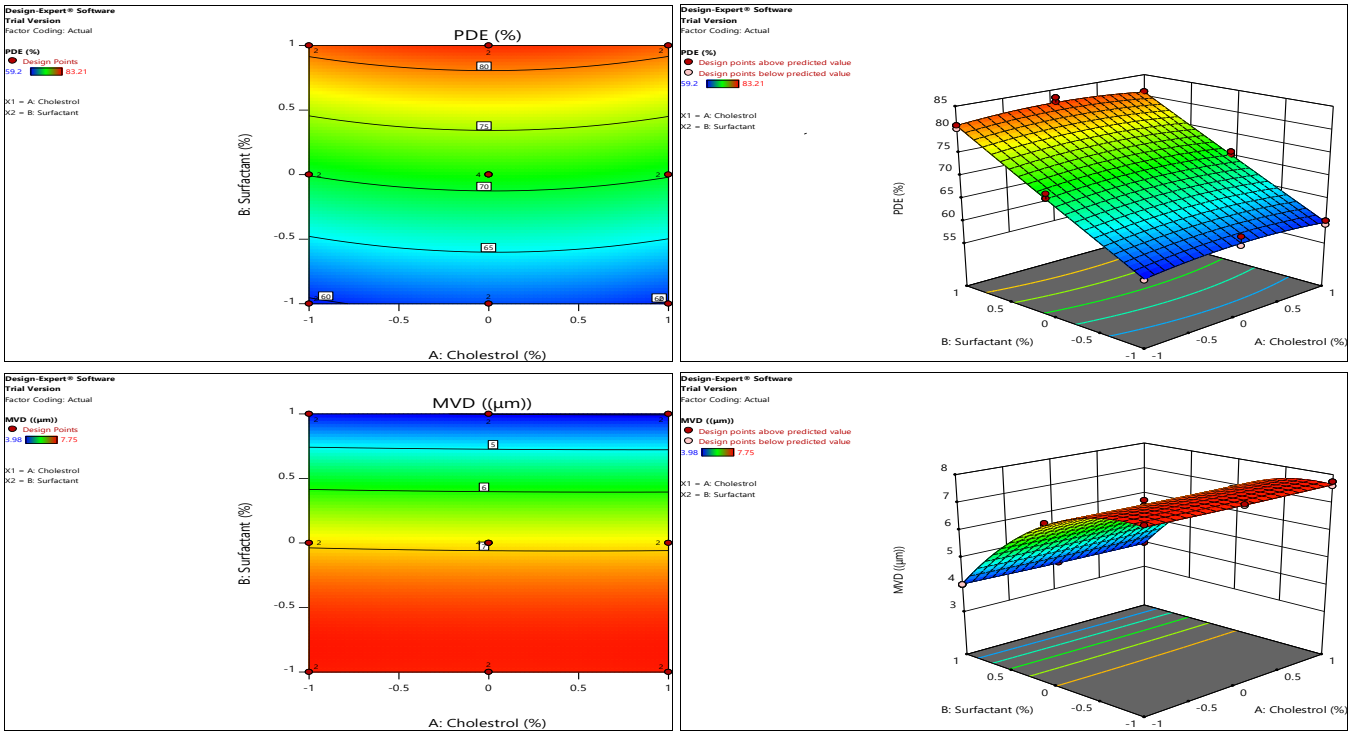

FIG. 4: THREE-DIMENSIONAL AND CONTOUR RESPONSE SURFACE PLOT IMAGE SHOWING INFLUENCE OF INDEPENDENT VARIABLES ON PDE AND MVD

After studying the effects of the independent variables on the responses, the levels of these variables that give the optimum responses were determined. The optimum formulation is one that gives a high value of PDE (≥70%) and is constrained to a low MVD (≤ 5 µm) as well as having a high total amount of drug entrapped and low amount of carrier present in the resultant niosomes.

Using a computer optimization process and the contour plots are shown in **Fig. 4**, the levels selected for both X_1 and X_2 were -0.77 and -0.8 respectively, which gives the theoretical value of 84.12% and 4.12 µm for PDE and MVD, respectively. Decreasing the level of X2 from the optimum level resulted in a significant increase in the amount of carrier but an insignificant increase in the PDE value.

However, an increase in the level of X_1 above the selected level led to an increase in the PDE value but as well an increase in the vesicle size above the desired value. Hence, -0.77 level of the molar ratio of drug: lipid (X_1), -0.8 level of surfactant loading (X_2) were selected as optimum. For confirmation, a fresh formulation was prepared at the optimum levels of the independent variables and the resultant proniosomes were transformed to niosomes and evaluated for the responses. The observed values of PDE and MVD were found to be 84.21% and 4.12 µm respectively, which were in close agreement with the theoretical values.

CONCLUSION: The slurry method was found to be simple and suitable for laboratory scale preparation of Capecitabine proniosomes. The statistical approach for optimization of the formulation is a useful tool when several variables

are to be studied simultaneously. The polynomial equations and contour plots developed by using central composite design allowed us to prepare proniosomes with optimum characteristics.

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CONFLICT OF INTEREST: Authors declare that they have no competing interests.

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