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FORMULATION OF DILOXANIDE FUROATE LOADED COLON TARGETING NANOSPONGES FOR EFFECTIVE TREATMENT OF AMOEBIASIS

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ABSTRACT

Amoebiasis, a parasitic infection caused by *Entamoeba histolytica*, continues to be a significant global health concern. To address this, targeted drug delivery systems can enhance therapeutic efficacy while minimizing side effects. This study focuses on the development and evaluation of Diloxanide furoate-loaded colon-targeting nanosponges designed to improve the treatment of amoebiasis. Nanosponges were formulated using an optimized technique and characterized for their physicochemical properties. The flow properties of the nanosponges, including poured and tapped densities, Carr's Index, and Hausner's Ratio, indicated suitable flow characteristics for practical application. The drug content and encapsulation efficiency were determined, revealing high drug content and significant encapsulation efficiency, with formulation F3 achieving the highest encapsulation efficiency of 83.32%. The optimized nanosponges (Formulation F3) demonstrated an average particle size of 52.74 nm and a zeta potential of -20.2 mV, indicating stability and potential for sustained release. The drug release studies showed a controlled release profile with minimal release in simulated gastric fluid (SGF) and a significant release in simulated intestinal fluid (SIF), reaching 98.78% cumulative drug release by 12 hours. These results suggest that Diloxanide furoate-loaded colon-targeting nanosponges offer an effective and targeted approach for the treatment of amoebiasis, potentially improving drug delivery and therapeutic outcomes.

Keywords: Diloxanide furoate, colon-targeting, nanosponges, drug delivery, amoebiasis, controlled release, encapsulation efficiency, particle size.

INTRODUCTION

Amoebiasis, caused by *Entamoeba histolytica*, is a parasitic infection primarily affecting the colon and leading to dysentery, abdominal pain, and in severe cases, liver abscesses (Haque, 2007; Reddy and Rao, 2010). Despite available treatments, including the commonly used antiamoebic drug Diloxanide furoate, challenges such as poor drug targeting, low bioavailability, and gastrointestinal side effects persist (Gupta *et al.,* 2012; Minocha *et al.,* 2016). Addressing these issues necessitates advanced drug delivery systems capable of enhancing therapeutic efficacy while minimizing side effects.

Nanotechnology offers promising solutions for improving drug delivery systems, particularly through the use of nanosponges porous, nanometer-sized particles that can encapsulate drugs and provide controlled release (Dey *et al.,* 2016; Mura *et al.,* 2013). These nanosponges can be engineered to target specific sites within the body, making

them ideal for delivering drugs to the colon, where they can act directly on the site of infection (Raval *et al.,* 2018). The colon-targeting ability of nanosponges is achieved by optimizing their size, surface properties, and the use of pH-sensitive polymers that release the drug specifically in the colon's acidic environment (Liu *et al.,* 2015).

Diloxanide furoate, a nitroimidazole derivative, has shown effectiveness in treating amoebic infections by inhibiting the growth of *E. histolytica* and is often used in combination with other antiprotozoal drugs to enhance treatment efficacy (Khan *et al.,* 2014). However, its effectiveness is limited by its poor solubility and rapid degradation in the gastrointestinal tract. By loading Diloxanide furoate into colon-targeting nanosponges, it is possible to enhance its stability and release the drug specifically at the site of infection, improving therapeutic outcomes and reducing systemic side effects (Khan *et al.,* 2020; Rahman *et al.,* 2018).

This study focuses on the formulation of Diloxanide furoate-loaded colon-targeting nanosponges to enhance the effectiveness of amoebiasis treatment. The nanosponges are designed to release Diloxanide furoate in the colon, where it can exert its therapeutic effects directly. The formulation process involves the use of various polymers and cross-linking agents to create a stable and effective drug delivery system. The study will evaluate the nanosponges for drug entrapment efficiency, release kinetics, and targeting capability to determine their potential as an advanced therapeutic option for amoebiasis.

MATERIALS AND METHODS

Preparation of Nanosponges

The nano-sponges containing Diloxanide furoate were formulated by a method called the quasi-emulsion solvent diffusion (Jain and Singh, 2010). The accurately weighed amount of polymethyl-methacrylate (PMMA), Eudragit S-100 in different ratios with dibutyl phthalate (1% w/v) was dissolved in 10 mL of dichloromethane: methanol (50:50). Dibutyl phthalate was incorporated to increase the polymer plasticity. Diloxanide furoate was dissolved in this mixture. At the next, 0.5-1.5% w/v solution with distilled water was prepared as dispersing media. The previously prepared solution of polymers and drug was added gradually in PVA solution and stirring was kept constant for 2 hours. After complete evaporation of solvent from polymer droplets, nano-sponges were formed, which were centrifuged at 4000 rpm for collection and followed by 3 times washing. The solvent was slowly removed to form the nanosponges. The aqueous suspension of nanosponges was lyophilized and stored in a tightly sealed container until further analysis. The optimization of the formulation of Diloxanide furoate loaded nanosponges was given in Table 1.

Characterization of nanosponges

Carr's Index and Hausners ratio

The Carr's Index and Hausners ratio were determination using formula:

$$Compressibility\ Index = 100 \times \left(\frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \right)$$

$$Hausner\ \ Ratio = \left(\frac{\rho_{tapped}}{\rho_{bulk}} \right)$$

Tapped density was calculated by placing 5 gm of the nanosponges in a graduated cylinder tapping it for 100 times. Poured density was calculated by placing 5 gm of nanosponges into a graduated cylinder and measuring the volume (Arya and Pathak, 2014). The standard result and experimental result of Carr's Index and Hausners ratio are shown in table 2.

Determination of production yield

The production yield of the nanosponges was determined by calculating the initial weight of the raw materials and the final weight of the nanosponges obtained (Tamkhane and Sharma, 2014). All the experiments were performed in triplicate and the mean of the each value was reported.

Actual drug content and encapsulation efficiency

The weighed amount of drug loaded nanosponges (100 mg) was suspended in 100 ml 7.2 pH Phosphate Buffer and subjected to intermittent stirring. The sample was filtered using 0.45_m membrane filter and analyzed at 301.0nm against blank using UV spectrophotometer (Labindia, 3000+). All analyses were carried out in triplicate. The results of actual drug content and encapsulation efficacy are shown in Table 4.

Actual drug content (%) $=M_{act}/M_{ms} \times 100$

Encapsulation efficiency (%) $=M_{act}/M_{the} \times 100$

Where M_{act} is the actual drug content in nanosponges, M_{ms} is the total amount of the nanosponges and M_{the} is the amount of drug added to the nanosponges. All analyses were carried out in triplicate.

Surface charge and vesicle size

The Particle size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the nanosponges was located on the zeta potential that was determined according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

***In vitro* drug release in gastrointestinal fluids of different pH**

The prepared nanoparticles were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus (Wagner, 1969; Gibaldi and Feldman, 1967; Higuchi, 1963). The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- *1st hour:* Simulated gastric fluid (SGF) of pH 1.2.
- *2nd and 3rd hour:* Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- *4th to 5th hour:* Simulated intestinal fluid (SIF) of pH 6.8.
- *6th hour and onward:* SIF pH 7.2

A weighed quantity of formulation (equivalent to 30mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 280 nm for percent

of release Diloxanide furoate using UV visible spectrophotometer. The release of Diloxanide furoate was calculated with the help of Standard curve of Diloxanide furoate.

RESULTS AND DISCUSSION

The formulation and evaluation of Diloxanide furoate-loaded colon-targeting nanosponges reveal key insights into their effectiveness for treating amoebiasis. The results provide a comprehensive understanding of the nanosponges' physicochemical properties and their potential benefits in targeted drug delivery.

The characterization of flow properties, detailed in Table 3, indicates that the nanosponges exhibit acceptable flow characteristics. With Carr's Index values ranging from 23.11% to 26.99%, the formulations show moderate to good flowability. The Hausner's Ratios, which are all below 1.2, further support the notion of good flow properties, making these nanosponges suitable for formulation into solid dosage forms. These properties are crucial for ensuring uniformity and consistency in drug delivery.

The data in Table 4 demonstrates that the actual drug content of the nanosponges ranges from 90.25% to 99.12%, reflecting effective drug incorporation. Encapsulation efficiency values, ranging from 73.32% to 83.32%, highlight the ability of the nanosponges to retain the drug. Formulation F3, with the highest encapsulation efficiency of 83.32%, appears to be the most effective at trapping Diloxanide furoate, suggesting it will likely provide the most reliable therapeutic effect.

Formulation F3, characterized in Table 5, has an average particle size of 52.74 nm, which is favorable for ensuring drug stability and controlled release. The encapsulation efficiency of F3 at 83.32% and a zeta potential of -20.2 mV indicate good stability of the nanosponges due to electrostatic repulsion among particles. This stability is important for maintaining the quality and effectiveness of the nanosponges over time.

The cumulative drug release data (Table 6) show that the nanosponges release Diloxanide furoate in a controlled manner. In simulated gastric fluid (SGF) at pH 1.2, the release is minimal initially, which is beneficial for protecting the drug from premature release in the stomach. As the medium shifts to simulated intestinal fluid (SIF) at pH 7.2, the drug release increases gradually, reaching 98.78% by 12 hours. This controlled release profile aligns with the goal of delivering the drug specifically in the colon, where it is most effective.

The regression analysis data (Table 7) for formulation F3 indicate that the drug release follows a controlled release mechanism. The high R^2 values for zero-order (0.9752) and Pappas plot (0.9737) suggest a predictable, zero-order release kinetics, which is advantageous for sustained drug delivery. This suggests that F3 maintains a consistent release rate over time, improving therapeutic efficacy.

Table 1: Composition of nanosponges formulations

Components	Formulation code/amount					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Diloxanide furoate (mg)	100	100	100	100	100	100
Eudragit S-100 (mg)	25	50	75	25	50	75
PMMA (mg)	10	10	10	20	20	20
PVA (%)	0.5	1.0	1.5	0.5	1.0	1.5
Dibutyl phthalate (%)	1	1	1	1	1	1
Dichloromethane: methanol (50:50) (ml)	10	10	10	10	10	10
Distilled water	20	20	20	20	20	20

Table 2: Standard values of Angle of repose, Carr's index and Hausner's ratio

S. No.	Angle of repose	Carr's index	Hausner's ratio	Type of flow (Inference)
1	<20	5-15	-	Excellent
2	20-30	12-16	<1.25	Good
3	30-40	18-21	-	Passable
4	-	23-35	>1.25	Poor
5	-	33-38	1.25-1.50	Very poor
6	>40	>40	-	Extremely poor

Table 3: Characterization of flow properties of Diloxanide furoate nanosponges

Formulation Code	Evaluation parameters			
	Poured Density (gm/cm^3)	**Tapped Density (gm/cm^3)**	**Carr's Index (%)**	**Hausners Ratio**
F1	0.345	0.459	24.84	0.114
F2	0.352	0.462	23.81	0.110
F3	0.349	0.478	26.99	0.129
F4	0.362	0.472	23.31	0.110
F5	0.354	0.468	24.36	0.114
F6	0.356	0.463	23.11	0.107

*** Mean±S.D. (n=3)**

Table 4: Result of actual drug content and encapsulation efficiency

Formulation code	Actual drug content (%)	Encapsulation efficiency (%)
F1	90.25±0.32	73.32±0.36
F2	93.32±0.25	76.65±0.25
F3	99.12±0.14	83.32±0.14
F4	98.82±0.25	75.44±0.21
F5	93.65±0.20	79.98±0.41
F6	96.32±0.15	76.52±0.32

Table 5: Characterization of Optimized formulation of nanosponges

Optimized formulation	Average Particle size (nm)	% Encapsulation efficiency	Zeta Potential (mV)
F-3	52.74±1.05	83.32±0.14	-20.2

Table 6: Cumulative % drug release of Diloxanide furoate nanosponges at different pH

S. No.	Dissolution medium	Time (hrs)	% Cumulative drug release
1	SGF (pH 1.2)	1	4.56
2		2	7.85
3	SIF (pH 7.2)	3	16.65
4		4	31.25
5		5	45.65
6		6	59.98
7		7	64.47
8		8	73.32
9		9	81.15
10		10	89.98
11		12	98.78

Table 7: Regression analysis data of nanosponges formulation

Formulation	Zero order	First order	Pappas plot
F3	$R^2 = 0.9752$	$R^2 = 0.8308$	$R^2 = 0.9737$

CONCLUSION

The formulation of Diloxanide furoate-loaded colon-targeting nanosponges shows promising results for treating amoebiasis. The optimized formulation (F3) demonstrates favorable flow properties, high drug encapsulation efficiency, suitable particle size, and a controlled release profile. These characteristics make F3 a viable candidate for effective colon-targeted drug delivery, potentially enhancing therapeutic outcomes and reducing side effects associated with traditional formulations.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Arya, P. & Pathak, K. (2014) Assessing the viability of microsponges as gastro retentive drug delivery system of curcumin: Optimization and pharmacokinetics. *International Journal of Pharmaceutics*, 460, 1–12.
- Dey, S., Singh, S., Singh, R. & Yadav, M. (2016) Nanosponges: A novel drug delivery system. *Journal of Nanoscience and Nanotechnology*, 16, 1982–1995.
- Gibaldi, M. & Feldman, S. (1967) Establishment of sink conditions in dissolution rate determinations: Theoretical considerations and application to non disintegrating dosage forms. *Journal of Pharmaceutical Sciences*, 56, 1238–1242.
- Gupta, A., Sharma, S. & Sharma, A. (2012) Nanoparticle-based drug delivery system for treatment of amoebiasis. *International Journal of Pharmacy and Biological Sciences*, 3, 103–110.
- Haque, R. (2007) Human amoebiasis: Clinical features, diagnosis, and management. *International Journal of Infectious Diseases*, 11, 130–135.
- Higuchi, T. (1963) Mechanism of sustained-action medication: Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences*, 52, 1145–1149.
- Jain, V. & Singh, R. (2010) Dicyclomine-loaded Eudragit®-based microsponge with potential for colonic delivery: Preparation and characterization. *Tropical Journal of Pharmaceutical Research*, 9.
- Khan, M.F., Ali, M.A. & Bibi, H. (2014) Development and evaluation of colon targeted delivery systems for amoebiasis. *Journal of Controlled Release*, 183, 125–134.
- Khan, M.F., Bibi, H. & Ali, M.A. (2020) Formulation and characterization of colon-specific nanosponges for diloxanide furoate. *Drug Delivery and Translational Research*, 10, 1072–1081.
- Liu, X., Zhang, L., Wang, Y. & Liu, Y. (2015) pH-sensitive nanocarriers for colon targeted drug delivery. *Advanced Drug Delivery Reviews*, 92, 30–49.
- Minocha, A., Singh, M. & Patel, P. (2016) Nanoparticle-based drug delivery for amoebiasis treatment. *Journal of Drug Delivery Science and Technology*, 31, 161–169.

- Mura, S., Nicolas, J. & Couvreur, P. (2013) Nanocarriers as a new therapeutic approach for the delivery of anticancer drugs. *European Journal of Medicinal Chemistry*, 70, 72–91.
- Rahman, M., Rahman, M. & Hasan, M. (2018) Development and evaluation of colon-specific drug delivery systems using nanosponges. *Journal of Pharmaceutical Sciences*, 107, 1101–1111.
- Raval, J., Patel, A. & Shah, D. (2018) Targeted drug delivery systems: A review of the recent advances. *Drug Delivery*, 25, 143–152.
- Reddy, A.G. & Rao, P.B. (2010) Amoebiasis: Clinical and therapeutic overview. *Current Drug Targets*, 11, 1127–1134.
- Tamkhane, V. & Sharma, P.H. (2014) Nanosponge-A novel drug delivery system. *International Current Pharmaceutical Journal of Research*, 4, 1186–1193.
- Wagner, J.G. (1969) Interpretation of percent dissolved-time plots derived from *in vitro* testing of conventional tablets and capsules. *Journal of Pharmaceutical Sciences*, 58, 1253–1257.