Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M.P.), India

Influence of different generations of poly(propylene imine) dendrimers on human erythrocytes

V. MISHRA, U. GUPTA, N. K. JAIN

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Narendra K. Jain, Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar (M.P.) India – 470003
jnarendr@yahoo.co.in

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The unique characteristics of dendrimers make them attractive candidates as drug carriers. However, the toxicity associated with dendrimers is a basic hurdle in their biomedical application. To ensure development of dendrimer based safe and effective delivery systems, the effect of dendrimers on human erythrocytes (RBCs) must be studied. The present study explores the toxicological behavior of different generations of poly(propylene imine) dendrimers on human RBCs. Plain fifth generation PPI dendrimers (1 mg/mL) showed approximately 6.39% hemolysis which was an indication of their suitability in drug delivery. The study was conducted on all generations from 0.5 to 5.0G of PPI dendrimers.

1. Introduction

Considering the ineffectiveness of current drug delivery system there is a need to develop a suitable delivery system that distributes the therapeutically active drug molecule only to the site of action, without affecting healthy organs and tissues. "Nanomedicine" is the best solution to achieve this target, which not only helps in lowering doses required for efficacy but also increases the therapeutic indices and safety of the new drug. Various nanoscale systems are designed for drug delivery which involves mainly carriers constructed by the use of polymers. As polymers is the soul of this particular kind of carriers, possible application of several polymers has been extensively researched and dendrimer is one of the new class of nanoscopic polymeric containers used widely as drug delivery devices (Koo et al. 2005). There are several requirements for developing a device small enough to efficiently perform multiple functions.

Dendrimers are synthetic polymers with well defined biocompatible, spherical structures ranging from 1 to 10 nanometers in diameter. Dendrimers offer particular advantages including their nanoscale spherical architecture, lower polydispersity and the possibility to modify their surface. The empty intra-molecular cavity can be used for host entrapment providing opportunities for controlled drug release (Duncan et al. 1996; Malik et al. 2000; D'Emanuele and Attwood 2005). Any polymeric carrier, must be non-toxic, non-immunogenic, and biocompatible to be suitable as drug carrier. Dendrimers have proved their potential as drug delivery agents and in this sequence, understanding of biological compatibility of these carriers becomes essential for their ultimate acceptance as drug carriers (Duncan and Izzo 2005; Mishra et al. 2009).

To discuss dendrimer safety there is a need to consider the likely route and frequency of administration in the proposed application. Intravenous administration is one of the major routes used for parenteral targeted delivery application. In this case, when the carrier is administered it is likely to interact with the cells and other components present in the blood, which may affect the availability of the active molecules at the required site.

Red blood cell lysis is a simple method to study polymer-blood cell membrane interaction. It can give a qualitative as well as a quantitative indication of potential damage to RBCs by administered formulation of dendrimer. It gives a quantitative measure of haemoglobin (Hb) release (Sgouras and Duncan 1990).

This study was undertaken to examine hemolytic toxicity aspects of different generations of poly(propylene imine) (PPI) dendrimers.

2. Investigations, results and discussion

The present work was undertaken in order to study whether the poly(propylene imine) dendrimers have any effect on the morphology of human red blood cells. The PPI dendrimers were synthesized up to generation 5 taking ethylenediamine as core and were characterized.

Synthesis of 0.5G PPI was confirmed by IR peaks, mainly of nitrile at 2247 cm^{-1}. All the nitrile terminal 0.5G PPI got converted into full-generation dendrimer (amine terminal), which was confirmed by IR of PPI (1.0 G), that give major peak at 3363.5 cm^{-1} of amine (asymmetric stretch of N-H). Further synthesis of 5.0G PPI dendrimer was similarly confirmed by IR peaks for -CH_2 rocking (612.9 cm^{-1}); N-H bending vibrations (1651.8 cm^{-1}); C-H asymmetric stretch (2948.3 cm^{-1}). Weak peak of C-N stretch of nitrile (2130.0 cm^{-1}) and N-H stretch of 1^0 amine (3401.7 cm^{-1}) confirming most of the nitrile terminal groups of dendrimer were converted to amine terminals. The results matched with the reported synthesis of PPI dendrimers (De Brabander-Van den Berg and Meijer 1993).

The deformability of normal human erythrocytes and its ability to survive in the microcirculation depend on the geometry of the cell. Human erythrocytes, are, in the normal state regular biconcave discs. Changes in surface morphology of red blood cells after interaction with increasing generations (0.5G to 5.0G)

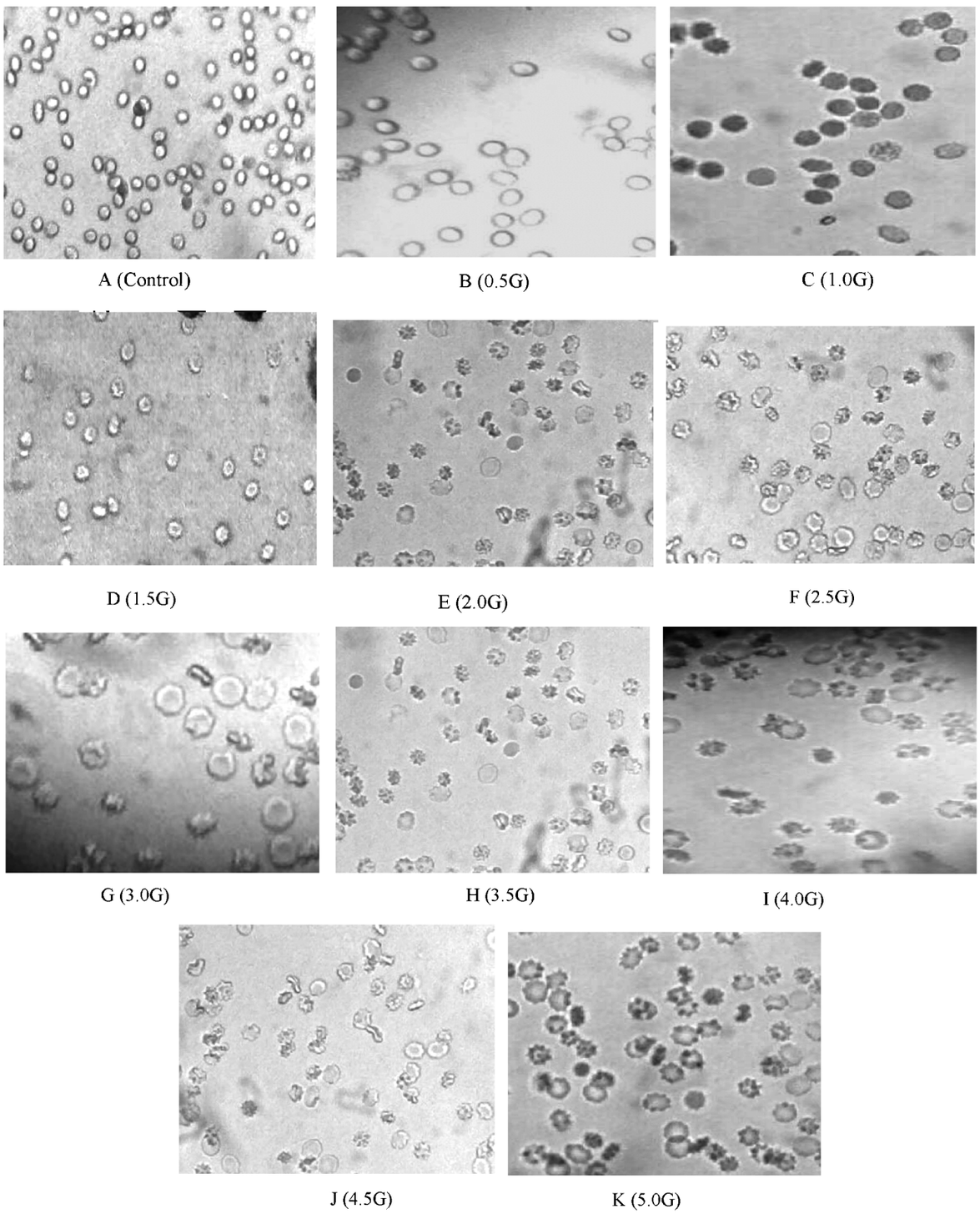

Fig. 1: (A-K) RBCs Photographs showing surface morphology under influence of different generations of PPI dendrimers in 1 mg/mL concentration

of synthesized PPI dendrimers were studied by keeping dendrimers concentration (1 mg/mL) constant. The results showed that when the generation number of PPI dendrimers increases, the cells display the changes in shape as compared with red blood cells in normal saline as shown in Fig. 1 (A-K).

Along with the changes in contour of RBCs, the hemolysis of erythrocytes was also studied at 10% hematocrit value and dendrimers solution of different generations (Fig. 2). Fifth generation of PPI dendrimers led to approximately 6.39% hemolysis which indicates that they can be used up to a concentration of 1 mg/mL. These findings are similar to the results of previously reported studies (Malik et al. 2000).

These outcomes suggest that with the change in shape and percent hemolysis values, some interaction between dendrimers (Figs. 3 and 4) and erythrocytes occurs as the consequence of charge present on cells as well as dendrimers. It has already been reported that cationic dendrimers are more cytotoxic than anionic ones. Dendrimer-mediated hemolytic toxicity is influ-

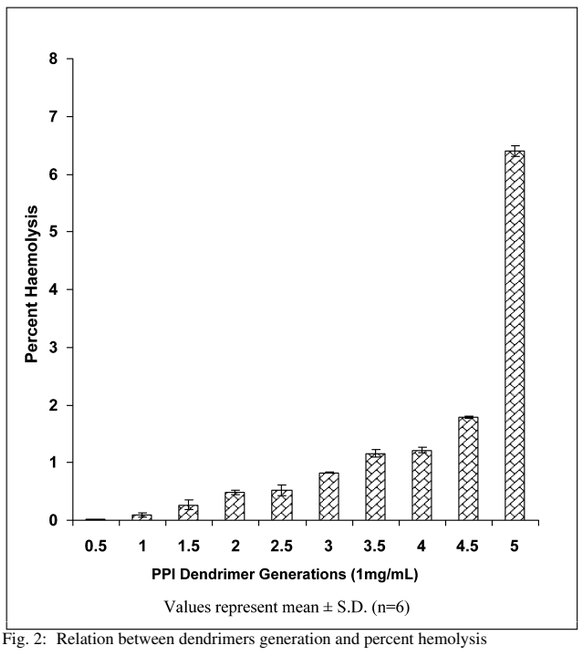

Fig. 2: Relation between dendrimers generation and percent hemolysis

enced by the nature of terminal groups on dendrimer periphery. Further, the change in shape of red blood cells may also be attributed to the interaction with proteins.

On the basis of effects on surface morphology of erythrocytes and hemolytic toxicity investigations, it can be concluded that, 5.0G PPI dendrimers (up to a concentration of 1 mg/mL) showed promising performance to be developed as a carrier system for exploring its further biomedical application in intravenous drug delivery.

This study explores the potential of PPI dendrimers for their use as drug delivery vehicle. However further research can be concentrated on the surface engineered dendrimers for drug delivery. Surface engineering of dendrimers shields the cationic charge, which prevents the interaction of RBCs with dendrimers and thereby leads to a decrease or complete removal of the cationic surface, which reduces hemolysis and results in better hematological profiles as compared to parent dendrimers.

3. Experimental

3.1. Materials

Ethylenediamine and Raney Nickel were purchased from Merck, India. Acrylonitrile was purchased from Central Drug House (CDH), India. Methanol was purchased from Rankem, Chemical Division of Ranbaxy

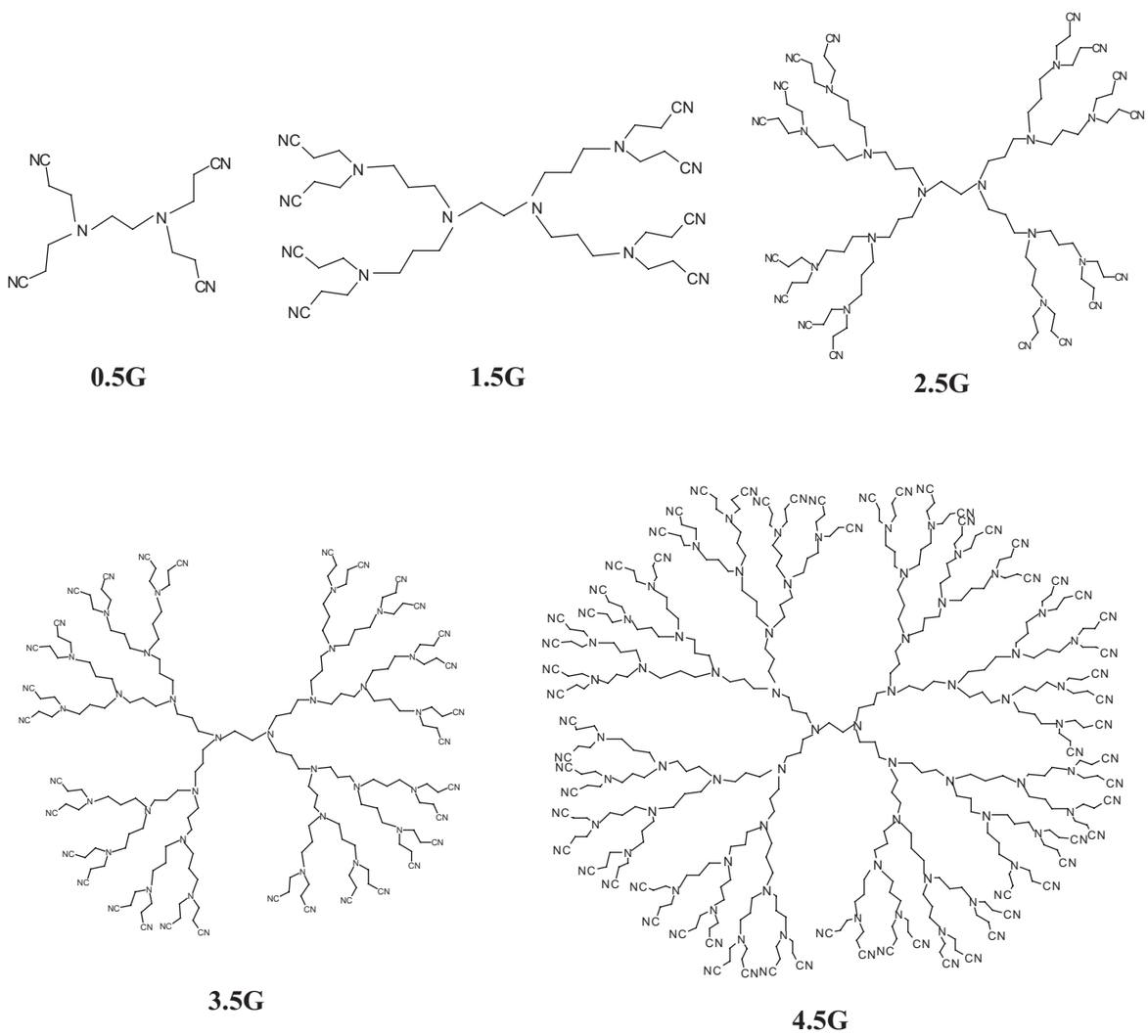

Fig. 3: Chemical structures of half-generation poly(propylene imine) dendrimers

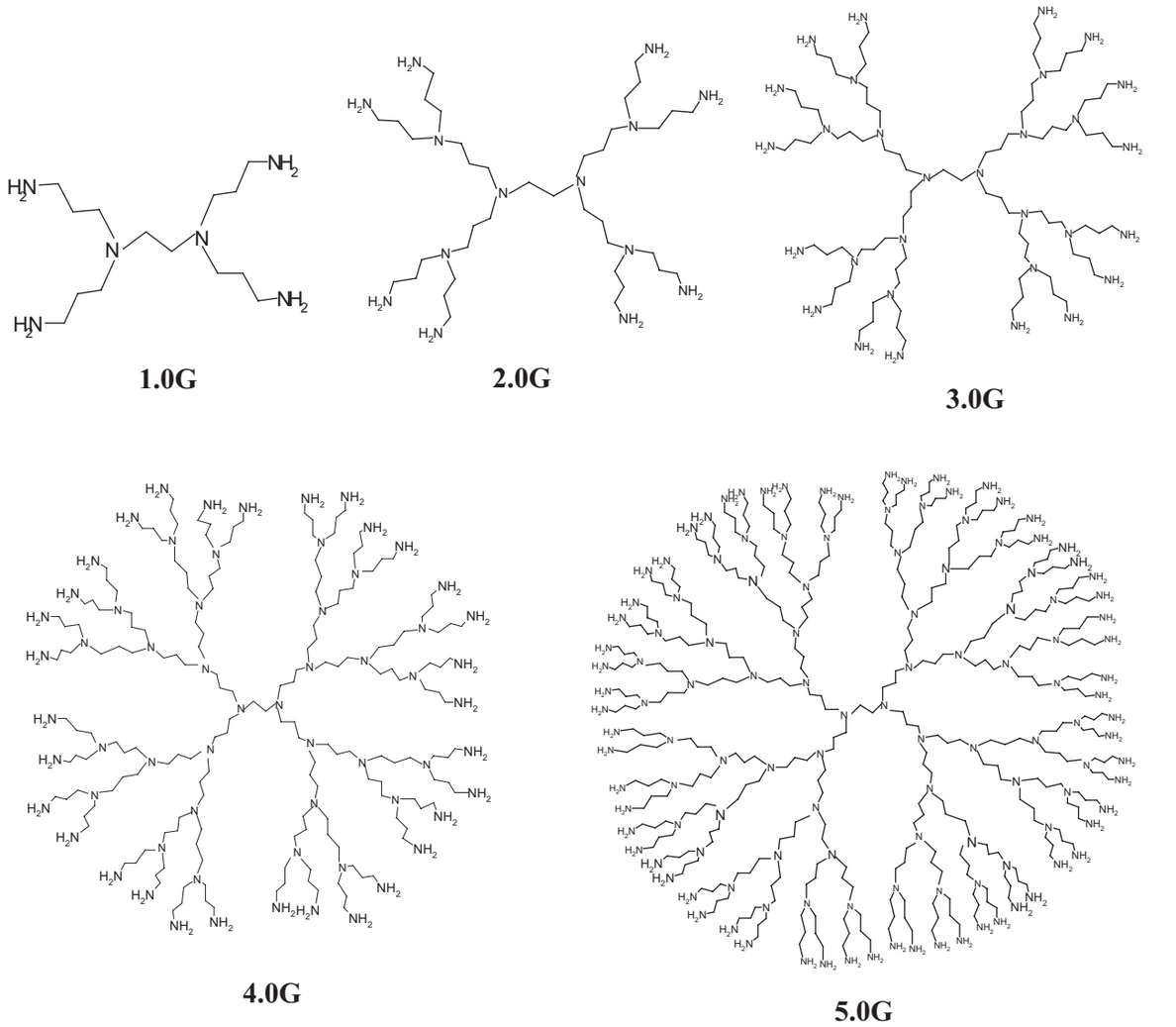

Fig. 4: Chemical structures of full generation poly(propylene imine) dendrimers

Labs, Mohali, India. All the other chemicals were purchased from Himedia Lab., India. All the chemicals used were of analytical grade.

3.2. Methods

Poly(propylene imine) dendrimers were synthesized following the scheme given by De Brabander-Van den Berg and Meijer (1993). In this sequence, ethylenediamine (EDA) is used as dendrimer core. The half-generation [EDA-dendr-(CN)4n], (where n is generation of reaction or reaction cycle) was synthesized by double Michael addition reaction between acrylonitrile and aqueous solution of ethylenediamine. The full-generation [EDA-dendr-(NH2)4n], was prepared by hydrogenation of the half-generation dendrimer. The PPI dendrimers up to 5.0G were synthesized by repetition of all the steps consecutively, with increasing quantity of acrylonitrile (De Brabander-Van den Berg and Meijer 1993).

The prepared PPI dendrimers were characterized by IR spectroscopy using the KBr pellet method (Perkin Elmer, USA) and by ^{1}H NMR spectroscopy (FT NMR Spectrometer model Avance-II Bruker, Germany) at a working frequency of 400.1324008 MHz. The characterization of dendrimers was done as reported earlier (De Brabander-Van den Berg and Meijer 1993; Jansen et al. 1994; Bhadra et al. 2005; Gupta et al. 2007). Characterization data of synthesized dendrimers is shown in the Table.

Hemolytic toxicity was studied as reported, with slight modification (Domanski et al. 2004; Singhai et al. 1997). Briefly, blood from a healthy donor was obtained and kept in a K_3EDTA blood collection tube (Piove Di Sacco, Italy) containing K_3EDTA as anticoagulant. RBCs were separated by centrifugation, washed with phosphate buffer saline (PBS) pH 7.4 and resuspended in PBS pH 7.4. Phosphate buffer saline was prepared following the official method (Indian Pharmacopoeia 1996). Disodium hydrogen phosphate (2.38 g), potassium dihydrogen phosphate (0.19 g) and sodium chloride (8.0 g) were dissolved in sufficient distilled water and volume was made up to 1 L with distilled water. The pH was adjusted to 7.4 prior to the experiment.

Erythrocytes were used immediately after isolation. Suspension of red blood cells in PBS was treated with different generations of dendrimers at a fixed concentration (1 mg/mL). Then cell samples were viewed under a Leica

Table: Characterization data of synthesized poly(propylene imine) dendrimers

Generations	No. of –CN terminal groups	No. of –NH_2 terminal groups	Molecular weight
0.0G	–	2	60
0.5G	4	–	272
1.0G	–	4	288
1.5G	8	–	713
2.0G	–	8	746
2.5G	16	–	1594
3.0G	–	16	1658
3.5G	32	–	3356
4.0G	–	32	3486
4.5G	64	–	6881
5.0G	–	64	7140

optical microscope using a magnification of 400x and 630x for the morphological condition of RBCs.

To study the effects of dendrimers on hemolysis of erythrocytes, the systems containing 0.1 mL of RBCs suspension (10% hematocrit value) and 0.9 mL of dendrimers solution of different generations having a concentration of 1 mg/mL in PBS (pH 7.4) were incubated at 37 °C for 30 min and then the mixtures were centrifuged at 3000 rpm for 10 min to remove nonlysed RBCs. The supernatant was analyzed spectrophotometrically (1601 UV-Vis spectrophotometer, Shimadzu, Japan) at 540 nm (n = 6). To obtain 0 and 100% hemolysis, 0.1 mL RBC suspension was added to 0.9 mL of 0.9% NaCl solution (normal saline) and 0.9 mL distilled water, respectively (Kumar et al. 2006; Agrawal et al. 2007). The degree of hemolysis was determined by the following equation:

$$\text{Hemolysis}(\%) = \frac{\text{Abs} - \text{Abs}_0}{\text{Abs}_{100} - \text{Abs}_0} \times 100 \quad (1)$$

where Abs, Abs_0 and Abs_{100} are the absorbance of sample, a solution of 0% hemolysis and a solution of 100% hemolysis, respectively.

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